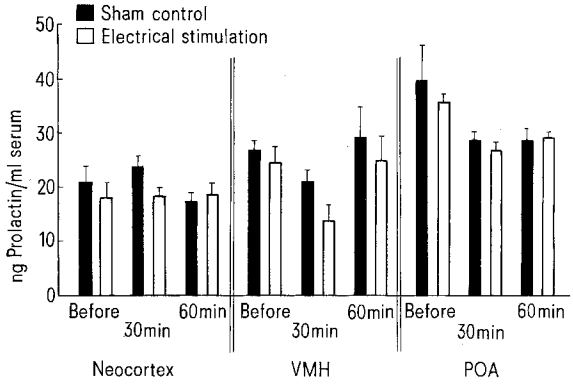
The Influence of Electrical Stimulation of the Rat Brain on Serum Prolactin Levels

The secretion of prolactin by the anterior pituitary is under the control of the central nervous system[1-4]. Evidence is accumulating to indicate that the anterior hypothalamic area plays a role in prolactin secretion[5-8]. We would like to report our results on the influence of brain electrical stimulation on serum prolactin.

Materials and methods. Female Sprague-Dawley rats (Spartan strain) were ovariectomized when they were 225–250 g in body weight. Seven to 20 days later the animals were anesthetized with sodium pentobarbital (42 mg/kg body weight, i.p.) and the tail artery cannulated using P.E. 50 size tubing to obtain blood samples. The animals were placed onto the stereotaxic instrument and a parallel electrode constructed from stainless steel enameled wire (Nilstain No. 304, 0.01 inch diameter) with a 1 mm separation at the tip, was placed into the neocortex, and the preoptic (POA) and the ventral medial (VMH) regions of the hypothalamus according to the atlas of PELLEGRINO and CUSHMAN[9]. The initial blood sample (0.8 ml) was obtained through the heparinized tail artery cannula after the electrodes were placed into the various regions of the brain. This was designated the 'before' blood sample and it was drawn 30–45 min after the animals were anesthetized Electrical stimulation consisted of a rectangular biphasic pulse with an intensity of 200 μA, a frequency of 100 cps, a duration of 1 m/sec, and an on-off time of 30 sec. The duration of stimulation was for 30 min. Immediately after stimulation a second 0.8 ml blood sample was taken and a third sample was taken 30 min after the second. In sham control animals no electrical current was passed through the electrode and it was left in the brain for the duration of blood sampling. The location of the electrode was marked using a 1 mA direct current for 30 sec. All brains were serially sectioned. Those animals found to have electrodes outside the desired region were discarded from the experiment. A total of 4–6 animals were included in each group. Serum prolactin levels were measured by radioimmunoassay using NIAMD-RP-1 as the reference standard. The details of the assay have been published elsewhere[10]. Statistical analysis of the data was performed using the analysis of variance for two-way classification as described by DIXON and MASSEY[11]. Differences among means were determined by KRAMER's modification[12] of Duncan's multiple range test. Data from the VMH area was assessed using the *t*-test.

Results. There was no significant difference in serum prolactin content between sham control and electrical stimulation in any of the areas studied (Figure). The presence of electrodes in the neocortex did not alter serum prolactin levels from that af the 'before' value. Electrodes in the ventral medial hypothalamus resulted in a significant decrease in serum prolactin at the 30-min period (*t*-test, $P < 0.02$), but by 60 min the serum levels returned to the initial value. In contrast, the preoptic hypothalamic region responded to electrode placement with a sustained decrease in serum prolactin (Figure). The 'before' serum prolactin levels from the preoptic group were significantly higher ($P < 0.01$) than the 'before' levels from the neocortex and the ventral medial hypothalamic groups.

Discussion. The similarity in response of sham and electrically stimulated animals has been observed by us previously[13] as well as by others[14] but not by some investigators[15]. It is apparent that the duration or intensity of stimulation among the three laboratories does not account for the differences in response. Electrodes for both sham and stimulated animals were left in place during blood sampling in our studies. The transient decrease in serum prolactin levels with electrodes in the VMH area has been noted before by us but the temporal aspect of the return to the initial value was extended[13]. The initial high serum prolactin levels and the sustained decrease in serum prolactin when electrodes were placed in the POA has also been observed by others[14,15]. The present study indicates that in acute experiments the mere presence of mechanical disturbance in the hypothalamus can alter serum prolactin. Perhaps chronic electrodes should be the method of choice in neuroendocrine studies of this nature. A similar conclusion was reached by KAWAKAMI[16]. The data presented here also



Serum prolactin levels (NIAMD-RP-1 used as reference standard) of animals with electrodes placed in various regions of the rat brain. The 'before' sample was taken after electrode was placed in brain. The 30 min sample was taken immediately after a 30 min electrical stimulation for the stimulation group or 30 min after the 'before' sample in the sham group. The 60 min samples were taken 30 min after the 30 min samples. Areas of brain examined were neocortex, ventral medial hypothalamus-VMH, and preoptic area of the hypothalamus-POA.

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indicates that the POA as well as the VMH influence prolactin secretion.

Zusammenfassung. Prolaktin wurde mittels Radioimmunbestimmung in Seren gemessen, die 30 und 60 min nach elektrischer Stimulation des Gehirns abgenommen wurden. Ähnliche Ergebnisse wurden in Kontrolltieren und elektrisch stimulierten Tieren erhalten. Die Serumprolaktinspiegel sanken, wenn die Elektroden in der präoptischen Region lagen. Ein vorübergehender Abfall wurde gemessen, wenn die Elektroden im ventralen, medialen Hypothalamus lagen. Keine Änderung im Prolaktinspiegel wurde gefunden, wenn die Elektroden im Neocortex lagen.

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[17] Acknowledgment. This work was supported by an NSF Research Grant, No. GB 27359. The authors appreciate receiving as a gift the rat prolactin RP-1-1 used for radioiodination and the rat prolactin RP-1 used as the standard from the NIAMD Rat Prolactin Hormone Distribution Program.

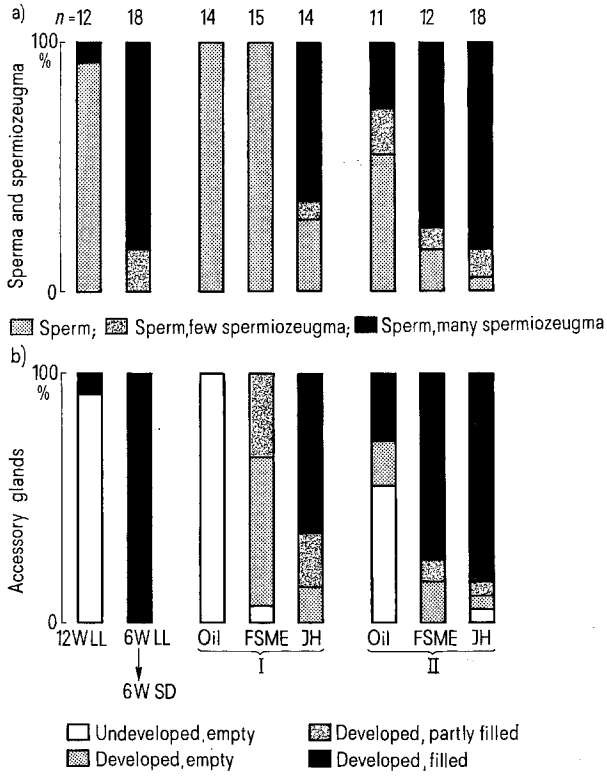
Juvenile Hormone-Initiated Sexual Maturity in Males of *Pterostichus nigrita* F. (Col. Carab.)

The formation of spermiozeugma (bundling of sperma) in *P. nigrita*[1] only takes place under short-day conditions (SD) (critical photoperiod 15.0 h), but spermiogenesis is almost independent of the applied photoperiods. To examine the hormonal processes underlying the effect of short-day conditions, 6-week-old males, enclosed in continuous light (LL), were given an injection of a juvenile-hormonomimetic substance [10.11-epoxy-farnesylmethyl-ester (FSME) Ro 8-4314, Hoffmann-La Roche, Basel, Switzerland] or a synthetic juvenile hormone (JH) (mixture of 8 possible isomeres, AY-22,342. Ayerst Research Laboratories, Montreal, Canada) dissolved in olive oil (5 µg per injection). After another 6 weeks under continuous illumination, the beetles were dissected.

The Figure shows that 1 injection of JH induces maturity in 65% of the males, a second injection does so in 83%. FSME has a weaker influence: 5 µg only stimulates growth and secretion in the accessory glands; with 10 µg the results are similar to those with JH.

Conclusion. It can be shown that JH initiates the formation of spermiozeugma in males of *P. nigrita*. Short-day conditions apparently stimulate the synthesis of JH within the organism. Inducing previtellogenesis was successful in females of *P. nigrita* by replacing the short day by injection of a juvenile-hormonomimetic substance[2] Histological investigations show an increased activity of the corpora allata in females under short-day illumination[3]. Further studies on the influence of the c.allata during short-day conditions on the formation of the spermiozeugma will be conducted.

Recent results show clearly that the c.allata and c.cardiaca are probably the hormone-producing organs: by transplantation of these organs from mature into immature beetles, sexual maturity of the immature males can be induced.



Formation of spermiozeugma (a) and contents of accessory glands (b) in correlation with continuous light (12 W LL), the change of continuous light to short day (6 W LL → 6 W SD), and the injection of olive oil, 10,11-epoxy-farnesylmethyl-ester (FSME) and juvenile hormone (HJ); I, 1 injection; II, 2 injections spaced 2 weeks apart.

Résumé. Les journées courtes (short-day) induisent la maturité sexuelle chez les mâles de *Pterostichus nigrita*. L'influence de la journée courte peut être remplacée par une injection d'un hormone juvénil synthétique.

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