Acute Effect of Neural Deafferentation on Timing of Gonadotropin Secretion Before Proestrus in the Female Rat

MASAZUMI KAWAKAMI AND EI TERASAWA

2nd Department of Physiology
Yokohama City University School of Medicine, Yokohama

Synopsis

An attempt was made to investigate a role of the brain on release of gonadotropin responsible for the events of proestrus and estrus in the 4-day cyclic rats. Rats were acutely deafferented at the level of anterior commissure (AC) with three kinds of the L-shaped knives at the different time of day, and autopsied to examine the uterine distension and ovulation on the days of expected proestrus or estrus. Serum and pituitary level of LH, FSH and prolactin in the deafferented rats were measured by radioimmunoassay. The results are as follows: 1) With an L-shaped knife of 2.5 mm of the horizontal blade (knife A) the fornix (Fx) and the stria terminalis (ST) were interrupted and simultaneously the tract of diagonal bundle of Broca (TDB), the bed nucleus of stria terminalis (BST), the nucleus of accumbens (ACC), the nucleus of anterior commissure (NAC) and parolfactory area were commonly damaged. The deafferentation at 20:00 on the day of diestrus I prevented vaginal cornification, uterine distension and ovulation on the expected estrous day. Full cornification was observed in all examined rats with transection after 10:00 on the day of diestrus II, while uterine distension was not observed in the rats with transection before 20:00 on the same day. None of rats bearing the transection ovulated on the day of estrus, even the transection was performed at 8:00 on the day of proestrus. 2) With an L-shaped knife of 1.4 mm of the horizontal blade (knife B) the Fx were interrupted and the BAC and the TDB were commonly damaged, however, the ST, the BST and the ACC were only partially damaged. Most of results following to the neural transection with knife B were similar to those observed with knife A except for ovulation being observed in the rats transected at 8:00 on the day of proestrus. However, transection at the mid-thalamus (knife placed at 2 mm caudal to the AC) did not interfere any normal cyclic changes. 3) Estrogen injection into the rats transected with knife B at 20:00 on the day of diestrus II resulted in inducing ovulation, while with knife A did not. 4) Transection either Fx or medial cortico-hypothalamic tract (CHT) with an L-shaped knife of 1.0 mm of the horizontal blade (knife C) on the day of diestrus I partially prevented the occurrence of events of proestrus and estrus. 5) Transection of the septal complex with knife B at 20:00 on the day of diestrus I prevented a rise of pituitary LH, pituitary prolactin and serum prolactin, which used to be observed in normal proestrus. Both Fx and CHT transection resulted in an increase of pituitary FSH. From the ovariectomy experiment the ovary of the our colony's rats must be in situ at 15:00 for vaginal cornification and at 24:00 for uterine distension. Thus, following facts are suggested 1) the septal complex or higher brain such as the limbic structure may be involved in the release of gonadotropin responsible for the events of proestrus and estrus, 2) timing of release of gonadotropin (folliculotropin) might occur preceding to the ovarian changes and it must start sometime before early morning of diestrus II.

It has been shown that the ovarian steroids responsible for the proestrous rise in uterine weight, an ovulatory surge of gonadotropin, and for estrous vaginal cornification, start to...
secrete on the afternoon before proestrous
day (Schwartz, 1964; Lawton and Sawyer, 1968; Kobayashi et al., 1969a). Furthermore,
this ovarian secretion due to gonadotropin
release on the day before proestrus is also
reported (Lawton and Sawyer, 1968; Kobayashi
et al., 1969b). However, susceptible role
of the brain on basal gonadotropin secretion
on the day before proestrus is not observed
(Lawton and Sawyer, 1968; Schwartz and
Lawton, 1968), in contrast to the neural
mechanism regulating the ovulatory surge of
gonadotropin on the day of proestrus (Everett
et al., 1949).

Previous works from our laboratory in-
dicate that the hippocampus is a neural struc-
ture responsible for release of FSH in normal
cycling rats and prepuberal rats (Kawakami
et al., 1971, 1972; Kawakami and Terasawa,
1972), in which FSH may induce the secretion
of estrogen, at least, at the initiation of
cyclicity or onset of puberty. Therefore, it is
of interest to deafferent the neural pathway
from the higher brain including the hip-
pocampus with a small L-shaped knife and to
investigate whether the basal gonadotropin
secretion was inhibited or not. The present
experiment was, further, designed to in-
vestigate the timing of neural stimulation
responsible for the gonadotropin secretion.

Materials and Methods

Wistar female rats were maintained in a room
illuminated from 5:00 to 19:00. Four day cyclic
animals were selected by taking vaginal smears every
morning, at least 3 consecutive cycles.
Experimental design was as follows.

Experiment 1:
To test the timing of ovarian hormone secretion
in the rat of our colony, ovariectomy was performed
under ether anesthesia at various times of day between
17:00 on the day of diestrus I and 24:00 on the day of
diestrus II. The animals were autopsied at proestrus
or estrus to determine whether the expected vaginal
and uterine changes occur.

Experiment 2:
The effect of acute deafferentation was investigated
with an L-shaped knife, which was originally proposed
by Halasz and Pupp (1965) and modified by Taleisnik
et al. (1970). Deafferentation surgery was made at the
different time of day between 20:00 on the day of
diestrus I and 8:00 on the day of proestrus under ether anesthesia. Rats were autopsied on the day of
estrus and inspected ovulation and uterine distension.
Uterus was weighed with or without intraluminal
fluid. Three kinds of neural deafferentation were
performed to interrupt the neural input to the anterior
basal hypothalamus coming from amygdala and the
hippocampus. Deafferentation A: The interruption of
both limbic afferents coming from the hippocampus
and the amygdala through the fornix and the stria
terminals were inserted by rotating an L-shaped
knife with a horizontal blade 2.5 mm long (Knife A).
The knife was inserted at a = 7.0 (center) and was
lowered into the brain through the superior sagittal
with the aid of a stereotaxic apparatus until the level
of anterior commissure (v = 4.5) by means of atlas by
Albe-Fessard et al. (1966). The handle was then
rotated through 90° at each side of mid-line. De-
afferentation B: The interruption of frontal part of
fornical afferents were intended with a knife of
horizontal blade 1.4 mm long. Knife was inserted at
a = 7.5 and was lowered into the brain at the level of
above the anterior commissure (v = 4.8). The handle
was then rotated through 90° for each side. For this
experimental series some animals were transected at
a = 6.0, v = 5.0 or at a = 5.0, v = 4.0. The former
transection included partially in the fornical fibers
but was mostly in the mid-thalamus and the latter
transection was in the mid-thalamus. Deafferentation
C: Fornix was intended to interrupt with a smallest
knife, of which horizontal blade was 1.0 mm long
(Knife C), by rotating 90° of each side at the level of
a = 7.0, v = 5.0. One of hippocampal afferents as
called “Tractus cortico-hypothalamus medialis” were
intended to interrupt with an L-shaped knife, of
which horizontal blade was 1.0 mm long (knife C).
The knife was inserted at a = 7.0 and lowered at the
level of $v = 4.0$ and rotated through $90^\circ$ for each side. Sites and sizes of transection were shown in Figure 1 and Figure 2.

**Experiment 3:**
Effect of estradiol-17$\beta$ on induction of ovulation was examined in the rat deafferented with an L-shaped knives of type A and type B. Transection was performed at 20:00 on the day of diestrus II under ether anesthesia, and 200 $\mu$g of estradiol-17$\beta$ was administered subcutaneously at 21:00 on the same day. Ovulation was inspected on the day of expected estrus. [Estradiol-17$\beta$ was chosen for estrogen to induce ovulation, because it was suggested by Kobayashi et al. (1969a), as the most efficient estrogen for induction of ovulation.]

**Experiment 4:**
Pituitary and serum level of gonadotropin were measured in the rats of deafferentation B and C. Transection of the brain were performed under ether anesthesia at 20:00 on the day of estrus or diestrus I and autopsied on the day of proestrus between 11:00 and 12:00. Rats were anesthetized with ether, and blood samples were collected from the femoral vein. The head was guillotined and anterior pituitary gland was quickly removed. Radioimmunoassay procedure of LH, FSH and prolactin was based on the methods described by Niswender et al. (1968) and by NIAMD instructions. The amounts of assayed hormones were expressed in terms of NIH-LH-SI, NIH-FSH-SI and prolactin-RP-1.

Ovulation was determined microscopically by the presence of ova in oviducts on the morning of expected estrus.

Histological sections were made to determine the sites and the size of transected area.

**Results**

**Experiment 1:**
Ovariectomy at 8:00 and earlier on the day of diestrus II prevent the events of proestrus day. Ovariectomy at 15:00 on the day of diestrus II did not prevent the vaginal cornification on the day of expected estrus, while ovariectomy before 15:00 on the day of diestrus II prevented the uterine distension of expected proestrus. However, most of rats who received ovariectomy at 24:00 on the
Table 1. Effect of ovariectomy at diestrus I and diestrus II on uterine distension and vaginal cornification

<table>
<thead>
<tr>
<th>Treatments</th>
<th>On the day of expected proestrus</th>
<th>On the day of expected estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>uterine distension</td>
<td>vaginal cornification</td>
</tr>
<tr>
<td>Sham Ovariectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>diestrus I 17:00</td>
<td>2/2*</td>
<td>2/2</td>
</tr>
<tr>
<td>diestrus II 06:00</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>10:00</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>15:00</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>19:00</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>22:00</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>diestrus I 17:00</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>24:00</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>diestrus II 06:00</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>08:00</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>10:00</td>
<td>0/3</td>
<td>2/3</td>
</tr>
<tr>
<td>15:00</td>
<td>0/4</td>
<td>4/4</td>
</tr>
<tr>
<td>20:00</td>
<td>2**/4</td>
<td>4/3</td>
</tr>
<tr>
<td>24:00</td>
<td>3/4</td>
<td>4/4</td>
</tr>
</tbody>
</table>

* No. of rats with positive response/ no. of rats examined
** Uterus was distended partially in these rats

The cyclic change of vaginal smear and uterine change were not disturbed by sham ovariectomy at any time period examined (Table 1).

Experiment 2:

The effect of neural deafferentation on vaginal smear, uterine changes and ovulation were summarized in Table 2, 3, 4 and 5.

Deafferentation A (Table 2): The fornix and the stria terminalis were interrupted. Simultaneously, the tract of diagonal bundle of Broca, the bed nucleus of stria terminalis, the nucleus of accumbens, the nucleus of medial septum, the bed nucleus of anterior com-
misasure and the parolfactory area were commonly damaged. The deafferentation at 20:00 on the day of diestrus I prevented vaginal cornification, uterine distension and ovulation on the expected estrous day. Cornified cells in vaginal smear were observed in all examined rats with the transaction after 10:00 on the day of diestrus II, while uterine distension was not observed in the rats with transection before 20:00. For example, 2 out of 4 rats showed vaginal cornification with the transection at 6:00 on the day of diestrus II and all 4 rats had a distended uteri with the transection after 20:00 on the day of diestrus II. However, none of rats ovulated on the day of estrus in this experimental series, even the transection was performed at 8:00 on the day of proestrus. Deafferentation B (Table 3): Fornical afferents were interrupted, and the bed nucleus of the anterior commissure, the tract of diagonal bundle of Broca, the nucleus of medial septum were commonly damaged. However, the stria terminalis, the bed nucleus of stria terminalis and the nucleus of accumbens were only partially damaged in this series. The effect of this deafferentation for vaginal cornification and uterine distension were similar to those in observed deafferentation A. That is, the deafferentation at 20:00 on the day of diestrus I resulted neither vaginal cornification nor uterine distension on the expected estrous day. The deafferentation at 20:00 on the day of diestrus II resulted in uterine distension in 7 out of 9 rats, while it still prevent ovulation in all examined rats. However, most of animals ovulated, when the deafferentation were performed 8:00 on the day of proestrus. In contrast, if the knife placed at posterior to the fornix (a = 5.0), ovulation as well as vaginal cornification were observed in most of rats, which were transected at any time between 20:00 on the day of diestrus I and 20:00 on the day of diestrus II. Uterine distension in these rats

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Table 3. Effect of neural deafferentation by a small L-shaped knife (knife B) (Autopsied on the day of expected estrus)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vaginal cornification</th>
<th>Uterine distension</th>
<th>Weight of uterus</th>
<th>Weight of intraluminal water/distended uterus</th>
<th>No. of ovulating rats</th>
<th>Mean No. of ovulating rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>a = 7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrus</td>
<td>20:00</td>
<td>0/5*</td>
<td>0/5*</td>
<td>272 ± 27**</td>
<td>0/5*</td>
<td></td>
</tr>
<tr>
<td>Diestrus</td>
<td>20:00</td>
<td>0/7</td>
<td>0/7</td>
<td>268 ± 16</td>
<td>0/7</td>
<td></td>
</tr>
<tr>
<td>Diestrus II 6:00</td>
<td>2/2</td>
<td>1/2</td>
<td>407 ± 51</td>
<td>370</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>Diestrus II 20:00</td>
<td>9/9</td>
<td>7/9</td>
<td>470 ± 42</td>
<td>454 ± 38</td>
<td>0/9</td>
<td></td>
</tr>
<tr>
<td>Proestrus 8:00</td>
<td>6/6</td>
<td>1/6</td>
<td>422 ± 25</td>
<td>310</td>
<td>5/6</td>
<td>9.4</td>
</tr>
<tr>
<td>Diestrus II 20:00</td>
<td>11/11</td>
<td>6/11</td>
<td>444 ± 19</td>
<td>549 ± 25</td>
<td>4/11</td>
<td>8.5</td>
</tr>
<tr>
<td>+ estradiol 17β</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a = 6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diestrus I 20:00</td>
<td>2/2</td>
<td>1/2</td>
<td>389 ± 25</td>
<td>136</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>Diestrus II 6:00</td>
<td>1/1</td>
<td>0/1</td>
<td>421</td>
<td>—</td>
<td>1/1</td>
<td>16.0</td>
</tr>
<tr>
<td>Diestrus II 20:00</td>
<td>2/2</td>
<td>1/2</td>
<td>418 ± 31</td>
<td>246</td>
<td>1/2</td>
<td>11.0</td>
</tr>
<tr>
<td>a = 5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrus</td>
<td>20:00</td>
<td>4/6</td>
<td>4/6</td>
<td>480 ± 12</td>
<td>171 ± 75</td>
<td>2/6</td>
</tr>
<tr>
<td>Diestrus I 20:00</td>
<td>4/5</td>
<td>0/5</td>
<td>383 ± 30</td>
<td>—</td>
<td>4/5</td>
<td>11.0</td>
</tr>
<tr>
<td>Diestrus II 20:00</td>
<td>2/2</td>
<td>0/2</td>
<td>370 ± 23</td>
<td>—</td>
<td>2/2</td>
<td>9.5</td>
</tr>
<tr>
<td>intact estrous control</td>
<td>5/5</td>
<td>0/5</td>
<td>345 ± 15</td>
<td>—</td>
<td>5/5</td>
<td>10.4</td>
</tr>
</tbody>
</table>

* No. of rats with positive response/no. of rats examined
** Mean ± SE
Table 4. Effect of neural deafferentation
(Animals were deafferented at 20:00 and autopsied in the late morning of proestrus)

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. of rats</th>
<th>No. of vaginal proestrus</th>
<th>No. of distended uterus</th>
<th>Weight of uterus</th>
<th>Intra luminal water/distended uterus</th>
<th>Weight of APG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>628 ± 40*</td>
<td>224 ± 25</td>
<td>7.8 ± 0.5</td>
</tr>
<tr>
<td>Knife B deafferented a = 7.0 v = 4.8 (Septal complex)</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>240 ± 27a</td>
<td>—</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>Knife B deafferented a = 5.0 v = 4.0 (Thalamus)</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>540 ± 38</td>
<td>180 ± 20</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td>Knife C deafferented a = 7.0 v = 5.0 (Fornix)</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>443 ± 51</td>
<td>139 ± 40</td>
<td>6.3 ± 0.5</td>
</tr>
</tbody>
</table>

* Mean ± SE
  a: P < 0.05

Table 5. Effect of neural deafferentation on gonadotropin secretion
(Animals were deafferented at 20:00 on the day of estrus and autopsied in the late morning of proestrus)

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. of rats</th>
<th>No. of vaginal proestrus</th>
<th>No. of distended uterus</th>
<th>Weight of uterus</th>
<th>Intra luminal water/distended uterus</th>
<th>Weight of APG</th>
<th>Pituitary FSH Total /mg</th>
<th>Serum FSH ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>580 ± 62*</td>
<td>211 ± 33</td>
<td>8.8 ± 0.9</td>
<td>72.8 ± 4.8</td>
<td>1410 ± 157</td>
</tr>
<tr>
<td>Knife B a = 7.0 v = 4.0 septal complex</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>312 ± 45</td>
<td>—</td>
<td>7.3 ± 0.7</td>
<td>68.5 ± 4.4</td>
<td>1410 ± 157</td>
</tr>
<tr>
<td>Knife B a = 5.0 v = 4.0 Thalamus</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>485 ± 20</td>
<td>193 ± 36</td>
<td>7.0 ± 0.3</td>
<td>50.7 ± 4.2</td>
<td>1687 ± 264</td>
</tr>
<tr>
<td>Knife C a = 7.0 v = 4.0 medial cortices hypothalamic tract</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>343 ± 56</td>
<td>516 ± 51</td>
<td>6.5 ± 0.8</td>
<td>75.4 ± 8.8</td>
<td>782 ± 149(A)*</td>
</tr>
</tbody>
</table>

* Mean ± SE  A: Three rats of vaginal diestrus  B: Two rats of vaginal proestrus
  a: p < 0.05  (A and B were separately calculated because of two different level)
on gonadotropin secretion on the day of diestrus I and proestrus.

Deafferentation C (Table 4): The transection was limited to the fornix alone or the medial cortico-hypothalamic tract alone. In contrast to the results of transection including septal complex, the transection of the fornix, or the medial cortico-hypothalamic tract on the day of diestrus I prevented partially the events of proestrous or estrous day.

Experiment 3:
The rats which had the neural deafferentation with knives of type A and type B at 20:00 on the day of diestrus II, were received 200 \( \mu \text{g} \) of estradiol-17\( \beta \) at 21:00. In the rats deafferented with knife A did not ovulate after estrogen treatment (Table 2). The rats had neural transection with a knife B resulted in 4 ovulating rats out of 11. Since none of rats ovulated without estrogen, estrogen could induce ovulation apparently in these rats (Table 3).

Experiment 4:
Neural transection of the septal complex with the knife B on the day of diestrus I resulted in the low level of pituitary LH, pituitary prolactin and serum prolactin on the day of expected proestrus, while the transection of the mid-thalamus with knife B did not induce any significant changes in pituitary or serum level of gonadotropin (Table 4). Transection of the septal complex on the days of estrus and diestrus I did not induce any changes in FSH of both pituitary and serum (Table 4 and 5).

However, either transection of the fornix with knife C or transection of the medial cortico-hypothalamic tract resulted in an increase of pituitary FSH. Serum level of FSH was low in the rats of vaginal diestrus and high in the rats of vaginal estrus, although the latter was not statistically significant (Table 4 and 5).

Discussion

Present experiment clearly demonstrated that the destruction of the septal complex including the hippocampo-fornical pathway...
on the day of diestrus I in 4-day cyclic rats prevented the gonadotropin secretion, which may be responsible for the proestrous change of uterine distension and estrous change of vaginal cornification as well as ovulation. Gonadotropin secretion at diestrus II is responsible for an increase of estrogen secretion (Bourdel and Li, 1963; Lostroh and Johnson, 1966), which has been shown to increase on the afternoon of the same day (Hori et al., 1968). Therefore, it is possible that a neural structure, which is responsible for the gonadotropin release on the day of diestrus II, may stimulate the anterior pituitary gland sometime between 18:00 on the day of diestrus I and 6:00 on the day of diestrus II. This assumption is, further, supported by the fact that the rats bearing the destruction in the septal complex including the fornical pathway are lower in the pituitary LH on the day of expected proestrus than as are those of thalamic transected rats or intact controls. Since the highest content of pituitary LH on the day of proestrus and higher content of the pituitary hormone on the day of diestrus II were observed (McClintock and Schwartz, 1968; Schwartz and Bartosik, 1962; Caligaris et al., 1967), the deafferentation at the level of above anterior commissure on the day of diestrus I prevents the increase of pituitary gonadotropin on the day of proestrus. Lawton and Sawyer (1968) and Kobayashi et al., (1969b) demonstrated that hypophysectomy before noon on the day of diestrus II blocked the vaginal cornification in estrus, while the cornification occurred when the operation was delayed until late afternoon. From these facts they assumed that a minute of gonadotropin must occur for the uterine distension on the following day (Kobayashi et al., 1969c). The interruption of the neural pathway for release of folliclrotropin and/or estrogen at 20:00 on the day of diestrus II did not result in ovulation in spite of distended uteri on the day of estrus, while interruption at 8:00 on the day of proestrus resulted in almost normal ovulation (Table 3). Thus, failure of ovulation in the deafferented rats with knives A and B before proestrus may be due to the estrogen deficiency, instead of damage of neural pathway for the release of ovulatory hormone. This assumption is, further, supported by the fact that an injection of estrogen to the deafferented rats restored the estrogen deficiency, which was induced by the neural transection. However, the negative result for the ovulation in the rats deafferented with knife A, was due to the damage of neural structure responsible for the release of ovulatory hormone, such as the bed nucleus of stria terminalis, the nucleus of accumbens, or the stria terminalis as a path-
way from amygdala. Induction of ovulation by electrical stimulation of the bed nucleus of stria terminalis, the accumbens or the amygdala was reported (Bunn and Everett, 1957; Everett et al., 1964; Terasawa and Sawyer, 1969; Kawakami et al., 1970). Previous works from our laboratory showed that electrical stimulation of the dorsal hippocampus in 4-day cyclic rats elevated the pituitary content of FSH throughout the estrous cycle (Kawakami et al., 1971), facilitated the release of FSH on the day of estrus (Kawakami et al., 1972a) and increased biosynthesis of estrogen in the ovarian homogenates on the day of estrus (Kawakami et al., 1972b). Even in the prepuberal rats the hippocampal stimulation increased pituitary and serum FSH as well as estrogen synthesis in the ovary, and therefore, the hippocampus was considered as an important part of the brain for initiation of FSH release and estrogen secretion before puberty (Kawakami and Terasawa, 1972). At the beginning of this experiments authors hypothesized that the transection of hippocampal efferent to the hypothalamus might block the onset of estrous cyclicity which was prepared by folliculotropin (Rothchild, 1960) and presumably estrogen. However, not only transection of the fornix at the level of caudal to the anterior commissure, but also interruption of the medial cortico-hypothalamic tract did not prevent entirely the vaginal cornification on the day of estrus. The medial cortico-hypothalamic tract was suggested as a pathway from hippocampus to the basal hypothalamus (Grudjian, 1927; Krieg, 1932; Nauta, 1956; Raisman et al., 1966). Recently Velasco and Taleisnik (1971) have reported that the transection of this tract, but not the fornix longus, resulted in the loss of the inhibitory function of the hippocampus on LH release. In the present experiment the destruction of the septal complex, including the parolfactory area, the nucleus of accumbens, the bed nucleus of stria terminalis, the bed nucleus of anterior commissure, but not rather simply the fornix or the medial corticohypothalamic tract, revealed blocking effect of an increase of folliculotropin and estrogen responsible for the events of proestrus and estrus. Therefore, it is assumed that the septal complex except for the proper nuclei of septum, may be related for the release of folliculotropin.

An importance of preoptic anterior hypothalamic area for triggering ovulation was suggested by Köves and Halász (1970) that the chronic interruption of the bilateral, anterior and superior connection to the preoptic anterior hypothalamic area resulted in ovulation in 9 out of 13 animals, in which some rats showed nearly normal vaginal cycle and others were predominantly in diestrus smear. This observation further indicates that the outside area of the preoptic anterior hypothalamus, such as the septal complex, the amygdala and the hippocampus may be involved in triggering release of folliculotropin in the normal cycling rats, although the medial preoptic anterior hypothalamus seems to be sufficient for inducing ovulation under the neural disconnected condition. In fact, related experiment of our laboratory supports this hypothesis that all of the fornical transected rats showed normal 4-day cycle as well as normal ovulation by about 2 weeks from surgery, however, both pituitary and serum level of FSH in the chronically transected rats on the day of diestrus II were higher (about 1.4 times) than those of normal controls (Kawakami et al., 1972b). Thus, the transection of the major afferent fibers from the hippocampus may result in a disinhibition to the hypothalamus and therefore, neural transected animals differ from normals, even through the estrous cycle being resumed after transection. Because of disinhibition, an increase of pituitary FSH following to the fornical transection or medial cortico-hypothalamic transection were observed in the present experiment.

The different effect of estrogen on ovulation in the rats transected with the knife
A and the knife B suggests the sites of estrogen action in the brain. Negative response with the knife A indicates that estrogen which is responsible for the release of ovulatory hormone may not feed back to the preoptic basal hypothalamus, at least when neural deafferentation was performed acutely, as this knife places above the preoptic area and basal hypothalamus. Positive response with knife B, however, suggests that estrogen may feed back to the stria terminalis, the bed nucleus of stria terminalis, the amygdala and or others in the septal complex. Whether estrogen feeds back to the amygdala itself or rather the septal complex in inducing ovulatory hormone release, was not clear in the present experiment. Although Velasco and Taleisnik (1971) showed that estrogen acts to the preoptic basal hypothalamus to release LH in the rats with amygdaloid lesions, author observed facts that the bed nucleus of stria terminalis or nucleus of accumbens could originate the neural triggering for the ovulatory hormone release under estrogen influences. That is, characteristic increases of multiunit activity was observed in the bed nucleus of stria terminalis during the critical period of the proestrous day (Kawakami et al., 1970), and estrogen induced remarkable increase of multiunit activity in the same nuclei (Kawakami et al., unpublished observation). Furthermore, electrical or electrochemical stimulation of all these area could induce ovulation with low threshold current in inducing ovulation (Terasawa and Sawyer, 1969; Kawakami et al., 1970). Therefore, the septal complex may have an active role in controlling gonadotropin release rather than being as a neural pathway.

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