side of the partition, a filter paper was deposited, one with the isoamyl acetate solution (2 µl) and the other with the whole blend. The number of bees recruited by each paper disk was recorded for the length of 1 min. Another evaluation was carried out 30 min later with new paper disks and fresh solutions but in reversed positions.

The results obtained with the 323 workers recruited by the 2 solutions were as follows: the whole blend recruited 197 (61%) bees, but only 126 (39%) were recruited by isoamyl acetate (table 3).

Discussion and conclusion. The testing method, carried out with outdoor colonies, has been previously and successfully used for ethological studies with *Apis mellifica* L.[2,3,7] and with the Asian species of the genus *Apis*[8]. However, in the 1st experiments with colonies A and B, the bees gradually became accustomed to the presence of the filter paper disk and reacted even to a control paper disk or to hexane alone. This lead us to reduce number of tests carried out with the same colony. The compounds identified on the sting[5] have been detected on the setaceous membrane[9] which releases the sting alarm pheromone of the worker[2]. Accordingly, an artificial mixture of these compounds was tested; it efficiency had been compared to the stings of bees collected at random on the combs. These stings containing an average of 1 µg of isoamyl acetate were used as reference. Although they are not rich in volatile substances, the stings or their extracts in hexane exert a recruiting effect. The whole blend showed a similar effect and may be considered as an active alarm pheromone. Like freshly excised stings, its recruiting effect is stronger than that of isoamyl acetate alone. Both light and heavy fractions have a recruiting effect showing that the 2 fractions participate and give to the whole blend its full activity. However, this blend seems to be an incomplete alarm pheromone: n-octyl acetate[5] and other unidentified compounds were not incorporated. All the compounds of the blend and n-octyl acetate have been separately tested in a laboratory test with small groups of young caged honeybee workers[11]. Two substances (n-decyl acetate and benzyl alcohol) were found inactive; 2 nonanol and benzyl acetate caused the longest reaction times, but this had never been proved with outdoor colonies. The effect of the blend differs with bees from different colonies. Behavioral differences between colonies A and B can be attributed to internal factors (genetics): aggressivity and sensibility threshold to the pheromone. Like most pheromones of the honeybee (mandibular gland pheromone of the queen, Nasanoff gland pheromone of the worker), the alarm pheromone appears as a blend. Functional connections between the Koschewnikow gland ant the setaceous membrane have been established by light microscopy[12]; volatile compounds of the blend have been detected in the Koschewnikow gland. The alarm pheromone is produced by the Koschewnikow gland, stored on the setaceous membrane and released when the guard bees are disturbed[2]. Other stimuli (visual, olfactory) induce the attack of the recruited bees[2,10].

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Vasodilatation on preoptic heating in capsaicin-treated rats

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Summary. Rats treated with 50 or 300 mg/kg capsaicin were less tolerant of a warm environment than controls. However, preoptic thermoregulatory impairment, as shown by a decreased vasodilatation on preoptic heating, was not found for the lower dose, suggesting a deficiency in extrahypothalamic thermoreceptive structures.

In rats treated with capsaicin, an irreversible deficit of thermoregulation has been reported; when exposed to heat, the animals develop severe hyperthermia due to an impaired functioning of the thermolytic mechanisms[2-8]. Although several lines of evidence indicate that the drug affects the preoptic/anterior hypothalamic (POAH) warmth detectors[3,4,8-13], some observations suggest that a deficiency of the extrahypothalamic thermoreceptive structures may also contribute to the thermoregulatory impairments[3,14-16]. Moreover, in rats treated with capsaicin 2 days after birth only peripheral morphological alterations without damage of the POAH were reported[17-19]. To obtain information on the role of the POAH in thermoregulatory impairment by capsaicin, we studied the tail skin vasodilatation response, an important thermolytic reaction, to local heating of the POAH.

Methods. Male rats of the CFY strain were used. Capsaicin (32.7 mmoles · l^{-1}) was dissolved in saline with the aid of Tween 80[20]. Three groups of animals were injected s.c. with the drug under light ether anesthesia. Newborn rats received a single injection of 50 mg/kg capsaicin 2 days after birth (group ND-50). Two-month-old rats were treated with either 50 mg/kg (group AD-50) or 300 mg/kg (group AD-300) capsaicin. The latter dose was administered in 6 daily fractions (10, 20, 20, 50, 100 and 100 mg/kg). The vasodilatation experiments were carried out with 3-4-month-old animals.

Before the POAH heating experiments, the rats kept at

24 °C were placed in an environment of 38 °C for 1 h to test their tolerance to heat. Rectal temperatures were taken before and at the end of the heat exposure. POAH heating was performed in rats anesthetized with urethane (1 g/kg), an anesthetic that is satisfactory for studying POAH thermodetectors and thermoregulatory tail skin vasodilatation in response to both POAH and scrotal thermal stimulations[21,22]. A heating coil (resistance 400 ohm) wound around the tip of a stainless steel pin was inserted into the POAH. Direct current (180 mW) was used to heat the coil. Local temperature increased by 3.0 °C in 5 min and remained at this level while the power was on. Only rats meeting the 3 following conditions were evaluated: a) histologically verified location of the coil in the medial preoptic region, b) an increase of at least 0.5 °C in tail temperature induced by heating, and c) return or tendency to return of tail temperature to the baseline level after the heating period. Thus, the number of animals in the 4 groups was: controls, 11; ND-50, 8; AD-50, 8; and AD-300, 11. Colon temperatures were recorded continuously by means of a platinum resistance probe inserted 6.5 cm into the colon. In order to maintain a normal body temperature in the anesthetized rat and to ensure a constant reduction of peripheral cold input throughout the experiment, water of 39 °C was circulated in tubes around the body leaving the tail free. The rats' tails were in air regulated at 24–25 °C. In this way colon temperature was kept constant at 38.0±0.2 °C. Since the ambient temperature did not change tail skin vasodilatation could be regarded as the function of POAH temperature. Tail skin temperature was measured by a disc thermistor taped to the dorsal surface of the tail at $^2/_3$ of its length. Colon and tail skin temperatures were taken at 60-sec intervals. After 15-min baseline recordings the POAH was heated for 20 min. Measurements were continued for another 14 min after the heating was switched off.

Results. Each capsaicin-treated group reacted with a significantly larger hyperthermia to an ambient temperature of 38 °C than the control animals (table). In the POAH heating experiments, the average colon and tail temperatures (±SEM) in the 4 groups, calculated for the minute prior to the thermal stimulation, were as follows: controls, 37.9±0.09 °C and 25.9±0.4 °C, ND-50: 38.0±0.08 °C and 25.8±0.4 °C, AD-50: 38.0±0.03 °C and 25.9±0.3 °C, AD-300: 38.1±0.09 °C and 25.6±0.4 °C, respectively. In response to the preoptic heating the tail skin temperatures in the control, ND-50 and AD-50 animals rose rapidly, resulting in a 3–4 °C increase by the end of the 20-min stimulation period (fig.). A decline of colon temperatures was noted in these groups. In the AD-300 group the vasodilatation was slow, the increase in tail temperature being approximately half of that found in the controls. The difference between the control and AD-300 groups was significant throughout the heating period and in the first half of the postwarming period (fig.).

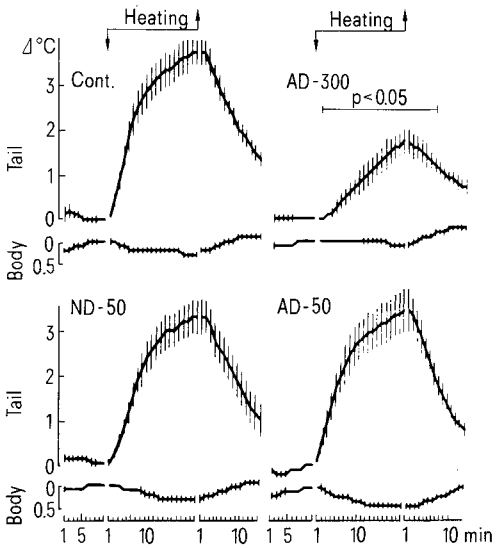
It seemed that the impairment of the POAH thermoregulatory function studied in our experiments depended on the dose of the drug: only 300 mg/kg capsaicin brought about a vasodilatation deficiency, and the reaction was not completely blocked even in this case. Although the POAH warmth detectors are generally accepted as the targets of the action of capsaicin, it is worth emphasizing that ultrastructural alteration in the POAH[9] and a loss of preoptic thermodetectors[12] was reported only after large capsaicin doses (at least 150 mg/kg). Unlike reports on rats injected with capsaicin as adults, neonates (treatment 1–2 days after birth) were not mentioned as having alterations in the POAH, while massive degeneration of the unmyelinated afferent fibers running in the peripheral and cranial sensory nerves were found[17–19]. Accordingly, the ND-50 animals reacted to POAH heating in the same way as the controls in our experiments. Nevertheless, capsaicin treatment with huge doses in 6–8-day-old animals may induce a large reduction of POAH thermodetectors[23,24].

Though the AD-50 and ND-50 rats reacted to POAH heating as readily as the controls, they showed a hyperthermia as severe as the AD-300 animals at an ambient temperature of 38 °C. Moreover, thermoregulatory disturbances have been reported after doses as low as 20–30 mg/kg[15]. It is tempting to assume that an impairment of extraPOAH thermoregulatory structures was responsible for the deficiencies in these animals. Capsaicin treatment results in a long-term decrease of substance P and somatostatin in rat dorsal root ganglion and spinal cord[25,26]. While this effect might be the basis for the diminished nociception, no evidence has been reported concerning the involvement of these putative transmitters in heat reception. Similarly, morphological or electrophysiological evidence regarding the loss of extraPOAH warmth sensors in capsaicin-treated rats has not yet been reported. Nevertheless, there are some indications that such defects may exist at least in the peripheral thermoreceptors[3,4,15,16].

The effect of a 1-h exposure to an ambient temperature of 38 °C on the average colon temperature (±SEM)

Group	Colon temperature	
	Before heat exposure	After heat exposure
Control	37.4±0.1	39.9±0.1
ND-50	37.5±0.2	41.0±0.2*
AD-50	37.5±0.2	41.3±0.1*
AD-300	37.2±0.1	41.5±0.1

*Significantly different from the control at a level of at least $p < 0.05$ (Student's t-test).



The effect of POAH heating on body and tail temperatures in controls and in 3 groups of capsaicin-treated rats (50 and 300 mg/kg). Continuous line marks significant differences with respect to the control group (Student's t-test). (AD, rats treated as adults; ND, rats treated as neonates.)

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Neuronal and glial localization of acetylcholinesterase and GABA transaminase in organized cultures of developing rat spinal cord

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Summary. Acetylcholinesterase (AChE) and γ-aminobutyrate transaminase (GABA-T) were localized in organized cultures of rat embryo spinal cord. AChE was seen in neurones of the ventral horn and intermediate nucleus and in the dorsal horn neuropil. AChE was also present on the soma membrane of neuroglia, even in areas devoid of AChE-stained neuronal processes. GABA-T activity was present chiefly in dorsal horn neurones, and was absent from neuroglia.

Acetylcholinesterase (AChE) and γ-aminobutyric acid aminotransferase (GABA-T) are enzymes whose function is the inactivation of the neurotransmitters acetylcholine and GABA respectively. Both enzymes are trans-synaptic, i.e. they are present both in presynaptic neurones containing the transmitter and in the post-synaptic membrane[1,2]. Thus these enzymes serve as markers for the existence, or the potential for formation, of functional synapses where acetylcholine and GABA are transmitters.

Organotypic cultures were prepared from transverse sections of spinal cord from Hooded Lister rat embryos of 14–17 days' gestation as described elsewhere[3,4] and were maintained for 7–28 days. The anatomical arrangement of cultures was similar to that seen in spinal sections. For acetylcholinesterase staining, intact cultures were fixed in 2% buffered formol calcium for 10 min at 4 °C. The reaction was carried out using the thiocholine technique[5] as adapted by El Badawi and Schenk[6]. The reaction was carried out in semi-solid phase in some experiments by making up the reaction media in 3% ion agar No.2. The preparations were counterstained with Giemsa stain. Specificity of the AChE reaction was tested by the inclusion of 10^{-4} M iso-OMPA, an inhibitor of pseudocholinesterase. GABA-T was demonstrated using the procedure of Hyde and Robinson[7] on unfixed cultures at an incubation temperature of 37 °C. Controls for each technique were prepared by the omission of the substrate.

Enzyme reaction products reached their maximum intensity after 10 days in vitro for AChE and 21 days in vitro for GABA-T. The positions of groups of neurones stained with each method was noted in 20–30 cultures. Neurones were all stained evenly in the cytoplasm with both methods. Heaviest deposits of AChE were seen over large ventral horn neurones[8] (fig. 1,a). Lighter deposits were seen in the dorsal pole of the explant as a diffuse staining of the neuropil (fine axonal and dendritic processes) and of some small neurones (fig. 1,b). AChE was also present in the intermediate nucleus and commissural bundles connecting the two halves of the cord. Glial cells were easily distinguished from other cell types on morphological criteria[9,10], having very fine, copiously branched cytoplasmic processes and a small phase-dull cell body. The same cells were heavily stained on the soma membrane even in areas of the cultures devoid of AChE-stained neurones or their processes (fig. 1,c and d). AChE reaction product was seen in all of the above sites when iso-OMPA was included in the medium. Diffusion of reaction product did not take place since no change in its distribution was seen either in neurones or glia when the reaction was carried out in agar.

GABA-T was observed in large numbers (up to 50 per