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Neuronal Substrate of Electrically Induced Grooming in the PVH of the Rat: Involvement of Oxytocinergic Systems?

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VAN ERP, A. M. M., M. R. KRUK, J. G. VEENING, T. A. P. ROELING AND W. MEELIS. *Neuronal substrate of electrically induced grooming in the PVH of the rat: Involvement of oxytocinergic systems?* PHYSIOL BEHAV **57**(5) 881–885, 1995.—Electrical stimulation of the paraventricular (PVH) and adjacent hypothalamic area evokes self-grooming behaviour. Current intensity thresholds for grooming can be obtained depending on the exact localization of the electrode site. Sites localized at greater distance of the center of the grooming area evoke grooming at greater latencies and higher current intensity, or no grooming at all. Results are compared with injections of neuroactive substances into the PVH from previous studies, which showed a similar site specificity for grooming. We found similarity in the distribution of electrode sites in the paraventricular and anterior hypothalamic areas at which grooming is induced, and hypothalamic immunoreactive oxytocinergic neurons and fibres. In addition, we reported earlier that oxytocin infusions into the PVH in resting animals induce grooming, in contrast to other grooming-related peptides, such as α-melanocyte-stimulating hormone. We hypothesize that electrical stimulation may induce grooming by activation of oxytocinergic systems originating from the PVH.

Electrical stimulation Hypothalamus Oxytocin Paraventricular nucleus Self-grooming

ELECTRICAL stimulation of the hypothalamus induces distinct behavioural responses. Depending on the precise site of activation, different responses are evoked: for example, stimulation of the intermediate hypothalamic area (IHA), situated between the lateral hypothalamus and ventromedial nucleus, may induce attack behaviour (13,15), and stimulation of the paraventricular area may induce self-grooming (14,29). Depending on the current intensity used, physical properties of the electrode, and physiological properties of the brain tissue, a larger or smaller population of neurons and/or fibres may be activated (12). Therefore, a detailed analysis of evoked behavioural responses has to be combined with precise histological verification, to identify the neuronal substrate that is activated during elicitation of a particular response. In the present report, the hypothalamic distribution data from several experiments, in which electrical stimulation was used to evoke grooming responses, have been pooled and their distribution reexamined. Some of these data have been published previously (27,28).

Grooming can also be induced by the intracerebroventricular administration of peptides, such as adrenocorticotrope hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH), β-endorphin, oxytocin, or bombesin (5,9,21,32). In previous reports we have shown that local injection of some of these peptides into or near the PVH also may induce grooming (24,25,28–30). Interestingly, we found that slow infusion of oxytocin into the PVH in resting animals, after they had settled down, induced grooming, whereas α-MSH had no effect at all (30). This suggests that oxytocin-receptive systems may be involved in the initiation of grooming. It has been described that, apart from the axons, the dendrites of the oxytocinergic cells in the PVH may release oxytocin as well (2). Therefore, we decided to compare the distribution of electrode sites at which grooming was induced with the general distribution of oxytocin-immunoreactive neurons and fibres in the PVH and rostral hypothalamus.

METHOD

Subjects and Surgery

One hundred five male rats (Wistar/Harlan, Zeist) weighing 400–500 g were implanted bilaterally with in total 210 bipolar electrodes (150 μm) under Hypnorm (10 mg fluanison and 0.315 mg fentanyl citrate per ml; Janssen Pharmaceutica, Tilburg, The Netherlands) anesthesia (0.8 ml/kg b.wt.). Electrodes were aimed at the coordinates AP 7.40 mm, ML 0.50 mm, and DV 1.90 mm

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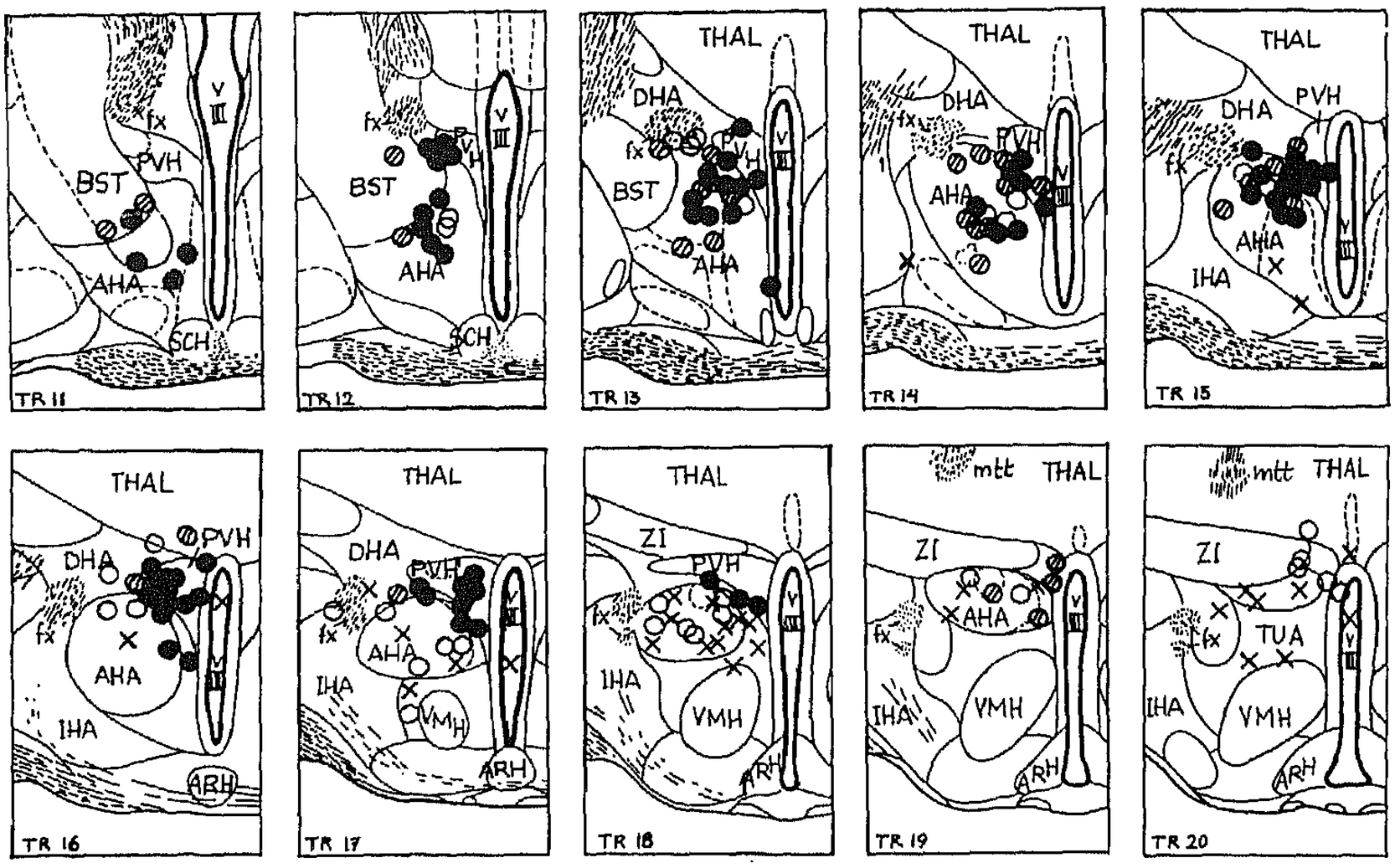

FIG. 1. Localization of electrodes in the PVH area. Sites are drawn on adjacent sections (150 μm) of a detailed cytoarchitectonic atlas of the hypothalamus (7,8). Upper left panel: anterior part; bottom right panel: posterior part. Each dot represents one electrode tip. Crosses: no grooming at all; open circles: grooming response but no stable thresholds; hatched circles: stable thresholds for grooming within 20 s; filled circles: stable thresholds for grooming within 10 s. Abbreviations: AHA, anterior hypothalamic area; ARH, arcuate nucleus; BST, bed nucleus of stria terminalis; DHA, dorsal hypothalamic area; fx, fornix; IHA, intermediate hypothalamic area; mtt, mammillothalamic tract; PVH, paraventricular hypothalamic nucleus; SCN, suprachiasmatic nucleus; THAL, thalamus; TUA, area of the tuber cinereum; vIII, third ventricle; VMH, ventromedial hypothalamic nucleus; ZI, zona incerta.

according to the atlas of Paxinos and Watson (20). For details about the electrodes and connectors used see Kruk et al. (13). After surgery, rats were housed individually in macrolon cages in low-noise rooms at 22°C and 75% relative humidity. Food and water were available ad lib and an inverted day/night cycle was installed, with lights on from 1500 to 03.00 h.

Behavioural Testing

Behavioural testing was started 1 week after surgery. Rats were stimulated with trains of 40-Hz biphasic square-wave pulses with a phase duration of 0.2 ms and a phase interval of 12.5 ms. At the first test, train duration was 30 s with 60-s intervals; at subsequent tests, train duration was decreased to 10 s. Threshold current intensity needed to evoke a grooming response was determined using the up-and-down method of Dixon and Mood (4) as modified by Wetherill (33). At a preset frequency of stimulation (40 Hz), current is raised in small steps (of 10 μA to a maximum of 100 μA), until a response is obtained. Then the current is decreased until the response is lost, raised again, and so on, until six change points are determined. The average of these six change points is called the threshold current intensity needed to evoke a particular response. To avoid interference with spontaneously occurring grooming, current was applied only if animals were not grooming at that time. If necessary, stimulation was postponed until the animal had stopped grooming. Subsequent threshold determinations on consecutive days were used to assess the stability of baseline thresholds.

Histology

After completion of the experiment, the rats were perfused with physiological saline followed by a 4% formaldehyde solution. Sections (20 μm) of the brain were stained with luxol fast blue and cresyl-violet. The electrode tips were localized precisely and drawn on a detailed cytoarchitectonic atlas of the rat hypothalamus (7,8).

Oxytocin Staining

Another four rats were perfused with Somogyi fixative (4% paraformaldehyde, 0.05% glutaraldehyde, 0.05% picric acid in 0.1 *M* PB, pH 7.4). After 2 h of postfixation, brains were removed from the skull and rinsed overnight in TBS. Vibratome sections with a thickness of 75 μm were cut. Two brains were sectioned transversally and two brains sagittally. Sections were rinsed in TBS and preincubated with incubation fluid [TBS, 0.5% Triton X-100, 0.1% bovine serum albumin (BSA), 5% normal swine serum] for 1 h. Incubation with antioxytocin (polyclonal, IncStar, dilution 1:4000 in preincubation fluid) occurred for 16 h at room temperature. After rinsing, sections were incubated with peroxidase-conjugated swine anti-rabbit antiserum (1:100) for 2 h. Sections were stained with DAB (20 mg/100 ml Tris buffer, 0.05 *M*, pH 7.6). Staining intensification with ammonium nickel sulphate (600 mg/100 ml Tris buffer, 0.05 *M*, pH 7.6) was used; 10 μl H_2O_2 (30%) was added to start the staining reaction.

RESULTS

Electrode sites at which the best grooming responses (with low thresholds and short latencies) were evoked were localized within the paraventricular hypothalamic nucleus itself and in the adjacent anterior hypothalamic area (AHA). Electrode sites at which slower responses were evoked were mostly found along the PVH border, as well as more ventrally in the AHA (Fig. 1).

TABLE 1

SUMMARY CLASSIFICATION OF RESULTS: NUMBER OF IMPLANTATIONS MEETING CRITERION

Criterion	Yes	No
Implanted	210	—
Tested	198	12
Grooming Induced	160	38
Threshold Obtained	117	43
Final Latency < 20 s	117	0
Final Latency < 10 s	82	35
First Latency < 10 s	22	60

Electrode sites at which grooming was induced, but where no stable thresholds could be determined, were observed even at greater distances from the PVH. In Fig. 1 the localization of all electrode sites have been combined as follows: no grooming at all (or other behavioural responses evoked, e.g., locomotion); grooming responses without stable thresholds; stable thresholds for grooming within 20 s; stable thresholds for grooming within 10 s (see Table 1). In a selection afterwards, those electrode sites at which a threshold within 10 s could be obtained in the first test already were mostly placed in the PVH itself (Fig. 2). At other electrode sites it always took more tests to obtain a grooming response within 10 s.

The localization of effective electrode sites in the dorsal and anterior hypothalamic area coincides remarkably with the localization of oxytocin-containing neurons, as well as with the efferent fibre streams (Fig. 3). The most effective stimulation sites (grooming response within 10 s in the first test) appear to surround the magnocellular part of the PVH, where oxytocinergic neurons are numerous, or to occur in the caudal part of the PVH, where slightly smaller oxytocinergic neurons are still present in considerable quantities (compare Figs. 2 and 3). Other effective sites (stable thresholds for grooming within 10 or 20 s) were localized in those parts of the anterior hypothalamic area that are being traversed by the descending oxytocinergic fibres. In between these fibres, local condensations of magnocellular oxytocinergic neurons are present (accessory groups) (Fig. 3). Because histology of electrode placements was done in Klüver–Barrera-stained sections, we cannot relate the electrode positions to the accessory oxytocinergic cell clusters in individual rats. However, the similarity in distribution over the entire group of electrodes is striking.

DISCUSSION

The distribution of electrode sites at which grooming can be evoked with short latencies and at low threshold current intensity is concentrated in the hypothalamic paraventricular area. This area coincides with previous observations (14). Moreover, it is very similar to the area where grooming could be elicited by microinjection of several neuroactive substances (24,25,28–30). No grooming responses can be evoked from other areas of the hypothalamus, as has been shown in an extensive distribution study by Lammers et al. (14–16). The best responses are consistently obtained from the PVH (i.e., for electrical stimulation: a threshold for grooming within 10 s determined in the first test; for peptide-induced grooming: grooming within 1 min after injection, accompanied by yawning and leading to a grooming score of more that 70% in the first 15 min after injection). In addition, grooming can be induced reliably from areas adjacent to the PVH: around the fornix, along the wall of the third ventricle, and in the anterior hypothalamic area. In these sites, grooming may be induced by activation of afferent or efferent pathways to the PVH.

There are many different peptides present in the PVH (17,26), of which several have been reported to be involved in the regulation of grooming, such as oxytocin, corticotropin-releasing hormone, ACTH, and α-MSH (5,6,9,11,21). However, there are differences in the grooming patterns induced by different manipulations. Grooming induced by electrical stimulation increases the frequency and duration of face washing and body grooming, at the expense of scratching (28). Grooming induced by oxytocin infusion into the PVH of resting rats also increases body grooming, but not face washing, at the expense of tail and paw grooming (30). Interestingly, we observed that at sites in the PVH yawning was often induced, after both electrical and mechanical stimulation. Yawning has been reported to occur after injection of oxytocin into the PVH (1,18). Direct microinjections into the PVH of low doses of excitatory amino acids (kainic acid, NMDA) or peptides (ACTH, α-MSH) also induce grooming (24,25,28–30). However, in a previous study we have shown that grooming induced by peptides like ACTH and α-MSH can be separated in two phases. The initiation of grooming may be the result of mechanical stimulation of the PVH and/or handling procedures, because saline injections have a similar effect. The administration of peptides leads to a considerable prolongation of these initial effects (29). Probably, the initiation phase is an effect of tissue compression or damage in or near the target area. This leads to the release of endogenous substances from damaged cells, which have an effect on neighbouring cells. It is known that damaged cells release large amounts of excitatory amino acids (EAAs) (34). This might lead to the activation of the area

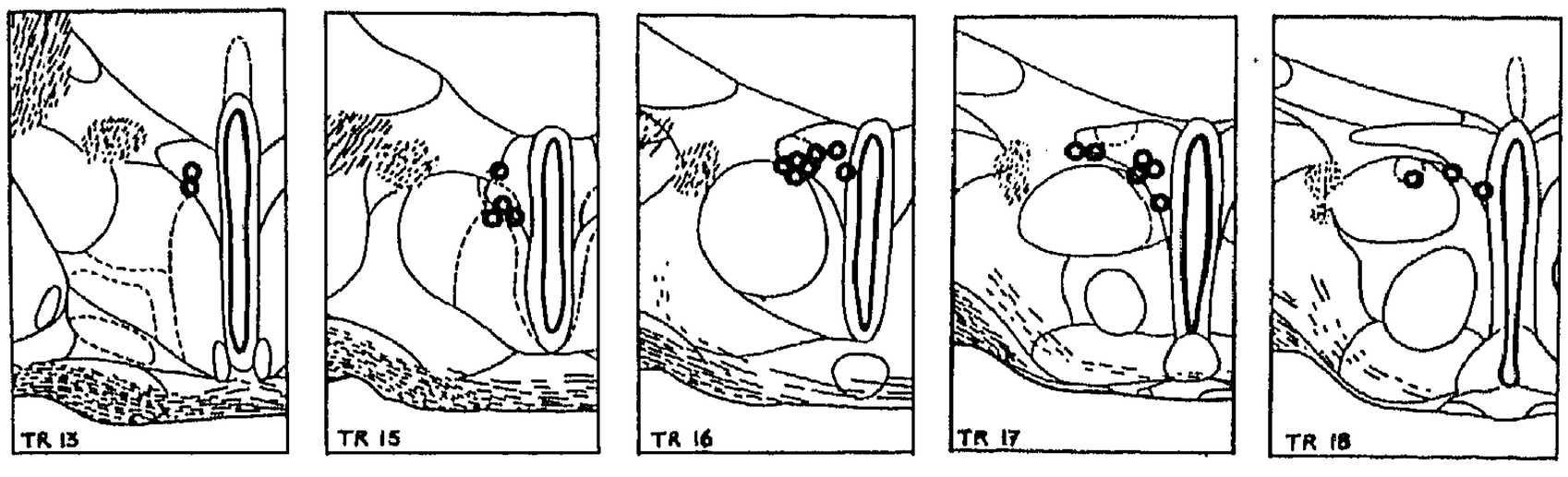

FIG. 2. Localization of electrodes at which a current threshold for grooming within 10 s was determined at the very first test after surgery. Each dot represents one electrode tip. Also see Fig. 1.

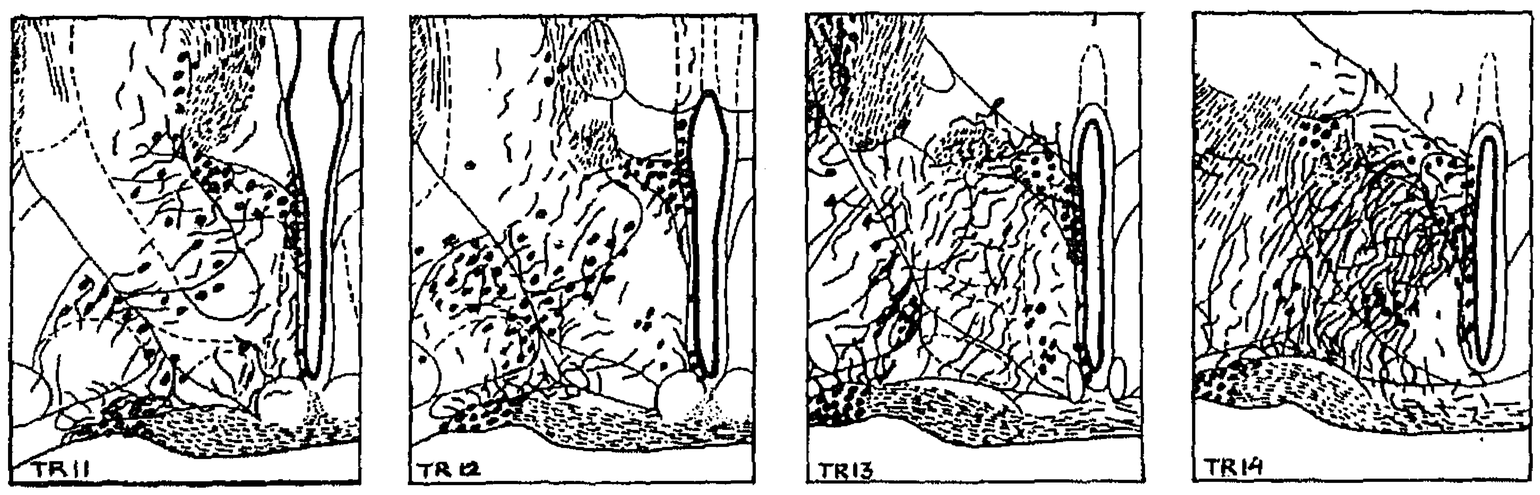

FIG. 3. Distribution of oxytocinergic neurons and fibres in the hypothalamus. Note the accessory nuclei containing oxytocin neurons in the anterior hypothalamic area, as well as oxytocin neurons in the periventricular area, and in between oxytocin fibres bending laterally around the fornix. Also see Fig. 1.

surrounding the needle tip. Interestingly, slow infusion into the PVH via a remote control cannula system (23) does not lead to a biphasic grooming effect. In a resting animal, α-MSH appears to be ineffective, whereas oxytocin infusion induces a clear-cut grooming response (30). This supports the suggestion that oxytocinergic systems are involved in PVH-induced grooming. Oxytocin infusions may activate oxytocinergic neurons via putative autoreceptors (3,19), whereas electrical stimulation may activate oxytocinergic neurons and fibres simultaneously.

In an extensive anatomical study, Roeling (22) showed that pathways originating from the hypothalamic grooming area (HGA) are very similar to the oxytocinergic pathways in the brain, in contrast with projections from other neighbouring parts of the hypothalamus. One of the descending efferent pathways from the HGA runs via the ventral tegmental area (VTA) through the brain stem; another pathway runs via the periaqueductal gray (PAG) and central tegmental field. In a pilot study, we found that PAG lesions, completely interrupting the descending hypothalamic fibres, had no effect on grooming responses evoked by electrical stimulation of the HGA (27). This suggests that the major pathway involved in hypothalamic grooming runs ventrally via the VTA. Interestingly, injection of oxytocin into the VTA has been reported to induce grooming (10). Other findings support the suggestion that the VTA, which is one of the origins of the dopamine system, is more important for the execution of hypothalamic grooming than the opioid-rich PAG: the opiate antagonist naloxone does not inhibit electrically induced grooming, whereas the dopaminergic antagonist haloperidol does (Van Erp, unpublished data). Recently it has been confirmed that the PAG is important for ACTH-induced, but not oxytocin-induced, grooming (31).

We conclude that there are interesting similarities in the distribution of electrode sites at which grooming can be induced and oxytocinergic neurons and fibres in the hypothalamus. Together with anatomical, pharmacological, and lesion studies in and outside the hypothalamus, we hypothesize that electrical and mechanical stimulation of the hypothalamic grooming area—including the PVH and some closely surrounding parts of the rostral hypothalamus—initiates a direct grooming response, probably by involvement of oxytocinergic mechanisms. However, more research is needed to test this hypothesis (e.g., by applying oxytocin antagonists during PVH stimulation).

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