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Egg–Oviduct Interaction Initiates Reproductive Behavior

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The experiments reported in this paper provide evidence that eggs must pass through the oviducts in order for receptivity to occur after ovulation in the female frog, *Rana pipiens*. In one experiment, oviductectomized frogs remained unreceptive after ovulation was induced by administration of exogenous pituitary glands, while sham-operates became receptive within 48 hr. Another experiment had four groups of subjects: ovariectomized females, females with oviducts ligated at the ostial end, females with openings in the uteri that prevented eggs from accumulating there, and sham-operated females. Only the last two groups, groups in which eggs could pass through the oviducts, became receptive. In these experiments, receptivity was indicated by absence of the release call during manual clasping of the trunk. Earlier experiments have shown that eggs have to pass through the oviducts in order to become fertilizable. Thus, the passage of eggs through the oviducts provides a mechanism which links the onset of reproductive behavior to the availability of fertilizable gametes.

The anuran female *Rana pipiens* is unreceptive before ovulation, and if she is clasped by a sexually active male, she emits a vocalization, the release call. The trunk contractions which accompany this call cause the male to loosen his grasp (Noble and Aronson, 1942).

Ovulation is initiated by pituitary gland action. Eggs are released into the body cavity, pass through the oviducts, and enter the uteri. By the time ovulation is complete, there are several thousand eggs stored in the uteri and the female has become receptive. She no longer croaks when clasped, so amplexus can be maintained and oviposition and spawning can occur (Rugh, 1935; Noble and Aronson, 1942).

It has been shown that eggs are prepared for fertilization as they pass through the oviducts. The evidence for this is that eggs retrieved from the body cavity cannot be fertilized *in vitro*, while uterine eggs at equivalent meiotic stages can (Aplington, 1957; Smith and Ecker, 1970).

The two experiments reported in this paper address the question of whether or not the association of eggs with the reproductive tract also

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influences the onset of breeding behavior in female *Rana pipiens*. Experiment 1 tested the hypothesis that oviducts need be present after ovulation for the occurrence of receptivity, and Experiment 2 tested the hypothesis that eggs need to pass through the oviducts in order for receptivity to occur.

EXPERIMENT 1

This experiment compared oviductectomized ($n=6$) and sham-operated ($n=9$) female frogs for their tendency to emit release calls in response to tactile stimulation. Subjects were tested before and after they ovulated. The experimental protocol was the following.

Day 1: arrival in laboratory and preoperative test for the release call.

Day 1 or 2: Oviductectomy or sham-operation.

Day 3: two preinjection tests for release calls, then injection with exogenous pituitary glands at 3:00 PM to induce ovulation.

Day 4: starting 16 hr after the pituitary injection of the previous day, subjects were tested every 4–8 hr (7 AM, 11AM, 3PM, 7PM, 11PM) until 72 hr after injection.

Procedures

Subjects and housing. All subjects were female Northern leopard frogs, *R. pipiens*, which were collected in Vermont in the spring as the animals were migrating to breeding ponds.

On receipt, median and range weight were 61.6 g (51.4–96.3 g). Subjects were housed individually in plastic containers (30 × 17 × 21 cm) containing a dilute solution of agristrep (streptomycin sulfate) in tap water. Laboratory temperature was 22–26°C and the room was illuminated 6AM–8PM EST with fluorescent ceiling lights.

Surgery. Animals were anesthetized by immersion in Finquel anesthesia (0.2 g/30 ml tap water) until swimming and respiratory movements stopped. Both oviducts were removed through a single, ventral, parasaggital incision. The oviducts were grasped and cut at the rostral ends, and attachment ligaments were torn as the oviducts separated from the body wall. The uteri were ligated just caudal to the uterine–oviductal junction and oviducts were cut at this junction. Incisions were closed with 4.0 surgical silk. There was no bleeding during the operations. For sham operations the animals were incised and sewn. Animals were rinsed with tap water to facilitate emergence from anesthesia.

Pituitary injection. Each subject was injected ip with one whole pituitary gland in approximately 1 ml Ringer solution using an 18-gauge needle. The pituitary donors were gravid females collected at the same time as the subjects. Pituitaries were removed from the donors less than 1 hr before injection.

Testing. Frogs were held on either side of the vertebral column posterior to the pectoral girdle between the thumb and forefinger of the experimenter. The number of release calls elicited in the first 30 sec of clasping was recorded (Diakow, 1977). The person performing the test did not know the group membership of the subjects.

Autopsy. Immediately after the last postinjection test, subjects were pithed and their ovarian and oviductal condition was examined. The data included in the results of the experiment are taken only from females in which ovulation was complete or almost so, i.e., from nine sham-operated and six oviductectomized females whose ovaries were estimated to be more than two-thirds empty. One sham-operated female and three oviductectomized females had ovaries that were less than half empty and their data were not included with the results. A rough subjective estimate of the degree to which the ovaries empty as ovulation proceeds is easy in these animals. The ovaries have several lobes. Before ovulation, each lobe contains several hundred black and yellow eggs (animal and vegetal poles are differently colored), held together by transparent membranous tissue. Once ovulation begins in a lobe, it usually goes to completion; an ovulated lobe is easily recognized as a small grey mass of shrunken membranes. In the sham-operates the ovulated eggs were found in the uteri but in the oviductectomized female they filled the body cavity.

At autopsy, the median and range oviduct weights for the sham-operated females were 0.4 g (0.3–2.9 g) for the left oviduct and 0.5 g (0.4–0.5 g) for the right oviduct.

Statistics. The median numbers of release calls were calculated each day for each female. The median of the medians for each female is plotted on the graphs.

The Mann–Whitney U test was used for between-group comparisons, and the Wilcoxon matched-pairs signed-ranks test for within-group comparisons. The level of significance was $P \leq 0.05$ using two-tailed tests.

Results and Conclusion

Before injection, there was no significant difference in the median number of release calls of the two groups. Both were unreceptive. After injection, the sham-operates became receptive, but the oviductectomized group did not. The median number of release calls was significantly higher in the oviductectomized females than in the sham-operates by 48 hr after injection (Fig. 1).

We conclude that oviducts are necessary participants in the onset of receptivity after ovulation. The results are consistent with the idea that neither ovulation nor the presence of ovulated eggs in the body cavity are sufficient for the onset of reproductive behavior, and raise many other questions about the internal factors which affect reproductive behavior: Are the eggs necessary at all? If so, need they pass through the

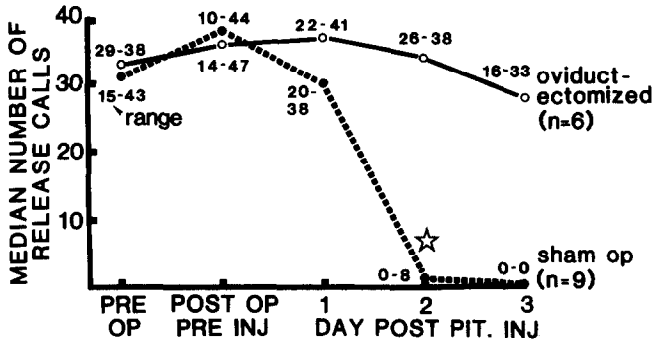

FIG. 1. Receptivity of female *Rana pipiens* is indicated by a decline in their tendency to emit release calls when grasped in a manner simulating amplexus. Oviductectomy prevented the onset of receptivity after ip pituitary injection. The star indicates a significant difference using a Mann–Whitney U test, $P \leq 0.05$, two-tail.

oviduct? Need eggs accumulate in the uterus? Is the presence of oviducts sufficient for inducing receptivity without the eggs? These questions are addressed in Experiment 2.

EXPERIMENT 2

This experiment compared four groups: ovariectomized females ($n = 7$), females in whom the oviducts were ligated at the rostral (ostial) end so that eggs could not pass through (oviduct-ligated group, $n = 3$), females in whom the uterus was cut open so that most eggs passing through the oviduct passed out again into the body cavity and did not accumulate in the uterus (uterus-open group, $n = 6$), and sham-operated controls ($n = 7$). These females were tested for the release call on the second day after arrival in the laboratory; thereafter, they followed the same schedule of testing, injection of exogenous pituitary glands, and surgery as the subjects in Experiment 1.

Procedures

Subjects and housing. The median and range weights of the females in this experiment were 58.5 g (48.1–70.2 g). Except for their being housed in groups until testing, their housing conditions were the same as those in Experiment 1. Laboratory temperature was 17–24°C.

Surgery. For oviduct ligation, 4.0 silk thread was passed around the straight, ostial end of the oviduct which is located on the dorsal body wall near the head. For ovariectomies, the ovarian ligaments were tied and cut distal to the body wall. In order to cut open the uterus, the walls of the uteri were cut along the impressions of hemostats applied to the ventral uterine wall. As much of the uterine wall as possible was removed, with care to avoid damage to the rectum and bladder. All

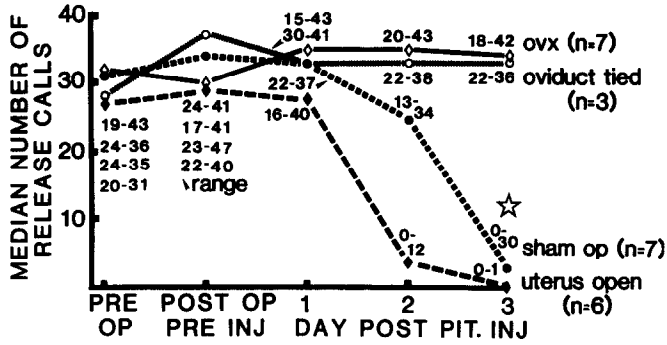

FIG. 2. Females in which eggs passed through the oviducts became receptive after ip injection of pituitary glands. These included sham-operated females (sham op) and those in which the uterus was perforated so eggs could not accumulate within (uterus open). Females did not become receptive if eggs did not pass through the oviduct. These were ovariectomized (ovx) females and those who ovulated but had oviducts ligated at the ostial end (oviduct tied). The star indicates a significant difference among groups with the Kruskal–Wallis one-way analysis of variance, $P \leq 0.05$, two-tail.

operations were performed through single parasaggital ventral incisions. Sham operations, closure, and postoperative care were the same as in Experiment 1, except that ether, which has a shorter term of action than Finquel, was used for anesthesia. This was possible because the investigators were more experienced with oviduct surgery and could work more quickly.

Testing and statistics. These were the same as in Experiment 1. In addition, the Kruksal–Wallis one-way analysis of variance was used for comparison among groups.

Autopsy. Group membership was confirmed and ovaries and oviducts were examined and weighed after the last test. As in Experiment 1, only females who were nearing completion of or who had completed ovulation contributed to the final data. These were 3/8 oviduct-ligated, 6/8 uterus-open and 7/8 sham-operated females. The data of all ovariectomized females, except one which became sick during the experiment, were used.

Results

There was no significant difference in the tendency to emit the release call among groups before pituitary injection, either preoperatively (H = 1.539) or postoperatively (H = 0.761) (Fig. 2). By the third day after pituitary injection there was a significant difference (H = 15.690) among the groups. Sham-operated and uterus-opened females showed receptive behavior, oviduct-ligated and ovariectomized females did not. The number of release calls had declined significantly from preinjection values in both

TABLE 1
Oviduct Weight at Autopsy (Experiment 2)

Group	Weight in grams (median and range)
Ovariectomized	3.4 (2.8–4.9)[a]
Oviduct tied	3.7 (3.3–3.8)[a]
Sham operated	0.8 (0.8–2.3)
Uterus open	1.0 (0.9–5.5)

[a]Significantly different from sham-operated group (Mann–Whitney U test, $P \leq 0.05$, two-tail).

the sham-operated ($T = 0$) and uterus-opened females ($T = 0$). The number of release calls of the oviduct-ligated and ovariectomized females was significantly higher than that for the sham-operates ($U = 1$ and $U = 3$, respectively). Thus, the presence of the oviducts alone without eggs was not sufficient to induce receptivity. Eggs had to pass through the oviducts. Eggs did not have to accumulate in the uterus in order to cause receptivity.

There was a significant difference in oviduct weights among groups at autopsy ($H = 12.966$), with oviduct weights significantly higher in oviduct-ligated ($U = 0$) and ovariectomized ($U = 0$) groups than in sham-operates (Table 1). Thus, the oviducts were small only in females in which eggs had completed transit though the oviducts.

We conclude that passage of eggs through the oviducts is necessary both for onset of receptive behavior and for regression of the oviducts after ovulation.

DISCUSSION

Most studies of the physiological bases of receptive behavior of female anurans have focused on electrophysiological correlates of behavior, on parts of the brain that mediate behavior, or on hormones under central nervous system control that potentiate behavior (Diakow and Raimondi, 1981). For example, the antidiuretic hormone, arginine vasotocin (Diakow, 1978), and luteinizing hormone releasing hormone (Kelley, 1982) were shown to affect receptivity. This study extends this conceptual framework by focusing on an endogenous peripheral influence, one whose source is outside the central nervous system, as an internal signal for the initiation of sexual behavior in female frogs. We propose that, because the frog's eggs must pass through the oviducts in order for receptivity to occur, there are probably responses in the oviducts, eggs, or both which bring about the change in behavior.

The marked morphological change in the oviducts certainly indicates that there were major physiological changes in them. The eggs might have stimulated the oviducts to emit neural or hormonal signals which

silence the females. Prostaglandins inhibit the release call (Diakow and Nemiroff, 1981), and we have suggested (Diakow, Scharff, and Aronow, 1983) that they might be released from the oviducts as eggs pass through. Prostaglandins have been demonstrated in the rabbit oviduct (Saskena and Harper, 1975), and we have found them in high concentration in the oviducts of *R. pipiens* (Rodriguez-Sierra and Diakow, unpublished). Prostaglandins have been implicated in the physiological bases of receptivity of many species (see Diakow and Nemiroff, 1981) including other cold-blooded vertebrates such as the goldfish (Stacey, 1976, 1981; Stacey and Goetz, 1982; Stacey and Peter, 1979; Villars, Hale, and Chapnik, 1985) and African clawed toad (Weintraub, Kelley, and Bockman, 1985).

The eggs were also clearly influenced during their passage through the oviducts, as they acquired a jelly coating at this time. They, too, could have provided a hormonal or neural stimulus that silenced the females. Stacey and Liley (1974) have presented evidence for the participation of ovulated eggs in the onset of spawning behavior of female goldfish. In the frog, transfer of material from eggs into the bloodstream or body fluid could normally occur through the very thin, transparent, highly vascularized wall of the uterus. Noble and Aronson (1942) proposed that neural stimuli which silence the female are produced when eggs stretch the wall of the uterus. The results of the present experiment are inconsistent with this hypothesis.

At this point in our study of the physiological basis of reproductive behavior of female *Rana pipiens*, we have evidence that many factors are involved in initiating receptivity. These include distension of the body, water imbibition, the antidiuretic hormone (arginine vasotocin), prostaglandin, and now, an interaction between the eggs and oviducts. We might generate a plausible scheme that links these factors. For example, vasotocin can inhibit the release call because it causes distension with fluid (Diakow, 1978) and prostaglandin synthesis (Diakow and Nemiroff, 1981). Perhaps the egg–oviduct interaction is also responsible for the release of prostaglandin into the bloodstream and provides an effective pressure stimulus.

We conclude this discussion with the proposal that an interaction between eggs and oviducts synchronizes the onset of the female's reproductive behavior with the availability of fertilizable eggs. This proposal is based on the observation that eggs must pass through the oviducts for both these events to occur. This form of synchronization seems very different from that in birds, reptiles, and mammals, wherein the primary agents for bringing about both receptivity and availability of eggs are hormones of hypothalamic and ovarian origin, but it is consistent with our early studies (Diakow, 1977; Diakow, Wilcox, and Woltmann, 1978) which indicated an independence from ovarian hormones for receptivity in *R. pipiens*. Our perceptions of the similarities and differences of the

system in birds, reptiles, and mammals and that in amphibians will, of course, be sharpened as we learn more about the physiological factors which underlie reproductive behavior in female amphibians.

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REFERENCES

Aplington, H. W. (1957). The insemination of body cavity and oviductal eggs of *Amphibia*. *Ohio J. Sci.* **57**, 91–99.

Diakow, C. (1977). Initiation and inhibition of the release croak of *Rana pipiens*. *Physiol. Behav.* **19**, 607–610.

Diakow, C. (1978). A hormonal basis for breeding behavior in female frogs: Vasotocin inhibits the release call of *Rana pipiens*. *Science* **199**, 1456–1457.

Diakow, C., and Nemiroff, A. (1981). Vasotocin, prostaglandin and female reproductive behavior in the frog, *Rana pipiens*. *Horm. Behav.* **15**, 86–93.

Diakow, C., and Raimondi, D. (1981). Physiology of *Rana pipiens* reproductive behavior: A proposed mechanism for inhibition of the release call. *Amer. Zool.* **21**, (1), 295–304.

Diakow, C., Scharff, C., and Aronow, L. (1983). The importance of oviducts for the onset of reproductive behavior in female frogs. *Soc. Neurosci.*, **9**, 536 (Abstract).

Diakow, C., Wilcox, J. N., and Woltmann, R. (1978). Female frog reproductive behavior elicited in the absence of the ovaries. *Horm. Behav.* **11**, 183–189.

Kelley, D. (1982). Female sex behaviors in the South African clawed frog, *Xenopus laevis:* Gonadotropin releasing, gonadotropic and steroid hormones. *Horm. Behav.* **16**, 158–174.

Noble, G. K., and Aronson, L. R. (1942). The sexual behavior of *Anura*. I. The normal mating pattern of *Rana pipiens*. *Bull. Amer. Mus. Nat. Hist.* **80**, 127–142.

Rugh, R. (1935). Pituitary-induced sexual reactions in the *Anura*. *Biol. Bull.* **68**, 74–81.

Saskena, S. K., and Harper, M. J. K. (1975). Relationship between concentration of prostaglandin F (PGF) in the oviduct and egg transport in rabbits. *Biol. Reprod.* **13**, 68–76.

Smith, L. D., and Ecker, R. E. (1970). Uterine supression of biochemical and morphogenic events in *Rana pipiens*. *Dev. Biol.* **22**, 622–637.

Stacey, N. E. (1976). Effects of indomethacin and prostaglandins on the spawning behavior of female goldfish. *Prostaglandins* **12**, 113–126.

Stacey, N. E. (1981). Hormonal regulation of female reproductive behavior in teleosts. *Amer. Zool.* **21**, 306–316.

Stacey, N. E., and Goetz, F. W. (1982). Role of prostaglandins in fish reproduction. *Canad. J. Fish Aquat. Sci.* **39**, 92–98.

Stacey, N. E., and Liley, N. R. (1974). Regulation of spawning behavior in female goldfish. *Nature (London)* **247**, 71–72.

Stacey, N. E., and Peter, R. E. (1979). Central action of prostaglandins in spawning behaviour of female goldfish. *Physiol. Behav.* **22**, 1191–1196.

Villars, T., Hale, N., and Chapnick, D. (1985). Prostaglandin-F_α stimulates reproductive behavior of female paradise fish (*Macropodus opercularis*). *Horm. Behav.* **19**, 21–35.

Weintraub, A. S., Kelley, D. B., and Bockman, R. S. (1985). Prostaglandin E_2 induces receptive behaviors in female *Xenopus laevis*. *Horm. Behav.* **19**, 386–399.