



Pergamon

Journal of Insect Physiology 46 (2000) 1509–1515

*Journal
of
Insect
Physiology*

www.elsevier.com/locate/jinsphys

Mini review

Organizational and activational effects of hormones on insect behavior

Michelle M. Elekonich ^{a,*}, Gene E. Robinson ^b

^a Department of Psychology and Department of Entomology, University of Illinois at Urbana-Champaign, 320 Morrill Hall, MC118, 500 S Goodwin, Urbana, IL 61801, USA

^b Department of Entomology and Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Received 14 March 2000; accepted 5 June 2000

Abstract

The concepts of hormone organization and activation provide a framework for thinking about the influence of hormones on development, brain, and behavior in vertebrates. There is good evidence for activational effects of hormones on the nervous system and behavior in insects, but organizational effects are almost never discussed in the insect literature. This paper explores the utility of the concepts of hormonal organization and activation of behavior in insects. We describe the two concepts as developed from studies of vertebrates, review some insect examples that appear to fit this classification scheme, and consider how explicit use of the concept of organization might benefit studies of the insect brain and behavior. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Behavior; Juvenile hormone; Ecdysone; Activational effects; Organizational effects

The connected concepts of organization and activation, common to the vertebrate neuroendocrine literature, serve as a framework for understanding the influence of hormones on development, brain, and behavior. There is good evidence for activational effects of hormones on the nervous system and behavior in insects, but they are usually called releaser or modifier effects (Truman and Riddiford, 1974). Organizational effects are almost never discussed in the insect literature (c.f. Truman 1992, 1995) and the term is not even mentioned in the most recent authoritative review of insect hormones (Nijhout, 1994). There are, however, several examples of hormone-mediated polymorphisms and polyphenisms that imply organizational effects (reviewed in Applebaum and Heifetz, 1999; Nijhout, 1999a,b). Sometimes they are termed morphogenic or primer effects (Truman and Riddiford, 1974). But the organization concept goes beyond these descriptive terms to suggest behavioral effects of hormone exposure early in life that are manifested later in life.

In this paper we explore the utility of the concepts of hormonal organization and activation of behavior in insects. We focus on the insects because they are one of the most-well studied invertebrate classes from the perspective of behavioral endocrinology. We begin with a brief review of the two concepts as developed from studies of vertebrates. We then review some insect examples that appear to fit this classification scheme, and we end by suggesting how explicit use of the concept of organization might benefit studies of insect brains and behavior.

1. Background and history

The development of species-typical and sex-specific adult behaviors in vertebrate animals is influenced by gonadal steroid hormones, non-gonadal hormones, and non-hormonal factors working on the underlying neural circuitry (reviewed in Diamond et al., 1996; Kawata, 1995; Schlinger, 1998). Effects of hormones on brain and behavior occur through three mechanisms: (1) behaviors both organized and activated by hormones, (2) behaviors only organized by hormones, and (3) behaviors only activated by hormones (reviewed in Arnold and Breedlove, 1985; Diamond et al., 1996).

* Corresponding author. Tel.: +1-217-333-6843; fax: +1-217-244-3499.

E-mail address: elekonic@life.uiuc.edu (M.M. Elekonich).

The terms organization and activation are used to describe hormonal effects specifically on behavior and its neural substrates, and not on development or morphology in general. According to the classical definition (Phoenix et al., 1959; Young et al., 1964), organizational effects of hormones on behavior are those that occur during a specific critical period early in development and create permanent changes in the neural substrates that underlie behaviors. These changes include reorganization of neural pathways by creation of new synapses and alteration of thresholds for neural response. Organizational effects of hormones cause the development of the nervous system and behavior to proceed along separate developmental pathways, such as masculine and feminine. They may also cause variation in the development of the nervous system and behavior of individuals within a single developmental pathway.

Activational effects by definition occur at a later stage than organizational effects, usually during adulthood. They modify neural activity within already existing pathways, some of which were previously sculpted by organizational effects. Activational effects may include changes in the production or release of neurotransmitters or upregulation of hormone receptors. Activational effects serve to alter the behavioral state of the animal in response to a variety of changes in environmental or social conditions.

Organizational effects are not reversible, but activational effects typically are. For example, a female rat gonadectomized as a neonate will not display female-typical sexual behavior (lordosis) when exposed to a male as an adult, even if treated with the relevant hormones, estrogen and progesterone. A female rat gonadectomized as an adult also will not display lordosis when exposed to a male. However, she will if injected with estrogen and progesterone (reviewed in Pfaff et al., 1994; Takahashi, 1990). Performance of lordosis requires the presence of hormones during adulthood but the neural circuitry underlying the behavior, previously “organized” under the influence of perinatal hormones, is not permanently lost in the absence of adult exposure to hormones.

The terms organization and activation first appeared in the literature in 1959 in a study that compared the female offspring of guinea pigs injected with testosterone propionate during pregnancy with the female offspring of untreated individuals. The offspring were gonadectomized, hormone-treated, and assayed for male-like and female-like sexual behavior (Phoenix et al., 1959). Gonadectomized females exposed to testosterone in utero displayed significantly lower levels of lordosis when injected with estradiol and progesterone than gonadectomized females injected with the same hormones but not exposed to testosterone in utero. Charles Phoenix, Robert Goy, and colleagues concluded that hormone exposure during the perinatal period had an “organizing

or differentiating action on neural tissues mediating mating behavior....during adulthood the hormones are activational” (Phoenix et al., 1959). Later work identified neural effects of hormonal organization, such as sexual dimorphism in neuroanatomy and the functioning of steroid-sensitive neural circuits controlling sexually dimorphic behaviors (e.g. Breedlove and Arnold, 1980; Gurney and Konishi, 1980).

As research in this area continued the distinction between organizational effects and activational effects has become less obvious. Critical periods may not be limited to early life, activational effects are sometimes permanent (reviewed in Arnold and Breedlove, 1985), and some neuroanatomical and neurochemical systems show chronic hormone-induced plasticity. For example, changes in the titer of circulating ovarian steroids in female rats that occur during the estrus cycle are associated with variation in the density of synapses among pyramidal neurons in the CA 1 region of the hippocampus, a brain area involved in learning and memory (Wolley and McEwen, 1992). This type of change was previously considered typical of an organizational effect, but in this case it occurs in adults and over a time scale more typical of an activational effect.

A second recent development in this area is the growing appreciation of more subtle organizational effects. Early work emphasized big differences in hormone exposure, such as hormone presence or absence, and a resulting dramatic effect on nervous system and behavior, such as “male-like” or “female-like”. Recent work indicates that smaller differences in hormone exposure during development can also have effects (e.g. Fitch and Denenberg, 1998; Moore et al., 1998). For example, differences in intrauterine position in rodents cause relatively small variation in fetal exposure to hormones; overall hormone levels are themselves quite low. Even this variation in hormone exposure is sufficient to cause behavioral variation among littermates (reviewed in Clark and Galef, 1998). A third recent development in the vertebrate literature is the growing appreciation of the role of non-steroidal hormones, especially peptide hormones, in the organization and activation of behavior (e.g. McKinley and Oldfield, 1998; Weiger, 1997).

2. Activational effects in insects

The insect literature provides numerous examples of hormone activation of behavior. Some of the behaviors in which hormone activation occurs include: mating, oviposition, foraging and flight, wandering, and ecdysis (reviewed in Atkins and Stout, 1994; Ewer et al., 1997; Rankin, 1991; Zera et al., 1989). We will briefly review a few examples in the following paragraphs, chosen because the effects are understood at both behavioral and neural levels.

Hormones activate various aspects of reproductive behavior, in some cases by the interaction of different hormones. This has been especially well studied in the cricket, *Acheta domesticus* (reviewed in Strambi et al., 1997). As circulating levels of juvenile hormone (JH) increase, female crickets become more responsive to male calls. This is apparently due to JH-related decreases in the response thresholds of prothoracic auditory interneurons. Response thresholds increase with allatectomy (removal of the JH-producing corpora allata glands, the CA), an effect that is reversed with transplants of CA or hormone treatment. Varying the timing of allatectomy relative to the imaginal molt revealed that JH initiates oviposition behavior but is not required for it to continue. Other hormones also appear to be involved in cricket oviposition behavior. Prostaglandin E₂ injection increases the frequency of oviposition behavior in both immature virgin and ovariectomized females, and amplifies the behavioral effects of JH injection in allatectomized females. In addition, both JH and ecdysteroids are involved in neurogenesis in the mushroom bodies of the cricket brain. Because these brain regions are involved in the integration of complex behaviors, it is likely that the mushroom bodies also play a role in oviposition behavior.

Another example of hormone-activated mating behavior involves control of responsiveness to pheromones. JH controls the responsiveness of male *Agrotis ipsilon* moths to female sex pheromones (Anton and Gadenne, 1999). Using the electroantennogram assay, the antennae of newly eclosed male moths were shown to be responsive to sex pheromones upon adult emergence, but intact males themselves were not. This is apparently because hemolymph titers of JH are low in newly eclosed adults. Age-related increases in JH biosynthesis are involved in the onset of male responsiveness to pheromone, due to a JH-dependent reduction in the response thresholds of neurons in the male specific macroglomerular complex of the olfactory lobes. Allatectomy of older males decreases responsiveness, while JH treatment of newly emerged or allatectomized males increases responsiveness (Anton and Gadenne, 1999).

JH also influences the division of labor among workers in colonies of the European honey bee, *Apis mellifera*. It does so by affecting the rate of behavioral development, i.e. the age at which bees switch from working in the hive to foraging outside for nectar and pollen. Circulating JH titers increase over the course of adult behavioral development. Young adults caring for brood and performing other tasks in the hive for the first 2–3 weeks of life typically have low circulating levels of JH. Foraging bees, which as a class are the oldest bees in the colony, have the highest JH titers (reviewed in Huang and Robinson, 1999). There is no evidence for an involvement of ecdysteroids in division of labor among workers; circulating titers are undetectably low in both

hive bees and foragers (but are measurable in queens and egg-laying workers; Robinson et al., 1991). JH also influences age-related responsiveness to alarm pheromone in honey bees (Robinson, 1987); in workers less than 5 days of age treatment with the JH analog methoprene induces a precocious sensitivity to alarm pheromones, in a manner similar to the induction of precocious foraging following treatment with methoprene, JH or JH mimics. In contrast, isolated antennae do not show the same age-related increase in alarm pheromone response and are not sensitive to hormone treatment (Robinson, 1987). These results led to the idea that JH influences behavioral development in honey bees by modulating central nervous system response thresholds to task-related stimuli (Robinson, 1987); this would be consistent with an activational effect. However, allatectomy does not prevent foraging, but rather causes bees to begin foraging at an older age. This delay in foraging onset is eliminated with methoprene treatment (Sullivan et al., 2000). The nature of the allatectomy effect is difficult to interpret as evidence for hormone activation. The organization and activation concepts were originally described as dichotomous (Phoenix et al., 1959). However, more recent data from vertebrates suggests that they actually define the extremes of a continuum that describes the time course and intensity of effects of hormones on the neural substrates which underlie behavior (see Arnold and Breedlove, 1985; Berenbaum, 1998). Perhaps the effects of JH on honey bee division of labor provide an example of hormone effects that blur the distinction between activation and organization in insects.

The best understood example of an activational effect on insect behavior is larval ecdysis in the hawk moth, *Manduca sexta* (Ewer et al., 1997). Ecdysis, the shedding of the old cuticle following a molt, depends on positive feedback between eclosion hormone and ecdysis triggering hormone. The release of the neuropeptide eclosion hormone from neurosecretory cells in the central nervous system causes the peripherally located epitracheal glands to release ecdysis triggering hormone. Ecdysis triggering hormone in turn causes the release of more eclosion hormone. Injections of either eclosion hormone or ecdysis triggering hormone will induce ecdysis. In addition to facilitating release of ecdysis triggering hormone, eclosion hormone also causes release of crustacean cardioactive peptide which directly triggers motor output (Gammie and Truman, 1999). When both hormones are released together through this positive feedback mechanism a neural network is activated that supports the sustained performance of ecdysis (Ewer et al., 1997). Recent work with mutant *Drosophila* lacking the eclosion hormone gene (Baker et al., 1999) suggests that additional factors are involved in ecdysis including other peptide hormones (most likely crustacean cardioactive peptide) and release of inhibition by descending neurons.

3. Organizational effects in insects

Similarities between the endocrine systems of insects and vertebrates suggest that insects should also show organizational effects on behavior. Organizational effects were first shown for vertebrate steroid hormones as described above. Insects, of course, have steroid hormones, and recent molecular evidence indicates common mechanisms of steroid action on neural development in both insects and vertebrates (Kawata, 1995; Mangelsdorf et al., 1995; Thummel, 1995). In fact, much of the research that has elucidated steroid action has relied on insect preparations. Regulation of gene expression by steroid binding to nuclear receptors was first shown by Clever and Karlson (1960) in larvae of the midge *Chironomus tentans* because their giant polytene chromosomes were more easily directly visualized than those of vertebrate preparations (reviewed in Ashburner, 1990).

Nijhout and Wheeler (1982) and Nijhout (1994) have developed a conceptual model to explain how JH controls insect polymorphism and polyphenism. Both polymorphism and polyphenism are characterized by striking patterns of intraspecific variation in behavior, morphology, and physiology. Polymorphism usually describes variation between individuals that is associated with variation in genotype and polyphenism describes variation among genotypically similar individuals induced solely by environmental factors. Nijhout and Wheeler proposed that JH controls polymorphisms and polyphenisms by influencing gene expression during “JH sensitive periods” that occur periodically during the life of an insect. These proposed sensitive periods for JH function are similar to the critical periods for organizational effects of steroid hormones in vertebrates. JH is known to regulate the expression of various genes (reviewed in Jones, 1995; Wyatt and Davey, 1996), but these analyses have not yet been extended to polymorphisms or polyphenisms.

Some of the best examples of endocrine effects that are compatible with the organization concept come from the literature on caste determination in social insects. Caste determination processes are thought to be polyphenisms (c.f. Kerr and Nielsen, 1966), but there has been little research on the issue of whether genotypic variation contributes to different probabilities of developing into one caste or another. The first example is queen-worker determination in honey bees. A female honey bee egg is totipotent, and can develop into either a worker or a queen. A larva that experiences suprathreshold levels of JH during an early sensitive period develops into a queen (reviewed in Hartfelder and Engels, 1998). Starting in the third larval stadium, individuals destined to become queens are fed royal jelly, a substance high in both sugars and proteins. This added nutrition causes an increase in JH production. Larvae developing into queens have a higher metabolic rate than

worker-destined larvae, as indicated by increased oxygen uptake (Shuel and Dixon, 1968) and higher levels of mitochondrial gene expression (Corona et al., 1999). Starting in the last larval instar queens and workers also show differential rates of ovarian protein synthesis which correlate with differences in circulating ecdysteroid titers. In fact, treatment with makisterone A, the major ecdysteroid hormone found in the hemolymph of honey bee larvae and pupae, induces queen-typical protein synthesis in worker honey bee ovaries cultured in vitro (Hartfelder et al., 1995). Other differences in gene expression between worker and queen larvae have been reported (Evans and Wheeler, 1999), in some cases with worker larvae showing higher levels of gene expression. These caste-specific differences in gene expression (and many others no doubt awaiting discovery) are reasonably interpreted as direct or indirect consequences of differential exposure to hormones. This is reminiscent of steroid-induced differential gene expression during vertebrate sexual differentiation (reviewed in Fitch and Denenberg, 1998; Berenbaum, 1998).

The result of caste determination in honey bees, like sexual differentiation in vertebrates, is two types of adult individuals that show extreme differences in behavior, morphology, and physiology (reviewed in Hartfelder and Engels, 1998). For example, workers perform all the tasks required for colony growth and maintenance, while queens are occupied solely with oviposition. Workers and queens both possess numerous morphological adaptations for their specialized roles (Winston, 1987). Workers show an age-related increase in JH titer for about the first three weeks of adulthood that influences the rate at which they make the transition from working in the hive to foraging outside. In contrast, the JH titer in queens peaks within the first few days of adult life and then drops (Fahrbach et al., 1995); the function of JH in adult queens is not well understood (Robinson and Vargo, 1997). The volume of the mushroom bodies, areas of the brain associated with behavioral integration and learning, is much larger in adult workers than in queens (Fahrbach et al., 1995). Increases in dendritic arborization of neurons in the mushroom bodies during the life of the adult worker bee (Farris et al., 1999) do not appear to require adult exposure to JH (Sullivan et al., 1996). However, this does not preclude an organizational role of JH in the development of the mushroom bodies. The plausibility of this suggestion is supported by recent evidence indicating that many cells in the mushroom bodies of honey bee larvae survive metamorphosis and persist into adulthood (Farris et al., 1999).

JH also influences caste determination in ants and termites. This is a striking example of convergent evolution because the holometabolous ants, in the order Hymenoptera, are only distantly related to the hemimetabolous termites, in the order Isoptera. In the ant, *Pheidole bica-rinata*, exposure to JH between the fourth and sixth days

of the final instar causes larvae to continue to grow instead of pupating. These larger larvae then become soldiers, behaviorally and morphologically specialized for defense, while larvae that did not experience increased JH become minor workers, specialized for brood care (Wheeler and Nijhout, 1984). Caste-specific differences in brain structure similar to those observed in honey bees have recently been reported for ants (Gronenberg and Liebig, 1999). In *Zootermopsis angusticollis* termites, JH influences worker-queen differentiation, but in this case high levels of JH during a critical period induce the development of workers, rather than queens (Lüscher, 1974). Caste specific differences in gene expression have been reported recently in termites (Miura et al., 1999), but possible differences in the brain have not yet been studied.

In vertebrates, organizational effects can depend not only on the internal state of the animal during an early critical period, but also on exposure to specific environmental stimuli. Density-dependent, endocrine-mediated polyphenism in the locust, *Locusta migratoria*, is reminiscent of temperature-dependent, steroid-mediated sex determination in crocodiles, turtles, and lizards (reviewed in Crews, 1993). High population density experienced by the mother, embryos, and nymphs leads to development of gregarious adult locusts with yellow body color and reduced fecundity. Low population densities lead to development of adults that behave in a more solitary manner, have green coloration and higher fecundity (Pener, 1991; Pener and Yerushalmi, 1998). These differences are due, in part, to density-dependent effects on behavior (Applebaum and Heifetz, 1999) and the endocrine system (Pener and Yerushalmi, 1998).

Prototypical organizational effects in vertebrates involve steroid hormones like testosterone, which alter both the structure and function of neurons and neuronal circuits throughout development (e.g. Arnold and Gorski, 1984). In lepidopteran insects ecdysteroids regulate neuronal morphology and cell death during metamorphosis (e.g. Levine et al., 1991). For these processes to reflect organizational effects of ecdysteroids, there must also be permanent differences in the nervous system and behavior of adults who experienced different early hormone regimes; unfortunately this information is not currently available. But the following results from *Drosophila melanogaster* suggest just such effects of ecdysteroids.

The *dare* gene has recently been identified in *Drosophila*; sequence comparisons with vertebrate genes suggested it is an insect homologue of the gene that encodes adrenodoxin reductase, an enzyme involved in steroid hormone synthesis. In situ hybridization showed that *dare* mRNA expression was highest in fly tissues that synthesize steroids (Freeman et al., 1999). Transgenic adult flies with mildly reduced expression of *dare* exhibited decreased avoidance of normally repellent odors;

this deficit was rescued by the addition of transgenes that express adrenodoxin reductase. Transgenic adults with even lower levels of *dare* have widespread neural degeneration. In null mutants, normal development is disrupted at the second larval instar and is reinstated by treatment with ecdysteroids (Freeman et al., 1999). Given that some neurons survive from the larval through adult stages in the species of Diptera, Hymenoptera, and Lepidoptera studied to date (reviewed in Farris et al., 1999), these results suggest that ecdysteroids can exert organizational effects.

Another possible context for organizational effects of ecdysteroids involves the effects of this hormone on the differentiation of imaginal discs, patches of undifferentiated epidermal tissue found inside immature insects that develop to become adult appendages and sensory organs. Because imaginal discs are sensitive to ecdysteroids, perhaps variation in ecdysteroid levels at certain critical times could induce variation in sensory structures. This might then lead to variation in perception of environmental cues and subsequent behavioral variation.

4. Conclusions

The utility of the concept of hormonal organization of behavior is to suggest that there is a direct connection between variation in early hormone levels and variation in later behavior and its neural substrates. With this perspective researchers are more open to consider how various environmental and endogenous factors early in life influence individual variation in behavior in later life stages. Even relatively small differences in environment can exert strong effects, such as testosterone-mediated intrauterine position effects in female mice (e.g. Clark and Galef, 1998). In insects, it might be productive to consider more broadly the possible effects of variation in temperature, population density, and diet experienced during early life stages on later behavior.

Another possible benefit of using the concept of hormone organization in insect studies is to focus attention on the possibility of critical periods for behavior. Critical periods for morphological development in insects have been discussed (e.g. Hartfelder and Engels, 1998; Wheeler and Nijhout, 1984), but not explicitly in the context of behavior. These critical periods may occur sometime during larval development, which might be roughly analogous to the perinatal period in vertebrates. Or they may occur during the transitions between larval instars, like the JH sensitive periods for development proposed by Nijhout and Wheeler (1982).

Using both concepts of hormone organization and activation might suggest various strategies for identifying hormone effects on behavior. For example, there are many examples from vertebrates of both organizational and activational effects on the same behavioral

system. Perhaps some of the examples reviewed above, either in the category of activation or organization, actually involve some of both. For example, JH exerts an activational effect on responsiveness to sex pheromones in male *Agrotis ipsilon* moths; perhaps it also plays an organizational role for this sex-specific neuropil (e.g. Schneiderman et al., 1982). Another possibility is that brain regions involved in a particular hormone-mediated behavior might be identified by searching for regions that exhibit especially high concentrations of hormone receptor (e.g. Arnold et al., 1976). This remains problematic for JH (Jones, 1995; Wyatt and Davey, 1996), but not for ecdysteroids. Researchers also may consider organizational and activational effects of peptide or other non-steroidal hormones in invertebrates (e.g. Kravitz, 2000).

The classical concepts, organization and activation, now seen as poles along a continuum of hormonal effects on behavior, provide a comprehensive framework for thinking about the effects of hormones on behavior. While the insect literature contains numerous examples of hormone activation, explicit use of the organization concept provides a window into the developmental origins of phenotypic variation in behavior. It also broadens the time course over which hormonal actions on insect behavior are considered, from egg to adult.

Acknowledgements

We would like to thank S.N. Beshers, Y. Ben-Shahar, G. Bloch, E.A. Capaldi, S.E. Fahrback, D.J. Schulz, J.P. Sullivan and one anonymous reviewer for comments on the manuscript. MME was supported by a postdoctoral fellowship from the University of Illinois Developmental Psychology and Neurobiology Training Grant (PHS 5 T32 HDO7333-12) and a NRI/CSREES grant (USDA 99-35302-8187).

References

- Anton, S., Gadenne, C., 1999. Effect of juvenile hormone on the central nervous processing of sex pheromone in an insect. *Proceedings of the National Academy of Sciences USA* 96, 5764–5767.
- Applebaum, S.W., Heifetz, Y., 1999. Density dependent physiological phase in insects. *Annual Review of Entomology* 44, 317–341.
- Arnold, A.P., Gorski, R.A., 1984. Gonadal steroid induction of structural sex differences in the CNS. *Annual Review of Neuroscience* 7, 413–442.
- Arnold, A.P., Breedlove, S.M., 1985. Organizational and activational effects of sex steroids on brain and behavior: A re-analysis. *Hormones and Behavior* 19, 469–498.
- Arnold, A.P., Nottebohm, F., Pfaff, D.W., 1976. Hormone concentrating cells in vocal control and other brain regions of the zebra finch (*Poephila guttata*). *Journal of Comparative Neurology* 165, 487–512.
- Ashburner, M., 1990. Puffs, genes and hormones revisited. *Cell* 61, 1–3.
- Atkins, G., Stout, J., 1994. Processing of song signals in the cricket and its hormonal control. *American Zoologist* 34, 655–669.
- Baker, J.D., McNabb, S.L., Truman, J.W., 1999. The hormonal coordination of behavior and physiology at adult ecdysis in *Drosophila melanogaster*. *Journal of Experimental Biology* 202, 3037–3048.
- Berenbaum, S., 1998. How hormones affect behavioral and neural development: Introduction to the special issue on “Gonadal hormones and sex differences in behavior”. *Developmental Neuropsychology* 14, 175–196.
- Breedlove, S.M., Arnold, A.P., 1980. Hormone accumulation in a sexually dimorphic motor nucleus in the rat spinal cord. *Science* 210, 564–566.
- Clark, M.M., Galef, B.G. Jr., 1998. Effects of intrauterine position on the behavior and genital morphology of litter-bearing rodents. *Developmental Neuropsychology* 14, 197–211.
- Clever, U., Karlson, P., 1960. Induktion von puff-veränderungen in den speicheldrüsen chromosomen von *Chironomus tentans* durch ecdyson. *Experimental Cell Research* 20, 623–626.
- Corona, M., Estrada, E., Zurita, M., 1999. Differential expression of mitochondrial genes between queens and workers during caste determination in the honeybee *Apis mellifera*. *Journal of Experimental Biology* 202, 929–938.
- Crews, D., 1993. The organizational concept and vertebrates without sex chromosomes. *Brain, Behavior and Evolution* 42, 202–214.
- Diamond, M., Binstock, T., Kohl, J.V., 1996. From fertilization to adult sexual behavior. *Hormones and Behavior* 30, 333–353.
- Evans, J.D., Wheeler, D.E., 1999. Differential gene expression between developing queens and workers in the honey bee, *Apis mellifera*. *Proceedings of the National Academy of Sciences USA* 96, 5575–5580.
- Ewer, J., Gammie, S.C., Truman, J.W., 1997. Control of insect ecdysis by a positive-feedback endocrine system: roles of eclosion hormone and ecdysis triggering hormone. *Journal of Experimental Biology* 200, 868–881.
- Fahrback, S.E., Giray, T., Robinson, G.E., 1995. Volume changes in the mushroom bodies of adult honey bee queens. *Neurobiology of Learning and Memory* 63, 181–191.
- Farris, S.M., Robinson, G.E., Davis, R.L., Fahrback, S.E., 1999. Larval and pupal development of the mushroom bodies in the honey bee, *Apis mellifera*. *Journal of Comparative Neurology* 414, 97–113.
- Fitch, R.H., Denenberg, V.H., 1998. A role for ovarian hormones in sexual differentiation of the brain. *Behavioural and Brain Sciences* 21, 311–352.
- Freeman, M.R., Dobritsa, A., Gaines, P., Segraves, W.A., Carlson, J.R., 1999. The *dare* gene: steroid hormone production, olfactory behavior, and neural degeneration in *Drosophila*. *Development* 126, 4591–4602.
- Gammie, S.C., Truman, J.W., 1999. Eclosion hormone provides a link between ecdysis triggering hormone and crustacean cardioactive peptide in the neuroendocrine cascade that controls ecdysis behavior. *Journal of Experimental Biology* 202, 343–352.
- Gronenberg, W., Liebig, J., 1999. Smaller brains and optic lobes in reproductive workers of the ant *Harpegnathos*. *Naturwissenschaften* 86, 343–345.
- Gurney, M., Konishi, M., 1980. Hormone induced sexual differentiation of brain and behavior in zebra finches. *Science* 208, 1280–1282.
- Hartfelder, K., Engels, W., 1998. Social insect polymorphism: Hormonal regulation of plasticity in development and reproduction in the honeybee. *Current Topics in Developmental Biology* 40, 45–78.
- Hartfelder, K., Köstlin, K., Hepperle, C., 1995. Ecdysteroid-dependent protein synthesis in caste-specific development of the larval honey bee ovary. *Roux Archives of Developmental Biology* 205, 73–80.
- Huang, Z.-Y., Robinson, G.E., 1999. Social control of the division of labor in honey bee colonies. In: Detrain, C., Deneubourg, J.L., Pasteels, J.M. (Eds.), *Information Processing in Social Insects*. Birkhäuser Verlag, Basel Switzerland, pp. 165–186.

- Jones, G., 1995. Molecular mechanisms of action of juvenile hormone. *Annual Review of Entomology* 40, 147–169.
- Kawata, M., 1995. Roles of steroid hormones and their receptors in structural organization in the nervous system. *Neuroscience Research* 24, 1–46.
- Kravitz, E.A., 2000. Serotonin and aggression: insights gained from a lobster model system and speculations on the role of amine neurons in a complex behavior. *Journal of Comparative Physiology A* 186, 221–238.
- Kerr, W.E., Nielsen, R.A., 1966. Evidence that genetically determined *Melipona* queens can become workers. *Genetics* 54, 859–866.
- Levine, R.B., Fahrbach, S.E., Weeks, J.C., 1991. Steroid hormones and the reorganization of the nervous system during insect metamorphosis. *Seminars in the Neurosciences* 3, 437–447.
- Lüscher, M., 1974. Die Kompetenz zur Soldatenbildung bei Larven (psueduergaten) der Termiten *Zootermopsis angusticollis*. *Revue Suisse Zoologie* 81, 710–714.
- Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Manuel, M., Chambon, P., Evans, R.M., 1995. The nuclear receptor superfamily: The second decade. *Cell* 83, 835–839.
- McKinley, M.J., Oldfield, B.J., 1998. The brain as an endocrine target for peptide hormones. *Trends in Endocrinology and Metabolism* 9, 349–354.
- Miura, T., Kamikouchi, A., Sawata, M., Takeuchi, H., Natori, S., Kubo, T., Matsumoto, T., 1999. Soldier caste-specific gene expression in the mandibular glands of *Hodotermopsis japonica* (Isoptera: Termitidae). *Proceedings of the National Academy of Sciences USA* 96, 13874–13879.
- Moore, M.C., Hews, D.K., Knapp, R., 1998. Hormonal control and evolution of alternative male phenotypes: Generalization of models for sexual differentiation. *American Zoologist* 38, 133–151.
- Nijhout, H.F., 1999a. Control mechanisms of polyphenic development in insects. *Bioscience* 49, 181–192.
- Nijhout, H.F., 1999b. Hormonal control in larval development and evolution. In: Hall, B.K., Wake, M.H. (Eds.), *The Origin and Evolution of Larval Forms*. Academic Press, San Diego, pp. 217–254.
- Nijhout, H.F., 1994. *Insect Hormones*. Princeton University Press, Princeton, NJ.
- Nijhout, H.F., Wheeler, D., 1982. Juvenile hormone and the physiological basis of insect polymorphisms. *Quarterly Review of Biology* 57, 109–133.
- Pener, M.P., 1991. Locust phase polymorphism and its endocrine relations. *Advances in Insect Physiology* 23, 1–79.
- Pener, M.P., Yerushalmi, Y., 1998. The physiology of locust phase polymorphism: an update. *Journal of Insect Physiology* 44, 365–377.
- Phoenix, C., Goy, R., Gerall, A., Young, W., 1959. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology* 65, 369–382.
- Pfaff, D.W., Schwartz-Giblin, S., McCarthy, M.M., Kow, L.-M., 1994. Cellular and molecular mechanisms of female reproductive behaviors. In: Knobil, E., Neill, J. (Eds.), *The Physiology of Reproduction*, vol. 2. Raven Press, New York, pp. 107–220.
- Rankin, M.A., 1991. Endocrine effects on migration. *American Zoologist* 31, 217–230.
- Robinson, G.E., 1987. Regulation of honey bee age polyethism by juvenile hormone. *Behavioral Ecology and Sociobiology* 20, 329–338.
- Robinson, G.E., Vargo, E.L., 1997. Juvenile hormone in adult eusocial Hymenoptera: Gonadotropin and behavioral pacemaker. *Archives of Insect Biochemistry and Physiology* 35, 559–583.
- Robinson, G.E., Strambi, C., Strambi, A., Feldlaufer, M., 1991. Comparison of juvenile hormone and ecdysteroid hemolymph titers in adult worker and queen honey bees (*Apis mellifera*). *Journal of Insect Physiology* 37, 929–935.
- Schlinger, B.A., 1998. Sexual differentiation of avian brain and behavior: Current views on gonadal hormone-dependent and independent mechanisms. *Annual Review of Physiology* 60, 407–429.
- Schneiderman, A.M., Matsumoto, S.G., Hildebrand, J.G., 1982. Transsexually grafted antennae influence development of sexually dimorphic neurones in moth brain. *Nature* 298, 844–846.
- Shuel, R.W., Dixon, S.E., 1968. Respiration in developing honey bee larvae. *Journal of Apicultural Research* 7, 11–19.
- Strambi, A., Strambi, C., Cayre, M., 1997. Hormonal control of reproduction and reproductive behavior in crickets. *Archives of Insect Biochemistry and Physiology* 35, 393–404.
- Sullivan, J.P., Jassim, O., Robinson, G.E., Fahrbach, S.E., 2000. Juvenile hormone paces behavioral development in the adult worker honey bee. *Hormones and Behavior* 37, 1–14.
- Sullivan, J.P., Jassim, O., Robinson, G.E., Fahrbach, S.E., 1996. Foraging behavior and mushroom bodies in allatectomized honey bees. *Society for Neuroscience Abstracts* 22, 1144.
- Takahashi, L.K., 1990. Hormonal regulation of sociosexual behavior in female mammals. *Neuroscience and Biobehavioral Reviews* 14, 403–413.
- Thummel, C.S., 1995. From embryogenesis to metamorphosis: The regulation and function of *Drosophila* nuclear receptor superfamily members. *Cell* 83, 871–877.
- Truman, J.W., 1992. Hormonal regulation of behavior: Insights from invertebrate systems. In: Becker, J.B., Breedlove, S.M., Crews, D. (Eds.), *Behavioral Endocrinology*. MIT Press, Cambridge MA, pp. 423–450.
- Truman, J.W., 1995. Lepidoptera as model systems for studies of hormone action on the central nervous system. In: Goldsmith, M.R., Wilkins, A.S. (Eds.), *Molecular Model Systems in the Lepidoptera*. Cambridge University Press, London, pp. 323–339.
- Truman, J.W., Riddiford, L.M., 1974. Invertebrate systems for the study of hormonal effects on behavior. *Vitamins and Hormones* 35, 283–350.
- Weiger, W.A., 1997. Serotonergic modulation of behaviour: A phylogenetic overview. *Biological Reviews* 72, 61–95.
- Wheeler, D.E., Nijhout, H.F., 1984. Soldier determination in the ant *Phidole bicarinata*: Inhibition by adult soldiers. *Journal of Insect Physiology* 30, 127–135.
- Winston, M.L., 1987. *The Biology of the Honey Bee*. Harvard University Press, Cambridge MA.
- Wolley, C.S., McEwen, B.S., 1992. Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. *Journal of Neuroscience* 12, 2549–2554.
- Wyatt, G.R., Davey, K.G., 1996. Cellular and molecular actions of juvenile hormone II. Roles of juvenile hormone in adult insects. *Advances in Insect Physiology* 26, 1–155.
- Young, W.C., Goy, R.W., Phoenix, C.H., 1964. Hormones and sexual behavior. *Science* 143, 212–218.
- Zera, A.J., Strambi, C., Tiebel, K., Strambi, A., Rankin, M.A., 1989. Juvenile hormone and ecdysteroid titers during critical periods of wing morph determination in *Gryllus rubens*. *Journal of Insect Physiology* 35, 501–511.