

Multidisciplinary dissection of behavioral arousal: The role of muscarinic acetylcholine stimulation in grasshopper stridulatory behavior

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Contemporary neuroscience faces few limitations in its attempts to identify and characterize fundamental molecular, physiological, or biochemical constituents of brain systems. Such information alone, however, will ultimately be of little value unless we can also provide insights into their functional role and behavioral relevance (1). Although the latter is always a challenge, it is especially so when we aim to explore the proximate causal mechanisms underlying behavioral phenomena such as motivations (2), emotions (3), or the dynamic control of decision making (4). For such hypothetical constructs, values cannot be assigned directly; estimates of tendency can only be assessed once they manifest as observable behavior within a given context. In our search for neural substrates, then, it is essential that we combine studies of physiology, biochemistry, and molecular genetics, with quantitative behavioral analyses.

The paper by Heinrich *et al.* (5) in this issue of PNAS explores the neural underpinnings of arousal in the calling behavior of grasshoppers. Bridging different levels of analysis, it aims to both highlight and foster the search for a mechanistic understanding of arousal as a complex behavioral phenomenon. With a primary focus on the motivational components of behavior, these observations offer a significant and timely contribution in several respects. Firstly, the study provides a penetrating view into the molecular and physiological workings of a system in which behavioral states play a powerful role. Secondly, its truly interdisciplinary approach serves as a valuable case study illustrating how to combine investigation across diverse levels of organization to probe the underpinnings of behavior within an ethological framework. Finally, and perhaps most importantly, it highlights how expectations for complex interactions between behavior and its neural substrates have shifted from a view of neural mechanisms “for” a particular behavior, to a neural environment that

“fosters” the occurrence of a specific behavior (6).

A “simpler systems” approach initiated in the mid-1950s has been successfully used to explore the subcellular mechanisms contributing to control of behavior in a diverse range of invertebrate preparations (e.g., refs. 7 and 8). In line with this trend, pharmacological dissection of grasshopper stridulation has recently emerged as a viable model system for such investigations (9). Its advantages include a well defined functional context, sex and species differences in stridulation patterns, and the existence of a robust Caeliferan phylogeny (10). Natural stridulatory behavior in gomphocerine grasshoppers comprises a bidirectional and species-specific sequence of elaborate leg movements used in the contexts of mate location, courtship, and rivalry (Fig. 1). Focal injection of acetylcholine (ACh) into specific brain areas (viz., the upper and lower divisions of the central body complex, and a small neuropil situated posterior and dorsal to it) was found to induce stridulatory behavior identical to that of natural song, even in females who do not normally sing postmating (9, 11). In the grasshopper central nervous system, as in other insects, muscarinic ACh receptors (mAChRs) are concentrated on somata and in distinct neuropil regions. Coupled to intracellular signaling pathways, they modulate synaptic transmission. For instance, prolonged sensory stimuli have been shown to elevate the excitatory state of specific neurons by muscarinic effects on membrane potential and spike initiation thresholds (12).

Networks that generate the neuromuscular patterns driving the sound-producing hindleg movements are situated entirely in the metathoracic ganglion complex (13, 14). These hemiganglionic networks are activated through tonic discharges of descending protocerebral command neurons. In a given taxon, several identifiable types of command neuron each control one specific pattern of stridulatory movement, and are activated con-

secutively in an order appropriate to the particular behavioral situation (15). Thus, although neuronal networks that generate the stridulatory hindleg movements are located in the metathoracic ganglion complex, it is the brain that selects the time and type of stridulation, determining for instance the amplitude of hindleg movement (16). Precisely how this is achieved remains unclear.

The study of Heinrich *et al.* elegantly demonstrates that mAChRs are the basis for specific arousal in defined areas of the brain, affecting both the threshold for performance of a behavioral act, and the selection of a song pattern from a repertoire. The underlying mechanism appears to be neuronal excitation mediated by activation of the adenylate cyclase pathway. These findings are of particular interest in light of previous results implicating acetylcholine in arousal underlying acoustic vocalizations in several vertebrate taxa. Acetylcholine is reported to modulate vocal production in squirrel monkeys (17). The muscarinic agonist carbachol stimulates 22-kHz calls in rats, which are indistinguishable from normally occurring calls and prevented by pretreatment with choline antagonists scopolamine and atropine (18). Moreover, muscarinic acetylcholine receptor participation has been implicated in the control of separation-induced distress vocalizations in birds (19).

The study also raises further intriguing questions. Does arousal occur by modulation of contributions made by the central neurons to central pattern generators or through regulation of the transfer of sensory information? If stridulation is controlled by latent excitation, what is the role of inhibition in cephalic systems? Although the mechanisms of song production and recognition appear to be completely decoupled (20), is the cephalic

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Fig. 1. Adult male *Gomphocerus sibiricus* spontaneously singing in the laboratory, as seen by one of the optoelectronic cameras used for detecting the position of the reflector fixed to the hindleg of the grasshopper. Hindleg and reflector are blurred because of the stridulatory movement. (Photograph courtesy of R. Heinrich.)

control of mAChRs related to the cephalic filter for recognition of conspecific song in any way? However, the issue of higher sensory integration leading to choice and coordination of stridulatory behavior in orthopterans remains. Injection either activates command neurons directly, leading to one specific pattern of hindleg movements, or it activates structures that are presynaptic to the command neurons coordinating and controlling the timing of their activity. In insects, the central body complex and the mushroom bodies have long been implicated in such processing (7, 21). Although neuronal interconnections of the central body complex and the small neuropil areas suggest that the former is the major control center for stridulation in a hierarchically organized descending pathway, further analysis of the mushroom body is needed. Comparative studies of taxa with markedly different species-specific song patterns, especially those in which courtship involves both leg and wing stridulation (22), should prove illuminating.

Arousal or “motivation” are terms collectively referring to all reversible, short-term alterations in behavior not associated with fatigue or learning, and are used as intervening variables without regard to their precise implementation or underlying mechanisms. Although these are useful concepts, we have to accept that if we fully understood how a particular behav-

ior is produced, we would have no need for such terms at all (23). If we aspire to such a goal, it is clear that neither the classical ethological nor a traditional neurobiological approach will alone suffice. For instance, with respect to the control of behavior, it is significant that rivalry song, which uses the same hardware as that for calling and courtship, has never been induced either pharmacologically or electrically in any gomphocerine grasshopper (11). Furthermore, sensory input relevant to stridulation may come from the physical presence or acoustic signals of conspecific grasshoppers; both have a demonstrated effect on the occurrence of specific stridulatory behaviors. It is thus telling indeed that Heinrich *et al.* could elicit stridulation from males by injection of ACh doses below that required for behavioral response only when injections were accompanied by playback of female calls.

Arousal systems have an integrity that was created through evolutionary selection rather than simply through the life experiences of an organism (24). However, at any given moment there are presumably a variety of competing, parallel intrinsic arousal mechanisms, each of which fosters the emergence of a set of adaptive behaviors in a particular context. These mechanisms are thought to vie for access to what the animal will do next—c.f. Lorenz’s “great parliament of in-

stincts” (25). Because organisms can generally perform only one particular deed at a time, the observed stream of behavior is necessarily a sequential series of acts. The power of arousal mechanisms is thus not in determining, or producing, a behavior. Rather, they alter neural substrates to make the emergence of a particular act more likely; they modulate the system to bias the animal toward adaptive responses. The work of Heinrich *et al.* demonstrates how such an arousal mechanism functions, the levels of organization involved, and to what extent these are integrated. Although some may well resist our characterization of this work as affective neuroscience, preferring instead to identify with the cognitive-computational view of neural function, it nevertheless provides a solid foundation for questions concerning neuromodulation and the orchestration of behavior at the most fundamental level. How the dynamics of such circuits can express the full range of acoustic behaviors and states of arousal constitutes a worthy challenge to contemporary neuroethology.

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