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Control of Cell Proliferation in the Corpora Allata During the Reproductive Cycle of the Cockroach *Diploptera punctata*

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Unlike in *Blattella germanica* and *Supella longipalpa*, the corpora allata (CA) of *Diploptera punctata* exhibited cyclic changes in cell number during the reproductive cycle. In mated females, a wave of DNA synthesis followed by mitosis resulted in a significant increase in CA cell number from about 9,000 cells on day 0 to 12,000 cells at ovulation on day 8. Subsequently, the number of cells per CA underwent a gradual decline to about 10,000 cells by day 64. During this long period of gestation, mitotic activity was undetectable (by colchicine arrest) and pycnotic nuclei were frequently observed by transmission electron microscopy. Just before parturition on day 72 another mitotic wave was detected and CA cell number increased again. The early wave of CA cell proliferation could be postponed by delaying mating or abolished by maintaining females as virgins. Neural disconnection of the CA from the brain mimicked the effect of mating, suggesting that enhanced cell proliferation is permitted by the removal of inhibitory signals from cerebral neurosecretory cells. The proliferative activities after mating were neither abolished by ovariectomy, which suppressed the normal increase in JH synthesis, nor elevated by unilateral allatectomy, which doubled the rates of JH synthesis in the remaining CA. These data corroborate previous results (Szibbo and Tobe, 1981a; Tobe et al., 1984; Johnson et al., 1993) and suggest that waves of cell proliferation and JH synthesis, though simultaneous, are regulated independently by inhibitory signals from cerebral neurosecretory cells. © 1996 Wiley-Liss, Inc.

Key words: juvenile hormone, corpora allata, cell number, cockroach, *Diploptera punctata*

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INTRODUCTION

The corpora allata (CA*) synthesize and release juvenile hormones (JH), which regulate growth, metamorphosis, and reproduction in insects. In many adult insects, the CA have been shown to exhibit cyclic changes in volume coincident with cycles of JH synthesis and ovarian development (Novak, 1975; Tobe and Stay, 1985; Cassier, 1990). Volumetric growth of the CA in reproductive insects has been attributed to both cellular enlargement and cellular proliferation, and it has been suggested that increases in CA cell number are necessary for the CA to produce adequate amounts of JH for successful reproduction (Szibbo and Tobe, 1981b; Tobe et al., 1984). Nevertheless, while the relationship between CA cell proliferation and gland activity has been studied extensively, especially in adult cockroaches, it remains unclear whether these two events are mechanistically linked. Such a linkage was suggested by the coincidence of a rise and fall in CA volume and cell number with an increase and decline in JH biosynthesis during the ovarian cycle in the viviparous cockroach *Diploptera punctata* (Szibbo and Tobe, 1981b; Tobe et al., 1984; Johnson et al., 1993). On the other hand, evidence for a lack of linkage between changes in cell number and JH biosynthesis was provided by the absence of fluctuations in CA cell number during the ovarian cycle in two other cockroaches, *Blattella germanica* and *Supella longipalpa* (Chiang et al., 1991c; Chiang and Schal, 1994). In these cockroaches, fluctuation in CA volume and activity were mainly due to synchronous alterations in the size of CA cells (Chiang and Schal, 1991; Chiang et al., 1991a,b; Chiang and Schal, 1994).

Herein, we have re-examined the kinetics of CA cell proliferation in *D. punctata* during the first two ovarian cycles. Our results show that changes in CA cell number and JH biosynthesis are not necessarily linked and can occur independently. The pattern of change in CA cell number is consistent with the timing of cell birth examined with BrdU-incorporation and the timing of cell death observed with TEM. In addition, we have analyzed mechanisms involved in regulation of CA cell proliferation.

MATERIALS AND METHODS

Insects

The *D. punctata* colony was maintained as previously described (Chiang et al., 1993). Last instar and adult females were collected from the colony within 24 h of ecdysis and reared in groups of 2–10. Under our rearing conditions, most mated females oviposited on day 8. The age of parturition varied between days 72 and 76. The day of parturition was considered to be day 0 for second ovarian cycle. All measurements were derived from at least 3 animals.

Surgery

Ovariectomy and unilateral allatectomy were performed on day 0 last instar nymphs. In some females, sham surgery for unilateral allatectomy was

*Abbreviations used: BrdU = 5′-bromo-2′-deoxyuridine; CA = corpora allata or corpus allatum; JH = juvenile hormone; NCA-1 = nervus corporis allati-1.

also performed. Unilateral transection of the nervus corporis allati-I (NCA-I) was performed on day 0 adults. The right CA was always denervated or removed while the left CA was kept intact. Dissected CA were cleaned from adjacent tissues and separated from each other in cockroach saline (Kurtti and Brooks, 1976) modified to 360 mOsM.

CA Cell Number and Volume

Total cell number of a single corpus allatum was determined by hemocytometric sampling (Chiang et al., 1993); individual glands were desheathed with 0.1% collagenase, stained with 0.1% safranine, and dissociated into a nuclear suspension with 0.2% Triton-X 100 and 0.2 M citric acid in cockroach saline. The volume of a fresh CA was determined by the formula $V = 4/3\pi abc$, where a, b, and c were the radii of the three principal axes as measured with an ocular filar micrometer under a dissecting microscope at ×50. Means ± SEM are reported throughout.

Mitosis and DNA Synthesis

Mitosis was monitored with the colchicine block technique which arrests proliferative cells in metaphase. Each female was injected with colchicine (Sigma, St. Louis, MO) in saline (0.02 μg/mg body mass) through the base of the right metathoracic coxa. Four hours later, individual glands were desheathed with collagenase and spread into whole-mount nuclear monolayers under a coverslip (Chiang et al., 1989). The number of metaphase cells per corpus allatum was determined by examining all nuclei under a Nikon Optiphot microscope.

DNA synthesis was examined with a cell proliferation kit from Amersham (Arlington Heights, IL). For in vivo labeling of S-phase cells, insects were injected with 2 μl cockroach saline containing 6 μg 5′-bromo-2′-deoxyuridine (BrdU), a thymidine analog. Two hours after injection the corpus allatum was dissected and spread into a monolayer, and cells were treated with anti-BrdU followed by peroxidase anti-mouse-IgG2a. BrdU-labeled nuclei were visualized by incubation of monolayers in a solution of diaminobenzidine in PBS containing hydrogen peroxide and nickel chloride (Chiang et al., 1995). Alternatively, CA were fixed in Carnoy's fixative for 1 h and BrdU-labeled nuclei were observed in whole-mount preparations (Truman and Bate, 1988). Some CA were embedded in paraffin and serially sectioned at 5 μm.

Electron Microscopy

CA were processed for electron microscopic observation as described previously (Cheng and Chiang, 1995).

RESULTS

CA Development in Mated Adult Females

The CA of adult females that mated on day 0 underwent a cyclic change in volume in relation to oocyte development (Fig. 1A,B). In both virgin and mated *D. punctata* females both CA volume and basal oocyte length are accurate predictors of the level of JH synthesis by CA (Stay and Tobe, 1977; Szibbo

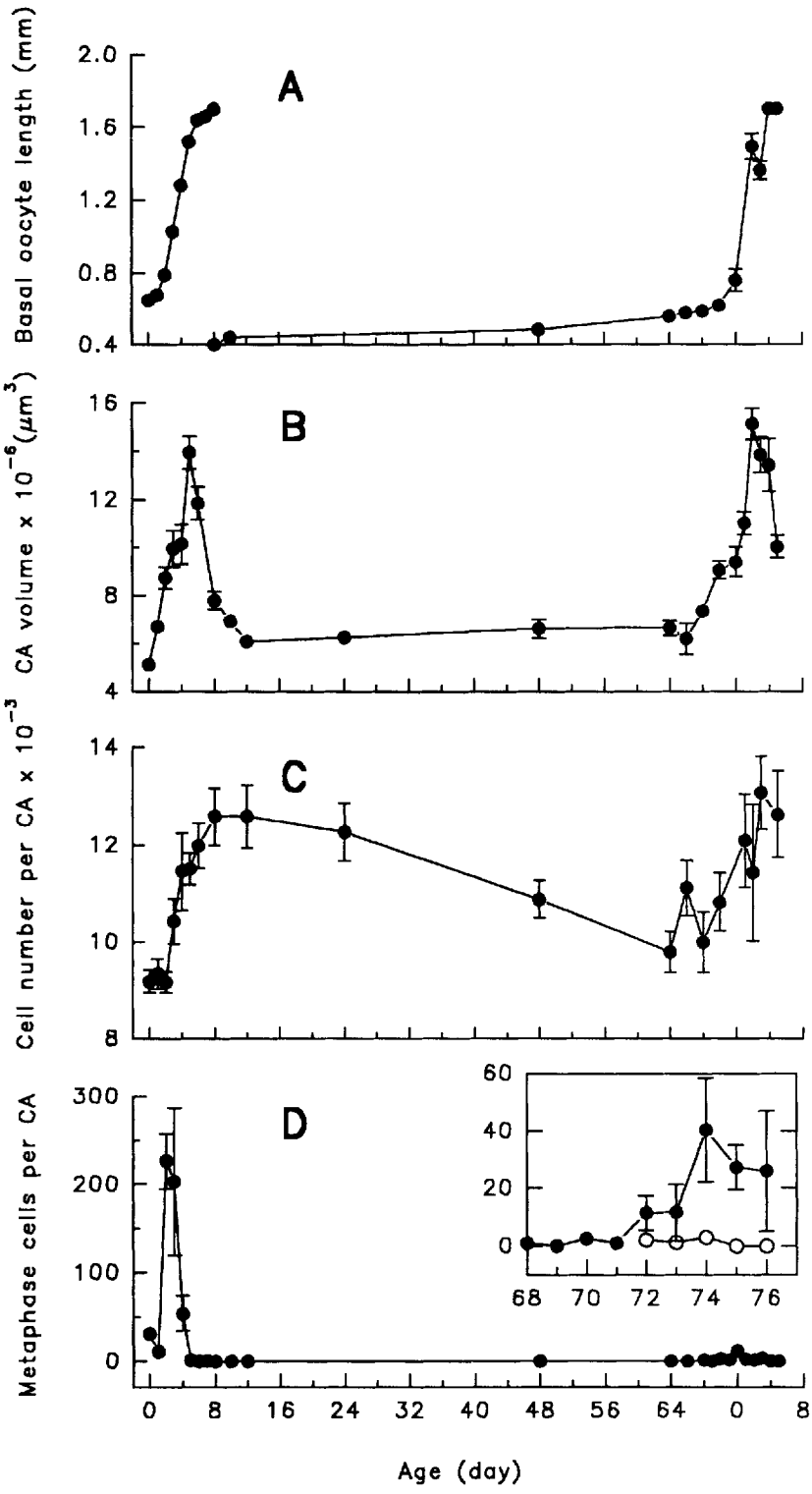
A
B
C
D
Basal oocyte length (mm)
CA volume x 10^{-6}(μm^3)
Cell number per CA x 10^{-3}
Metaphase cells per CA
Age (day)
2.0
1.6
1.2
0.8
0.4
16
12
8
4
14
12
10
8
300
200
100
0
60
40
20
0
68
70
72
74
76
0
8
16
24
32
40
48
56
64
0
8

Figure 1.

and Tobe, 1981b). The CA of newly emerged adult females contained approximately 9,000 cells (Fig. 1C). In females that mated on day 0, CA cell number increased abruptly after day 2 to approximately 12,000 cells by day 8. During the long gestation period (days 8–72), the number of CA cells declined gradually to about 10,000 cells by day 64. In the second gonotrophic cycle, CA cell number increased again to nearly 12,000 cells.

Since CA cell number varied greatly among females, cell number measurements alone were not sufficient to examine patterns or regulation of cell proliferation. Thus, mitotic activity was measured in addition to changes in CA cell number. Whole mount preparation of CA allowed for the counting of all cells within individual CA. Figure 2A shows a portion of a monolayer of CA cells taken from a day 2 mated female 4 h after colchicine injection. Most cells were in interphase while several proliferative cells were blocked at metaphase, characterized by chromosomal condensation and dispersion. During the first ovarian cycle, the increase in cell number that was especially evident between days 2 and 4 was a result of a sharp increase in DNA synthesis (Fig. 3) and a mitotic wave that peaked on days 2–3 (Fig. 1D). At peak DNA synthesis, approximately 3% of cells were labeled with BrdU 2 h after injection. Most BrdU-labeled cells were localized in the gland periphery but many were distributed randomly within the CA (Fig. 2B). This pattern of distribution of BrdU-labeled cells was observed in all three CA pairs examined.

Mitotic activity declined after day 4 and was undetectable during most of gestation (Fig. 1D). To explore if a second mitotic wave occurred before parturition, we examined the incidence of mitosis in females that remained gravid after day 72. In these females, the mean number of arrested metaphase cells increased with female age, suggesting that mitosis occurs just prior to parturition (Fig. 1D, inset). Regardless of the age of parturition, CA cells ceased to proliferate after parturition even when females were remated.

The pattern of change in cell number was validated by examination of cell birth and death in CA by TEM (Fig. 2C,D). Dying cells, characterized by pycnotic nuclei, were frequently observed in the CA of day 0 adult females. In virgin females, pycnotic nuclei were found sporadically throughout the first 12 days of adulthood (Fig. 2C). Cytoplasm in dying cells typically contained numerous vacuoles and evident perinuclear space. Pycnotic nuclei were not observed in the CA of more than 100 mated females aged from 1 to 10 days. Pycnotic nuclei were, however, observed at low frequency during pregnancy; days 11, 12, 24, and 48 were examined (Fig. 2D).

Fig. 1. Changes in **(A)** basal oocyte length, **(B)** CA volume, **(C)** total cell number, and **(D)** number of metaphase cells during the first two gonotrophic cycles of *D. punctata*. Females were mated on day 0 and remated again after parturition. Total cell number was determined by hemocytometric sampling. Number of metaphase cells per CA was determined by total count from whole-mount monolayers 4 h after colchicine injection. **Inset** in D is the mitotic activity in gravid females older than day 72, the earliest day of parturition. For each day after day 72, open circles denote females that gave birth on that day, while solid circles represent females in the same cohort of insects that were still gravid. Data show that mitosis precedes parturition. Each point is the mean ± SEM from 5–19 measurements.

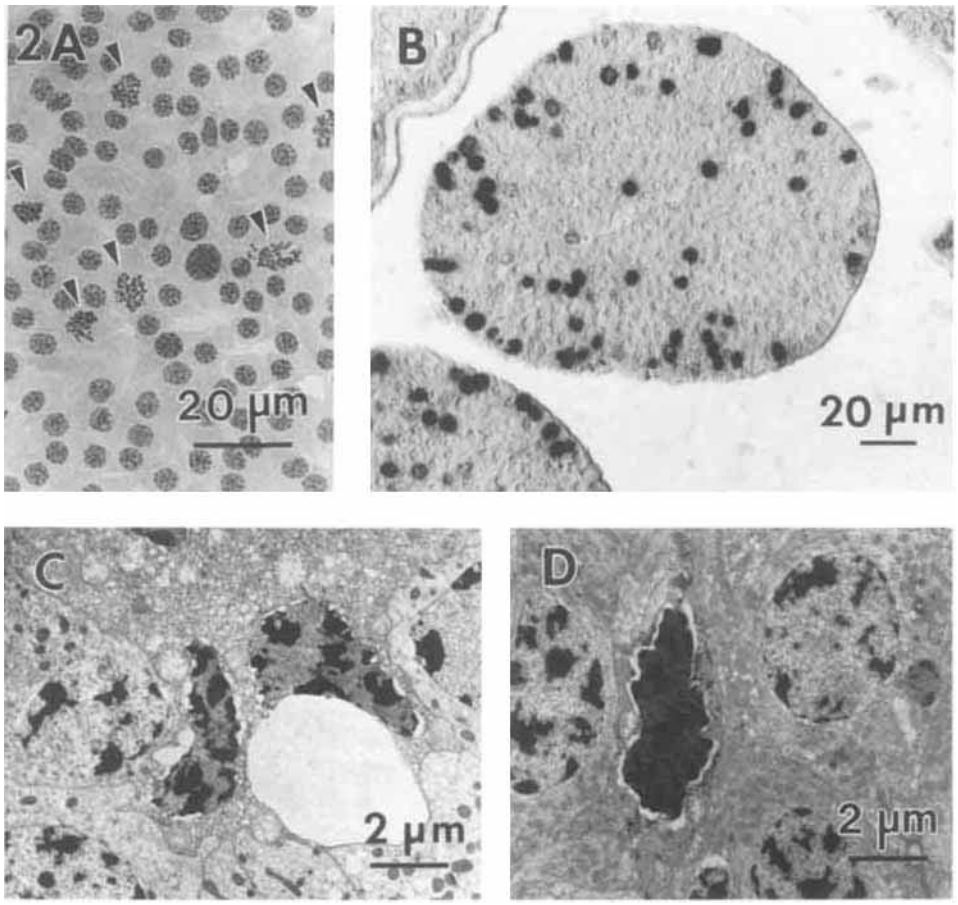

Fig. 2. Cell birth and death in CA. **A:** A portion of a whole-mount monolayer of CA cells taken from a day 2 mated female 4 h after colchicine injection showing six nuclei in metaphase (arrowheads). Note the size variation among nuclei which is common in CA of all ages without colchicine treatment (picture not shown). **B:** A paraffin section through CA center of a day 2 mated female 2 h after BrdU injection. Immunodetection shows distribution of BrdU-labeled cells in both peripheral and central areas. Left bottom is part of the other CA. **C:** An electron micrograph showing two pycnotic nuclei in CA of a day-12 virgin female. **D:** A pycnotic nucleus in the CA of a day-11 mated female.

Effect of Mating

Mitotic activity was relatively high in day 0 virgin females and, as in mated females (Fig. 1D), decreased to a low level on day 1 (Fig. 4D). Unlike in mated females, mitotic activity in CA from virgin females remained low from day 1 to day 12 (Fig. 4D), and CA cell number increased only slightly during this period (Fig. 4C). Basal oocytes in virgins exhibited little development (Fig. 4A) and the CA remained small (Fig. 4B) throughout the first 12 days of adulthood. Mating of virgins on day 8 resulted in a mitotic wave that peaked 2 days later at 72 ± 17 metaphase cells/CA (Fig. 4D). By day 12, basal oocytes

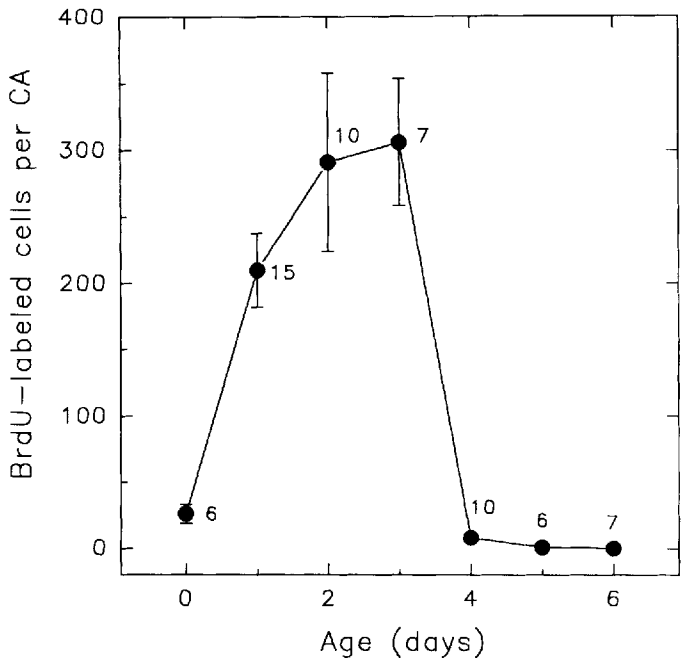

Fig. 3. The pattern of DNA synthesis in CA cells during oocyte maturation. Females were mated on day 0. Cells synthesizing DNA were labeled with BrdU for 2 h. The total number of nuclei containing replicated DNA was counted from a whole-mount monolayer of CA cells with BrdU immunodetection. Each point represents the mean ± SEM with the number of measurements indicated.

developed to 1.32 ± 0.03 mm (Fig. 4A), CA grew to 12.8 ± 0.7 $\times 10^6$ μm^3 (Fig. 4B), and cell number increased to 11,838 ± 410 cells/CA (Fig. 4C).

Effect of CA-Denervation

JH synthesis is stimulated in mated *D. punctata* females through a graded lifting of brain inhibition on the CA. Thus, while the CA of virgin females produce JH at low rates, denervation of the CA results in a cycle of JH synthesis (Stay and Tobe, 1977). To examine whether neural signals affect CA cell proliferation, the NCA-I of the right gland was transected and the intact left CA was used as an internal control in newly emerged adult females. These females were kept virgin and examined on each of the following 4 days (Fig. 5A). In unilateral NCA-I-transected virgin females, basal oocytes developed from 0.66 ± 0.02 (n = 5) on day 1 to 1.1 ± 0.04 mm (n = 6) on day 4, indicating that JH was synthesized at high rates by the denervated CA, as previously reported by Stay and Tobe (1977). The denervated gland exhibited a mitotic cycle with mitosis peaking 2 days after nerve transection and thereafter declining rapidly (Fig. 5A). In contrast, mitosis in the contralateral intact CA was nearly undetectable in the 4 days after surgery.

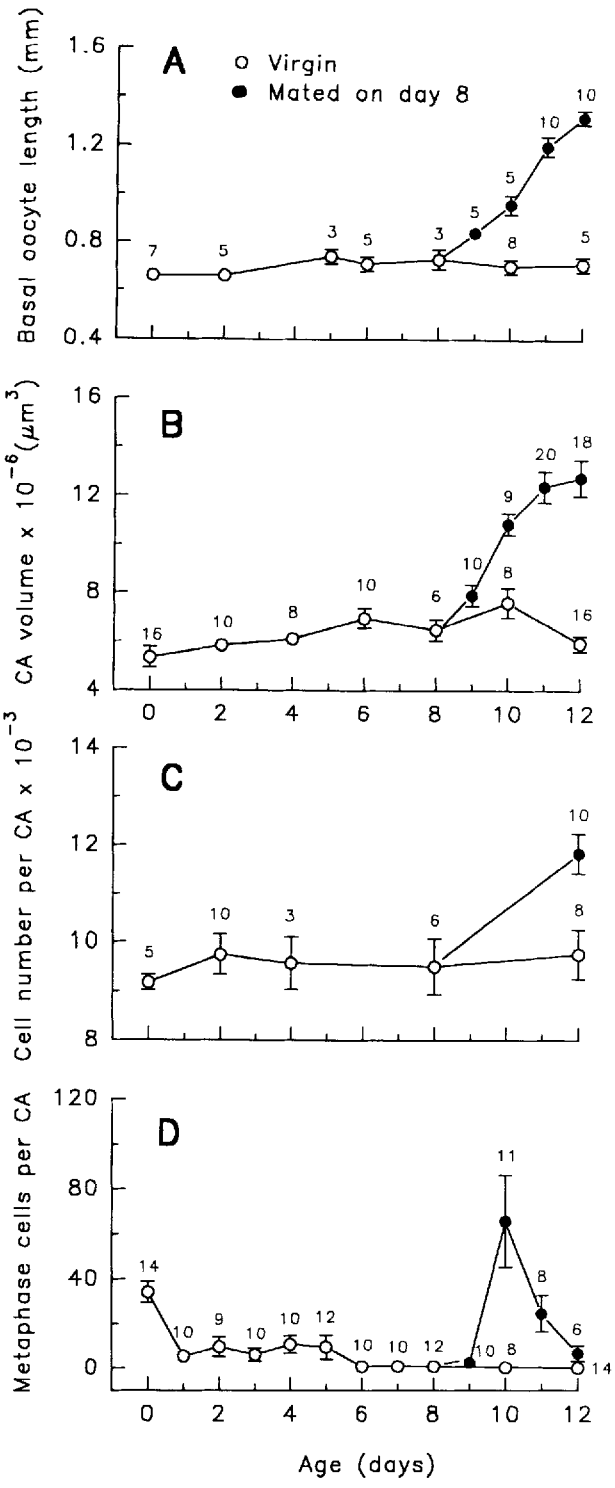

Fig. 4. The effect of delaying mating on (A) oocyte growth and (B–D) CA development. Oocyte length **(A)**, CA volume **(B)**, total cell number **(C)**, and number of metaphase cells **(D)** were determined by the same method described in Figure 1. Some females were mated on day 8. Each point represents the mean ± SEM with the number of measurements indicated.

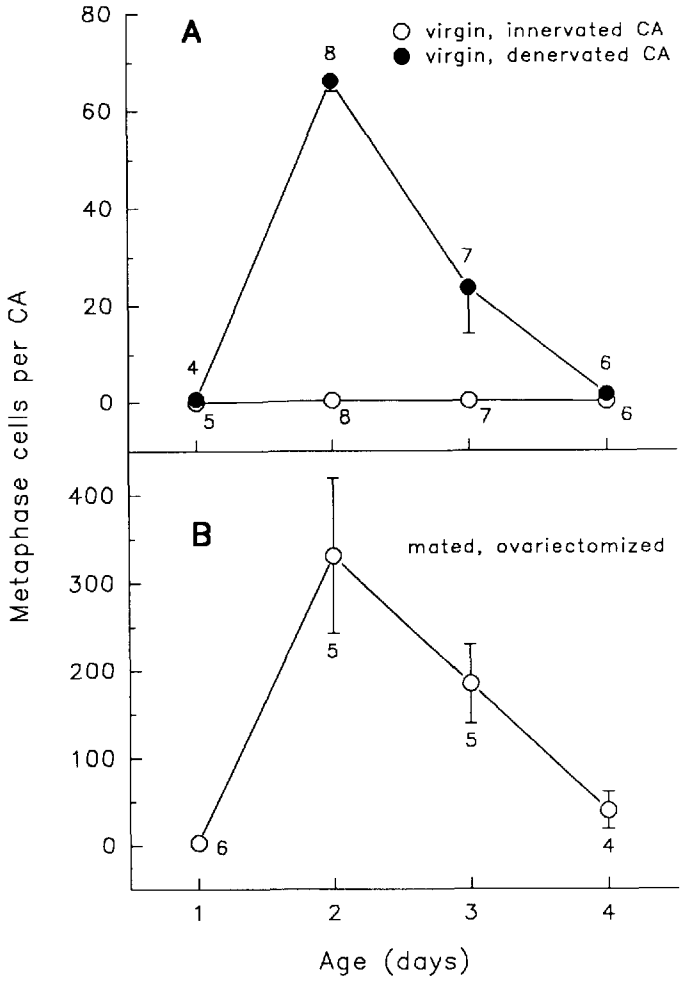

Fig. 5. Changes in mitotic activity following **(A)** unilateral transection of the nervus corporis allati-I or **(B)** ovariectomy. Females with unilaterally denervated CA were kept virgin while ovariectomized females were mated on the day they molted into the adult stage. The number of metaphase cells per CA was counted 4 h after colchicine injection. Each point represents the mean ± SEM with the number of measurements indicated.

Effect of Ovariectomy

The CA of ovariectomized females that mated on day 0 exhibited a mitotic wave similar to that in intact females (Figs. 1D, 5B), both with a peak on day 2. The peak level of CA cell mitosis was not statistically different between ovariectomized (331 ± 88 metaphase cells/CA, n = 5) and normal mated females (246 ± 56 metaphase cells/CA, n = 7) (Student's *t*-test, $P > 0.1$).

Effect of Unilateral Allatectomy

In *D. punctata,* unilateral allatectomy of day 0 mated females results in a compensatory doubling of JH production by the remaining CA, but peak JH

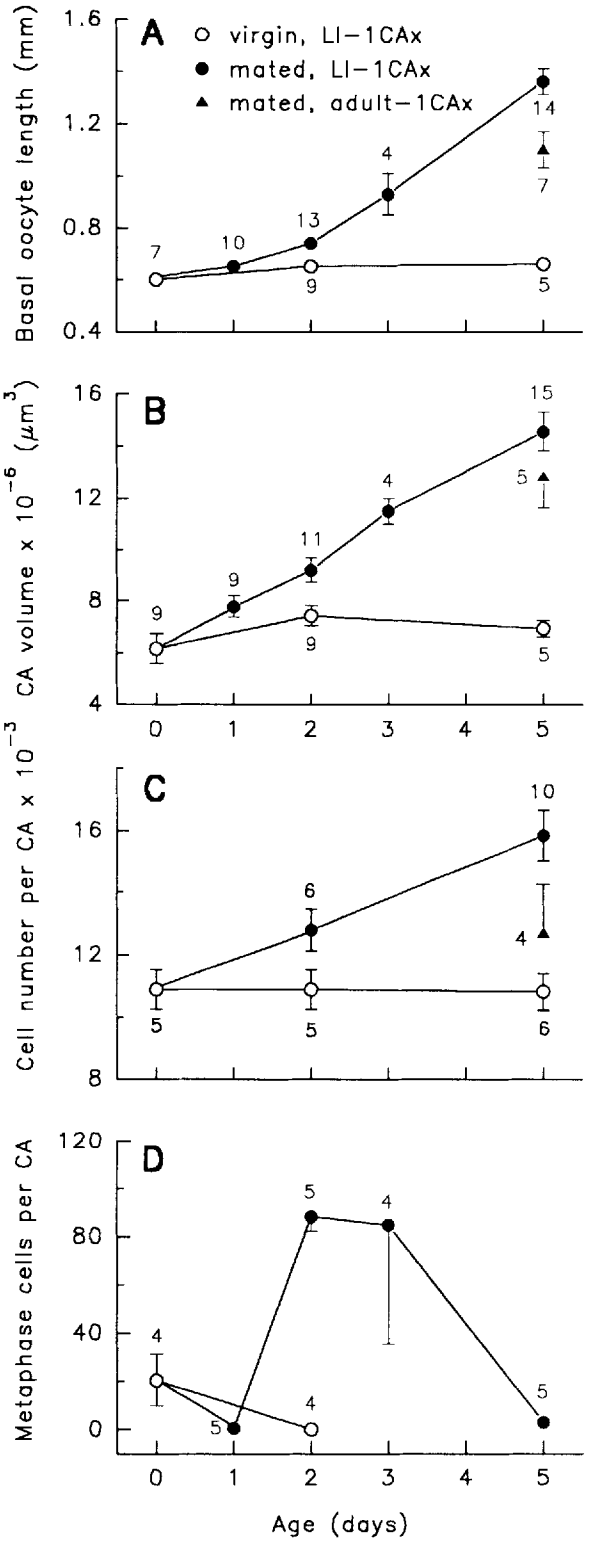
A
virgin, LI–1CAx
mated, LI–1CAx
mated, adult–1CAx
Basal oocyte length (mm)
1.6
1.2
0.8
0.4
7
10
13
4
14
7
9
5
B
CA volume x 10^{-6} (μm^3)
16
12
8
4
9
9
11
4
15
5
9
5
C
Cell number per CA x 10^{-3}
16
12
8
5
6
5
10
4
6
D
Metaphase cells per CA
120
80
40
0
4
5
4
5
4
5
0
1
2
3
4
5
Age (days)

Figure 6.

synthesis and maturation of basal oocytes are delayed by 1 day (Stay and Tobe, 1978; Figs. 1A, 6A). Our results following the same surgical procedure indicated that compensation in JH production by the remaining CA was not due to hypertrophy of the gland or hyperplasia of CA cells (compared between Fig. 1B,C and Fig. 6B,C), as was shown previously (Szibbo and Tobe, 1981b). When unilateral allatectomy was performed on day 0 of the last instar, oocyte development was not delayed after mating as in females operated on as day 0 adults (Figs. 1A, 6A). This suggested that the remaining CA in insects unilaterally allatectomized as nymphs was able to perfectly compensate for the absence of its partner. We did not measure JH synthesis. Compensation was not due to hyperplasia of CA cells after adult emergence. However, by the time of adult emergence, CA cell number was approximately 20% higher in unilaterally allatectomized females (11,810 ± 234 cells/CA, n = 5) (Fig. 6C) and in sham operated females (11,499 ± 517 cells/CA, n = 10) than in normal females (Fig. 1C). This was likely due to the longer duration of the last stadium in operated insects (26.2 ± 0.5 day, n = 80) as compared with normal insects (22.6 ± 0.3 day, n = 20). Thus, although mitotic activity during oocyte maturation in unilaterally allatectomized insect was normal (Fig. 6D), the CA was large (15.2 ± 0.8 $\times 10^6$ μm^3; Fig. 6B) and contained significantly more cells (15,840 ± 813 cells/CA, n = 10; Fig. 6C) than single CA in intact females (11,502 ± 326 cells/CA, n = 9; Fig. 1C) by day 5 (Student's *t*-test, $P < 0.001$). In contrast, in all treatments, oocytes did not mature in virgin females (Fig. 6A), and the CA in these females remained small (Fig. 6B), and did not undergo a significant increase in cell number (Fig. 6C) or a wave of cellular mitosis (Fig. 6D).

DISCUSSION

Changes in Cell Number

We have found that corpus allatum cell number increases rapidly from about 9,000 cells on day 0 to about 12,000 cells by day 4 in adult females of *D. punctata* mated on day 0. Both the number of cells and the pattern of their increase was in agreement with a recent report by Johnson et al. (1993) who counted fixed nuclei from suspensions of lysed CA cells, a technique similar to the one employed by Chiang et al. (1993) and in this report. Both we and Johnson et al. have found that CA contain approximately 2,000–3,000 more cells than in a previous report in which histological sectioning was employed and showed that CA cell number increased from 6,000 cells per CA on day 0 to 9,000 cells by day 5 (Szibbo and Tobe, 1981b). The discrepancies among separate studies are likely accounted for by differences in techniques employed to determine CA cell number. The technique of counting nuclei from

Fig. 6. The effects of unilateral allatectomy on (A) the growth of basal oocytes and (B–D) the development of the remaining CA. Oocyte length **(A)**, CA volume **(B)**, total cell number **(C)**, and number of metaphase cells **(D)** were determined by the same methods described in Figure 1. The right corpus allatum was removed (1CAx) from newly ecdysed (day-0) final instar nymphs (LI) or from mated day-0 adult females. Adult females were mated on day 0 or kept virgin. Each point represents the mean ± SEM with the number of measurements indicated.

histological sections has recently been criticized since estimates of cell number from this technique are based upon biased assumptions of constant nuclear size and shape (Bertram and Nurcombe, 1992). The considerable variation in nuclear size (Fig. 2A) in CA cells suggests that histological sectioning is inappropriate for estimation of CA cell number. Nevertheless, no matter what the technique employed to count CA cell number in *D. punctata*, all reports confirm that *D. punctata* CA are regulated differently from those of two oviparous cockroaches, *B. germanica* and *S. longipalpa*, wherein the number of CA cells remains constant during ovarian cycles (Chiang and Schal, 1994; Chiang et al., 1991c).

The increase in CA cell number in *D. punctata* during the first ovarian cycle was due to a surge of cell division preceded by DNA synthesis (Figs. 1–3). DNA synthesis in CA was previously reported by Szibbo and Tobe (1981b) who examined [^{3}H]-thymidine incorporation into nuclei 4 h after injection. They found that a low frequency of DNA synthesis (< 100 cells/CA) occurred only in the peripheral cells of CA from females aged 3 to 5 days. This level of DNA synthesis was too low to account for the rapid increase in CA cell number. In comparison with [^{3}H]-thymidine incorporation, our current technique using BrdU to detect S-phase cells has shown a 3-fold higher level of DNA synthesis in CA within just 2 h after injection. Furthermore, this technique showed that S-phase cells were not limited to the gland periphery; indicating that central cells also displayed proliferative capacity (Fig. 2B).

Our data indicated that the decline in JH synthesis before ovulation is temporally independent of the decrease in CA cell number which occurs during gestation. In support of our contention are observations that pycnosis, accompanied by reduction in CA cell number, is limited to gestation in *D. punctata* (Figs. 1C, 2D) and *Leucophaea maderae* (Scharrer and von Harnack, 1958). In contrast, results from Johnson et al. (1993) support earlier studies suggesting that the fall in hormone production before ovulation coincides with and may depend upon a reduction in CA cell number. Overall, the pattern of change in cell number that we determined was consistent with the timing of cell birth and death during the first two gonotrophic cycles (Figs. 1–3).

Neural Inhibition of Cell Proliferation

In vertebrates, neurotransmitters may stimulate or inhibit proliferation of postsynaptic non-neuronal cells (Lauder, 1993). In insects, the CA are innervated by brain neurosecretory cells which produce neuropeptides that influence JH production (Stay et al., 1992). Neural disconnection of the CA from the brain, or cauterization of brain neurosecretory cells, results in a significant elevation of JH synthesis in cockroaches (Stay and Tobe, 1977; Ruegg et al., 1983; Pipa, 1986; Gadot et al., 1989; Woodhead and Stay, 1989; Schal et al., 1993). However, the role of brain neurosecretory factors or neural signals in regulating development of CA cells has not been thoroughly examined (Tobe et al., 1984).

We have found that in adult females of *D. punctata*, a mitotic wave and an increase in CA cell number occur after mating and that both mitosis and cell proliferation are postponed by delaying mating or abolished by keeping females virgin (Fig. 4). Mating appears to exert its effect by removing brain

inhibition on CA cell proliferation, as it does on JH synthesis, since unilateral transection of the NCA-I in virgin females resulted in elevated mitotic activity in the denervated, but not in the contralateral, CA within the same insect (Fig. 5). Interestingly, the CA of females that mated during the second ovarian cycle did not subsequently undergo a cycle of mitosis. Rather, mitosis preceded mating (Fig. 1D). The ovaries do not appear to mediate the effect of mating during the first reproductive cycle, since the CA of ovariectomized mated females exhibited a cycle of mitotic activity (Fig. 5).

It is not clear what factors affect the decline in mitotic activity after day 2. Clearly, this decline is independent of neural connections, since it occurs in both innervated CA (Fig. 5) and denervated CA (Fig. 5). It is possible that the high amount of JH produced by day 3 or 4 CA may act as a negative autocrine regulator to prevent CA cells from re-entering the cell cycle. This speculation is supported by the inhibition of a normal increase in CA cell number in male adults (Chiang et al., 1995) and last instar nymphs (unpublished data) by treatment with a JH analog. However, this idea fails to explain the decline in mitosis in the low JH environment of ovariectomized adult females (Fig. 5). Overall, our results suggest that enhancement of CA cell proliferation is permitted by the removal of inhibitory signals from cerebral neurosecretory cells but that reduction of CA cell proliferation is regulated through a non-neural mechanism.

It is also not clear what factors are responsible for the gradual decrease in cell number during gestation (Fig. 1C). Tobe et al. (1984) suggested a role for ovarian ecdysteroids in reduction in CA cell number. This hypothesis is supported by the elevation in CA cell number that occurs in ovariectomized mated females (Johnson et al., 1993) that presumably lack a source (ovaries) of ecdysteroids.

Cell Proliferation in Relation to JH Synthesis

Our results show that a cycle of cell division occurs prior to the increase in JH synthesis (as indicated by the growth of basal oocytes) after mating (Figs. 1, 2) or after CA denervation (Fig. 5). In normal mated females, a wave of cell division peaked 2 to 3 days after mating and a second wave peaked just prior to parturition when basal oocyte started to increase in size and were 0.7–1.0 mm in length. Peak rates of JH synthesis have been shown to occur at oocyte lengths of 1.1–1.4 mm in both the first and second reproductive cycle (Rankin and Stay, 1985). Although it has been proposed that cell proliferation in the CA may be a prerequisite for maximal JH synthesis (Szibbo and Tobe, 1981b; Tobe et al., 1984), these events appear to be regulated independently as was previously shown by cell number counts after unilateral allatectomy (Szibbo and Tobe, 1981a) and ovariectomy in mated females (Tobe et al., 1984; Johnson et al., 1993). After unilateral allatectomy, the remaining intact CA doubles its production of JH (Stay and Tobe, 1978), yet its mitotic activity is similar to that in normal females (Fig. 6). After ovariectomy, mating causes a mitotic wave (Fig. 5) without a subsequent elevation in JH synthesis (Stay and Tobe, 1978). Comparative studies with other species also support these observations. Rates of JH synthesis elevate in both normal and ovariectomized *B. germanica* females without concomitant increases in CA cell number

(Chiang et al., 1989, 1991a; Gadot et al., 1991). A search is now underway for factors regulating CA cell proliferation in cockroaches.

LITERATURE CITED

Bertram JF, Nurcombe V (1992): Counting cells with the new stereology. Trends Cell Biol 2:177–180.

Cassier P (1990): Morphology, histology, and ultrastructure of JH-producing glands in insects. In Gupta AP (ed): Morphogenetic Hormones of Arthropods, New Brunswick, NJ: Rutgers University Press, pp 83–194.

Cheng H-W, Chiang A-S (1995): Autophagy and acid phosphatase activity in the corpora allata of adult mated females of *Diploptera punctata*. Cell Tissue Res 281:109–117.

Chiang A-S, Schal C (1991): Correlation among corpus allatum volume, cell size, and juvenile hormone biosynthesis in ovariectomized adult *Blattella germanica*. Arch Insect Biochem Physiol 18:37–44.

Chiang A-S, Schal C (1994): Cyclic volumetric changes of corpus allatum cells in relation to juvenile hormone biosynthesis during ovarian cycles in cockroaches. Arch Insect Biochem Physiol 27:53–64.

Chiang A-S, Gadot M, Schal C (1989): Morphometric analysis of the corpus allatum cells in adult females of three cockroach species. Mol Cell Endocrinol 67:179–184.

Chiang A-S, Burns EL, Schal C (1991a): Ovarian regulation of cyclic changes in size and activity of corpus allatum cells in *Blattella germanica*. J Insect Physiol 37:907–917.

Chiang A-S, Gadot M, Burns E, Schal C (1991b): Developmental regulation of juvenile hormone synthesis: Ovarian synchronization of volumetric changes of corpus allatum cells in cockroaches. Mol Cell Endocrinol 75:141–147.

Chiang A-S, Gadot M, Burns EL, Schal C (1991c): Sexual differentiation of nymphal corpora allata and the effects of ovariectomy on gland morphometrics in adult *Blattella germanica*. Experientia 47:81–83.

Chiang A-S, Holbrook GL, Schal C (1993): Postembryonic development of corpora allata in relation to juvenile hormone biosynthesis in cockroaches. In Mauchamp B, Cluillaud F, Baehr JC (eds): Fundamental and Applied Progress in Insect Juvenile Hormone Research. Paris, France: INRA Press, pp 219–230.

Chiang A-S, Tsai W-H, Schal C (1995): Neural and hormonal regulation of growth of corpora allata in the cockroach, *Diploptera punctata*. Mol Cell Endocrinol 115:51–57.

Gadot M, Burns E, Schal C (1989): Juvenile hormone biosynthesis and oocyte development in adult female *Blattella germanica:* Effects of grouping and mating. Arch Insect Biochem Physiol 11:189–200.

Gadot M, Chiang A-S, Burns EL, Schal C (1991): Cyclic juvenile hormone biosynthesis in the cockroach, *Blattella germanica:* Effects of ovariectomy and corpus allatum denervation. Gen Comp Endocrinol 82:163–171.

Johnson GD, Stay B, Chan KK (1993): Structure-activity relationships in corpora allata of the cockroach *Diploptera punctata:* Roles of mating and the ovary. Cell Tissue Res 274:279–293.

Kurtti TJ, Brooks MA (1976): The dissociation of insect embryos for cell culture. In Vitro 12:141–146.

Lauder JM (1993): Neurotransmitters as growth regulatory signals: Role of receptors and second messengers. Trends Neurosci 16:233–240.

Novak VJA (1975): Insect Hormones. London, UK: Chapman & Hall.

Pipa RL (1986) Disinhibition of oocyte growth in adult, virgin *Periplaneta americana* by corpus allatum denervation: Age dependency and relatedness to mating. Arch Insect Biochem Physiol 3:471–483.

Rankin SM, Stay B (1985): Regulation of juvenile hormone synthesis during pregnancy in the cockroach, *Diploptera punctata*. J Insect Physiol 31:145–157.

Ruegg RP, Lococo DJ, Tobe SS (1983): Control of corpus allatum activity in *Diploptera punctata:* Roles of the pars intercerebralis and pars lateralis. Experientia 39:1329–1334.

Schal C, Chiang A-S, Burns EL, Gadot M, Cooper RA (1993): Role of the brain in juvenile hormone synthesis and oocyte development: Effects of dietary protein in the cockroach *Blattella germanica* (L.). J Insect Physiol 39:303–313.

Scharrer B, von Harnack M (1958): Histophysiological studies on the corpus allatum of *Leucophaea maderae*. I. Normal life cycle in male and female adults. Biol Bull 115:508–520.

Stay B, Tobe SS (1977): Control of juvenile hormone biosynthesis during the reproductive cycle of a viviparous cockroach. I. Activation and inhibition of corpora allata. Gen Comp Endocrinol 33:531–540.

Stay B, Tobe SS (1978): Control of juvenile hormone biosynthesis during the reproductive cycle of a viviparous cockroach. II. Effects of unilateral allatectomy, implantation of supernumerary corpora allata, and ovariectomy. Gen Comp Endocrinol 34:276–286.

Stay B, Chan KK, Woodhead AP (1992): Allatostatin-immunoreactive neurons projecting to the corpora allata of adult *Diploptera punctata*. Cell Tissue Res 270:15–23.

Szibbo CM, Tobe SS (1981a): The mechanism of compensation in juvenile hormone synthesis following unilateral allatectomy in *Diploptera punctata*. J Insect Physiol 27:609–613.

Szibbo CM, Tobe SS (1981b): Cellular and volumetric changes in relation to the activity cycle in the corpora allata of *Diploptera punctata*. J Insect Physiol 27:655–665.

Tobe SS, Stay B (1985): Structure and regulation of the corpus allatum. Adv Insect Physiol 18:305–432.

Tobe SS, Clarke N, Stay B, Ruegg RP (1984): Changes in cell number and activity of the corpora allata in the cockroach *Diploptera punctata:* A role for mating and the ovary. Can J Zool 62:2178–2181.

Truman JW, Bate M (1988): Spatial and temporal patterns of neurogenesis in the central nervous system of *Drosophila melanogaster*. Dev Biol 125:145–157.

Woodhead AP, Stay B (1989): Neural inhibition of corpora allata in protein-deprived *Diploptera punctata*. J Insect Physiol 35:415–421.