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Invertebrate neuroethology: Guiding principles

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Key words. Field neuroethology; model systems; research constraints; single neuron approach; behavioral changes; evolutionary design.

Pillars upon which neuroethology should rest

Nervous systems are computational structures designed to produce behavior in natural surroundings. For neuroethological research, natural behavior serves as the substrate for which an understanding has to be obtained of sensory, central nervous, and effector events that underly behavioral strategies shaped by nature's abiotic and biotic forces to improve survival and reproductive fitness of animals during the course of evolution[1,9].

According to the dual roots of neuroethology, concepts, methods and results derived from studies of behavior, and those emerging from the entire field of neuroscience, have to be incorporated. Neuroethologists have to concentrate on molecular (subcellular), cellular, and network (circuitry) levels, which form the building blocks for hard- and software computational operations[3]. Our everyday work should focus on animals which are suitable for bridging the gap between distinct behavioral tactics and their underlying neural basis.

A call for quantitative and comparative field studies

Progressive neuroethology needs quantitative field work that teaches how behavior is executed and modified under the constraints of habitat conditions and optimized through intra- and interspecific interactions between animals and between animals and plants. Quantitative studies should reveal how behavioral components are coordinated and timed, when exposed to the relevant stimulus configurations in daily life. Such studies, especially when subsequently transferred to and simulated within the laboratory, require sophisticated methods adapted to the animal's design to pose questions that the unrestrained (or partially restrained) individual can answer, even when confined by the strait-jacket of a microelectrode.

Questions of principle should guide our research[1,9]. At the behavioral level, we might focus on how animals orient within their environments to terrestrial and astronomical cues using different forms of locomotion, how they select and find their mates, defend their territories, avoid predators, hunt for prey or cooperate as members within a social group. Such strategies demand from the nervous system at the input side, that it should detect and recognize signals produced by con- and heterospecifics, to discriminate and localize them in a noisy habitat. On the integrative and output side, the nervous system has to decide among different behaviors, how it triggers, generates and times its components, and how it controls behavior by peripheral and central feedback devices. Moreover, we should consider actions of the nervous system that are not dependent, or are only weakly dependent on external stimuli – actions which reflect the nervous system's internal world. We should engage in a search for neural and humoral factors that determine the behavioral state and look for the causes.

Such fundamental questions often arise from, or are brought into better focus by, comparative studies. They elucidate species-specific and even individual solutions, and they open our eyes to general principles that govern larger taxonomic groups. Let us not forget, even when searching for the most basic principles, that a snail differs from a cockroach, and a frog from a bird in its demands. Thus, the exploration of an animal's natural behavioral repertoire, and the comparative study of species with different specializations, are indispensable guides for the conception of meaningful physiological experiments, particularly when approaching integrative central nervous mechanisms.

How to select a proper model system?

Comparative and evolutionary studies provide the framework for a search for those model systems which best address the desired question. But even deeper insight into the behavior in question does not necessarily help to select the best 'system' to work with on the neural level. Evolution is indifferent to the needs of students of animal behavior and to neurobiologists and it is rare that animals are equally suitable for both behavioral and nervous system analysis. Quite on the contrary, the most fascinating strategies we observe in the field are at present, and perhaps for a long time, out of reach for cellular and network analysis. The signal code of a flashing firefly and its mimicking potential are as interesting as the acoustic code of a frog, but the former is much harder to crack than the latter, when considering the neuronal implementation.

There is no unique solution for selecting the proper model system. For me, an important guideline has always been to search for an animal that will execute the strategy in question under somewhat restrained conditions which allows also the application of the instrumentarium for a cellular and circuit approach. Thus, it is not only the kind of question we want to answer that restricts progress in neuroethology. The constraints most often lie in the degree of tolerance an animal exhibits in a somewhat artifical environment, and how fast we make progress in our technical designs to reduce such limitations.

Constraints in our research

The limitations mentioned are reasons why only certain species and strategies were favored in the past, and why neuroethologists have concentrated on building blocks of behavior, such as reflexes and fixed action patterns, rather than on variable and modifiable acts and sequences. Experimental restrictions have also dictated why stereotyped behaviors were chosen rather than others shaped and modified by the animal's own individual experience. We have often focussed on 'sign stimuli' rather than on complex stimulus configurations because of our restricted knowledge, and also because we could not mimic the complex natural stimuli for the animal. Our research was also guided by most valuable but nevertheless simplified concepts, such as that of a central pattern generator or of a command neuron. We originally considered nervous systems and behaviors as hierarchically organized, but soon we learned that parallel processing and multifunctional operations are widely used.

Several articles in this volume, and elsewhere[1, 2, 4, 6], show what kind of questions, with what relationships to behavior, can be asked and solved with present day methods and concepts. Most often we have to explore the behavior first and look for the neurons and circuits afterwards, independently in the same or a different conspecific individual. This two-step approach is also a reason why at present most neuroethological studies end up with a neuronal or network *correlate* rather than with a *causal relationship*. But there is hope, justified by recent results listed in this volume, and elsewhere[5, 13, 18], that causal interpretations may be approached. Phonotactic behavior in flying and walking crickets can now be studied while recording and manipulating simultaneously the activity of single identified neurons, with predictable consequences for the behavior.

Laboratory work can also guide further studies in the field. Again, one example comes from crickets, which are known to use their moving forelegs for hearing and phonotactic tracking. When the cricket is walking towards a sound source the auditory receptors and auditory neurons are additionally stimulated or even suppressed when a foreleg touches the ground or hits an obstacle, and song-copying properties are camouflaged at low sound intensities reflecting the situation with a far distant singer[19]. This calls for field work to analyze in detail the stop-orient-and-go strategy of conspecific receivers in response to distant senders.

Is our research being sidetracked?

We are indeed in a situation of conflict. The deeper we enter the nervous system, the more we have to restrain the animal, and the further we deviate from its natural behavior. Without question, a search for molecular properties of nerve cells, the study of ionic channels or of discrete synaptic actions, and also the investigation of division of labor in cells and networks often requires the isolating of cells and ganglia, or even the use of tissue cultures. At first glance, such studies might seem to be sidetracks in neuroethology, but they are not.

To understand how rhythmic behaviors are produced, we need detailed knowledge of the cellular components, of synaptic efficacies and of the interactions of cells within a circuit[20]. To estimate the role of feedback in behavior, we must have precise knowledge of the kind of sensory or even central information which is fed back, whether it is phase-related to ongoing movement or not, and whether it acts briefly or over time. Only sufficient insight into circuits helps us to evaluate how local networks function, and eventually allows us to bridge the gap from neuronal implementations to computational algorithms as postulated on the basis of behavioral experiments. Decision-making is even within reach at the level of single neurons or neuronal assemblies, when studying the action of discrete neuromodulator cells. Finally we need to learn how cells and circuits are formed and modified during ontogeny and postembryonic development, and the consequences of previous sensory experience for behavior[10].

As outlined by J. M. Camhi in his introduction to this volume, neuroethology at its neurobiological roots is a reductionist field of science. How could one disagree? But as long as our research strategy is merely centered around cells and networks, without an attempt to connect them to behavior, we will not reach the goal of synthesis. Experimental designs and techniques have to be improved, to avoid pitfalls such as inappropriate stimulus configurations or false cellular environments. Even work with deafferented preparations or with isolated parts of the nervous system that lack relevant feedback or central modulation may guide us wrongly. Without concentrating on stimulus regimes that mimic those to which the animal is adapted to respond in its habitat, one is very unlikely to discover neuronal specializations. Without knowing the natural forms of input and the conditions under which they come into play, we may waste time and effort and perhaps only study side effects. Insight into central nervous specializations, as revealed by comparative studies, provides a path for determining the neuronal hardware of abstract computational algorithms[3].

But there are a few aspects beside those mentioned which I have experienced in my own work with insects. I shall discuss them briefly in the following paragraphs.

How far can one proceed with the single neuron approach?

When searching for cellular and network causes of behavior the single identified neuron approach seems impossible to renounce, and without it we would still be in the stone age of invertebrate neuroethology[6]. Even vertebrate neuroethologists, interested in the detailed operation of circuits often established by larger assemblies of neurons, must finally focus on single cells[8]. At least for some invertebrates, with their smaller number of neurons and perhaps a reduced redundancy in function, rather straightforward connections can be made to behavior, as outlined in some of the articles in this volume. Selective cell-killing by photoinactivation or enzyme treatment may help to illuminate the contribution of a single neuron to behavior. However, since plasticity at the cellular and network level is a common feature in all nervous systems from hydra to man, and parallel processing a widely used principle, the inactivation of a single neuron may not cause a change in the behavior. This calls for studies at the neuronal level to investigate short- and long-term changes in synaptic strengths and connectivities that may compensate for the loss of neurons[10].

Certainly the most powerful way to manipulate single neurons is to de- or hyperpolarize them and to look for the immediate consequences such procedures have upon behavior. But again, manipulating single neurons within a network may not be sufficient; yet, one sometimes finds lucky cases[13, 18].

Vertebrate neuroethologists are confronted with larger neuron assemblies which cannot be studied simultaneously with single cell microelectrode techniques. Here, labelling of sets of neurons with radioactive tracers was particularly successful in localizing central substrates involved in complex and higher order sensory processing, and in uncovering diverse sensory maps within the brain[12, 21]. Since labelling procedures can be combined with behavior, they open another avenue, even for an assembly approach in invertebrates. There, one has the great chance to combine labelled assemblies with single neuron studies in order to evaluate their potential in a known network. In addition, there are assemblies of nerve cells in invertebrates, especially in the head ganglia, with thousands of small neurons which so far have escaped the single cell approach. At least in a first step labelling these small neurons can explore areas of behaviorally relevant sensory processing, even of multimodal input. Furthermore, areas involved in learning and arousal might be found. Therefore, I urgently call on neuroethologists to use such techiques even in systems where a single cell approach is within reach.

Immunocytochemical methods, now being used more and more in both invertebrates and vertebrates, are tools which characterize transmitters, neuropeptides, and membrane receptors. With sufficient knowledge of neuroanatomy, we can open new insights into the distribution of specialized neurons. I am particularly eager to see more work done with selective pharmacological stimulation and treatment at the cellular level, in correlation with behavior.

Exploration of the basis for behavioral changes

Even the most stereotyped behaviors in invertebrates exhibit variations. Input-output relationships change with time and stimulus treatment, and are indicated by changes in behavioral thresholds. At one extreme, complete absence of behavior is observable in spite of the relevant stimulus being present. Moreover, behavioral changes can be locked to circadian clocks, specific neuromodulator cells and to circuits, and to hormonal systems, although it is often rather difficult to find the proper stimulus-response paradigms.

Adult female grasshoppers need a certain hormonal titer to become ready for stridulation and subsequent mating. When deprived of certain groups of neurosecretory cells in the brain, or after removal of the corpora allata (glands which produce juvenile hormone III), such females remain in or return for the rest of their life to the state of primary defence when confronted with a singing male. Their own stridulatory response to the male's song is completely suppressed. After implantation of the glands into allatectomized females, sexual responsiveness gradually reappears, as indicated by the female's song response and mating[11].

Male crickets, while courting, exhibit a strongly reduced escape behavior in response following cercal stimulation, to the extent that a female may even bite off part of the male's cercus without eliciting defence from the male[7].

I have chosen these examples to draw attention to neurons and neuromodulator cells within reach of cellular and network analysis. Studies within the brain and ventral nerve cord should concentrate on physiological and pharmacological properties of neuroendocrine cells. Little is known about their individual structure and connectivity, and about their target cells. Nearly nothing is known about whether such cells encode sensory information and interface to behavioral output, and if so how. These elements could be involved in decision making, as indicated in female grasshoppers. Moreover, sex specific differences may be found. Thus, I am sure that when the first neuroendocrine circuit is unravelled we may be launched on a journey to a new shore.

A call for the study of evolutionary design at the neuronal level

It is often claimed, especially by vertebrate neurobiologists, that redundancy is built into the nervous system, expressed, for instance, by the many ways of parallel processing or by the compensation for deprivations and lesions. One may ask: Does natural selection operate only on nerve cells that are disadvantageous, and leave out those which remain to some extent neutral during the course of evolution? Are there redundant neurons and assemblies which merely copy the function of others besides having special tasks? For invertebrates with an apparently economical organization of the nervous system, such questions can be approached. By comparing the structure and function of homologous motoneurons or even interneurons[8], we see specializations of functional types related to the behavior in which they are involved. Evolution may only have changed the inputs they receive or their relative weighting, or may have changed their outputs with the result that a single interneuron present in different species or even genera – as a stable element – may now fulfill different tasks. Therefore, I call for single neuron studies to explore such 'evolutionary designs' by concentrating on homologous or serially homologous nerve cells in combination with behavior.

A call for neuroethological research in the field

The vast improvement in neurophysiological techniques in semi-restrained animals opens the gate for outdoor neuroethology. This is particularly appropriate when studying sensory processing in the natural habitat. Let me list a few examples from recent work with insects.

For bushcrickets, which are known to communicate from shrubs and trees, Rheinlaender and Römer[14], have developed an outdoor experimental setup to study, at the single identified neuronal level, how the habitat influences the response to conspecific and heterospecific sound signals, and what constraints determine spacing of conspecific males and mate recognition[16]. One of the discoveries was that distances between sender and receiver are encoded in different parts of the receiver's auditory neuropile[17]. Pattern-copying properties of the neurons were best from locations where the animals normally broadcast.

Flying crickets are known to avoid ultrasonic sounds, as do noctuid moths (see Fullard, this issue). With the elegant flight paradigm developed by Hoy and co-workers[5, 13], combined with the single cell approach, it should be possible to analyze sensory capacities and processing in combination with a tethered flight regime in the habitat. One may then experience how far ultrasonic signals produced by echolocating bats are heard, and how precisely they are encoded, despite the noisy habitat reflecting nocturnally calling insects and frogs. Distances of perception could then be estimated and studied with motor performances to see whether the tactic used is the best.

On the other hand, outdoor neuroethology may even reveal that a single sensory channel is not sufficient to cope with the behavior seen. For instance, crickets track visual targets with a special walking mode, different to that in phonotactic tracking, when placed on a walking compensator[22]. With the walking compensator positioned in the field while simultaneously recording from single neurons, we might get new information about how conspecific acoustic stimuli are processed alongside visual and chemical stimuli, and in changing habitats. In neotropical grasshoppers, particularly those occupying the canopy area of large rainforest trees, Riede[15] found many species that lack stridulation and intraspecific sound communication but have functional ears. Here, outdoor neuroethological research could tell us reasons why the ears have remained functional and what kinds of interspecific signals are encoded.

Is there a chance of bridging the gap between the cellular and the computational level?

It is certainly true that behavioral experiments may provide us with an opportunity to determine successfully the particular neuronal implementation of computational operations within the nervous system[3]. But we know that the more we deviate from the primary sensory neuron, or from the motoneuron via a chain of interneurons to the brain, the more difficult it is to explore how the proper stimulus is encoded and a motor pattern performed. As shown by Schildberger (this issue), some local auditory neurons within the cricket brain act as elements intimately related to conspecific song recognition. When compared with primary sensory and ascending auditory neurons they lose both intensity dependence and synchronization of their activity to phonotactically essential temporal parameters. What is the neural parameter for which we have to look, and what does it tell us about the computation used? Even though we may end up with a set of neurons called 'complex feature detectors' solely responding to distinct stimulus configurations, our task is not at an end; it is just beginning.

As Heiligenberg[3] has pointed out so rightly, one may indeed wonder whether a coherent theory of brain and behavior can ultimately be formulated, even for invertebrates, at the level of individual neuronal activities. It may well turn out that we face some kind of higher-level language, detached from single neurons, as the only possible solution. It is my strong feeling that the relationship between neuronal implementation and the computational level will not be found soon, and that this gap is perhaps the strongest challenge for neuroethology in the future.

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Full Papers

Pharmacodynamic profile of CQP 201-403, a novel 8α-amino-ergoline*

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Summary. The profile of action in animals of CQP 201-403, a novel 8α-amino-ergoline, is in most aspects that of a very potent dopaminomimetic, both as a prolactin secretion inhibitor, and at the levels of the CNS and the cardiovascular system. Qualitatively CQP 201-403 differs slightly from bromocriptine and apomorphine in its effects on the CNS (no influence on serotonin metabolism in the rat cortex; induction of masculine mounting behavior in rats) and the cardiovascular system of the dog (reflex tachycardia in response to a blood-pressure fall). In man the new compound proved to be highly active in lowering prolactin serum levels and to be more potent than bromocriptine (Parlodel®).
Key words. CQP 201-403; 8α-amino-ergolines; ergot pharmacology; D-2 agonist; endocrine; CNS; cardiovascular actions.