# *Interneuron Diversity series*: Interneuron research – challenges and strategies

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**The field of interneuron research has come of age. An influx of new data has shed light on many areas, but has also highlighted major challenges. The articles in this review series will address several of these challenges, including developing a standardized classification scheme, defining how the integrative properties of interneurons shape their functional roles (including the generation of oscillatory activity), and identifying molecular mechanisms of synaptic plasticity. New technologies can help us address these problems in ways not previously possible. To coordinate the vast amount of data being generated, we propose the creation of a world-wide-web Interneuron Database that will facilitate inter-laboratory comparisons and collaborative studies. A well-crafted database has the potential to bring new insight by standardizing and organizing data collected in physiological, anatomical and molecular studies.**

The interneuron field is no longer a 'bright young thing' eagerly courted by granting agencies and journal editors. Instead, it is maturing into a more classic figure as a wider variety of genetic and imaging technologies are brought to bear on the issues of form and function. Since 1995, the interneuron field has been growing more than three times as fast as the whole field of biomedical research, as judged by the number of interneuron-related papers appearing in MEDLINE (http://medline.cos.com/) (bars in Fig. 1) compared with the growth of MEDLINE itself (solid line in Fig. 1). What are these cells? An interneuron is commonly thought of as a neuron that does not project outside the brain region in which its cell body is located – that is, a so-called 'local circuit neuron' – a definition a bit fuzzy at the edges but still useful. Not all interneurons are inhibitory (witness the excitatory cholinergic interneurons of the striatum), nor are all inhibitory neurons interneurons (e.g. dopaminergic projection neurons in the substantia nigra); finally, not all inhibitory interneurons are GABAergic (e.g. the glycinergic Renshaw cell of the spinal ventral horn). However, this series of reviews will focus mainly on GABAergic inhibitory interneurons of the cortex and hippocampus. Some of the first electrophysiological recordings from interneurons were made by Renshaw [1] in the spinal cord, and later by Andersen *et al.* [2] in the hippocampus. Development of the ability to make whole-cell patch recordings from identified neurons under visual control [3] provided a quantum advance that greatly opened the study of those neurons residing outside the main cell laminae of the brain.

The purpose of this article is to lay the groundwork for the series of reviews that follows. These reviews address many different aspects of interneuron research; however, broadly speaking, they can be divided into four central topics: developing a standardized classification scheme for interneurons, defining how the integrative properties of interneurons shape their functional roles, understanding how diverse populations of interneurons generate and pace oscillatory activity, and identifying molecular mechanisms of synaptic plasticity. Addressing each of these issues represents a major challenge for interneuron researchers today. In the sections that follow we discuss these challenges. We also identify what we believe to be an even greater challenge looming on the horizon: to translate the ever-increasing amount of data in this field into a form that allows a more global understanding of their roles in brain function. We propose the creation of a world-wide-web-accessible Interneuron Database. Such a database could foster collaboration and insights not possible today and bring order and consistency to physiological and anatomical studies.

## Classification schemes

A common theme in interneuron research is that the diversity of interneuron 'types' is far larger than that of principal neurons in the same brain region, a theme variously presented as a lament or a cheer depending on the views and needs of the author. Multiple interneuron types interact and function within unique circuits that execute complex functions including learning, memory, emotion, motivation, perception and motor behaviors. Identifying the molecular basis of these higher-order functions is a major goal of neuroscience and has provided an especially difficult challenge for those studying interneurons.

Current interneuron classification schemes are anecdotal in the sense that useful facets or elements of classification, and even the vocabulary used to distinguish one subtype from another, have not been agreed upon by

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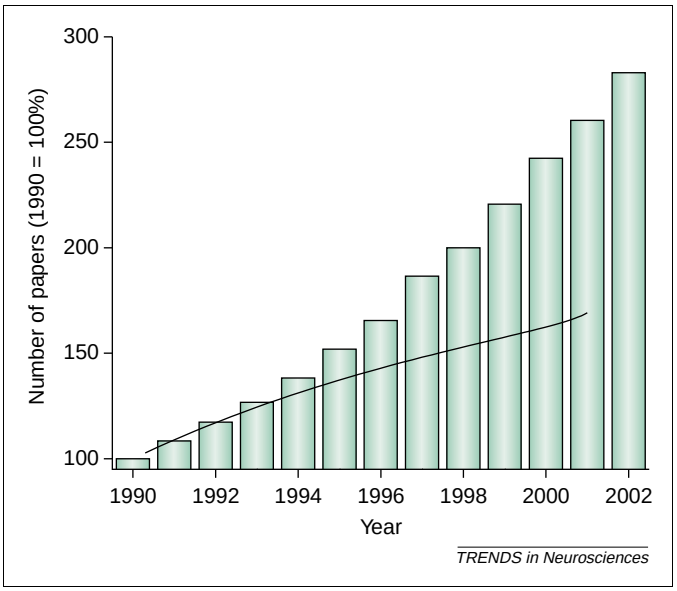


**Fig. 1**. Rapid recent growth of interneuron-related publications. The bars indicate the cumulative number of publications in MEDLINE having the word 'interneuron' in the title or abstract, with the number of papers up to 1990 (460) set to 100%. The solid line represents percentage growth in the total number of papers in MEDLINE over the same period.

different laboratories. Attempts have been made to classify interneurons based on axonal trajectory and location of cell body, on the expression of a limited number of neurochemicals expected to be functionally discriminating (e.g. calbindin, calretinin, parvalbumin, somatostatin, vasoactive intestinal peptide or nitric-oxide synthase) or on some notion of the physiological function of the cells (e.g. incrementing or decrementing responses to an incoming train of excitatory impulses). Interneurons can be grouped differently depending on the particular task under study or the developmental stage of the animal. Articles in this series will review these different classification schemes. No classification scheme has yet proved entirely satisfactory [4]. Towards the end of this article we propose a different approach to the classification problem.

## Integrative properties

Interneurons exhibit physiological properties that are different from those of principal cells. In particular, interneurons are tuned to fire rapid and precisely timed action potentials and many are capable of following high-frequency afferent input with fidelity. The ability to respond quickly to afferent input is likely to result from the expression of specific voltage-gated [5] and ligand-gated [6] ion channels. The rapid firing of interneurons appears to be important for the role of these cells in establishing long-range coherence of oscillatory activity in cortical networks [7], allowing interneurons to function with temporal precision and act as coincidence detectors of principal neuron activity. The role of ligand-gated and voltage-gated ion channels in defining the integrative properties of interneurons will be addressed in detail in reviews in this series.

Despite advances in recent years in our understanding of the integrative properties of interneurons, many questions remain. Which proteins are expressed uniquely in interneurons? How is the expression of these proteins regulated by internal and external signals? Which molecular mechanisms govern the dendritic or axonal targeting of specific proteins? How do the integrative properties of interneurons shape their functional roles? Molecular approaches could shed light on several these questions. As will be reviewed, techniques such as single cell reverse-transcriptase polymerase chain reaction (RT–PCR) combined with patch-clamp recording and anatomical reconstruction of the recorded cell can be used to identify the functional roles of specific proteins in identified interneuron subpopulations. Electrophysiological study of interneuron subpopulations can be eased by labeling these subpopulations *in vivo* with fluorescent proteins. Transgenic techniques and homologous recombination in stem cells can be used to alter receptor expression in GABAergic interneurons. Key molecules in interneurons can be tagged to allow study of protein trafficking and subcellular localization. The development of novel pharmacological agents would also facilitate interneuron research. Toward this end, several major universities are fostering collaborations between organic chemists, pharmacologists and others with the intent of using high-content screening strategies to develop new research compounds. Bringing these tools to bear on questions in the interneuron field promises to generate a wealth of new data providing novel insight into these issues.

## Functional roles of interneurons

Inhibitory interneurons serve a wide variety of functions in the brain. Interneurons have been described that control the generation of $Na^+$-dependent and $Ca^{2+}$-dependent action potentials, regulate synaptic transmission and plasticity, and generate and pace large-scale, synchronous oscillatory activity [8–10]. Anatomical and physiological heterogeneity contribute to this diversity of function. For example, the localization of inhibitory synapses to specific somato–dendritic domains on principal cells determines, in part, the integrative properties of interneurons in the network [11]. As will be reviewed, both presynaptic and postsynaptic mechanisms can impact the integrative function of interneurons. It is now possible to understand the immediate cellular function of interneurons in terms of their detailed anatomical structure and the complement of ion channels they express. This capability allows the development of ever more realistic computer simulations of network behavior, models that are crucial for our understanding of the role of interneurons in networks containing large numbers of neuronal elements.

Evidence from both computer simulations and experiments predicts that interconnected networks of interneurons can entrain large populations of principal cells to fire in a rhythmic pattern [9]. Rhythmic, synchronous firing provides the associative activity needed to trigger changes in synaptic strength, both during development and in the adult. The observation that interneuron behavior is linked to the generation of brain rhythms has brought into focus several important questions. How does synchrony within interneuron networks regulate information processing within the network? What is the

role of different interneuron populations in brain oscillations and in cognition? Finally, what role do interneurons play in brain pathologies, and can manipulation of interneuron firing patterns be used therapeutically? For example, can interneuron-selective compounds be developed that would, by virtue of their effect on brain rhythmicity, improve attention and cognition or decrease anxiety? Articles in this series will address each of these questions.

A better understanding of the answers to these questions might also require the development of novel strategies and tools with which we can address the questions. For example, a method for studying the behavior of groups of interneurons would be beneficial, as is routinely performed with field recordings of populations of principal cells. Imaging techniques might prove useful in this regard. Also useful would be genetics-based methods and technologies used to map functional interneuron interconnectivity, to monitor functional changes, or to drive functional changes within interneuronal circuits. For example, a fluorescent tracer protein that is transported across synapses orthogradely or retrogradely and that is expressed from an interneuron-specific promoter could be used to map the distribution of targets of an interneuron population. Alternatively, the use of gene targeting to kill subsets of interneurons might help us understand the functions of these interneurons in network behavior [12]. We can expect that interneuron research will be one of the earliest beneficiaries of such technological developments.

## Synaptic plasticity

Both short-term and long-term changes in the strength of excitatory synapses on interneurons are thought to be key factors maintaining the balance between excitation and inhibition in neuronal networks. During repetitive activation, glutamatergic synapses on interneurons in the cortex and hippocampus exhibit several types of short-term plasticity, with some synapses showing only facilitation, others only depression, while yet others show combined facilitating–depressing responses [13]. The mechanisms underlying these short-term behaviors could involve both presynaptic and postsynaptic processes. Recent studies have also described long-term plasticity at some excitatory synapses onto interneurons. In the hippocampus, LTD and LTP have been evoked using stimulation protocols different from those used to evoke plasticity at neighboring excitatory synapses onto pyramidal cells. These differences suggest that interneurons might possess distinct mechanisms for induction of long-term plasticity. Indeed, LTD that depends on $Ca^{2+}$-permeable AMPA receptors has been described [14,15], as have both NMDA-receptor-dependent [16] and NMDA-receptor-independent LTP [17,18]. These studies indicate that interneurons are heterogeneous with respect to their ability to undergo Hebbian plasticity, in addition to plasticity through mechanisms apparently not used by principal cells. The different forms of plasticity, the lack of dendritic spines on most interneurons [8] and the presence of different intracellular interneuronal signaling networks [19] raises the question of whether distinct molecular mechanisms might underlie synaptic plasticity in interneurons and pyramidal cells.

Given the role of interneurons in synchronizing oscillatory behavior in the brain, mechanisms of both short-term and long-term plasticity could play significant roles in information processing. Modeling studies suggest that plasticity at interneuron synapses might be important for learning and recall by hippocampal networks [20]. Study of the molecular mechanisms underlying both short-term and long-term synaptic plasticity could be greatly facilitated by the development of genetics-based tools, such as transgenic mice expressing different fluorescent receptor proteins driven by selected promoters in specific interneuronal populations. Alternately, animals genetically engineered to lack key molecules in plasticity signaling pathways might prove useful in defining the role of this plasticity in synchronizing oscillatory behavior. Understanding the nature of this role, in addition to the effect of heterogeneity in both short-term and long-term plasticity, remains a challenge.

## Interneuron database: a foundation for order

As interneuron research moves forward, a major challenge is to translate the ever-increasing amount of molecular and synaptic data into an understanding of functions of the various interneuron types in the brain. This is particularly difficult when dealing with an unknown, but high, degree of heterogeneity among the interneuron population. A method for classification of these cells that encompasses all phenotypic aspects of these cells (i.e. anatomical, physiological, pharmacological, developmental and phylogenetic) might serve as a foundation to help standardize and organize data and hopefully to bring new insight.

Classification schema are formalized and well developed in molecular biology. For example, GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html) and Gene Ontology (GO; http://www.geneontology.org) [21] come to mind as increasingly useful relational databases. Imagine modern molecular studies today without GenBank, or progress in any field without MEDLINE. These databases were conceived to systematize a growing knowledge base. Neuroscience needs a comparable suite of online databases that facilitate inter-laboratory comparisons and collaborative studies. The complexity and hierarchical organization of the brain calls for a suite of inter-operable databases, one of which should be an Interneuron Database. We foresee a time when each neuron type in the mouse brain, eventually in many species, is represented by a code tied to a complete phenotypic description of that neuron. If well-crafted, such a tool would, over time, bring order and consistency to physiological and anatomical studies. There are several attributes of interneurons that need to be represented systematically. One would like answers to questions such as the following. Where is the cell located (i.e. where are its cell body and dendritic and axonal arborizations)? What are the immediate cellular targets of the interneuron? What higher-order functions does the cell control? By what processes (e.g. circuitry, hormonal or circadian) are the firing pattern and transmitter-release probability of the interneuron controlled? What are the molecular underpinnings of higher-order circuit behaviors?

Which interneurons in species X and Y have the same functions?

To minimize false starts, the logistics of creating the Interneuron Database would have to be carefully considered by a stakeholder group of neuroscientists and database engineers. However, to initiate the discussion, we suggest consideration of the following steps (Box 1), modeled after another multidimensional database, GO.

First, create a structured vocabulary of terms describing different aspects of neuronal phenotype (e.g. anatomical data, including molecular structure, 3D images, location within the brain, phylogenetic data, age of the animal, neural connectivity and circuitry; electrophysiological and pharmacological properties; diseases of the brain associated with interneuron malfunction; and an mRNA expression profile). A controlled vocabulary is needed to impose regularity in digital databases and to permit development of algorithms and computational tools that operate on the data. Certain aspects of this vocabulary are relatively straightforward (e.g. text describing a brain region), whereas others involving raw or processed data (e.g. 3D images or neuronal connectivity) require a good deal of creative thought. To be most useful for future analyses, the Interneuron Database would have to contain raw or minimally processed data. Data in a form that is easiest to read in a journal format might not be the most useful in a computer format. Finding a practical way to represent physiological data [e.g. excitatory postsynaptic currents (EPSCs) or firing patterns] and images (e.g. morphological or $Ca^{2+}$ images) in a digital format amenable to queries is itself a formidable challenge. Progress is being made in the development of databases that hold and manipulate images [22] but, to our knowledge, structured databases of neuronal connectivity or electrophysiological properties have yet to be introduced.

Second, describe each individual interneuron studied using these terms. Authors might submit entries to the Interneuron Database much as they do to GenBank, with each interneuron being assigned a unique identifier similar to an accession number and the author filling in as many of the blanks as possible. The minimum amount of data constituting a valid entry would have to be carefully considered to avoid filling up the database with small fragments of data but updates to each entry would be encouraged as new data emerge. It will be a major challenge to organize such a wide assortment of information in standardized database formats that are accessible via the internet and interactive with other databases [e.g. MEDLINE, Online Mendelian Inheritance in Man (OMIM) and GO] in a manner that allows it to be navigated in a user-friendly manner. There has been a lot of work in the informatics field on the creation of ontology-like structures and it is well known that consistency issues in such structures pose a problem that might not be completely solvable. The database structure must be designed to minimize such problems.

Third, create informatics tools for querying and manipulating these vocabularies and interneuron descriptions. One can envisage search engines and Basic Local Alignment Search Tool (BLAST)-like comparator engines, in addition to numerous clustering algorithms that weight anatomical, functional or molecular aspects of a description differently. We predict that, over time, categories of entries (i.e. interneuron types) will emerge from the data, much as the 22 million GenBank sequence records converge on 30 000 to 100 000 genes. If the Interneuron Database is to serve investigators as a research tool rather than simply as a data repository, the database must be flexibly designed so that it can be mined for knowledge nuggets in ways we cannot foresee today. As a reference, GO was introduced publicly in 2000, and most of the 57 publications that have since appeared describe the creation of novel data-mining algorithms applied to this database.

Undoubtedly one of the most useful descriptor dimensions will be the mRNA-expression profile, as determined by microarray analysis. This is a more systematic approach than classification based on expression of a single protein (e.g. whether an interneuron is somatostatin-positive or parvalbumin-positive). Although the reproducibility of microarray hybridization is probably adequate today given sufficient starting material, the reliability of mRNA obtained from single cells remains a significant challenge. An interesting initial exercise would be to determine the expression profile of 100 interneurons harvested from a single brain region by laser capture microscopy and to compare it with that of a similar number of adjacent principal cells. A mouse expressing green-fluorescent protein (GFP) under control of a glutamic-acid decarboxylase (GAD) promoter could be used for this purpose. Morphological analysis of each interneuron before mRNA harvest would enable association of the structural properties of that cell with its expression profile. Clustering strategies can then be used to determine (1) whether interneurons segregate from principal cells based on their molecular fingerprints, (2) whether diversity is larger in interneurons than in principal neurons, and (3) how many clusters of interneurons exist within this population. To determine the number of interneuron subtypes based on expression profiles alone, a program called Autoclass (www.openchannelfoundation.org/projects/AUTOCLASS_III) can be used to predict the optimum number of clusters in an unknown population, and then

**Box 1. Steps to creation of an interneuron database**

1. Create a structured vocabulary (ontology) of terms describing all aspects of neuronal phenotype, including:
   (a) Anatomy
   (b) Cellular physiology
   (c) Neural connectivity
   (d) Associated brain diseases, if any
   (e) mRNA-expression profile
2. Describe each interneuron using these terms, to provide a relational database that is:
   (a) World-wide-web accessible
   (b) Inter-operable with other databases
3. Create tools to query and manipulate the ontology, such as:
   (a) Search engines
   (b) Basic Local Alignment Search Tool (BLAST)-like comparator algorithms
   (c) Clustering algorithms to identify interneuron classes

traditional k-means clustering can be used to group the interneurons.

The Human Genome Project has provided experience in the organization of large-scale projects in biology. The importance of internationalizing the effort, the need for setting explicit milestones, the value of rapid rollout of data to the scientific community, and the necessity for new informatics tools, are some of the lessons learned [23]. Creation of a new database of information related to interneurons is no small endeavor and, to be successful, will require cooperation among normally competing laboratories and funding agencies such as NIH institutes on a scale not yet seen.

## Looking forward

In this review we have touched upon several topics that will be considered in more detail by the series of articles that follows, including classification, integrative properties, roles in brain oscillations, developmental aspects and roles in disease. We anticipate that this series will stimulate discussion and promote interneuron research. There has been explosive interest in interneurons in recent years, but there is more to come. Understanding the roles of interneurons in brain function requires an integrative, systematic approach that inevitably involves modeling. We expect that interneurons, like ageless beauties, will be center stage for some time to come, only to be displaced by efforts to achieve systematic global understanding of brain function, perhaps the next bright young thing.

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