Effects of Vaginal Stimulation on Hypothalamic Single Units in Unanesthetized Rabbits

In the rabbit, ovulation occurs naturally in response to mating but can be induced by mechanical stimulation of the vagina. The stimulus is relayed through the central nervous system and reaches the median eminence in a few seconds; release of gonadotropin by hypophysis is then activated. Rupture of follicle occurs 10 h after stimulation[1]. Since ovulation no longer occurs if the pituitary stalk is cut before the 25th min after stimulation[2], the nervous mechanism of ovulation can be limited to the first $^1/_2$ h that follows stimulation. Our purpose is to study modifications of single unit firing in the hypothalamus during that critical period.

Since ovulation can be disturbed by anesthesia we have used a chronic preparation for the unanesthetized animal[3]. On the other hand, coïtus or vaginal stimulation induces general changes in behavior and in the sleep-wakefulness state in the rabbit[4,5]. We also know that most of the cells in the hypothalamus[6] exhibit changes in their pattern of firing according to the level of arousal. It is therefore of great importance to identify the specific part the ovulatory function plays in a subsequent change observed in single unit activity apart from the non-specific influence of sleep-wakefulness variations.

Methods. We fitted 30 female 'fauve de Bourgogne' rabbits (3.5–4 kg) with a stereotoxically implanted cylinder which allowed us to record single units in the freely-moving animal; after each animal had recovered from surgery, we recorded unit activity in the hypothalamus with a hydraulically driven glass-insulated platinum microelectrode[7].

When we detected a stable neurone, we first tested its sensitivity to change in the level of arousal and recorded from it during wakening, slow-sleep and paradoxical sleep. Then vaginal stimulation was performed with a lucite rod acted on by an electrovibrator (50 Hz) during 30 sec. The ovaries were examined after 48 h for ruptured ovarian follicles.

Results. The frequency of spontaneous discharge (F) was statistically analyzed for each unit in order to determine the stability of the discharge. A confidence interval of variation (CIV) was established for the 3 levels of arousal: $CIV = 2h\sigma/\sqrt{n}$ (h, coefficient of probability; σ, standard deviation; n, number of samples of measured frequency).

The changes of 95 cells analyzed from waking to slow sleep are summarized in the Table with respect to their anatomical location. Only 36 of those cells were studied after vaginal stimulation because of the difficulty of holding the unit for a sufficiently long period in the unanesthetized animal. We considered that unit discharge variation (UDV) was specifically related to vaginal stimulation when the UDV after stimulus exceeded the CIV or was dissociated from the normal variation corresponding to a sleep-wakefulness change.

Of those 36 cases, 17 failed to show an ovulation induced by vaginal stimulation. In those negative stimulations a non-specific UDV was observed in any hypothalamic units. In 19 cases, animals normally ovulated in response to vaginal stimulus. We observed specific UDV after those positive stimulations in 15 cells. Responses might be divided into 3 groups on the basis of anatomical location of the cells and on the pattern of UDV. The

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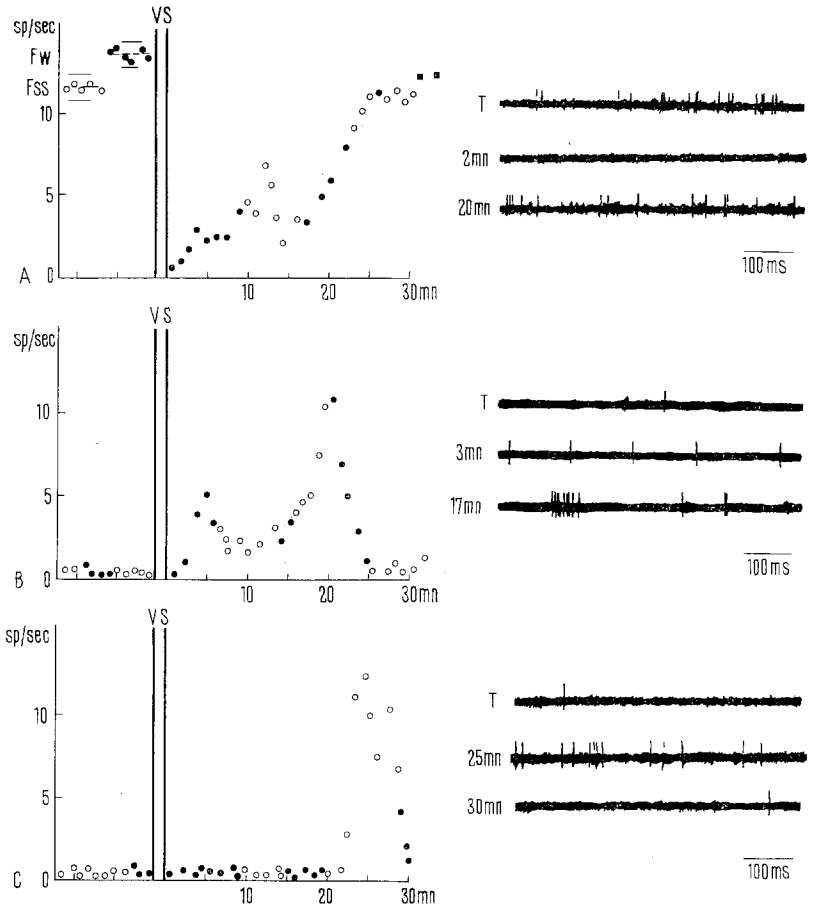
Brain site[a]	No. of cells[b]	Spontaneous changes from waking to slow-sleep			Response to vaginal stimulation		
		Decrease	Increase	No change	Ovulation[c]	Specific[d] UDV	Type[e]
MPO	19 (5)	13 (68%)	2 (11%)	4 (21%)	4	4	A
VMH	8 (6)	5 (62%)	2 (25%)	1 (13%)	3	3	A
DHA	32 (3)	23 (72%)	4 (12%)	5 (16%)	2	0	–
LHA	7 (2)	2 (29%)	1 (14%)	4 (57%)	1	0	–
SMA	6 (1)	5 (83%)	1 (17%)	–	0	0	–
PLHA	12 (8)	4 (33%)	3 (25%)	5 (42%)	2	2	C
PMA	8 (8)	–	–	8 (100%)	6	6	B
Others	3 (3)	1	–	2	1	0	–
Total	95 (36)	53 (56%)	13 (14%)	29 (30%)	19	15	

[a] Anatomical areas correspond to SAWYER et al.[9]. MPO, medial preoptic area; VMH, nucleus ventromedialis hypothalami; DHA, dorsal hypothalamic area; LHA, lateral hypothalamic area; SMA, supramammillary area; PMA, premamillary area; with the exception of PLHA: posterolateral hypothalamic area (p^3 limited inside by fornix and mamillothalamic tract); others correspond to anterior commissure and massa intermedia of thalamus. [b] Number in parenthesis corresponds to the number of the cells tested by vaginal stimulation. [c] Number of cases in which vaginal stimulation was followed by an ovulation. [d] Number of cases in which specific unit discharge variation was induced by vaginal stimulation. [e] Type of the cell response to vaginal stimulation described in text and figure.



3 types of significative UDV after positive vaginal stimulation (VS): A) Cell 24 (VMH); B) cell 90 (PMA); C) cell 78 (PLHA).
On the right: variations of F before and after VS; (●) correspond to waking state; (○) correspond to slow sleep; (■) correspond to paradoxical sleep; Fw, mean firing rate during waking; Fss, mean firing rate during slow sleep.
On the left: samples of spikes before (T) and after VS.

type A (7 cells Figure 1, A) was exclusively observed in MPO and VMH: positive vaginal stimulation was immediately followed by a large diminution of F independent of changes in arousal; then F returned to initial level 15–20 min after the end of stimulation. The opposite Type B (6 cells, Figure 1, B) was observed in PMA: raising of F began 1 or 2 min after the end of vaginal stimulation and was maximal between the 15th and 20th min, then F returned to the initial level after the 25th min. A response of type C was only observed in 2 cells located in PLHA: the raising of F after vaginal stimulation was more dramatic, shorter and occurred later than the response of type B.

Discussion. Since variations in electrical activity of some hypothalamic cells are recorded during the period in which pituitary activation is presumed to occur and failed to occur when vaginal stimulation does not induce ovulation, those unit firing changes could presumably be related to a neuro-endocrine phenomena. The lack of cell response in negative vaginal stimulation is also an argument for blockade of ovulation at a hypothalamic level. The short latency and the time course of cell response suggest a direct effect of vaginal stimulation. Since the decreased activity observed in VMH and MPO after positive vaginal stimulation was dissociated from changes in arousal, it is difficult to assume that this result could be due to a primary inhibition by progestin which is released within minutes following mating[8]. Nevertheless, a feedback effect of hormones released by vaginal stimulation (LH, oxytocin, progestin) cannot be eliminated. The cell activity observed in PMA has to be compared with the results of HAYWARD et al.[9] which induce ovulation by electrical stimulation of the same area and suggest that activation observed in those neurons is related to the secretion of releasing factor (LRF) into the portal system. The opposite UDV in VMH and APM suggests also the possibility of a functional antagonism of those area to the nervous control of ovulation.

Résumé. Certains neurones hypothalamiques présentent dans la demi-heure qui suit une stimulation vaginale des modifications de leur décharge spontanée indépendantes de l'état de vigilance de l'animal. Ces variations pourraient être en rapport avec les mécanismes nerveux de l'ovulation provoquée chez la Lapine.

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