a0005 1.27 Aggression in Invertebrates: The Emergence and Nature of Agonistic Behavioral Patterns

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s0005 1.27.1 Introduction

p0005 The study of behavior generally begins with watching. It is hard to say it better than Yogi Berra in his famous quote "You can observe a lot just by watching!" With repeated observations of social rituals, patterns often emerge that are easily recognizable and highly stereotypical. Like the digits on a hand that differ from each other even though all are called fingers, however, behavioral patterns differ each time they are performed dependent on the social context. In addition, variability exists in (1) when during social rituals patterns appear and (2) how one pattern links to the next. Despite such variability, statistical techniques can reveal behavior's inherent structure and show when patterns are most likely to appear and how predictably they link to each other. But is all behavior of all animal species modular and does this extend to human behavior? Moreover, if behavior is modular, how do patterns of this sort get established within nervous systems and what rules govern the likelihood that one will transition to the next? Modular construction implies that superimposed on the hard wiring diagrams of nervous systems, codes exist specifying that particular combinations of neurons and neuronal circuits transiently link together to form recognizable behavioral patterns at appropriate times during behavioral rituals. The observed behavioral patterns overlap each other, can utilize identical sets of muscles in different ways, are species- and/or genus-selective, and must be understandable by conspecifics in communicating with each other. In this article, we examine studies of aggression using invertebrate models: (1) to illustrate the highly organized nature of this complex behavior; (2) to explore the roles of neurohormones such as amines in the behavior; and (3) to begin an exploration of the role of specific genes in establishing behavioral patterns in nervous systems. A comprehensive review of aggression in invertebrates has appeared recently (b0115 Kravitz, and Huber, 2003). In this article, therefore, the focus will be solely on studies with fruit flies and with two decapod crustacean species, crayfish and lobsters.

1.27.1.1 A Short History: Nature vs. Nurture s0010

Although the presence of stereotyped elements in behavior has been recognized for more than 50 years, the interpretation of how these come about has given rise to acrimonious debate. The presence of stereotyped behavioral elements and their formation became an ultimate battleground when it became inextricably linked with the dispute over nature vs. nurture. Early ethologists noticed that many distinct, species-specific displays appear to develop fully intact, even in individuals raised without access to tutors (c.f., b0075 Heinroth and Heinroth, 1933; b0155 Lorenz, 1941). They attributed the action of p0010 inherited developmental programs to fixed action patterns and argued that such behavior would lend itself to phylogenetic analysis, akin to the use of morphological or physiological characteristics. [b0125]Lehrman (1953) delivered a stinging critique, arguing that any discussion about the genetic basis of behavioral traits is a distraction from learning more about the developmental processes that produce them. Subsequently, the emergence of any sort of comprehensive view with respect to behavioral phenomena was held up for decades by this dispute. J. P. Scott's work on the roles of genetic components in mammalian social behavior highlighted the genetic contributions to core processes in behavior and helped to establish the field of behavioral genetics ([b0220]Scott and Fuller, 1965). The practical solution now posits that behavioral development inevitably forms as a complex interchange of genes and environment and that the emergence of some traits may owe more to the contributions of one than the other. Attempts at an either/or classification between nature and nurture simply lack heuristic value due to the complexity of their entanglement ([b0170]Marler, 2004). These notions have been adopted by researchers in evolution, genetics, and neuroscience with their fields moving on to more important questions. Studies of behavior in ethology, psychology, and sociology, however, are only now beginning to emerge from this counterproductive clash of extreme positions. The present review aims more to discuss the characteristics and functioning of stereotyped elements in behavior that occur linked to specific elements in the nervous system, and less to contributions of how these systems got there.

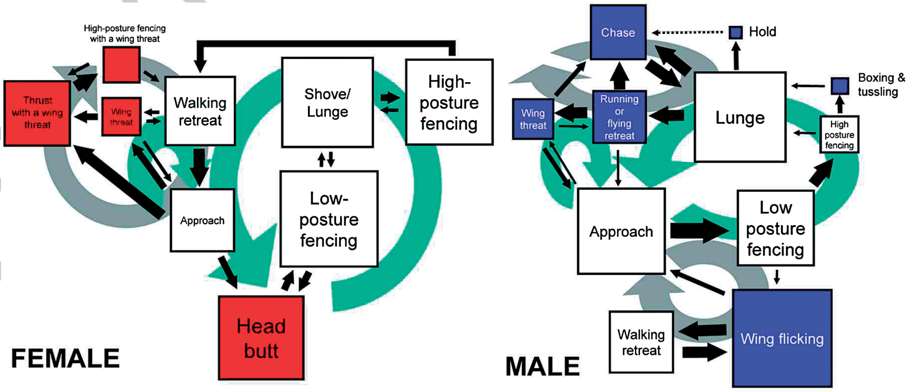
1.27.1.2 Why Aggression? s0015

p0015 Intraspecific aggression is a behavior that is particularly well suited to a comprehensive examination of its component structure and underlying mechanisms. In many taxa, agonistic encounters are readily evoked in dyadic interactions, making possible studies ranging from behavioral through physiological and ultimately to molecular and genetic levels. Animals with no previous social experience or tutors often are able to perform and interpret agonistic behavioral patterns and their structure. With a clear ability to decode the communication components of the behavior, animals are effectively able to take part in coordinated, agonistic encounters. Even with these innate abilities, however, the behavioral patterns are malleable and can be molded by experience.

1.27.2 The Fruit Fly Model of Aggression s0020

1.27.2.1 Characterization s0025

p0020 Male and female fruit flies (*Drosophila melanogaster*) both show aggression. Ethograms of the behavioral patterns involved have been constructed and first-order Markov Chain analyses have been performed to explore the dynamics of the fights ([b0040]Chen *et al.*, 2002; [b0175]Nilsen *et al.*, 2004). In comparing the patterns of fighting behavior between pairs of male and pairs of female flies, some of the patterns seen are the same in the male and female fights, while others appear selective to male or female fights (Figure 1; [b0175]Nilsen *et al.*, 2004). Thus certain mid- or high-intensity components of fighting such



f0005 **Figure 1** Behavioral patterns and transitions seen in fights between pairs of male and pairs of female *D. melanogaster*. Females and males share five common behavioral patterns (white boxes) while several gender-selective behavioral patterns also are seen (blue-male and red-female boxes). The box sizes represent the numbers of transitions to and from a pattern. The arrows between boxes represent the likelihood of transitions, with the addition that the dashed line between *hold* and *chase* in males illustrates a transition that approaches statistical likelihood. The large light blue arrows highlight similar transition loops, and the gray arrows highlight gender-selective transition loops. The female data is from 2597 behavioral transitions and the male data was collected from 2526 transitions from 376 encounters in 19 trials. Reprinted from [b0175]Nilsen *et al.*, 2004, Proc. Natl. Acad. Sci. U. S. A.

as chasing, tussling, lunging, and boxing along with extended wing threats (greater than 2 s and up to several minutes) are seen predominantly in male fights, whereas head butting and very short wing threats (all under 2 s) are found mainly in encounters between females. Moreover, male fights are more likely to lead to hierarchical relationships than female fights (Nilsen *et al.*, 2004).

p0025 These patterns appear in full complexity without requiring a tutor, but also are malleable by experience. In all experiments, male and female flies are placed singly in isolation vials containing fly food when they first emerge as adults or as pupae that emerge as adults in isolation. Thus the first time that these flies see another adult fly is when they are paired for fights, yet they use all the components that make up fighting behavior, they change intensity levels in appropriate ways in response to their opponents, and they establish hierarchical relationships (at least in males). This and genetic evidence suggest that the responsible neural circuits are established during embryonic, larval, or pupal life, and are already fully functional when flies emerge as adults. While the patterns are established early in development, they can be modified by experience that both alters the way male flies fight and changes the likelihood of their winning subsequent fights (Yurkovic, A. unpublished observations).

s0030 1.27.2.2 Genes and the Establishment of Behavioral Modules

p0030 In addressing the issue of how patterns of behavior get established in nervous systems, one approach is to ask how the patterns of female fighting behavior get established in female brains and the male patterns in male brains. Logical candidate genes for this purpose are the already established genes of the sex determination pathway (c.f. Goodwin, 1999; Baker *et al.*, 2001). The X-autosome ratio determines gender in fruit flies (females are XX and males XY), and early genes involved in the determination are: *sex lethal* (*sxl*), a splicing factor; *transformer* (*tra*), a second splicing factor that works in conjunction with a second transformer gene (*tra*-2), and *fruitless* (*fru*), a gene that codes for expression of members of the bric-a-brac-tramtrac-broad (BTB) complex family of zinc finger transcription factors (for reviews see Goodwin, 1999; Baker *et al.*, 2001). The genes *sxl* and *tra* are transcribed and translated in females but not in males. This leads to splicing of *fru* to male- and female-specific transcripts, all of which are generated from the first of four promoter sites found in the gene. The male-specific transcripts generate Fru^{M} proteins, while no proteins are generated from the female-specific mRNAs (see Lee *et al.*, 2000; Goodwin *et al.*, 2000). Mutant *fru* male flies cannot distinguish between male and female flies and will actively court both.

An indication that the emergence of behavioral modules might be influenced by genes is the report by Lee and Hall (2000), demonstrating enhanced levels of head-to-head interactions in *fru* mutant male flies. Such interactions are rarely seen in fights between pairs of wild type male fruit flies, but they are commonly seen in fights between pairs of wild type female flies (Nilsen *et al.*, 2004). Thus it is possible that *fru* mutant male flies not only cannot distinguish between males and females during courtship rituals, but perhaps one of the behavioral patterns associated with female fighting behavior might have been introduced into the brains of male flies in the absence of *fru* transcripts. Recent studies by Nilsen (unpublished observations) suggest that *fru*-null male flies fight more like females than like males in that extended wing threats and mid- and high-intensity components of male fighting behavior are not observed in these animals, whereas head butting is common. p0035

1.27.2.3 Genes and Mating Behavior s0035

Mating behavior has been well studied in fruit flies, and as mentioned above, the genes of the sex-determination pathway have been defined (c.f. Goodwin, 1999; Baker *et al.*, 2001). Mating behavior too appears largely modular, with distinct recognizable components including approach and orienting, tapping, singing, licking, attempted copulation, and copulation taking place in a well-defined sequence that is compatible with a first-order Markov Chain (Markow and Hanson, 1981). As mentioned above, *fru* mutant males cannot distinguish between males and females. The *fru* gene is expressed in about 20 groups of neurons in the fly brain and nerve cord (Goodwin *et al.*, 2000; Lee *et al.*, 2000). Using an RNAi technique, Manoli and Baker (2004) eliminated the FRU^{M} protein isoforms in one subgroup of about 60 *fru*-expressing central nervous system neurons located in the subesophageal ganglion. These neurons send processes from that site in a major projection through the median bundle to terminate in the dorsal protocerebrum of the brain. The phenotype of the resultant male flies was that they attempted to court females, but the first two modules associated with courtship behavior were not observed (orienting and tapping): instead flies immediately began singing, licking females, and attempting to copulate all at the same time and at an accelerated rate. These male flies were p0040 unsuccessful at copulating with females. In some thus far unknown way, the absence of FRU^M expression in this one subgroup of neurons leads to the absence of two of the early components of the courtship ritual, and the speeding up and disordering of the later ones.

s0040 1.27.2.4 Hormones and the Modulation of Behavioral Modules

p0045 Singing is usually not observed during aggression between pairs of males in *Drosophila melanogaster*. In other species, however, such as *Drosophila pseudoobscura*, singing is part of the fighting ritual (Nilsen, S. unpublished observations). Singing also can be introduced into fights between male *D. melanogaster* by altering levels of the amine octopamine (Certel, S. J. *et al.* unpublished observations). Using fly lines with mutations in the enhancer region for the gene tyramine-β-hydroxylase (the key enzyme in the biosynthesis of octopamine) generated by Monasterioti *et al.* (1996), it was found that flies with low or normal levels of octopamine would not sing during fights, while flies with double the level of octopamine would. This effect seemed related to selective effects of the amine on all modules of behavior associated with wing movements, as wing threats also increase in occurrence with increases in the levels of octopamine in the flies. These studies raise the interesting possibility that different neurohormones might have selective effects on different patterns of behavior and that switching between patterns during behavioral interactions might depend on the release of such substances associated with the social context at the time.

s0045 **1.27.2.4.1 The role of genes, hormones, and experience in molding behavior in fruit flies**

p0050 There is little doubt that genes play an important role in laying down behavioral patterns in fruit fly central nervous systems. In addition, with fruit fly studies leading the way, much recent work has focused on defining the sequences of genes involved in specifying neuronal identity and in establishing pathways of neuronal connectivity in the developing nervous system (c.f., Skeath and Thor, 2003). Much less work, however, has been done on the role of genes in establishing the behavioral patterns that are of concern here. Here again, however, elegant work with fruit flies is leading the way. For example, Bate and colleagues (Suster and Bate, 2002; Langraf *et al.*, 2003) have explored how the central pattern generator (CPG), which governs movements of larval fruit flies, gets established. Their findings are that rather than motor neuron cell body position determining the organization of these motor systems, the dendritic arbors of motor neurons appear to form a myotopic map within the larval ventral nerve cord. These endings form a central representation of the peripheral musculature and they do not require the muscles themselves, properly differentiated glial cells or sensory input to form their organizational domains. While sensory input is not required to form the central motor patterns, without sensory input the polarity of the movement patterns is abnormal. They found further that even though muscles are organized segmentally, the arbors of endings of the motor neurons are in the embryonic parasegmental organization lined up with the anterior margin of *engrailed* gene expression in the nervous system. They suggest that neurons of the ventral nerve cord can “differentiate autonomously to produce the CPG for larval peristalsis in Drosophila,” and that this may be an essential feature of the formation of patterns within the fruit fly central nervous system (Suster and Bate, 2002).

1.27.3 Crustaceans as Model Organisms for the Study of Aggression: Exploring the Role of Amines in Aggression s0050

1.27.3.1 Characterization of the System s0055

Crustacean species, including crayfish and lobsters, represent excellent study systems to explore the structure of complex behaviors and their causation. Paired fighting between socially naïve animals features an exchange of highly stereotypical behaviors that escalate through different intensity levels and that ultimately result in a decision with behavioral consequences for both winners and losers (Bruski and Dunham, 1987; Huber and Kravitz, 1995; Pave and Fielder, 1996; Barki *et al.*, 1997). Quantitative analysis of agonistic (fighting) behavior in lobsters and crayfish allowed the construction of ethograms of the common behavioral patterns and their temporal structure (Huber and Kravitz, 1995; Huber *et al.*, 2004). Fighting proceeds according to strict rules of conduct. All animals exhibit a series of common behavioral patterns in a stereotypical manner. A temporal sequence of these patterns was evident, representing an increase in intensity during confrontations. The typical scenario of an encounter begins with extensive threat displays upon first contact, continues with periods of ritualized aggression and restrained use of the claws, and terminates in a brief session of unrestrained combat. Predictions of game theory (i.e., assessment strategies) provide a useful framework for the understanding of fighting in lobsters p0055 ([b0195]Parker and Rubenstein, 1981; [b0130]Leimar and Enquist, 1984). The presence of a highly structured behavioral system may reduce the potential for damage in fights among conspecifics, and may prove useful in attempts to study the neurobiological causes of complex behavioral patterns such as aggression.

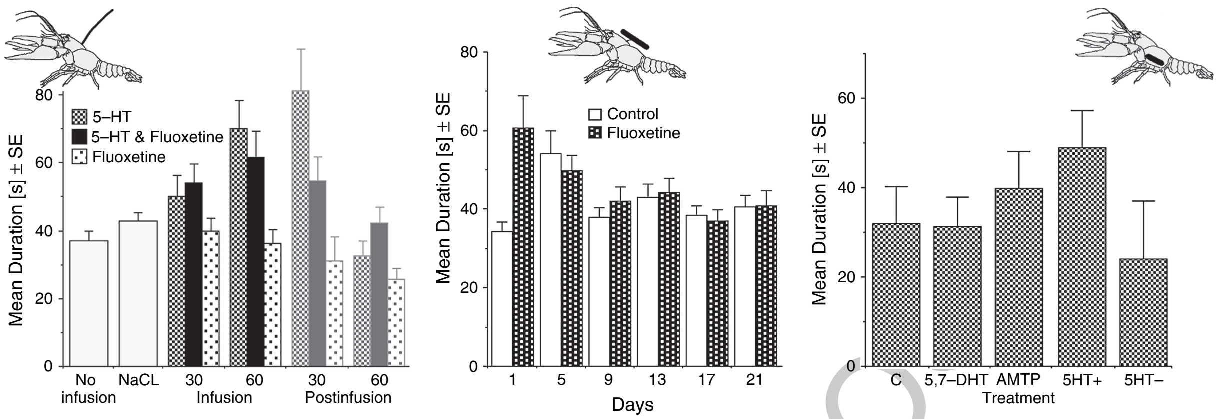
s0060 1.27.3.2 Neurohormones and Aggression

p0060 A common theme in these studies is that amines, peptides, and steroid hormones, substances that function as neuromodulators and as neurohormones, serve as important modulators of aggression at the behavioral level. Amine systems have been extensively studied in crustaceans. Beginning with studies showing that injections of octopamine and serotonin into animals triggered the appearance of opposing postures that resembled those seen in winning and losing animals during fights ([b0145]Livingstone *et al.*, 1980), and continuing with demonstrations of the localization of amine neurons in the nervous system ([b0025]Beltz and Kravitz, 1983; Schneider *et al.*, 1983), learning how these neurons function ([b0030]Beltz and Kravitz, 1987; [b0160]Ma *et al.*, 1992; [b0065]Heinrich *et al.*, 2000; [b0110]Kravitz, 2000), and recent demonstrations of changes in the functioning of these neurons with changes in social status ([b0245]Yeh *et al.*, 1996, [b0250]1997; [b0055]Edwards *et al.*, 1999), much has been learned about the functioning of amine neurons in aggression in crustacean systems. Several recent reviews describe the results of these experiments ([b0110]Kravitz, 2000; [b0190]Panksepp *et al.*, 2003; [b0115]Kravitz and Huber, 2003; [b0090]Huber *et al.*, 2004; [b0080]Huber, 2005). The focus here, therefore, will be on the behavioral effects of amine infusion and/or on the consequences of pharmacological manipulations that alter amine levels in these species.

p0065 In one set of studies, fighting behavior has been examined in crayfish pairs with large size asymmetries (>30%), where the smaller (subordinate) animal received acute serotonin infusions via a fine-bore, fused silica cannula (Huber *et al.*, 1997a, 1997b; [b0095]Huber and Delago, 1998). During such treatment, infused animals re-engaged their larger opponents, resulting in longer fights that reached higher levels of intensity compared with controls. Multivariate techniques (e.g., discriminant function analysis) revealed that serotonin treatment specifically altered the decision to retreat from an opponent, without affecting how likely the animal was to initiate fights, how individual fights progressed to higher intensities, or in the case of large size asymmetries, the eventual social rank that was achieved. In these studies, therefore, serotonin appears to act on key sites for agonistic decision making in the crayfish central nervous system. It is likely that serotonin reuptake mechanisms play an essential role in this behavioral change as the effects associated with acute serotonin infusion were significantly reduced in the presence of fluoxetine (Huber *et al.*, 1997a, 1997b; [b0095]Huber and Delago, 1998). This finding suggests that a functional high-affinity serotonin reuptake mechanism ([b0150]Livingstone *et al.*, 1981) is involved, and that serotonin-associated behavioral plasticity requires the reloading of synaptic terminals (which can be blocked by fluoxetine). Little information is available on the categories of serotonin receptors involved.

In studies with juvenile lobsters, although arriving at similar empirical outcomes after injections of serotonin, other investigators offered alternative interpretations of the observed effects, suggesting that an inhibition of retreat simply reflects global down-regulation of motor activity or motor coordination (see [b0210]Peeke *et al.*, 2000) (Figure 2). To address such criticism and to further distinguish between specific aggression-enhancing effects of serotonin and more global effects on motor activity or coordination, levels of activity, movement patterns, and space utilization were studied in crayfish receiving serotonin in ways that corresponded in dose, mode, site, and time course of infusion to those used in the earlier fighting studies. Under such conditions and compared with controls, acute serotonin-infusion neither altered absolute levels of locomotion nor movement patterns ([b0080]Huber, 2005). p0070 AU2

Subsequent work using chronic augmentation or disruption of serotonin function explored to what degree considerations of time scales and dynamic properties enhance understanding of the links between amines and behavior. Pharmacological interventions that alter serotonin–neuron function appear to be accompanied by the rapid induction of compensatory mechanisms that counteract such treatments. Thus, the differences in fighting behavior resulting from chronic infusions of serotonin were initially accompanied by the anticipated effects on behavior, but these were followed by a steady decline in effectiveness of the amine (Panksepp and Huber, 2002; [b0190]Panksepp *et al.*, 2003). Infusions from silastic tube implants containing serotonin (Panksepp and Huber, 2002) and lasting up to several weeks initially boosted absolute amine levels in the nervous system. Within a week, however, it appeared that adaptations took place in the system that counteracted the effects of constant infusion and absolute levels of serotonin returned to pretreatment levels. Moreover, with continued serotonin infusion, some animals actually showed serotonin depletion likely resulting from overcompensation in p0075



f0010 **Figure 2** Composite figure illustrates differences in fight duration resulting from pharmacological manipulations of crayfish serotonin systems. Fight durations varied considerably with some lasting a few seconds and others several minutes. Encounters are graphed according to mean duration (in seconds) of fighting during the experimental period. a, Fine-bore fused silica capillaries were used to infuse serotonin, fluoxetine, or both substances together into freely moving, subordinate animals. Serotonin infusion (at 3μg/min) resulted in longer fighting that persisted well after the infusion pump was turned off. Multivariate statistical techniques (i.e., discriminant function analysis) revealed that longer bouts of fighting resulted from a decreased likelihood of retreat. Infusion of fluoxetine alone did not enhance aggression, however, coinfused with serotonin resulted in a reduction of the fight-enhancing effects of serotonin. b, Chronic infusion of fluoxetine via osmotic minipumps increased duration of fighting during the early stages of treatment compared to animals receiving vehicle only. As with acute serotonin infusion, these differences in fighting were due to a decrease in the probability for retreat. c, Duration of fighting in animals that received chronic silastic implants containing either 5-HT synthesis inhibitors (5,7-dihydroxytryptamine or alpha-methyltryptamine) or serotonin at one of two different rates. No significant differences in fight duration existed among these groups. The figure and text are reprinted from Huber *et al.*, 2001 with permission. Reproduced from Huber, R., Panksepp, J. B., Yue, Z., Delago, A., Moore, P. 2001. Dynamic interactions of behavior and amine neurochemistry during acquisition and maintenance of social rank in crayfish. Brain Behav. Evol. 57, 271–282, with permission from Krager AG, Basel.

the system. Such neuronal compensation could involve changes at many different levels, including altered synthesis ([b0235]Stachowiak *et al.*, 1986; Sivam 1995), amine release ([b0135]Lent 1984; [b0060]Hall *et al.*, 1999), metabolic activity ([b0010]Åse *et al.*, 2000), or receptor distribution or turnover ([b0200]Patel *et al.*, 1996; [b0240]Woo *et al.*, 1996). The studies utilizing chronic treatments did not generate increases in the aggression-enhancing effects of serotonin like those that result from acute infusions. Although this advises caution when discussing possible links between amines and behavior, it more likely reflects the constraints in working within a dynamic system in which critical components are the relative size of a signal relative to a given set point, and the timing of when a signal is triggered. Thus, the global systemic effects of pharmacological manipulations may be poor mimics of normal physiological processes in which neurons release neurohormones such as amines within nervous systems at precise times for actions on restricted sets of targets. To illustrate, raising or lowering levels of serotonin in lobsters by pharmacological manipulations generates the same behavioral phenotype of enhanced aggression ([b0050]Doernberg *et al.*, 2001). It may well be that the more precise and selective control of amine neuron function that is available utilizing genetic methods such as the GAL4/UAS system (Kitamoto 2001; Brand *et al.*, 1994), or recently developed methods for selectively activating neurons in behaving animals ([b0140]Lima and Miesenbock, 2005), may provide more accurate information on how neurohormones function in complex behaviors such as aggression.

1.27.4 Summary s0065

p0080 There is little doubt that in invertebrate animals, complex behavioral patterns exist that are stereotypical, recognizable by conspecifics, moldable by experience, and likely specified during development by genes. For the most part, there is little or no parental rearing in these species. Thus, as organisms emerge as adults, they must be fully capable of functioning on their own in order to survive and procreate. The fruit flies and crustacean species described in this article know all the rules of how to fight and make decisions the first time they meet a conspecific, and the decisions made as a result of agonistic interactions and probably other social experiences as well have profound consequences on their subsequent behavior. Neurohormonal substances such as amines serve important and essential roles in behaviors such as aggression in these species and much elegant experimental work has focused on defining the roles served by these substances. In vertebrate species, with their extended periods of rearing, extensive learning or refining of behavioral patterns undoubtedly takes place shortly after pups emerge. How much of the rearing builds on patterns already defined, at least in outline form, by genes, during embryonic life, and how much defines new behaviors and patterns, remains an area of great contention. This discussion gets even more contentious when one relates it to human beings and to higher human functions such as cognition. Our goal is not to engage in this debate in this article. Instead, we aim to establish that there is much to learn from simpler forms such as invertebrates about important and essential processes that relate directly to human life and its complexities. Aggression is a serious problem in human society, a problem that undoubtedly has a biological basis. Invertebrate models offer insights into how complex behavioral patterns get established in nervous systems that are difficult to impossible when addressed in higher forms with the methodologies available at the present time, and offer elegantly detailed information on how social experience in turn molds behavior and the nervous system at levels of detail that are equally inaccessible with vertebrate studies. Those facts alone justify and warrant continuing studies of the amazing invertebrate organisms and the rich worlds in which they function and have functioned for far longer periods of evolutionary history than has man.

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