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# Neural and Hormonal Regulation of Calling Behavior in *Blattella germanica* Females

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**Virgin females of the German cockroach, *Blattella germanica*, exhibit cycles of calling behavior in relation to the cycles of corpora allata activity and ootheca production. The roles of juvenile hormone, mating, and the ootheca in the regulation of calling behavior were investigated. The onset of calling in relation to increasing rates of juvenile hormone synthesis suggested involvement of the corpora allata. Allatectomy prevented the expression of calling behavior and treatment with the juvenile hormone analog fenoxycarb restored calling. Corpus allatum denervation through nervi corporis allati transection, which accelerates the synthesis of juvenile hormone by the corpora allata, also accelerated the onset of calling. Using castrated males, timed interruption of mating and ventral nerve cord transection, we demonstrated that, while insertion of the male genitalia and/or spermatophore in the bursa copulatrix caused an immediate suppression of calling behavior, presence of sperm and/or associated seminal fluid in the spermatheca was required for the complete inhibition of calling in the first as well as in the second ovarian cycle. Although inhibitory signals from the presence of an ootheca in the genital atrium would suppress calling behavior indirectly by inhibiting corpus allatum activity, treatment of gravid females with fenoxycarb suggested that signals from the ootheca also inhibit calling directly. The ventral nerve cord plays a critical role in the transmission of inhibitory signals from mating and from the presence of the ootheca.**

German cockroach  Calling behavior  Juvenile hormone  Neural inhibition  Mating

## INTRODUCTION

Calling, a behavior associated with release of pheromone in many lepidopterans (Charlton and Cardé, 1982; Schal and Cardé, 1985; Krasnoff and Roelofs, 1988), has been intensively studied in relation to various environmental regulatory factors such as light and temperature [review: McNeil (1991)]. With regard to its physiological regulation, juvenile hormone has been shown to play no role in pheromone production and release in most moth species; rather, calling behavior is regulated neurally (Tang *et al.*, 1987; Itagaki and Conner, 1986) while neuropeptides control pheromone production [Raina *et al.*, 1989; but see also Cusson and McNeil (1989)]. Mating results in total suppression of calling in moths [e.g. Webster and Cardé (1984)], but the signals involved and their mechanisms of action have been studied in only a few species. In *Manduca sexta*, the switch from calling to oviposition was mediated by interaction of sperm and/or seminal fluids with the bursa copulatrix (Sasaki and Riddiford, 1984; Stringer *et al.*, 1985). Recently, Giebultowicz *et al.* (1991) showed that in the gypsy moth, *Lymantria dispar*, insertion of the male genitalia into the bursa copulatrix resulted in a transient suppression of calling behavior, while copulation with sperm transfer terminated calling permanently.

Calling behavior has been observed in females of several cockroach species (Hales and Breed, 1983; Seelinger, 1984; Schal and Bell, 1985) but its physiological regulation has been studied only in *Supella longipalpa*. In this oviparous species that deposits an ootheca within 2 days after ovulation, juvenile hormone is involved in the regulation of calling behavior as well as pheromone production (Smith and Schal, 1990a). The calling behavior is transiently inhibited until the completion of one ovarian cycle by the insertion of a spermatophore during copulation, while presence of sperm in the spermatheca inhibits calling during subsequent gonotrophic cycles (Smith and Schal, 1990b).

The German cockroach, *Blattella germanica*, provides a unique model for studies of cockroach reproductive physiology. It exhibits an intermediate reproductive strategy between the oviparous and ovoviviparous species: the female retains and carries an ootheca in its bursa vestibulum from ovulation until the nymphs hatch (Roth and Stay, 1962). During this 3-week "pregnancy" period, the activity of the corpora allata and thus the

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development of oocytes is inhibited by neural feedback from the ootheca (Roth and Stay, 1962; Gadot *et al.*, 1991; Chiang *et al.*, 1991). The ootheca is easily accessible for experimental manipulations, such as removal and replacement. Recently, we have shown that virgin females of the German cockroach exhibit a characteristic calling behavior (Liang and Schal, 1993b) during which a volatile sex pheromone is released (Liang and Schal, 1993a). The incidence of calling shows a clear relationship with the gonotrophic cycle. Calling occurs only during the vitellogenic period when juvenile hormone biosynthesis by the corpora allata is increasing. However, while mating stimulates juvenile hormone synthesis (Gadot *et al.*, 1989b), it terminates the calling behavior, suggesting a complex neural and endocrine regulation of the behavior. In this paper, we investigate the involvement of juvenile hormone in the expression of calling and the mechanisms by which calling is inhibited by mating and presence of an ootheca.

## MATERIALS AND METHODS

### *Insects*

Insects were maintained at 27°C, RH 30–60%, under a photoperiod of 12D:12L with Purina Rat Chow No. 5012 and water provided *ad libitum*. Newly emerged adults (day 0) were collected daily and males and females were kept in separate groups under the same conditions [see Gadot *et al.* (1989b)].

### *Observation of calling*

From day 0 or after experimental manipulations, females were housed individually in 9 × 1.5 cm transparent plastic petri dishes with food and water and with two males whose left phallomere was ablated. Phallomerectomized males were used to prevent retarded development of females due to isolation (Gadot *et al.*, 1989b); such males court vigorously but are unable to copulate. Observations were conducted twice daily, at 2 h before and 2 h after lights-on, because the calling behavior, characterized by stilted legs and raised wings, shows a diel periodicity with a broad peak around lights-on (Liang and Schal, 1993b). A group of females was observed repeatedly during a 20 min observation period, and a female was considered calling if she exhibited the characteristic posture described by Liang and Schal (1993b). Fluorescent lights with red photographic safelight filters provided constant red illumination to facilitate observations during the scotophase.

### *Microsurgery*

Insects were immobilized on ice and, if necessary, secured on a wax dish with plasticene. Allatectomies were conducted on day 0 females by cutting an opening in the dorsal cervical sclerite and removing the corpora allata with a portion of the corpora cardiaca. Completeness of the operation was ascertained at the conclusion of each experiment. Sham control females were manipulated the same way except the corpora allata were not removed. The ventral nerve cord was transected by removing a portion of the ventral nerve cord between the third and fourth abdominal ganglia to ensure that no nerve regeneration occurred. The testes were removed from males soon after the last nymphal molt through incisions in the intersegmental membrane between the fifth and sixth tergites. Examination of the spermatophores produced by these males confirmed the success of the operations. All operations were conducted under a stereo dissecting microscope and antibiotics were not used.

### *Mating interruption and removal of spermatophore and ootheca*

Females were allowed to mate with 10–20 day-old males on day 5, unless stated otherwise. Mating in *B. germanica* lasts about 60 min with a spermatophore transferred in the last 20 min. The spermatophore is ejected by the female from the bursa copulatrix after 12–24 h. Some spermatophores were removed manually with forceps after squeezing the female's abdomen to expose the spermatophore. For mating interruption experiments, the female and male were separated 40 min after the initiation of mating. Subsequent examination of the females showed that no spermatophore was transferred.

In *B. germanica*, an ootheca is produced whether the female is mated or not. Mated females produce oothecae on day 8 and carry them for about 3 weeks until just before nymphs hatch. Virgin females produce oothecae around day 9. While many virgin females abort the ootheca within 3 days of oviposition, some retain it much longer [see Liang and Schal (1993b) for further detail]. Manual removal of the ootheca releases the corpora allata from brain inhibition and results in faster ootheca production (Roth and Stay, 1962).

### *Juvenile hormone analog treatment*

Fenoxycarb (ethyl [2-(4-phenoxyphenoxy) ethyl]-carbamate, 99% pure) was applied onto the bottom of each 9 × 1.5 cm plastic petri dish in 200 μl ethanol. After the solvent evaporated, insects were placed in the petri dish until the end of the experiment with food and water. Ten micrograms of fenoxycarb was used in each petri dish unless otherwise indicated.

## RESULTS

### *Allatectomy*

Allatectomies were performed to determine whether calling behavior occurred in the absence of juvenile hormone. None of the females that were allatectomized on day 0 exhibited calling behavior during the 12 day observation period, compared with 94% calling in sham-operated control females (Table 1). The juvenile hormone analog fenoxycarb induced 95% of allatectomized females to initiate the calling behavior (Table 1), and all females that called initiated the behavior before day 8.

TABLE 1. Effect of allatectomy and juvenile hormone replacement therapy on female calling behavior in *B. germanica**

| Treatment | *N* | %Females calling | Onset of calling day ± SEM |
|---|---|---|---|
| Sham-allatectomy | 17 | 94 | 6.31 ± 0.87 |
| Allatectomy | 16 | 0 | — |
| Allatectomy + fenoxycarb† | 21 | 95 | 4.75 ± 0.72 |

*Females that died during the 12 day observation period were excluded from analysis.
†Ten micrograms of fenoxycarb was applied in ethanol to the bottom surface of a petri dish housing each female.

*NCA-I transections*

Brain inhibition of juvenile hormone biosynthesis by the corpora allata can be removed by transecting the nervi corporis allati (NCA-I) which connect the corpora allata to the brain through the corpora cardiaca. Such corpus allatum denervation accelerates the rate of juvenile hormone biosynthesis and oocyte development in virgin *B. germanica* females (Gadot *et al.*, 1989b; Schal *et al.*, 1993). The initiation of calling was earlier in NCA-I transected females (5.21 ± 0.20, $N = 42$) than in control females (5.90 ± 0.17, $N = 41$) (*t*-test, $P < 0.01$) (Fig. 1).

*Treatment with juvenile hormone analog after oviposition*

Calling behavior did not occur for about 10 days after oviposition, even though in most virgin females the infertile ootheca was aborted within three days of its formation (Liang and Schal, 1993b). During this period the corpora allata are inactive, as evidenced by lack of oocyte growth (Roth and Stay, 1962) and low rates of juvenile hormone synthesis *in vitro* (Gadot *et al.*, 1989a). This provided a unique period of low juvenile hormone titer in sexually mature females. We tested whether the lack of calling during this period is due to the lack of juvenile hormone. When virgin females were exposed to fenoxycarb after their ootheca was removed on day 12 (2–3 days after oviposition), calling behavior was observed the next day in 21% of the females. Within 5 days, 100% of the females treated with fenoxycarb exhibited calling behavior, but none of the control females, whose ootheca was also removed, exhibited calling by this day (Fig. 2). In the control group, females began to call on day 18, in relation to increasing corpora allata activity and oocyte growth [unpublished, see also Gadot *et al.* (1989b)]. It thus appears that calling behavior is not expressed in sexually mature females when juvenile hormone titers are low. Calling can be stimulated with exogenous treatments of juvenile hormone or with endogenous activation of the corpora allata.

*Mating interruption and spermatophore removal*

The effects of mating on calling behavior in the first ovarian cycle were examined by observing calling in individual females that were housed with two phallomerectomized males until oviposition. Females were allowed to mate with normal males on day 4 and they usually did not oviposit until day 8. During the preoviposition period, none of the mated females exhibited calling behavior while 95% of virgin females placed with phallomerectomized males called (Table 2). Calling activity is totally suppressed by a single successful copulation which lasts approx. 60 min and terminates with the insertion of a spermatophore into the bursa copulatrix of the female.

Neither interruption of mating before spermatophore transfer, nor removal of the spermatophore within 5 min after mating resulted in total suppression of calling in the first ovarian cycle (Table 2). In both treatments, females did not exhibit calling behavior on the day of mating, but calling resumed on the following day and most of the females exhibited calling behavior before they oviposited (89% for mating interruption and 80% for sperma-

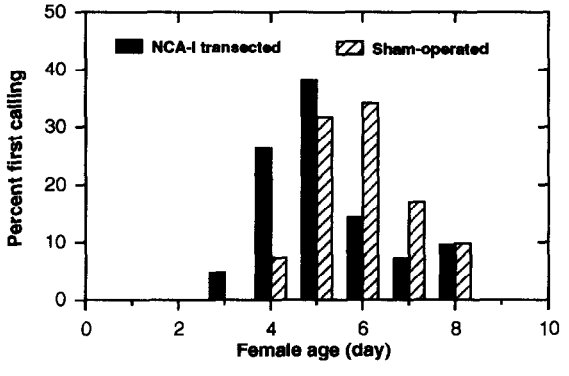


FIGURE 1. Effect of nervi corporis allati (NCA-I) transection on the initiation of calling behavior in virgin *B. germanica* females. The operation was performed on day 0. $N = 42$ and 41 for NCA-I transected and control groups, respectively.

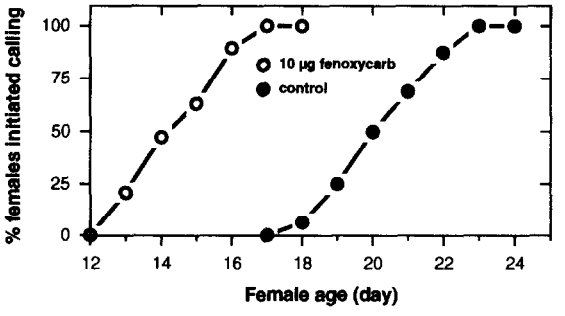


FIGURE 2. Advancement of the onset of calling in virgin *B. germanica* females treated with 10 µg fenoxycarb. All females had their oothecae removed on day 12 and then were treated with fenoxycarb ($N = 19$) or a solvent control ($N = 16$).

TABLE 2. Mating inhibition of female calling behavior in the first ovarian cycle in *B. germanica*

| Treatment | *N* | %Females calling before the first oviposition |
|---|---|---|
| Virgin control | 19 | 95 |
| Mated with normal males | 14 | 0 |
| Copulation interrupted 40 min after its initiation* | 18 | 89 |
| Spermatophore removed 5 min after insertion | 25 | 80 |
| Spermatophore removed 30 min after insertion | 19 | 5.3 |
| Mated with castrated male with spermatophore removed 60 min after its insertion | 16 | 100 |

*Before spermatophore transfer.

tophore removal respectively). These results indicated that insertion of the male genitalia and/or spermatophore during mating might temporarily inhibit calling, but females resumed calling before oviposition. In contrast, nearly complete suppression of calling before oviposition was achieved if the spermatophore was allowed to remain in the bursa copulatrix for 30 min. Calling was observed in only 5% of females whose spermatophore was removed 30 min after the completion of copulation (Table 2).

*Mating with castrated males*

That sperm transferred from the spermatophore plays a key role in the suppression of calling after mating was demonstrated by mating females with castrated males. Such males transfer a normal-sized spermatophore which contains no sperm, as confirmed by microscopic examination. While no calling was observed in the first night after removal of the spermless spermatophore, 100% of the females resumed calling on the next day (Table 2). This suggests that insertion of the male genitalia and/or spermatophore effected only a temporary inhibition of calling and it highlights the importance of sperm or associated seminal fluid in the complete suppression of calling behavior.

*Ventral nerve cord transection before mating*

The inhibitory signals from mating and sperm and/or associated seminal fluid could be hormonal, or neural signals transmitted via the ventral nerve cord. When the ventral nerve cord was transected between the third and fourth abdominal ganglia on day 0, the females exhibited a normal schedule of calling with 97% of females calling by day 8 (age for onset of calling was 6.63 ± 1.16 days). After the onset of calling, operated females were provided normal males for 2 h daily. Females with transected ventral nerve cords readily mounted the courting males, but fewer copulated compared with sham-operated females: only 62.5% of females with transected ventral nerve cord mated before oviposition while 93% of control females copulated. We considered only copulations longer than 50 min successful. Mating completely terminated calling in sham-operated females with intact ventral nerve cords. However, 75% of the females with transected ventral nerve cord continued to call after mating (Table 3), some within minutes after separating from the male. Examination of females indicated the successful transfer of a spermatophore in all females. Because the calling behavior involves mainly thoracic movement, ventral nerve cord transection in the abdomen did not disrupt the expression of calling. This result suggests that the signals associated with spermatophore insertion and sperm or associated fluid transfer inhibit calling after mating, and that they are transmitted neurally through the ventral nerve cord to the anterior portion of the central nervous system, most likely the brain.

*Inhibition of calling in gravid virgin females treated with juvenile hormone analog*

After oviposition, the ootheca of ovoviviparous cockroaches (Roth, 1973) and of *B. germanica* (Roth and Stay, 1962; Chiang *et al.*, 1991) inhibits juvenile hormone sythesis by the corpora allata. We have previously showed that virgin and mated *B. germanica* females do not call while carrying an ootheca (Liang and Schal, 1993b). When the ootheca is removed from virgin females on day 12, they readily express the calling behavior within a few days after treatment with fenoxycarb (Fig. 2). Because all treated females called before any of the control females, and the corpora allata remain inactive in control females (Gadot *et al.*, 1991), this unique oviparous model allowed us to examine other signals that inhibit calling in females that are hormonally in a ready state for calling. When virgin females with an ootheca were treated with fenoxycarb on day 12, no calling behavior was observed (up to 8 days) as long as the ootheca was retained (Table 4). This indicated that, in addition to inhibiting juvenile hormone synthesis, the ootheca also inhibits the expression of calling behavior in virgin females even in the presence of exogenous juvenile hormone.

TABLE 3. Effect of ventral nerve cord (VNC) transection on female calling behavior after copulation in *B. germanica**

| Treatment | No. females | No. calling | No. mated | %Calling after mating |
|---|---|---|---|---|
| Sham-VNC transection | 14 | 14 | 13 | 0 |
| VNC transection | 33 | 32 | 20 | 75 |

*A spermatophore was present in each mated female in both treatments.

TABLE 4. Roles of the ootheca and ventral nerve cord in calling behavior of *B. germanica* females treated with fenoxycarb*

| Treatment | *N* | %Females exhibited calling by day 20 |
|---|---|---|
| Virgin carrying an ootheca | 13 | 0 |
| Virgin with ootheca aborted | 18 | 94 |
| Virgin with ootheca removed† | 7 | 100 |
| Mated carrying an ootheca | 16 | 0 |
| Mated with ootheca removed† | 12 | 8.3 |
| Mated carrying an ootheca and VNC transected‡ | 14 | 93 |

*On day 12, each female was placed in a petri dish the bottom of which was treated with 10$\mu$g fenoxycarb.

†Oothecae were removed on day 15, after 3 days on fenoxycarb.

‡Ventral nerve cord transection was done on day 12. Two females that dropped the ootheca within 2 days of the operation were excluded from analysis.

To examine the interaction of neural inhibitory signals from the ootheca with endocrine signals, we monitored calling in virgin females that aborted their oothecae naturally and in females whose oothecae were removed manually (Table 4). Gravid virgin females that were treated with fenoxycarb on day 12 and aborted the infertile ootheca by day 18 initiated calling behavior shortly after dropping the ootheca: 30% called on the day they aborted the ootheca, another 40% the next day, and 94% of treated females exhibited calling behavior within 2 days after dropping the ootheca (Table 4). Similar results were observed in females whose oothecae were manually removed on day 15, 3 days after being treated with fenoxycarb: 43% initiated calling on day 15, another 43% the next day, and 100% of the treated females called within 2 days after the ootheca was removed (Table 4). When the ootheca was removed from 5 females on day 19, after 7 days on fenoxycarb, 3 called on day 19 and all called by day 20. Without fenoxycarb, no females called within 2 days of any of these treatments. These results indicate that the calling behavior in virgin females is inhibited by neural signals from the ootheca in the genital atrium even if the insect is hormonally ready to express the behavior.

*Inhibition of calling in gravid mated females treated with juvenile hormone analog*

In gravid mated females placed on fenoxycarb since day 12, no calling was observed as long as the ootheca was retained (Table 4), presumably due to the inhibitory effects of the ootheca. Removal of the ootheca on day 15 resulted in expression of calling behavior in only one of 12 mated females (Table 4). Since a similar operation resulted in 100% of virgin females exhibiting calling behavior, this result indicates that other factors, most likely the sperm or associated seminal fluid in the spermatheca, also exert independent inhibition on the calling behavior in gravid females. Both sets of signals, from the spermatheca and from the ootheca, are transmitted via the ventral nerve cord and they are disrupted by ventral nerve cord transection. Most (93%) gravid mated females that were treated with fenoxycarb immediately after ventral nerve cord transection on day 12 exhibited calling behavior within 3 days. These females also matured their basal oocytes and oviposited a new ootheca, some before dropping the first ootheca, as described by Roth and Stay (1962). After ventral nerve cord transection, females carrying two connected oothecae exhibit the characteristic calling posture.

## DISCUSSION

*Role of juvenile hormone*

Working with the communication systems of cockroaches and moths, Barth (1965) proposed that insects with prolonged imaginal lives and multiple cycles of sexual receptivity, which are punctuated with periods of sexual inactivity, should exhibit neuroendocrine control of pheromone production. In support of this hypothesis, pheromone production has been shown to be under juvenile hormone control in the ovoviviparous cockroaches, *Byrsotria fumigata* (Barth, 1962) and *Pycnoscelus surinamensis* (Barth, 1965), which exhibit distinct reproductive cycles with a long gestation period, and in an oviparous cockroach, *Supella longipalpa*, which deposits oothecae soon after ovulation (Smith and Schal, 1990a). However, in most of the early studies, pheromone production and pheromone release could not be separated in behavioral assays using live females or pheromone that adsorbed to filter papers. A distinction between production and release was possible only after the observation of a female calling behavior in *S. longipalpa* (Hales and Breed, 1983), a behavior that is associated with release of a volatile sex pheromone (Smith and Schal, 1990a). Both the production of pheromones and their emission are regulated by various environmental factors and by physiological intrinsic directives [Review: Prestwich and Blomquist (1987); McNeil (1991)]. It is likely that when pheromone production and oocyte development are under similar neuro-endocrine control, calling behavior should also be subjected to the same regulation because all three events must be coordinated. Pheromone production, female calling and oocyte maturation were shown to be controlled by juvenile hormone in the cockroach *S. longipalpa* (Smith *et al.*, 1989; Smith and Schal, 1990a) and in the moth *Pseudaletia unipuncta* (Cusson and McNeil, 1989; Cusson *et al.*, 1990).

Females of the German cockroach, *B. germanica*, exhibit a cyclic reproductive pattern in which copulation is physically impossible during a 3 week gestation period, as the ootheca is attached at the female's genital atrium. In this study, we showed that juvenile hormone is required for the expression of female calling behavior. Several lines of evidence support this conclusion:

(1) females initiate calling behavior concurrently with increasing corpus allatum activity over several ovarian cycles (Liang and Schal, 1993b),
(2) the schedule of calling was advanced by transection of nerves between the corpora allata and

the brain (Fig. 1), an operation that removes the corpora allata from brain inhibition and accelerates the increase in rates of juvenile hormone synthesis in *B. germanica* (Gadot *et al.*, 1989b; Schal *et al.*, 1993);

(3) non-calling virgin females whose corpora allata were inactive shortly after aborting an infertile ootheca were stimulated to call with the juvenile hormone analog fenoxycarb (Fig. 2); and

(4) allatectomized females fail to exhibit calling behavior and treatment with exogenous juvenile hormone restores the expression of calling behavior (Table 1).

While we have demonstrated conclusively that juvenile hormone is required for the expression of calling behavior, its mode of action remains unknown. In *B. germanica* and *S. longipalpa*, we have shown that females initiate calling when their basal vitellogenic oocytes reach a certain minimal size (Liang and Schal, 1993b; Smith and Schal, 1990a). Direct involvement of the ovaries in regulating calling was excluded by ovariectomies in both species [unpublished; Smith and Schal (1990a)]. Rather, oocyte size is an indirect measure of corpora allata activity. Because oocyte size is controlled by, and highly correlated with, juvenile hormone release from the corpora allata in both species (Gadot *et al.*, 1989a; Smith *et al.*, 1989), it suggests that expression of calling requires some threshold titer of juvenile hormone. Similarly in a migratory moth, *Pseudaletia unipuncta*, in which juvenile hormone is involved in calling behavior (Cusson and McNeil, 1989), calling females also have larger oocytes and higher rates of juvenile hormone biosynthesis than non-calling females (Cusson *et al.*, 1990).

*Role of mating*

Mating has been shown to result in drastic behavioral and physiological changes in female insects [Review: Barth and Lester (1973); Truman and Riddiford (1974); Barton Browne (1993)]. In the cockroach *S. longipalpa*, Smith and Schal (1990b) showed a two-stage regulation of termination of calling: The brief presence of a normal or spermless spermatophore is sufficient to completely suppress calling only in the first ovarian cycle, but sperm and/or seminal secretion in the spermatheca effect a complete suppression of calling. However, in *B. germanica*, while the insertion of genitalia and/or a spermatophore into the bursa copulatrix causes a temporary suppression of calling, sperm and/or associated secretions play a key role in the complete suppression of calling even in the first ovarian cycle.

In moths, inhibition of pheromone production and calling behavior after mating has also been reported. Involvement of sperm was excluded in *M. sexta* and *Heliothis* (*Helicoverpa*) *zea* because mating with normal or castrated males resulted in similar transient inhibition of calling behavior or pheromone production (Sasaki and Riddiford, 1984; Raina, 1989). In *H. zea*, in which pheromone production resumes on the first day after mating, it has been suggested that a male accessory gland secretion is responsible for the temporary suppression of pheromone production (Raina, 1989). Mechanical pressure from genital insertion into the bursa copulatrix appears to be responsible for such temporary inhibition in the gypsy moth, *Lymantria dispar*, since a 2 min interrupted copulation resulted in a decrease in pheromone titer in the pheromone gland (Giebultowicz *et al.*, 1991). Permanent suppression of pheromone production and receptivity in this species is effected by sperm in the spermatheca. In the moth *Epiphyas postvittana*, the permanent suppression of pheromone production is induced by a neural signal probably due to the presence of sperm (Foster, 1993). The ventral nerve cord is responsible for transmitting the signal from the abdomen to the central nervous system.

In *B. germanica*, the ventral nerve cord plays an important role in the inhibition of calling behavior (Table 3 and 4) and the regulation of the corpora allata (Gadot *et al.*, 1991) by signals associated with mating. An intact ventral nerve cord is required for the inhibitory signals from mating to be effective suppressers of calling behavior (Table 3). An intact ventral nerve cord was also required to turn off sexual receptivity after mating in *Nauphoeta cinerea* females (Roth, 1962); females with a transected nerve cord remained receptive after mating. Because calling behavior is only observed in virgin sexually receptive *B. germanica* females, this result suggests that calling and sexual receptivity may share common regulatory mechanisms.

*Role of the ootheca*

We previously observed that virgin females did not exhibit calling behavior while they carry an infertile ootheca (Liang and Schal, 1993b). This can be explained by inhibitory signals from the presence of the ootheca that ascend the ventral nerve cord (Table 4). When the ventral nerve cord was transected in gravid virgin or mated females, both exhibited calling behavior. However, the ventral nerve cord also transmits signals that suppress juvenile hormone synthesis in gravid females (Roth and Stay, 1962; Chiang *et al.*, 1991; Gadot *et al.*, 1991). Therefore, the occurrence of calling behavior in gravid females after ventral nerve cord transection could also be explained by activation of the corpora allata and a rise in the titer of juvenile hormone. To distinguish between direct suppression of calling by neural directives and indirect suppression through inhibiting a rise in juvenile hormone titer, we treated gravid females with fenoxycarb and either removed the ootheca or transected the ventral nerve cord. Intact gravid virgin females did not exhibit calling behavior even when treated with exogenous juvenile hormone (Table 4). However, such females initiated calling immediately after the ootheca was removed or the nerve cord cut. Therefore, the ootheca plays a dual function in controlling calling behavior: it inhibits calling directly, as well as indirectly

by suppressing juvenile hormone synthesis. Both signals ascend the ventral nerve cord and corpus allatum inhibition is effected by the brain (Gadot *et al.*, 1991). Oocyte development, on the other hand, occurs in gravid females whenever exogenous juvenile hormone is provided, even with an intact ventral nerve cord (unpublished).

We have demonstrated that calling behavior in the female German cockroach is regulated by juvenile hormone and by neural signals from the terminal abdominal ganglion. The production of the volatile sex pheromone that is emitted during this behavior is also controlled by juvenile hormone (Liang and Schal, unpublished), as are production of the contact sex pheromone (Schal *et al.*, 1991), female sexual receptivity (Schal, unpublished), and oocyte growth (Kunkel, 1973). However, while the neural signals from copulation terminate calling behavior and sexual receptivity, the rates of juvenile hormone synthesis are elevated after mating, inducing contact pheromone production and oocyte growth (Schal *et al.*, 1991). This suggests that juvenile hormone plays a "permissive" indirect role in the regulation of calling behavior. Juvenile hormone is necessary for the expression of calling, but is not sufficient in the presence of ascending inhibitory signals from the terminal abdominal ganglion. These neural signals might inhibit the release of humoral signals from the brain, whose release is possible only when juvenile hormone titers are high. Alternatively, neural signals that ascend the ventral nerve cord might inhibit a juvenile hormone-inducible motor program in the central nervous system.

In summary, it appears that the female calling behavior in *B. germanica* is an important event subjected to rigid physiological regulation. In order for a female to exhibit the calling behavior, the following requirements must be met:

(1) she is not carrying an ootheca, which would physically prevent copulation;
(2) she is in a virgin state; that is, she does not have enough sperm in the spermatheca;
(3) juvenile hormone exceeds a threshold titer, stimulating oocyte and colleterial gland maturation and sexual receptivity.

All these requirements strongly suggest a relationship between calling and mating. It seems that only when her physiological conditions permit and require mating to occur, would a female exhibit the calling behavior. During calling, the female releases a volatile sex pheromone to which males are attracted (Liang and Schal, 1993a). It has been observed that females exhibit the calling behavior only outside the shelter (Liang and Schal, 1993b). This, as well as the sex pheromone released, may place the females at high risk and thus impose ecological constraints to warrant a rigid regulation of the behavior.

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