

The organum vasculosum laminae terminalis in immune-to-brain febrigenic signaling: a reappraisal of lesion experiments

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Romanovsky, Andrej A., Naotoshi Sugimoto, Christopher T. Simons, and William S. Hunter. The organum vasculosum laminae terminalis in immune-to-brain febrigenic signaling: a reappraisal of lesion experiments. *Am J Physiol Regul Integr Comp Physiol* 285: R420–R428, 2003. First published April 24, 2003; 10.1152/ajpregu.00757.2002.—The organum vasculosum laminae terminalis (OVLT) has been proposed to serve as the interface for blood-to-brain febrigenic signaling, because ablation of this structure affects the febrile response. However, lesioning the OVLT causes many “side effects” not fully accounted for in the fever literature. By placing OVLT-lesioned rats on intensive rehydration therapy, we attempted to prevent these side effects and to evaluate the febrile response in their absence. After the OVLT of Sprague-Dawley rats was lesioned electrolytically, the rats were given access to 5% sucrose for 1 wk to stimulate drinking. Sucrose consumption and body mass were monitored. The animals were examined twice a day for signs of dehydration and treated with isotonic saline (50 ml/kg sc) when indicated. This protocol eliminated mortality but not several acute and chronic side effects stemming from the lesion. The acute effects included adipisia and gross (14% of body weight) emaciation; chronic effects included hypernatremia, hyperosmolality, a suppressed drinking response to hypertonic saline, and previously unrecognized marked (by ~2°C) and long-lasting (>3 wk) hyperthermia. Because the hyperthermia was not accompanied by tail skin vasoconstriction, it likely reflected increased thermogenesis. After the rats recovered from the acute (but not chronic) side effects, their febrile response to IL-1 β (500 ng/kg iv) was tested. The sham-operated rats developed typical monophasic fevers (~0.5°C), the lesioned rats did not. However, the absence of the febrile response in the OVLT-lesioned rats likely resulted from the untreatable side effects. For example, hyperthermia at the time of pyrogen injection was high enough (39–40°C) to solely prevent fever from developing. Hence, the changed febrile responsiveness of OVLT-lesioned animals is given an alternative interpretation, unrelated to febrigenic signaling to the brain.

fever; adipisia; third ventricle; median preoptic nucleus

THERE IS LITTLE DOUBT that the febrile response is regulated by the central nervous system (CNS), but the mechanisms by which peripherally originating pyro-

genic signals reach the brain remain unclear. At least four possibilities have been proposed. First, pyrogenic cytokines, such as IL-1 β , access the CNS by carrier-mediated transport across the blood-brain barrier (BBB) (2). Second, circulating pyrogens bind to the cerebral vascular endothelium and stimulate production of fever mediators, most importantly PGE₂, by endothelial cells; endothelial cells release PGE₂ into the brain tissue (10). Third, pyrogens act on neural terminals in peripheral tissues and convey febrigenic signals to the CNS via sensory fibers, e.g., those of the vagus nerve (7, 74). Fourth, peripheral immune signals enter the brain through the organum vasculosum laminae terminalis (OVLT) (4) and possibly other periventricular organs (69), in which capillaries are fenestrated resulting in a “leaky” BBB (36).

The significance of routes associated with a single neural structure (i.e., the vagus nerve or the OVLT) has been evaluated by assessing changes in febrile responsiveness after lesioning the respective structure. For the vagus nerve, experiments have involved surgical or chemical vagotomy (for review, see Ref. 48). For the OVLT, experiments have involved electrolytic, surgical (knife cuts), or thermal ablation of this structure (for review, see Ref. 40). However, a neural lesion may affect a physiological function not only by interrupting signaling along the corresponding route but also by causing undesired “side effects” that change the response of interest. For example, bilateral truncal vagotomy decreases fevers evoked by low doses of bacterial LPS (54), but it also leads to malnutrition, unless the animals are kept postoperatively on a liquid diet (1). Malnutrition itself attenuates febrile responsiveness (24, 62, 67). It was important, therefore, to determine that vagotomized animals have decreased febrile responsiveness even in the absence of malnutrition (51).

Lesions of the OVLT and its surrounding tissue in the anterior wall of the third ventricle also cause multiple side effects, including severe adipisia and emaciation (for review, see Ref. 8). In the fever literature, only a small number of studies (4, 6, 69) paid attention

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to these side effects. Blatteis et al. (4, 6) reported a tendency for polydipsia to develop in OVLT-lesioned guinea pigs in one study (4) and mentioned variable hypodipsia in another (6); the authors did not attempt to minimize these symptoms. Blatteis et al. (4, 6) also questioned whether lesions of the OVLT affect afebrile thermoregulation (6) or febrile responsiveness to intracerebral endogenous pyrogen (4), but found no gross deficiency. Takahashi et al. (69) tried to encourage resumption of normal drinking and eating patterns in lesioned rats by providing them with "palatable solutions," but the authors reported no data on the animals' eating, drinking, or body mass. In all other studies of fever in OVLT-lesioned animals (for references, see Table 1), side effects of the lesioning procedure were ignored altogether.

The results of fever experiments in OVLT-lesioned animals are contradictory (see Table 1). Electrolytic lesions of the lamina terminalis blocked or attenuated intravenous IL-1 β - or tumor necrosis factor (TNF)- α -induced fever in rabbits (23) and peripheral (intravenous, intraperitoneal, or intra-arterial) LPS-induced fevers in guinea pigs (4, 6, 26) and rats (9, 25). However, similar lesions did not affect the febrile response of rats to intravenous LPS in a study by Takahashi et al. (69) and enhanced the responses of rats and rabbits to intravenous administration of a crude preparation of IL-1 in a study by Stitt (63). It cannot be excluded that at least some of these results were "contaminated" by the uncontrolled, variable side effects of brain tissue lesions in the lamina terminalis. Therefore, we attempted to identify and minimize side effects of OVLT lesioning and then studied the febrile response of the lesioned rats to intravenous IL-1 β .

MATERIAL AND METHODS

Animals. Twenty-five male Sprague-Dawley rats (B & K Universal, Kent, WA) weighing ~180 g on receipt were housed individually in the institutional animal care facility on a 12:12-h light/dark (lights on from 7 AM to 7 PM) cycle and ambient temperature (T_a) of ~22°C. Standard laboratory rat pellets (Teklad Rodent Diet "W" 8604, Harlan Teklad, Madison, WI) and tap water were available ad libitum. The cage

space was enriched with artificial "rat holes" (cylindrical confiners made of stainless steel wire). In addition to spending time in the confiners voluntarily, the rats were systematically habituated to them (at least 5 training sessions, 4–5 h each). During some training sessions, the rats were instrumented with colonic thermocouples, and their colonic temperature (T_c) was recorded. Rats easily learn to stay in the enclosures and often prefer them to the open space of their home cages. Well-adapted, confined rats exhibit neither a stress fever nor any other signs of stress (52). The same confiners were used later in experiments. To minimize the effect of circadian rhythms on the experimental outcome, all experiments began at ~9 AM. On completion of the study, the animals were killed with a bolus intravenous injection of ketamine-xylazine-acepromazine cocktail (22.2, 2.2, and 0.4 mg/kg, respectively). The protocol was approved by the institutional animal care and use committee.

Electrolytic ablation of the OVLT. On the day of lesioning (*day 0*), the rats were treated with the antibiotic enrofloxacin (12 mg/kg sc) and anesthetized with a ketamine-xylazine-acepromazine cocktail (55.6, 5.5, and 1.1 mg/kg ip). The skin of the head over the frontal and parietal bones was shaved and scrubbed. The head was fixed in a stereotaxic apparatus (David Kopf, Tujunga, CA) in the de Groot (14) position, i.e., the incisor bar set 5 mm dorsal to the interaural line. The skin was incised over the sagittal suture, and the subcutaneous tissues were retracted and scraped off the skull. Two miniature stainless steel screws (1 on each side of the midline) were threaded into the bone ~5 mm caudal to the bregma and ~5 mm lateral to the sagittal suture. A 3-mm hole (centered on the midline, 8.2 mm rostral to the interaural line) was drilled through the skull to the dura mater. The dura was incised, and the underlying sinus was retracted to the side. At the center of the hole, a tapered (100 μ m) tungsten electrode, insulated except for 0.5 mm at its tip, was inserted 8.4 mm below the dura (i.e., 1.8 mm below de Groot's horizontal zero plane). Thus the de Groot coordinates of the electrode tip were as follows: rostral-caudal, 8.2 mm; medial-lateral, 0.0 mm; and dorsal-ventral, -1.8 mm. The coordinates were based on the atlas by Pellegrino and Cushman (45) and earlier work by Hunter (25). For a sham lesion, the first two coordinates remained the same, but the third was 0.6 mm (i.e., 6.0 mm below the dura). A second electrode (an "alligator" clip) was attached to the edge of the skin wound. The electrodes were connected to a Precision Lesion instrument (Stoelting, Wood Dale, IL). To lesion the brain tissue,

Table 1. *Effect of electrolytic lesion of the OVLT on the febrile response*

Species	Lesioning Parameters	Pyrogen, Dose	Effect on Fever	Reference
Rat	DC; 2 mA; 10 s	IL-1, (?) iv*	↑	63
Rat	DC; 3 mA; 20 s	LPS, 10 μ g/kg ia	↓↓	9
Rat	DC; 3 mA; 20 s	LPS, 50 μ g/kg ip	↓↓	25
Rat	DC; 1.5 mA; 30 s	LPS, 20 μ g/kg iv	→	69
Guinea pig	DC; 3 mA; 20 s	LPS, 2 μ g/kg ip	↓ ↓ ↓	4
Guinea pig	DC; 3 mA; 20 s	LPS, 2 μ g/kg ip	↓ or ↓ ↓ †	6
Guinea pig	DC; 2 or 3 mA; 10 or 20 s	LPS, 2 μ g/kg iv	↓ ↓ ↓	26
Rabbit	DC; 3 mA; 15 s	IL-1 β , 300 ng/kg iv	↓	23
Rabbit	DC; 3 mA; 10 or 15 s	IL-1, (?) iv*	↑	63
Rabbit	DC; 3 mA; 15 s	TNF- α , 10 μ g/kg iv	↓	23
Sheep	AC (radio frequency)‡ 40 mA; 15 s \times 3 sessions	LPS, 250 ng/kg iv	↓ or ↓ ↓ §	5

Effects are marked: ↑, exaggeration; →, no effect; ↓, "weak" (less than 2-fold) attenuation; ↓ ↓, "strong" (more than 2-fold) attenuation or complete blockade. *A crude preparation of "endogenous pyrogen" was used and dosed in ml/kg. †Magnitude of inhibition depended on the time elapsed after the lesion. ‡So-called thermal (not electrolytic) lesion. §Magnitude of inhibition depended on the location and size of the lesion. TNF, tumor necrosis factor; OVLT, organum vasculosum laminae terminalis.

constant anodal current (3 mA) was passed between the electrodes for 15 s. No current was passed in sham-lesioned rats. The electrodes were withdrawn, and the bone defect was filled with bone wax. Dental acrylic was applied over the exposed skull surface and screws. The acrylic was shaped to form a flat platform for later attachment of a plastic screw-capped container to hold the exteriorized end of the jugular catheter. When the acrylic hardened, the animal was released from the stereotaxic apparatus.

Postlesion care. Because animals with lesions in the lamina terminalis typically become adipsic but often accept palatable liquids (8), tap water was substituted with 5% sucrose to stimulate drinking for 1 wk after the surgery. During the same week, all operated animals were examined twice a day for signs of dehydration and general malaise; their body mass and sucrose consumption were measured daily. If a rat lost body mass >15% at 24 h postlesion, continued losing body mass at 48 h, or did not drink (0 daily consumption of sucrose) at any time after the surgery, it was hydrated with an infusion of isotonic saline (50 ml/kg sc) containing enrofloxacin (12 mg/kg). Postlesion care was conducted under close supervision of the staff veterinarian.

Jugular catheterization. On day 10, each rat was anesthetized a second time, and a silicone catheter filled with heparinized (20 IU/ml) saline was implanted in the superior vena cava through the right jugular vein (for details, see Refs. 28, 29). In brief, the catheter was tunneled under the skin to exit at the caudal edge of the cranial acrylic platform. A small plastic screw cap container was slipped over the cannula and affixed to the platform with fresh dental acrylic. The catheter was then coiled inside, and the container was capped. The catheter was flushed with heparinized saline on the next day and every other day thereafter.

Fever test. On days 18 and 22, the rats were placed in their enclosures and instrumented with copper-constantan thermocouple probes to measure T_c (10 cm beyond the anus) and tail skin temperature (T_{sk}). The thermocouples were fed to a data logger (model TCA-AI-24; Dianachart, Rockaway, NJ) and then to a microcomputer. The instrumented rats, in their confinements, were placed in an environment chamber (Forma Scientific, Marietta, OH) set to 50% relative humidity and a T_a of 30.0°C, which correspond to the midpoint of the rat's thermoneutral zone in our setup (50). The exteriorized portion of the jugular catheter was passed through a wall port and connected to a syringe. After a 1-h stabilization period, the measurements were begun, and T_c , T_{sk} , and T_a were recorded every 2 min from 30 min before to 180 min after an intravenous injection of either IL-1 β or saline. One-half of the rats received IL-1 β on day 18 and saline on day 21; the other one-half received the drugs in the opposite order. Recombinant mouse interleukin IL-1 β (Endogen, Boston, MA; 98% purity; LPS contamination <100 pg/ μ g) was freshly dissolved in pyrogen-free saline and infused at a dose of 500 ng/kg (1 ml/kg iv) over 1 min. Recalculated per body mass, LPS contamination was <50 pg/kg, which is substantially below the 1 μ g/kg minimal pyrogenic dose for the rat (54). The controls received saline (1 ml/kg) containing the same trace amount (50 pg/kg) of *Escherichia coli* 0111:B4 LPS (lot no. 35H4086; Sigma, St. Louis, MO).

Measurements of water-electrolyte balance. In addition to monitoring fluid consumption and body mass (see *Postlesion care*), drinking responses to hypertonic (5%) saline (4 ml/kg iv) were measured on day 14; plasma sodium concentration and osmolality were measured on day 40. For sodium and osmolality measurements (which were performed by flame photometry and freezing-point osmometry, respectively, by the Legacy Clinical Laboratory), 3 ml of blood was withdrawn

via cardiac puncture from ketamine-xylazine-acepromazine-anesthetized animals immediately before euthanasia.

Histological verification. The brains were removed from the euthanized animals, fixed in 10% neutral formalin, and dehydrated/cryoprotected in 20% sucrose. The hypothalami were sectioned (30 μ m) with a cryotome. The sections were mounted, stained with thionine, examined by light microscopy, and photographed.

Data processing and analysis. The change in T_c (deviation from the baseline) was used as a measure of the febrile response. The heat loss index (HLI) was used as a measure of the skin vasomotor tone. The HLI was calculated according to the formula: $HLI = (T_{sk} - T_a)/(T_c - T_a)$ (50); the theoretical limits for the HLI are 0 (maximal vasoconstriction) and 1 (maximal vasodilation). Single-point quantitative measurements (body mass, change in body mass, water consumption, plasma sodium and osmolality, basal T_c) were statistically compared between OVLT-lesioned and sham-operated rats by using Student's *t*-test. To compare the groups for the presence or absence of adipsia and for requirements for rehydration therapy (see *Postlesion care*), a nonparametric (Mann-Whitney *U*) test was used. As in the past (27, 49), time-function measurements (change in T_c , HLI) were converted into single numbers by subtracting the control curve (saline) from the corresponding experimental curve (IL-1 β) obtained in the same animal and integrating the resultant curve over the observation period; these numbers were averaged across groups (lesioned or sham) and for statistical comparisons treated as single-point measurements. All analyses were performed using Statistica AX'99 (StatSoft, Tulsa, OK). The data are presented as means \pm SE.

RESULTS

Seven rats in which histological examination revealed incomplete ablation of the OVLT were excluded from the study. In nine remaining rats, the OVLT was ablated completely; data obtained from these nine animals are presented below as data from lesioned rats. Although we attempted to produce relatively selective OVLT lesions (as opposed to larger lesions of the lamina terminalis; see Ref. 26), the small size of the OVLT (<100 \times 200 \times 300 μ m in the rat) and its proximity to the surrounding structures make involvement of adjacent areas almost unavoidable (8). Involvement of the median preoptic nucleus (MnPO), the rostral portion of which covers the OVLT from the dorsal side as a coat, is especially likely. In this study, the MnPO, anteroventral periventricular nucleus (AVPV), and ventromedial preoptic area (VMPO) were all partially damaged (although to a variable extent) in all nine lesioned rats (Fig. 1).

During the acute postoperative period, eight of the nine lesioned animals consumed no sucrose solution for at least 1 day, whereas none of the eight sham-operated rats exhibited adipsia ($P = 0.0002$). During the first 24 h after the surgery, the lesioned animals lost as much as 60 g (23% of body mass). The mean loss of body mass in this group was $14 \pm 2\%$, whereas the sham-operated group lost only $5 \pm 2\%$ ($P = 0.0009$; Fig. 2A). Despite access to a sucrose solution, eight of nine lesioned rats met the criteria for rehydration therapy on at least 1 day during days 1–3; five lesioned rats had to be treated with subcutaneous saline on 2 days.

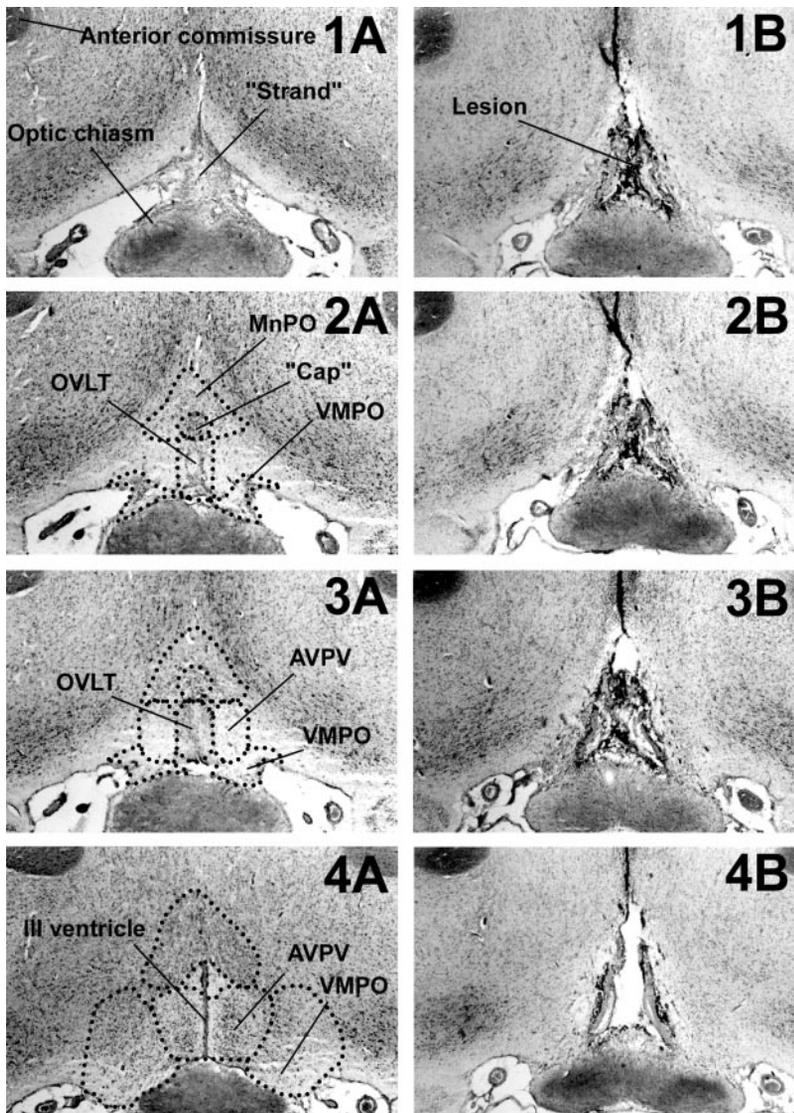


Fig. 1. Bright-field photomicrographs ($\times 40$) of serial coronal brain sections ($30\ \mu\text{m}$, thionine) of a sham-operated rat (1A–4A) and an organum vasculosum laminae terminalis (OVLT)-lesioned rat (1B–4B). Lesion shown was one of the most selective in the study. Thionine stain does not reveal the ventromedial preoptic area (VMPO) and poorly reveals the anteroventral periventricular nucleus (AVPV) and median preoptic nucleus (MnPO); approximate locations of these structures are marked on the basis of their relative position to the optic chiasm, third ventricle, and other anatomical landmarks with the help of maps by Elmquist and Saper (16).

Rehydration therapy was indicated for none of the sham-operated rats (sham-lesion difference: $P = 0.00003$). None of sham-lesioned or lesioned rats died.

After the acute period, the lesioned rats started to gain weight. At the time of the fever test, their body mass did not differ significantly from that of the sham-operated rats (324 ± 10 vs. 331 ± 10 g). However, rehydration therapy during the acute postoperative period did not prevent chronic impairments in the water-electrolyte balance. The lesioned rats were hyporesponsive to an osmotic stimulus. In a drinking test conducted on *day 14*, they consumed only 3.1 ± 0.7 ml of water over 1 h after a hypertonic saline load, whereas the sham-lesioned rats drank 5.5 ± 0.5 ml over the same period ($P = 0.005$; Fig. 2B). On *day 40*, the lesioned rats still showed hypernatremia (146.2 ± 0.7 vs. 144.0 ± 0.7 mmol/l in the sham-lesioned rats; $P = 0.002$) and hyperosmolality (313.8 ± 1.4 vs. 309.5 ± 1.3 mosmol/kgH₂O in the sham-lesioned rats; $P = 0.04$) under nonstimulated conditions (Fig. 2C).

During training sessions (*days 10–16* postlesion) and in both IL-1 β and saline experiments (*days 18–*

21), the basal T_c of the OVLT-lesioned rats was always $1.5\text{--}2^\circ\text{C}$ higher than that in the sham-operated animals. In the IL-1 β experiment, the initial T_c in the lesioned rats was 39.6 ± 0.4 compared with $37.7 \pm 0.1^\circ\text{C}$ in the sham-lesioned rats ($P = 0.0003$; Fig. 2D). In the saline experiment, these values were 39.3 ± 0.5 vs. $37.8 \pm 0.1^\circ\text{C}$ ($P = 0.008$). Extremely high values of T_c ($>40^\circ\text{C}$) were observed in several lesioned rats on several occasions. Despite such high levels of basal T_c , the lesioned rats showed no tail skin vasoconstriction. Their HLI (measured on *days 18* and *21*) was ~ 0.6 and did not differ from that of the sham-operated rats.

An intravenous injection of saline produced no thermal effects in either lesioned or sham-lesioned animals. A low (500 ng/kg) dose of IL-1 β caused a fever in the sham-operated rats but had no effect on T_c of the OVLT-lesioned rats (Fig. 3). This difference was highly significant ($P = 0.005$). Simultaneously with the onset of fever, the sham-lesioned rats developed a transient tail skin vasoconstriction (the HLI decreased from ~ 0.6 to ~ 0.4), but the magnitude of the response was

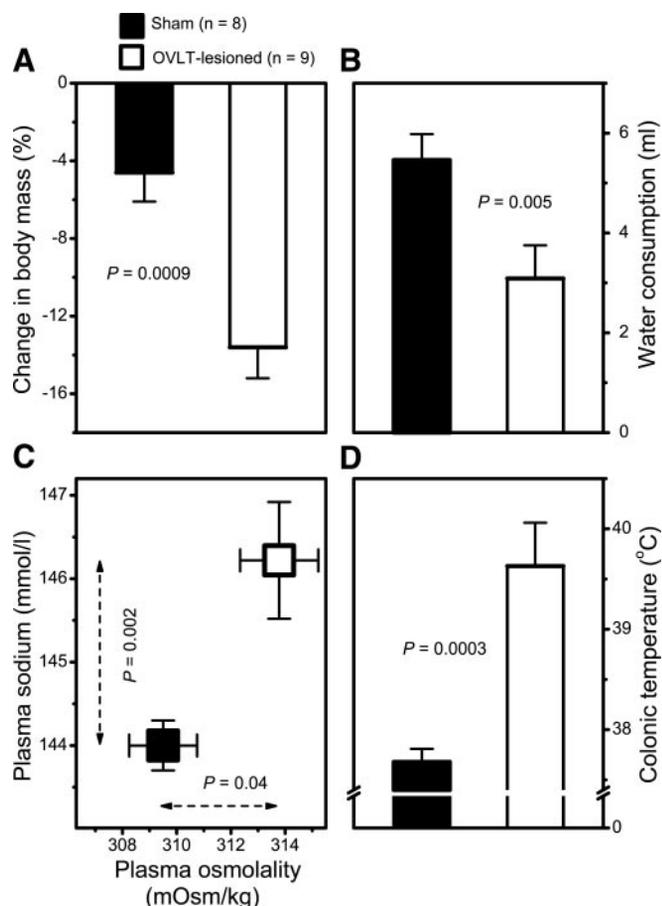


Fig. 2. Acute and chronic side effects of OVLT lesion. A: emaciation; shown as loss of body mass within 24 h postlesion. B: hyporeactivity to osmotic stimulation; shown as the water consumption over 1 h after an infusion of hypertonic (5%) saline (4 ml/kg iv); the test was conducted on *day 14* postlesion. C: chronic hypernatremia and hyperosmolality; blood plasma for measurements of sodium concentration and osmolality was collected under nonstimulated conditions on *day 40* postlesion. D: pronounced hyperthermia; basal colonic temperature was measured in on *days 18–21*.

not large enough to be significant. No vasoconstriction was observed in the lesioned rats.

DISCUSSION

Hyperthermia induced by OVLT lesion. The most dramatic finding of the current experiments is the elevated basal body temperature ($>39^{\circ}\text{C}$) found in OVLT-lesioned rats on *days 10–21* after lesioning. Such a pronounced hyperthermia was not seen in any of the earlier reports (listed in Table 1), and two of these studies (4, 23) did not find any increases of basal body temperature in OVLT-lesioned animals. However, body temperatures of at least some OVLT-lesioned animals were elevated in three other studies (5, 6, 26). For example, cases of long-lasting hyperthermia were registered in two experiments of the study by Blatteis et al. (6). In one experiment conducted on *days 14–17* after surgery, the lesion-control intergroup difference was both large (0.6°C) and statistically significant ($P < 0.05$). In another experiment, one of the lesioned animals had a basal temperature exceeding

40.5°C . In a different study by Blatteis et al. (5), two sheep with thermal lesions of the OVLT had the highest basal temperatures (39.5 and 39.6°C) among a group of eight (4 lesioned and 4 sham lesioned) animals studied on *days 11–14*. In experiments of Hunter et al. (26) conducted on *days 11–12* after lesioning, the lesioned-control temperature difference at the time of pyrogen administration was only 0.2°C , but it was substantially higher (0.5°C) 30 min before the injection. Four thermoregulatory studies (9, 25, 63, 69) did not report the values of basal body temperature of OVLT-lesioned animals at all, and, in one of them, the OVLT-lesioned group was the only group for which basal body temperature was not listed. Hence, the published studies do not establish how common long-lasting hyperthermia is after OVLT lesioning.

It is also unclear whether pronounced long-lasting hyperthermia is related to a certain location of the lesion within the lamina terminalis (many studies did not present a detailed anatomical description of the lesion) or whether it correlates with the presence or severity of any particular side effect. A potential role of intensive rehydration therapy should also be mentioned. The population of the lesioned rats in the present study likely included animals that would not have survived the procedure if they were not placed on rehydration therapy (8). Because such a therapy was not used in any thermoregulatory study in the past (Table 1), some animals were likely to die in those studies. Knowing that even mild hyperthermia strongly exaggerates neural damage and increases the related mortality rate (15), it is reasonable to suspect that hyperthermic animals were more likely to die after OVLT lesioning than normo- or hypothermic animals in the early studies.

The hyperthermia observed in the present experiments and the elevated body temperatures reported by Blatteis et al. (5, 6) and Hunter et al. (26) clearly differ from the acute fevers that often occur in response to preoptic hypothalamic tissue damage (13, 35, 53, 56). Such acute fevers are relatively short lasting and resolve within a few days (6, 35). They are thought to involve migration of leukocytes to the site of damage, local hemorrhage, and inflammation (13) and to be mediated by pyrogenic cytokines (35) and PGs (53, 56).

Although the exact genesis of the prolonged hyperthermia reported here is unknown, its duration is consistent with permanent damage to the thermoregulatory circuitry similar to the damage caused by knife cuts of brain tissue (21). That the lesioned rats had very high body temperatures but exhibited no tail skin vasoconstriction suggests an alteration in heat production, possibly a loss of warm-sensitive neurons that inhibit thermogenesis in the brown adipose tissue (BAT), the major source of heat production in the rat (19). The precise location of thermogenesis-inhibiting warm-sensitive neurons within the hypothalamus is unknown (41), but such neurons clearly exist (12, 76). Hypothalamic transections caudal to the presumed location of these neurons cause pronounced hyperthermia due to disinhibition of BAT thermogenesis (12, 72).

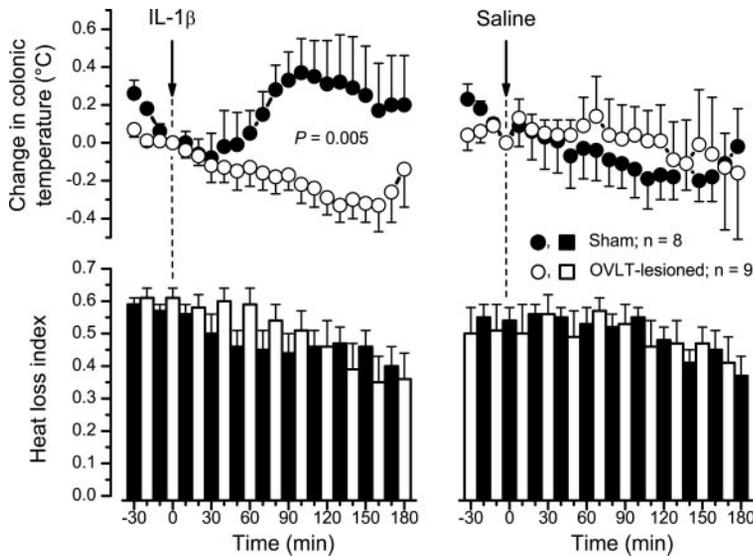


Fig. 3. Thermoregulatory response of the sham-operated and OVLT-lesioned rats to an intravenous injection (arrow) of IL-1 β (500 ng/kg) or saline (1 ml/kg) on days 18–21 postsurgery.

Several lines of evidence suggest that bodies of the thermogenesis-inhibiting neurons are located in the MnPO, a structure that was typically involved in the lesion in the present study (Fig. 1). Indeed, 20–30% of neurons in the MnPO are warm sensitive and have been proposed to play an important role in thermoregulation, including nonshivering thermogenesis (71). Efferent projections from the MnPO tend to be inhibitory (44), which is consistent with their GABAergic nature (43). Furthermore, MnPO neurons that project to the midbrain periaqueductal gray matter (which contains neurons generating excitatory signals for nonshivering thermogenesis) are activated by heat exposure (75), thus confirming their inhibitory role in the control of thermogenesis. Intriguingly, Nakamura et al. (43) recently identified projections from the MnPO to the rostral raphe pallidus nucleus (rRPa), a structure that also contains the excitatory efferent neurons for nonshivering thermogenesis. The authors have proposed that MnPO cells control BAT thermogenesis by tonically inhibiting rRPa neurons. Lesioning the MnPO would be consistent with disinhibition of thermogenesis and development of hyperthermia. Such a mechanism can also explain the very high values of body temperature observed in the present study. Indeed, disinhibition of BAT thermogenesis in the rat readily increases deep body temperature by several degrees, even under conditions of anesthesia and exposure to a subneutral T_a (61).

Other side effects of OVLT lesion. Lesions of the OVLT and its adjacent structures are known to severely disturb water-electrolyte balance and its hormonal regulation (39). Lesioned animals often become adipsic. When adipsia occurs, it is typically followed by anorexia. The result is both dehydration and undernourishment (8). Consistent with the literature, the lesioned animals in the present study exhibited acute adipsia and gross emaciation (14% mean loss of body mass within 24 h of lesioning; see Fig. 2A). Although this side effect is not mentioned in fever studies (Table

1), a higher degree of emaciation, sometimes incompatible with life, is considered typical after lesions of the anterior wall of the third ventricle (8). For example, Johnson and Buggy (30) observed severe adipsia lasting as long as 6 days after electrolytic lesions of the lamina terminalis; the mean weight loss of the adipsic rats on day 3 was 84 ± 5 g (~25%). The authors explained this loss by the rats' failure to compensate for their attenuated water intake by an appropriate antidiuresis. However, the magnitude of the postlesion emaciation recorded in their study (and even in the current work) substantially exceeds normal daily food and water consumption for rats; it also exceeds by several times the body weight loss in starving rats (65). Such a dramatic weight loss, especially combined with the high body temperature, likely results from intensive substrate "burning" caused by disinhibition of nonshivering thermogenesis.

Although the intensive postlesion care in the present study was successful in minimizing the acute effects of the procedure and eliminating mortality, it was proved ineffective in preventing chronic impairments of water-electrolyte balance, including hypernatremia, hyperosmolality (Fig. 1C), and sluggish drinking responses to hypertonic stimuli (Fig. 1B). Interestingly, the latter three symptoms are consistent with a loss of inhibitory neurons in the MnPO, which play an important role in water-electrolyte homeostasis (71).

Effect of OVLT lesion on the febrile response: a reappraisal. That the OVLT is important for febrigenic signaling to the brain was proposed by Blatteis et al. (4), and their innovative hypothesis has dominated the field for almost two decades. However, the more recent analysis by Blatteis and Sehic reveals several shortcomings of this hypothesis (for review, see Ref. 7). Furthermore, some findings that are typically cited in support of the signaling hypothesis (e.g., that microinjection of pyrogens or antipyretic substances in the vicinity of the OVLT causes a fever or antipyresis, respectively; see Refs. 37, 57, 58, 64) do not actually

support it. These findings show that the OVLT and/or other structures within the lamina terminalis are crucial for fever genesis, but they do not say by which of the four mechanisms (see Introduction) these structures are activated during systemic inflammation.

What is even more important in the context of the present work is that multiple studies designed to test the OVLT-signaling hypothesis produced contradictory results. Indeed, whereas several "lesion studies" (e.g., Ref. 4) and "nonlesion studies" (e.g., Refs. 22, 34, and 42) suggested an important role of the OVLT in immune-to-brain signaling, other lesion (e.g., Ref. 69) and nonlesion (e.g., Refs. 11 and 60) studies failed to support such a role, at least for the specific experimental conditions tested. In fact, lesion studies found all three possible effects on the fever response, namely, exaggeration, blockade, and no effect (see Table 1). When lesioning blocked the response, the blockade was attributed to interrupted pyrogenic signaling to the CNS (4–6, 9, 23, 25, 26). When a similar lesion exaggerated the response, the result was explained by stimulation of pyrogenic signaling (63). When lesioning produced no effect on fever in a study of Takahashi et al. (69), the authors did not eliminate the OVLT as a potential access point for circulating pyrogens and proposed that other routes of immune-to-brain communication (afferent nerves, the subfornical organ) may provide adequate access for circulating pyrogens, thus substituting for the role of the OVLT, in the lesioned animals. With the exception of two reports by Blatteis et al. (4, 6) and one by Takahashi et al. (69), all studies mentioned above failed to account for effects of the lesion unrelated to the processes of blood-to-brain pyrogenic signaling (side effects).

In the current study, OVLT-lesioned rats were found to have high basal body temperatures. After the original study of LPS fever in guinea pigs by Blatteis (3), it has been established for several pyrogens, including LPS (29, 66), yeast cells (33), PGE₁ and E₂ (18, 38, 68), and cholecystokinin-8 (68), that the maximal height of fever inversely correlates with basal body temperature. That is, animals readily respond to pyrogenic stimuli when their initial body temperature is low; no fever occurs when their basal body temperature is high. For example, rats do not respond to various pyrogens with fevers (68) and do not activate nonshivering thermogenesis in BAT (66) if basal T_c > 39°. Clearly, the febrile response can be exaggerated, attenuated, or even blocked completely by simply changing basal body temperature (for review, see Ref. 29), and the present results show that tissue lesions in the lamina terminalis can produce a strong and long-lasting effect on body temperature. A putative mechanism for this effect in lesioned animals is the loss of thermogenesis-inhibiting neurons in the MnPO. If the MnPO was involved in the OVLT lesion to a variable extent in the earlier studies, it could explain the contradictory effects of the lesion on the febrile response (Table 1).

In addition to the MnPO, autonomic circuits involved in thermoregulation and fever include two other structures adjacent to the OVLT, i.e., the VMPO and AVPV.

These structures show a sustained elevation of Fos-like immunoreactivity after intravenous challenge with LPS (17) and possess high sensitivity both to the pyrogenic action of locally administered PGE₂ (57) and to the antipyretic action of a locally applied inhibitor of PGE synthesis, ketolar (58). Thus damage to these efferent structures would be consistent with a weakened ability of the OVLT-lesioned animals to respond to pyrogens with fever. In fact, this explanation has been proposed in two previous studies (59, 69).

Other side effects of OVLT lesion also affect febrile responsiveness. Indeed, severe hypernatremia and hyperosmolality attenuate the febrile response (31, 47, 55). Malnourished animals respond to pyrogens with low fevers, no fever, or even with hypothermia (24, 62, 67). Interestingly, in the only study in which lesioned animals were maintained postoperatively on palatable solutions (to attenuate the above side effects), the febrile response was normal (69); in eight other studies in which the animals were maintained on a regular diet, the febrile response was affected (see Table 1). Lesions of the anterior wall of the third ventricle also influence secretion of and/or responses to melanocortins (32), glucocorticoids, and several other hormones involved in the regulation of water-electrolyte balance (for review, see Refs. 8, 20, and 39). The same hormones are involved in febrile pathogenesis, most (melanocortins, glucocorticoids, arginine vasopressin) as antipyretics (40, 46, 70) but some (angiotensin II) as fever promoters (73). Therefore, lesions of the OVLT and its surrounding structures may affect febrile responsiveness by interfering with the complex balance of anti- and pro-pyretic substances and not exclusively by modifying febrigenic signaling processes.

Conclusions and Perspectives

Lesions of the OVLT and neighboring structures cause severe side effects, such as acute dehydration, gross emaciation, and chronic impairments of water-electrolyte balance. The present work clearly shows that these side effects cannot be prevented by intensive rehydration therapy. This observation substantially limits the use of the electrolytic lesioning technique to study the physiological role of the OVLT.

This study also demonstrates a previously unrecognized phenomenon: high (> 39°C) and long-lasting (>3 wk) hyperthermia in the lesioned animals. The lesions performed in the present experiments appear to involve neurons that are crucial for the control of thermogenesis. On the basis of the recent studies by Yoshida et al. (75) and Nakamura et al. (43), we speculate that these neurons are located in the rostral MnPO, a structure that is adjacent to the OVLT and that was consistently damaged in our experiments. Further studies aimed at the identification of these neurons are required.

Starting with the pioneering works of Blatteis et al. (4–6), the fact that OVLT lesions can block the febrile response has been the most convincing argument favoring the physiological importance of the OVLT in

febrigenic signaling to the CNS. We offer an alternative interpretation of this fact. We argue that lesions of the lamina terminalis may affect the febrile response via multiple mechanisms unrelated to the passage of the immune signal across the BBB. Clearly, nonlesion techniques, such as those used by Harré et al. (22) and possibly highly selective chemical lesioning techniques should be used to decisively prove or disprove the OVLT signaling hypothesis.

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DISCLOSURES

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