# Comparative Neuroscience Holds Promise for Quiet Revolutions

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*Summary.* The brain has diversified and advanced in evolution more than any other organ; the variety of nervous systems and behaviors among animal species is thus available for our exploitation. Comparative neuroscience is likely to reach insights so novel as to constitute revolutions in understanding the structure, functions, ontogeny, and evolution of nervous systems. This promise requires pursuit on a wide front, in respect to disciplines and in respect to the species, stages, and states compared. It also requires deliberate concentration on the differences among animals, in addition to the prevailing concern for the basic and common. Neglect of these challenges would be costly. Without due consideration of the neural and behavioral correlates of differences between higher taxa and between closely related families, species, sexes, and stages, we cannot expect to understand our nervous systems or ourselves.

Long before the human species or even the vertebrates appeared, the greatest achievement of evolution was the brain. Within the natural order only systems of brains, constituting social systems, are more complex. The brain mediates virtually all that multicellular animals do in relation to each other and the outer world. We can look at it that everything else in the body has evolved to maintain and reproduce the behavior machine—that is to enable animals to *act*. The common conclusion that behavior and metabolism are no more than means to the end of reproducing and disseminating DNA deserves reformulation from the perspective of the animals that evolution has produced.

In the animal world of dozens of phyla, hundreds of orders, and millions of species, two primary features are the variety of brains and the variety of ways animals act. Three domains of differences are (i) those between distantly related animals such as goldfish and monkeys, or snails and squid; (ii) those between closely related taxa, such as species of monkeys; and (iii) those between the sexes and between ontogenetic stages. The range of complexity of the brain spans the greatest spectrum of any organ system. Between jellyfish and humans, nothing else has advanced as much as the nervous system and behavior.

This evolution was not in one line or ladder but in many radiations of descendant stocks. Nevertheless, it is a mistake to deny lower and higher forms, inasmuch as most members of later-appearing classes are more advanced than earlier classes, have more distinguishable parts and processes, and larger repertoires of behavior. Within the whole apparatus for behavior—receptors, effectors, endocrine glands, and nervous system—the last, and particularly the head ganglion of the central nervous system, has the principal complexity and diversity.

It is the thesis of this article that we cannot expect truly to comprehend either ourselves or how the nervous system works until we gain insight into this range of nervous systems, from nerve nets and simple ganglia in sea anemones and flatworms to the optic lobes of dragon flies, octopuses, and lizards, to the cerebral cortex in primates. Likewise, within a given level of complexity, such as the order Rodentia, we must gain some insight into the neural differences that underlie radiation in behavior—for example, the differences in the behaviors of beavers, kangaroo rats, flying squirrels, porcupines, capybaras, and mole rats. If we look only for the basic biological and general cellular mechanisms, it is in the nervous system where we will fall farthest short of explaining the full range of accomplishments, although discovering fundamental and valuable information. Comparison of diverse animals, states, or stages is essential, particularly for establishing structure-function relationships in the brain.

We will really be on the road to understanding how the brain achieves the functions for which it evolved when we ask two questions: (i) What are the neural correlates relevant to known behavioral differences among animals (such as slothfulness or tameness)? and (ii) What are the behavioral correlates relevant to known neural differences (such as lamination of the tectum and size of the cerebellum)? Though difficult, these two strategic questions are promising, drawing as they do on the reservoir of diversity of brains among taxa, developmental stages, and individuals.

### What Is Comparative Neuroscience?

The ancient question is still awaiting an answer: What features in our brain account for our humanity, our musical creativity, infinitely varied artifacts, subtlety of humor, sophisticated projection (in chess, politics, and business), our poetry, ecstasy, fervor, contorted morality, and elaborate rationalization? (The qualifiers are important; it is not the same to show that a chimpanzee can lie or laugh.) As anatomical features, the planum temporale and higher proportion of stellate cells in the isocortex may be better candidates than their predecessors: the hippocampus minor of Richard Owen, the pineal of Descartes, the spinal marrow of Plato, the third ventricle of Augustine, and the fourth ventricle of Herophilus of Alexandria. But it is not clear that they are adequate candidates. Our principal advance has been in excluding those earlier suggestions and in throwing serious doubt on others, such as either absolute or relative numbers of neurons or size of centers. Although there is no promise of an easy solution to the neural basis of humanity, we can work on many fronts toward neural bases of behavioral differences among a wide range of invertebrates and vertebrates.

Comparative neurology, quietly accumulating a formidable literature during the past century, has unfortunately acquired the connotation of comparative anatomy. It is time to emphasize the importance of all the relevant disciplines, including physiology, chemistry,

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pharmacology, ontogeny, ethology, and cognitive psychology from the perspective of exploiting differences among animals. Neuroscience is part of biology, more specifically of zoology, and it suffers tunnel vision unless continuous with ethology, ecology, and evolution.

### Aims of Comparative Neuroscience

Besides providing a broadening enrichment (stories of brain and behavior in octopus and owl are the most enthralling of mental trips), and unpredictable implications for artificial intelligence, education, and betterment of the human condition (*1*), an agenda for a first approximation for comparative neuroscience is easy to state. One is tempted to say, "We basically need to learn what kinds of nervous systems some representative taxa have anatomically, physiologically, and chemically." This might well give the data base for improved statements about what evolution has achieved—a large enough agenda for descriptive research, and embodying ample technical challenge. (But which fish or mammal or primate is representative?) There is the danger, however, that this agenda could preoccupy attention, delaying confrontation of some broad conceptual challenges. These challenges, like the real aims and promises of comparative neuroscience, are multiple.

I have elsewhere (*2*) grouped these aims under three headings—roots, rules, and relevance—which coexist and overlap in many studies. Some comparative neurology is looking for phylogenetic roots, for insights into evolutionary history: What is older and what is newer in our brain? What structures or processes in a shark or frog are homologous to our amygdala or to kindling? How is our brain similar to and different from that of other primates? What has evolution brought about?

Some comparative neurology is looking for rules, principles, or generalizations: Are there systematic correlates of the size of the optic lobe, cerebellum, or caudate nucleus? Are there general statements about the incidence or use of presynaptic inhibition, of spikeless neurons, of "recognition cells" selective for complex features, of particular transmitters or modulators?

Some comparative neurology is seeking clues of relevance to human conditions, such as normal or abnormal development or disease states, asking what specially favorable species reveal about how the brain works. Can we extrapolate to humans from kittens that require more or lambs that require less visual experience to develop their first stereoscopic cells? Many basic discoveries have been made on specially favorable species: sodium channels, electrical synapses, presynaptic inhibition, heterosynaptic facilitation, plateau potentials, and feature-selective cells—to name a few, picked from the cellular level and the physiological domain. The three headings underline the heterogeneity of approaches, questions and contributions in the face of a common monism: "*The* question is . . .".

The following examples of studies on various species are arbitrarily confined to the anatomy and physiology of cells or groups of cells. I have neglected membrane, metabolic, and molecular levels in this article.

### Examples from Invertebrates

Coelenterates have attracted a burst of activity in recent years which has markedly altered our understanding of the most primitive nervous systems. Dominated by a nerve net without a centralized nervous system and by neurons without axon-dendrite differentiation, they already show ganglia, inhibition, one-way transmission, electrical and chemical synapses with diverse facilitation requirements, tendencies to iterative discharge, endogenous patterned discharge, and other specialized properties permitting a considerable range of behaviors between hydroids, corals, anemones, and jellyfish. Puzzles remain that have significance for the neurobiology of other animals. One is the nonneural, epithelial, or muscular conduction systems that function in coelenterates in parallel with neural systems and that simulate them closely. A second is the recurring cycle of drastic changes in activity and responsivity reminiscent of moods in beings as simple as sea anemones.

Annelids, arthropods, and mollusks account for most of the dramatic advances in invertebrate neurology. Their availability and suitability, the great number of species and range of complexity from earthworms and leeches to bees, lobsters, slugs, and squids have contributed to the variety of fundamental issues they have illuminated. Indeed the cumulative changes they have induced in our view of the well-developed nervous systems of higher invertebrates can only be called revolutionary. I will mention only two such changes.

### Identifiable Neurons

One component of the newer view is the gradual realization, from the late 1960's onward, that at least major parts of the central nervous system of many members of each of these phyla (especially leeches, insects, crustaceans, pulmonates, and opisthobranch gastropods, but not all invertebrates) consist largely of neurons individually identifiable in every specimen of the species, by input and output connections, position, form, branching, chemical and pharmacological properties, and what may be called *personality*, that is, patterns of spontaneous activity and response to input. Most such cells are unique, and some researchers expect that the majority of central interneurons as well as motor neurons in these taxa will be identifiable. Such a result would imply that, given the normal developmental environment, not only the cell bodies but also the afferent and efferent processes and synapses are determined; the neuropil then is not a quasi-random tangle of fibers and synapses but is highly specified at the level of functional connections and dynamic properties. Study of branching patterns has revealed variation in detail, reminiscent of that between genetically identical trees, which indicates that the consistent specification at this level is relational rather than absolute. Like the isogenic willows, maples, and orange trees, each recognized by a trained eye as belonging to its variety even though individual leaves, twigs, and branches are not duplicated, a three-dimensional constellation of the hundreds or thousands of points of contact (the electron microscopist's synapse) between pre- and postsynaptic neurons that make up each physiological synapse must have characteristic statistical relations.

This radical concept of identifiable nerve cells, well established for certain annelids, arthropods, and gastropods and for a dozen or so neurons known so far in lampreys and bony fishes, would initially seem to be opposite to the usual view of the mammalian brain as a redundant and probabilistic array of large numbers of cells. A closer look suggests instead a continuum of intermediate situations in which relatively well-specified cells are not unique but occur in sets of 2, 3, 4, 5, to 50, 500, or more; these cells would be essentially indistinguishable from each other by any criterion, but differ in some appreciable way, even though slightly, from all other such *equivalence sets* (*3*). These sets can be estimated from the best-studied parts of

the mammalian cerebral cortex, thalamus, spinal cord, retina, cochlea, and skin to contain commonly between 5 and 40 nerve cells; in the granule cell layer of the cerebellum they may contain about 100. There seems to be no reason to assume any equivalence sets larger than about 1000 neurons (approximately the number in 0.01 $mm^3$ of our cortex). From the estimate of the probability distribution of set sizes one can calculate the total number of sets, which represents the number of distinguishable kinds of nerve cells, on any and all criteria, including input and output connections. For the gastropod *Aplysia* (400 g), this number of sets is approximately 600; for a lobster of this size, 4000; for a rat of the same size, $7 \times 10^4$; for the human, $5 \times 10^7$. These compel a distinction: they represent estimates of one form of redundancy, namely fully equivalent neurons (within our criterion of minimum appreciable difference). Another form of redundancy is at least as important, namely the partial overlap of input or output fields of neighboring units that are sufficiently different to belong to separate equivalence sets.

The crude guesses and large extrapolations in this exercise are justified by the interest of the conclusion that (far beyond the identifiable cells of some invertebrates, which probably reach a few thousand kinds in lobsters, insects, and gastropods) the number of specified neurons in small sets may reach tens of millions of kinds in man. The notion of comparing invertebrates and vertebrates, as well as lower and higher vertebrate brains has led to the tentative proposition that we do not have masses of millions of essentially equivalent cells. Note that this new view is based not only on static anatomy but also on dynamic "personality" properties that define cell types.

## Circuits and the Circuit Concept

A complementary aspect of the recent revolution is the success with which selected bits of behavior, such as feeding in a grazing snail, defensive withdrawal of mantle organs in *Aplysia*, swimming in a leech, stomach contractions in a lobster, escape swimming in a crayfish, and walking in a locust have been analyzed cell by cell and can be accounted for in terms of known connectivity and cell properties (*3*). Usually the sensory neurons are known only by type and region, but many motor and interneurons are identified. Some 20 cases are largely worked out. In consequence, the concept is now well entrenched that the nervous system works as a system of neuronal circuits with predetermined pathways, discrete pre- and postsynaptic fibers, discrete synapses, and discrete signals (impulses and synaptic events)—at least in invertebrates.

This success has encouraged the opinion that the nervous system can be understood and behavior explained in terms of cells and cellular processes. Reductionism flourishes, and this success extends the range of its hopes. Even in vertebrates the same success may be possible, although more difficult where whole classes of cells might function as a single, identified cell does in the invertebrate. The "jamming avoidance" and the startle response of fish, eye movements, monosynaptic and disynaptic reflexes, the functional anatomy of retina, cerebellar cortex, olfactory bulb, and spinal cord confirmed and extended the expectations from Cajal in allowing circuitry by generic cell types.

To this was added in the 1970's another large step forward, in the recognition of *local circuit neurons* (*4*) and *local circuits*. The latter, largely functioning without impulses, are connections within micrometers, which often comprise reciprocal and serial synapses with graded transmitter release and mutual effects of nearby chemical and electrical synapses on each other. Each situation on which this major new idea was based, depended on particularly favorable material, such as the rabbit olfactory bulb. Even the cerebral cortex, whose analysis in these terms had hardly progressed beyond the pioneering diagrams of Lorente de Nó (*5*), now seemed amenable with the intensive application of Golgi impregnation, electron microscopy, and newer experimental anatomical techniques. So remarkable have been the successes in identifying local circuits that a kind of euphoria has taken hold about the eventual explanatory power of circuitry, once we get the connectivity worked out, to the point that circuitry may fairly be said to be the current orthodoxy.

Yet further revolutions seem likely. The local circuit concept does not go far enough beyond the circuit concept. New terms, beyond circuitry, are required to take into account (i) the critical geometry suggested by the scores of characteristic forms of arborization of axonal endings and dendritic ramifications, remembering that one physiologist's synapse may involve thousands of electron microscopic synapses; (ii) evidence of dozens of neuroactive transmitters and modulators, (iii) transmitters that act over several micrometers, (iv) electric fields that may act over tens of micrometers [as in the Mauthner cell axon cap (*3, 6*)], and (v) many kinds of potentials besides spikes and classical synaptic potentials: plateau potentials, driver potentials, pacemaker potentials, slower and faster endogenous membrane shifts, and hyperpolarizations of very long duration with decreased conductance. There are further complexities, not qualitatively predictable from the forgoing considerations, among them the personality of each integrative locus, which means not only junctions but dendrites, preterminal axons, branch points, spike initiating loci, and the like. Properties of personality include the tendencies to regular or irregular firing, to patterned bursts, to afterdischarge, to rebound, to facilitation or its opposite, to synchronization of subthreshold oscillations with neighbors, and to long-term changes of responsivity (*6*).

When circuit analysis comes close to explaining behavior we are fortunate, but it encounters difficulties even in simple systems (*7*). Circuit analysis alone is unlikely to provide the major insights necessary to understand the emergent mechanisms present in complex systems. The close comparative study of simpler and more advanced exemplars provides the primary hope for understanding those mechanisms, including phenomena of large arrays of cells. For example, the significance of the size of the cerebellum and of other cell groups, and of lamination and other forms of differentiation, will be illuminated mainly with the aid of comparative data. Likewise, an understanding of the mechanisms of recognition of ethological sign stimuli and of hierarchical control of locomotor gait will depend on study of more than a few species. Other examples abound: The electroencephalogram and evoked potentials, as well as pathological or experimental phenomena such as seizures and kindling, differ among taxa in ways that seem potentially insightful. These and a long list of properties manifest emergent evolution in the brain.

## Comparative Anatomy Raises Questions

A battery of new techniques has stimulated a renaissance of interest and new work in comparative neuroanatomy. The result of reexamining long-studied material as well as additional taxa has led to major changes in interpretation. For ex-

ample, the telencephalon of elasmobranchs and teleosts, instead of being the vague and little differentiated "association" center for feeding and migration, with emphasis on olfaction, now seems to have one or even two specific projection areas for each sensory modality, just as in amniotes. As this information is consolidated we will have to ask how much uncommitted forebrain is left over for convergence of modalities. The forebrain of fish is subdivided into many regions homologous to parts of the septum, hippocampus, striatum, and cortex of mammals. The opportunity now presents itself to begin studying the evolving hippocampus with respect to its physiological, chemical, pharmacological, and behavioral role, just to name one example among regions neglected in nonmammalian groups.

Primary afferent nuclei and pathways in the brain stem of elasmobranchs and teleosts are also much more like those in advanced vertebrates than was formerly believed. New evidence suggests that each afferent system, as in mammals, uses parallel pathways with different physiological specialization and maintains their discreteness through the midbrain or farther. Classes, orders, and even families may differ widely in the relative emphasis on one or another subsystem—for example among the six or seven subsystems of optic nerve fiber targets; this variety offers opportunities for functional and behavioral correlations as levers for sorting out their different meanings.

The cerebellum of rays and bony fishes segregates sensory modalities into almost nonoverlapping areas (for tactile, visual, acoustic, electroreceptive, mechanoreceptive lateral line, otolithic, and semicircular canal inputs); most Purkinje cells are specialists in one modality. The tectum opticum, torus semicircularis (equivalent at least in part to the inferior colliculus) and dorsal thalamus are also more subdivided, receive a greater variety of inputs, and project to more targets than was appreciated earlier.

However, each of these advances raises new puzzles. What does it mean for their functional roles that the lateral geniculate nucleus and nucleus rotundus (roughly equivalent to the pulvinar) of the dorsal thalamus of reptiles appear to have no intrinsic neurons, that all their neurons project to the telencephalon; or that mormyrid fish have a corollary discharge to their electric organ command and gymnotid fish do not (*8*)?

An example of the ferment of interpretive questions raised by the new influx of information is Ebbesson's (*9*) proposed theory that a major way in which vertebrate brains have evolved is a parcellation involving chiefly the selective loss of connections by cell aggregates in more advanced taxa. Axons do not, according to this theory, invade unknown territories during either phylogenetic or ontogenetic development, but follow ancient paths to targets of their ancestors. Primitive taxa are said to have diffuse and undifferentiated connectivity, hence a greater variety than advanced taxa of afferent and efferent connections for each nucleus; ancestral forms presumably had all the connections now found in surviving groups. Advance is by loss, selective enlargement and subdivision into larger numbers of discrete cell groups. The theory has stimulated a fundamental debate over parsimony in phylogenetic dendrograms and over the plausibility of different evolutionary scenarios (*10*). The classical challenge, how to establish homologies of cell groups and pathways in the brain, has come to life after long dormancy. We now have the advantages of new data and methods and the rigor of refined distinctions between *homoplasy* (resemblance not due to inheritance from a common ancestry, whether based on parallelism, convergence, analogy, mimicry, or chance similarity) and *homology* (inheritance from a common ancestry, with or without resemblance in form or function).

Even where homology is difficult to analyze, comparison may reveal potentially instructive differences in anatomy and physiology. In songbirds (canaries, finches) and psittacines (parrots, budgerigars), but not in gallinaceous birds (chickens) or doves, a forebrain structure known as the *hyperstriatum ventrale, pars caudale*, is large, discrete, and histologically delineated from adjacent areas. This structure plays a role in both producing and recognizing learned song. It is remarkable for its plasticity in songbirds and possibly in psittacines: seasonally it expands and shrinks in some species; the size correlates with the complexity of the song of the individual in male canaries; in the male, it is approximately three times the size in the female; testosterone makes dendrites longer and new synapses form. Evidence points to cell turnover in the forebrain (*11*). Hemispheric asymmetry is physiologically apparent in swamp sparrows but not, by the same criteria, in song sparrows (*12*). This structure is the prime example thus far of a distinct structure in the telencephalon whose development is correlated with a distinct behavior. To our knowledge, only the auditory cortex of the mustache bat (*13*), better known in respect to organization and cell types, but less known in respect to plasticity and correlation with species differences in behavior, rivals it.

Differences in anatomy may be conspicuous though not yet correlated with function. The most advanced type of cerebral cortex in Cetacea (dolphins) is called a *proisocortex* because, although large in surface area and deeply convoluted, it is apparently less complex than the true isocortex in familiar mammalian orders. It is thin and less differentiated; the range of sizes of pyramidal cells is less; stellate cells are less common; koniocortex is lacking [a granular layer (IV) is missing even in sensory areas]; afferent axons end principally in layers I and II; layer I is thick, as in primitive mammals; and extroverted cells of layer II are abundant and have only apical dendrites, splitting close to the soma and projecting into layer I, as in primitive groups. Vertical striation is less developed (*14*). Cytoarchitectonic areas are fewer and the transitions between them are more gradual. These features are regarded as characterizing a protoneocortical stage in evolution. The proposition that cetaceans have intellectual achievements approaching or surpassing the human level has not been supported by evidence. However, the meager facts available might justify a tentative conclusion that their cognitive abilities approach those of advanced nonhominid primates. If so, they are mediated by a very different type of cortex.

The forgoing arguments largely concern comparison of higher taxa. Comparison of species within the same order presents another kind of challenge. The dorsal cochlear nucleus of the medulla differs drastically among species of rodents (*15*) and species of primates (*16*). Among more than 15 species of rodents examined, guinea pigs have a moderately differentiated nucleus; pocket gophers (Geomyidae), a markedly larger one, with a much thicker granule cell layer; and mountain beavers (*Aplodontia*, in a different family from the aquatic beaver, *Castor*), a grotesquely large nucleus with a huge granule cell mass. Prosimians such as galago and loris have the most differentiated nucleus among primates, well laminated and with a conspicuous granule cell layer; apes (gibbons) and humans have a virtually unlaminated nucleus with no granule cell layer. So far no correlates are known in behavior or physiology. The lateral geniculate nucleus differs in details both within and between orders of mammals (*17*); convergent development of parcellation and different kinds of lamination are said to

correlate with behavior that requires rapid evaluation of spatial relations (for example, whether the species flies, glides, is arboreal, or is a fast runner). The visual cortex in squirrel monkeys and perhaps other New World monkeys seems to lack the ocular dominance "columns" so well developed in the Old World species studied (*18*).

In trying to interpret such differences and similar variations in other systems (for example, convergent development of the corticomotoneuronal system), we are constantly reminded that evolution of major groups has proceeded by radiation, not in a ladder-like sequence. We have to eschew the facile use of series, such as rat, cat, monkey, and man. Often it turns out that some rodent is more like a monkey than a cat is, or that another carnivore is quite unlike the cat.

## Prospects and Challenges

Comparative neuroscience has at least the potential and perhaps bright prospects, for uncovering insights, given the great reservoir of species and higher taxa, given the variety of nervous systems, given the new techniques and new conceptual issues. I have mentioned some of the recently recognized issues: identifiable cells and equivalence sets, circuits and metacircuits, the evolution of integrative variables and coding, of cytological, chemical, and physiological differentiation, of emergent properties with larger systems, of pathways and projections; issues of homology and homoplasy, rules of brain evolution, reinterpretation of cerebellum, striatum, hippocampus, and the like.

To be specific, we may hope to understand a little better, from having the comparative perspective, even such derivative mysteries as brain waves and cognitive event-related waves, seizures, and kindling, and deficits from vestibular lesions and central compensation. Possibly more tractable and certainly of interest will be studies at the level of distribution of transmitters and transmitter enzymes. Neurons of the locus coeruleus of some species (rat and monkey) are all norepinephrine cells, of others (rabbit and cat) mixed, some containing norepinephrine and others serotonin (*19*). Substance P is in a single type of amacrine cell in the retina of pigeons (out of about eight types), but in three or four types of amacrines in rabbits and monkeys (*20*). Gamma amino decarboxylase is more widespread among neurons in the thalamus of the galago and the cat than of the opossum and is intermediate in the rabbit (*21*). We must now ask whether some interspecies behavioral differences may have their basis in such chemical differences, and whether those can cast light on the differences between Parkinson patients and healthy humans, or the differences between control animals and those in which bits of brain tissue have been implanted.

The conceptual and methodological challenges are demanding. Not the least of the conceptual challenges is what can be inferred from correlation, especially when an untestable evolutionary hypothesis is at stake. We must not assume that the main features distinguishing the brains of fish from those of birds are immediately attributable to the main differences between them in habitat and behavior. The problems are even serious in trying to infer a causal relation when the data are simply neuronal activity coincident with or partly preceding and partly overlapping behavior (*22*).

To go beyond a descriptive story, the general problem has two faces which may be formulated: What brain correlates (in anatomy, physiology, and chemistry) are relevant to observed differences in behavior? And what behavioral correlates are relevant to observed differences in the brain?

Besides the needs for further improvement in anatomical, chemical, and physiological techniques, these questions underline the urgent need for better methods for assessing behavior in ways that will permit its variety to be better correlated with brain variables. We need to know not merely the food and habitat preferences and the ethogram or repertoire of behaviors but also to have quantitative measurements of skills such as navigation, leaping, righting in midfall, or trail following. Relative ratings are also necessary, even if only subjective estimates of experienced observers, of qualities or tendencies such as exploration, aggression with conspecifics, wildness or tameability with humans, complexity of social organization, and cognitive capacities. Even the list of significant variables is not yet adequately formulated. Macphail (*23*), for example, after reviewing the data on "intelligence" in fishes, amphibians, reptiles, birds, and mammals, concluded that there is still no adequate evidence to support any general difference in intelligence among vertebrates, even between classes—except for the human species! To me the facts point to inadequate means of assessment or selection of variables. Elsewhere, I have ventured an agenda for research on cognitive differences among animals (*24*).

A significant likelihood that can be examined only in unusually well-known species is that many crucial behaviors correlated with brain adaptations are those used only rarely, during life crises, not the everyday behavior we are familiar with. Special demands during reproductive behavior, escape from predators, or uncommon climatic episodes may have a disproportionate influence on brain evolution.

In the face of the formidable problems of research strategy, and the many degrees of freedom in choice of technique and species, one might well regard comparative neuroscience as Mission Impossible. However, in our drama we do not know how the story will come out; many opportunities exist for intermediate successes; findings may have multiple meanings. They may illuminate the roots of different taxa, suggest rules and principles with favorable species, and imply insights relevant to human development, learning, disease, and recovery. The most certain predictions are that our view of the nervous system today will appear naïve tomorrow and that a vigorous comparative neuroscience can accelerate this hoped-for obsolescence.

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**RESEARCH ARTICLE**

# Endogenous Ionic Currents Traverse Intact and Damaged Bone

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Abstract. *Living bone drives an electric current through itself and into sites of damage. Such "fracture currents" consist of two components: an intense, decaying current dependent on bone deformation and a stable, persistent current driven by a cellular battery. The latter is carried by chloride ions and, to a lesser extent, by sodium, magnesium, and calcium ions. Endogenous fracture currents are of the same polarity and similar magnitude as clinically applied currents that are successful in treating chronic nonunions in fractured bones. This suggests that the defect in biological nonunions may reside in the electrophysiology of repair.*

Bone is a structurally dynamic tissue. It modulates its shape in response to changes in load and can heal itself spontaneously. Bone is also electrically dynamic. Steady voltages have been reported along intact and damaged bone (*1*, *2*) and short-lived voltages have been measured in response to loading (*3*). It has been widely suggested that such electrical phenomena underlie the physiology of adaptive remodeling and repair, even though experimental evidence for this is scant. Most electrical measurements of bone have not been made under physiological conditions, and, to my knowledge, no measurements of endogenous electrical currents in living bone have been described. I report here my measurements of a steady ionic (electric) current traversing living bone at physiological temperature and the changes in current pattern and density induced by damage.

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## Methodology

The ultrasensitive vibrating probe system precisely measures the density and direction of current traversing cells or tissues immersed in a natural medium. Essentials of its design and construction have been reported elsewhere (*4*), as has its use in a variety of biological studies (*5*). Current densities are routinely measured on the order of nanoamperes per square centimeter, with a spatial resolution of about 20 μm. The electrode is manipulated with a micromanipulator and viewing is performed with an inverted microscope.

Metatarsals of weanling mice were chosen as experimental material because they can be dissected from the digit intact, with little damage to the surface tissue ensheathments. The small size of the metatarsal (about 4 to 7 mm in length and 1 mm in diameter) makes it ideal for mapping with the vibrating electrode. Freshly dissected bones were immediately immersed in mammalian Ringer solution fortified with 5.5 m*M* glucose. Electrical recordings were usually begun within 2 to 3 minutes because of the necessary manipulations of the probe and specimen (Fig. 1). All bones were maintained at 37° ± 2°C, except where noted.

### Intact Bone

Current flow was mapped in 64 undamaged bones. Current densities ranged from 0.5 to 12 μA/cm$^2$; the densest current entered the articular surface of the epiphyses while less dense current entered the remaining epiphyseal regions. The terminal cartilaginous regions of any bone showed current densities two- to sixfold larger than the diffuse current observed along the diaphysis. The latter current both entered and left the diaphysis, and no consistent pattern could be observed in this region.

### Fracture Currents

Twenty-three of the 64 bones were also studied after being experimentally damaged. Ten of them were incompletely fractured with forceps (Fig. 1), and the balance were notched with a fine needle (the notch being 75 to 200 μm in width and penetrating the marrow cavity).

In two bones, the probe was placed in the fracture within 30 seconds after damage. Intense currents (129 and 102 μA/cm$^2$) were observed entering the lesion. In all other cases the electrical records were begun 2 to 3 minutes after damage. By this time, current densities had declined to 20.2 to 86.3 μA/cm$^2$. The decline reached an endpoint within 8 to 30 minutes after the injury (Fig. 2A). Plateau currents of 4.9 ± 0.5 μA/cm$^2$ (for all 23 bones) were exceptionally stable even after several hours (Fig. 2B).

Undamaged areas were mapped to determine whether any changes in outcurrent were associated with the increased densities of current entering the fractures. Foci of outcurrent were observed, but their position and pattern were high-