Effects of Brain Piqure on the First and Second Ovipositions of the Hen's 2-Egg Sequence. (32164)

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A previous study(1) has shown that electrical stimulation of the hen's preoptic brain evoked premature lay of the terminal (C_t) egg of a sequence during a 2-hour period on the day of C_t lay. Tests within the time that preoptic stimulation was completely effective disclosed that the response could be obtained by the mere insertion of electrodes (piqure) into any of several brain sites.

In apparent conflict with my observations on C_t lay, Juhàsz and van Tienhoven(2) reported that electrical stimulation of the telencephalon delays oviposition of the first (C_1) egg of a sequence. Since major differences in technique and in brain areas examined preclude meaningful comparisons of the two studies, and because endocrine mechanisms governing C_1 and C_t lay are not entirely comparable(3), it seemed important to extend my earlier experiments to the C_1 egg and to include tests of the telencephalon.

Materials and methods. White Leghorn hens were caged individually in laying batteries under electric lights from 6 a.m. to 8 p.m. Hourly records of lay, maintained from 8 a.m. to 4 p.m. for a flock of 800-900 hens, allowed selection of hens laying in 2-egg sequences with a lapse of 1 day between sequences. In such sequences, C_1 lay occurs between 9 a.m. and noon, C_t ovulation occurs within 1 hour after C_1 lay and C_t lay takes place between 1 and 4 p.m. on the next day.

Operations. The general operative procedures, type of electrode and parameters of electrical stimulation used are described elsewhere(1). Electrodes were placed stereotaxically with the aid of a brain atlas(4). The electrical stimulus was applied for 10 minutes. For piqure, electrodes were inserted for 1 minute.

Experiment 1. To establish a basis of comparison with available data on C_t lay(1), piqure and electrical stimulation were tested for effect on C_1 lay, and possible correlations between these effects and effects on the associated C_t ovulation were examined. Electrodes were placed bilaterally at sterotaxic coordinates $A_{9.5}H_{4.5}L_{0.5}$ in that area of the preoptic brain used as standard in the study on C_t lay(1). Groups of 8 hens were subjected to piqure or stimulation during 1 of 5 consecutive 3-hour intervals covering the period from 6 p.m. on the night before to 9 a.m. on the morning of C_1 lay. Operated hens were checked hourly for C_1 and C_t lay. The hour of C_t ovulation was estimated by appropriately scheduled digital palpations for the oviducal egg. All hens were sacrificed on the day of normal C_t lay for macroscopic examination of the ovaries and preparation of the brains for histological verification of the sites of electrode placement by methods previously described(1).

Experiment 2. In this experiment, the effects of time and site of brain piqure on C_1 and C_t lay were compared. Brain sites tested were the standard preoptic site(1), a site in the thalamus ($A_{7.0}H_{6.5}L_{1.0}$) used in the study on C_t lay and a site in the telencephalon ($A_{8.5}H_{7.5}L_{3.0}$) where Juhàsz and van Tienhoven(2) found electrical stimulation to delay C_1 lay. Times used were 9 to 11 p.m. on the night before lay, an optimal time of preoptic piqure for effect on C_1 lay, and 6 to 8 a.m. on the morning of lay, an optimal time for piqure for effect on C_t lay. Groups of 10 hens were subjected to piqure of 1 of the 3 sites during 1 of the 2 times for effect on *either* C_1 or C_t lay. Postoperative observations were identical with those outlined for Experiment 1.

Controls. Estimates of lay and ovulation were subject to certain naturally occurring errors, previously described in detail(1), arising out of the failure of an occasional hen, no matter how regular her previous record, to lay the expected 2-egg sequence. The probable frequency of such errors was estimated by following the performance at 100

TABLE I. Effects of Piqure of the Preoptic Brain on C_1 Oviposition, the Associated C_t Ovulation and C_t Lay.

Time of piqure	Hr before period of C_1 lay	No. of hens	No. of C_1 ovipositions premature*	No. of C_t ovulations delayed*	No. of C_t ovipositions premature*
6–9 p.m.	12–15	16	5^b	6^a	2^a
9 p.m.–12 m.	9–12	16	9^a	8^a	1^b
12 m.–3 a.m.	6–9	16	6^b	7^a	0^c
3–6 a.m.	3–6	16	5^b	3^b	2^a
6–9 a.m.	0–3	16	2^c	2^b	2^a
Unoperated controls		100	2^d	11^b	2^c

* Values in each column not having the same superscript differ significantly ($P < .01$).

unoperated hens selected on the same basis as experimental hens.

The data were analyzed by analysis of variance with significant differences between means determined by the method of Duncan (5).

Results. Experiment 1. No significant differences were found between the effects of piqure and electrical stimulation of the preoptic brain on C_1 lay, the associated C_t ovulation and subsequent C_t lay. The data are therefore pooled and presented in Table I as effects of piqure.

Piqure during any interval tested advanced C_1 lay. The greatest incidence of premature ovipositions, 56%, occurred with treatment between 9 p.m. and midnight; the frequency of the response then fell off gradually with advancing time of piqure (Table I). These results are in contrast with the earlier results on C_t lay, where the frequency of premature lay increased with advancing time of treatment from 9 p.m. to 9 a.m., attaining 100% with piqure between 6 and 9 a.m.

Independently of the time of piqure, 25 of 27 premature C_1 ovipositions occurred between 5 and 8 a.m. Based on past records of the individual hens, eggs laid during this period were premature by 1 to 6 hours (mean 2.5 hours). The two exceptions noted were laid between 1 and 4 a.m. In 2 other hens, C_1 lay was delayed by 20-22 hours.

Preoptic piqure before 3 a.m. induced a significant number ($P < .01$) of delayed ovulations (Table I). The period of delay was 3-6 hours in 6 hens and 24-48 hours in 15 hens. Such delays are believed to result from interference with LH release for ovulation (6). Nine hens treated before 3 a.m. did not ovulate the C_t egg. Atresia of large ovarian follicles was seen in these hens at autopsy as well as in 16 hens showing normal or delayed ovulation.

When C_t ovulation was not affected by treatment, C_t lay either occurred as expected (31 hens) or was premature by 1-13 hours (7 hens). If ovulation was delayed, the ensuing interval from ovulation to C_t lay was normal. No statistically significant correlations were found among the effects of preoptic piqure on C_1 lay, C_t ovulation and C_t lay.

Experiment 2. Piqure of the thalamus or telencephalon had no effect on C_1 lay. The frequencies of premature C_1 lay following preoptic piqure between 9 and 11 p.m. and 6 and 8 a.m. were similar to those recorded for the same treatment in Experiment 1 (Table II). Seven of the 8 premature eggs were laid between 5 and 8 a.m.

As a final check on the foregoing results, 10 hens were subjected to electrical stimulation of the telencephalic site between 9 and 11 p.m. on the night before C_1 lay. The results were negative.

TABLE II. Effects of Site and Time of Brain Piqure on C_1 and C_t Lay.

Time of piqure	Brain site	Incidence of premature lay C_1	Incidence of premature lay C_t
9–11 p.m.	Preoptic brain	5/10*	3/10*
	Thalamus	0/10	0/10
	Telencephalon	0/10	0/10
6–8 a.m.	Preoptic brain	3/10*	10/10*
	Thalamus	0/10	6/10*
	Telencephalon	0/10	2/10*
—	Unoperated controls	2/100	2/100

* Significantly different from controls ($P < .01$).

The effects of brain piqure on C_t lay were clearly time dependent. Piqure of the thalamus or telencephalon had no effect when applied between 9 and 11 p.m., but advanced C_t lay in significant numbers of hens ($P<.01$) when applied between 6 and 8 a.m. (Table II). Preoptic piqure was only 30% effective between 9 and 11 p.m., but was completely effective bewteen 6 and 8 a.m.

Of the 21 premature C_t ovipositions evoked by all treatments, 19 occurred between 9 a.m. and noon. Eggs laid during this time were premature by 2-6 hours. The facilitory period for C_t lay was about 1 hour longer than previously reported(1). This discrepancy may reflect the use of different strains of hens or of seasonal differences in the two studies.

Discussion. The results confirm and extend the previous finding(1) that brain piqure evokes premature oviposition during restricted hours of the day. Further, they establish clearly that electrical stimulation does not accentuate the response. How simple mechanical injury to the brain can lead to premature oviposition during restricted hours remains unknown.

My negative findings on piqure or stimulation of the telencephalon cannot be taken as evidence against the report of Juhàsz and van Tienhoven(2) that stimulation of this area delays C_1 lay. The discrepant results may be traced to major differences in the timing and techniques of stimulation.

In the 2-egg sequence, C_1 lay normally occurs between 9 a.m. and noon and C_t lay ensues during 1 to 4 p.m. on the next day. Irrespective of the time of piqure, 96% of the premature C_1 ovipositions in Exp. 1 occurred between 5 and 8 a.m. and 95% of the premature C_t ovipositions in Exp. 2 occurred between 9 a.m. and noon. That this resulted from restriction in the hours of piqure-evoked lay, rather than from a uniform advance in the time of lay, is shown by the fact that the interval from premature to normal lay in the individual hens ranged from 1 to 6 hours for the C_1 egg and from 2 to 6 hours for the C_t egg.

The fact that the periods of premature lay stand in the same relationship to each other as the periods of normal lay indicates that the dominant factor in the timing of the piqure-evoked oviposition, as in normal oviposition(7), is the cyclic release of pituitary gonadotrophin for ovulation. The lack of any correlation between the effects of preoptic piqure on C_1 lay and the associated C_t ovulation argues against a direct action of brain piqure on mechanisms controlling the ovulatory cycle. An alternate possibility is that piqure activates some secondary mechanism whose influence can be expressed only after some diurnally recurrent change in the cycle.

The data thus far show several important differences in the effect of brain piqure on C_1 and C_t lay. C_t lay was advanced by piqure of any brain site tested, but C_1 lay was advanced only by piqure of the preoptic brain. In general, preoptic piqure was far more effective on C_t than on C_1 lay. Further, the relationships between time of preoptic piqure and frequency of premature lay were markedly different for the two ovipositions. A possible explanation of these differences is that threshold of brain sensitivity to piqure depends on circulating levels of ovarian steroids. Steroid feedback on brain centers modulating gonadotrophin release has been used to explain cyclic ovulation and oviposition in the hen(7). Experiments by Rothchild and Fraps(8) suggest that C_1 lay is influenced by secretions from both the last ruptured and ovulating ovarian follicles; while C_t lay, which occurs on a day of no ovulation, is timed primarily by secretions of the ruptured follicle. It is not illogical to assume, therefore, that higher blood steroid levels and consequent lowered thresholds of brain sensitivity to piqure on the day of ovulation may account for the differences in the effects of piqure on C_1 and C_t lay. If the assumption is correct, the piqure-evoked oviposition should be readily blocked by appropriately timed steroid injections.

Finally, the results suggest that the zone of the preoptic brain previously implicated in ovulation(6) may also be a primary center in the control of oviposition, but that the mechanisms involved in the two processes are not necessarily the same. However, much

additional work, particularly in delimiting the areas in which brain manipulations evoke C_1 lay, is needed before these notions can be accepted.

Summary. Piqure of the hen's brain evoked premature lay of the C_1 and C_t eggs of a 2-egg sequence during restricted periods which stood in the same relationship to each other as the periods of normal lay for the respective eggs. C_t lay was advanced by piqure of any of several brain sites, but C_1 lay was advanced only by piqure of the preoptic brain. The frequency of premature lay in relation to time of preoptic piqure differed markedly for the two eggs.

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Comparative Evaluation of the Influence of Opsonins on Hepatic, Splenic and Pulmonary Phagocytosis.* (32165)

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The ability of the reticuloendothelial system (RES) to phagocytize circulating foreign particulate material is well established(1,2). However, the diffuse anatomical nature of the RES, as well as the multiplicity of factors(3-5) which can independently or collectively alter RE activity have enabled this system to elude complete quantitative analysis. Quantification of RE phagocytosis has been most successfully accomplished by determining the intravascular clearance rate of previously injected particulate material (6-8). In conjunction with such studies, tissue distribution of the injected colloids(9) has provided some insight as to relative participation of various organs. Studies of this nature have demonstrated that the greatest phagocytic activity resides in the cell populations comprising the fixed macrophages of the liver, spleen, and lung which collectively represent the major segment of the reticuloendothelial system.

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That phagocytic activity can be enhanced by non-specific serum factors called "opsonins" has been well established(10-15). Indeed, the interaction of a specific serum component(s) with foreign particulate material appears to be a prerequisite for cellular recognition and subsequent phagocytosis of foreign macromolecules(16,17). Early experiments by Manwaring and Coe(18) as well as Manwaring and Fritschen(19) have demonstrated that serum can augment hepatic phagocytosis of bacteria. Filkins and Smith (12) have shown that opsonic activity will enhance hepatic phagocytosis of colloidal carbon. In addition, recent studies from our laboratory(14,15) have established the existence of an opsonic system which can stimulate *in vitro* Kupffer cell phagocytosis. In contrast to these observations on liver, very little is known concerning the role of serum factors or "opsonins" in the phagocytic activity of fixed macrophages localized in the lung and spleen. In view of this apparent lack of information, the present studies were conducted to evaluate the influence of op-