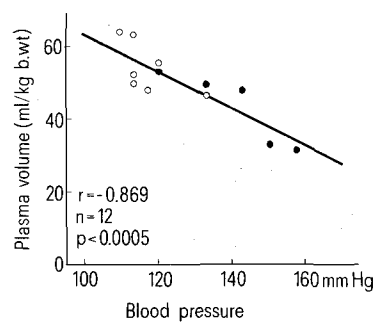


animal, obtained before dye injection and used for haematocrit determination. ECFV was estimated as sodium ferrocyanide space using a single injection technique<sup>5</sup> (10% Na-ferrocyanide solution w/v, 1 g/kg b.wt) from plasma concentrations found 80, 100, 120 and 140 min after ferrocyanide administration. The exact amount of both indicators administered was estimated by weighing the syringe before and after injection. IFV was calculated as the difference between PV and ECFV. Systolic and diastolic BP were measured immediately after awakening from anaesthesia in unrestrained animals by Korotkoff's method. Mean BP was calculated as previously described<sup>3</sup>. Results were statistically evaluated using Student's t-test. Coefficients of linear correlations were calculated in the usual manner.



Correlation between blood pressure and plasma volume in control (open circles) and salt-fed (closed circles) monkeys. *r*, coefficient of linear correlation.

**Results and discussion.** In salt-fed animals, the mean values of systolic, diastolic and mean arterial BP were significantly elevated (table), demonstrating the hypertensogenic effect of increased salt intake in *Macacus rhesus*, previously shown in *Pappio hamadryas*<sup>1</sup>. The BP increase was associated with a decrease of ECFV in both its compartments. However, only the PV reduction accompanied by a slight increase in haematocrit ( $0.05 > p < 0.1$ ) was significant. Since there was no correlation between PV and IFV, the ECFV decrease apparently resulted from a disproportionate reduction of both these compartments. A slight though nonsignificant decrease of PV/IFV ratio indicates that the PV reduction was predominant. The PV decrease was inversely related to the BP level, as may be deduced from the negative correlation between PV and mean BP values ( $r = -0.899$ ,  $n = 5$ ,  $p < 0.05$ ). This was also the case when control and experimental animals were pooled (figure). With IFV, no such correlation was found. It is concluded that in salt-hypertensive monkeys the PV, but not IFV, decreases inversely to BP levels. This resembles the situation observed in essential hypertension<sup>1</sup>, and thus supports the view that high salt intake may play a role in the pathogenesis of this disorder<sup>4</sup>.

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Spider odor receptor: Electrophysiological proof

K. Dumpert

Fachbereich Biologie der Universität Frankfurt, Siesmayerstr. 70, D-6000 Frankfurt/Main (Federal Republic of Germany), 13 December 1977

**Summary.** By means of electrophysiological single-cell recordings, it was shown that the tarsal organ of *Cupiennius salei* is an olfactory chemoreceptor. One cell type in the organ of males responds to the odor of living females.

It has been shown several times that spiders perceive odorous substances<sup>1</sup>. In the hunting spider *Cupiennius salei*, behavioral reactions to fatty acids, terpeneole, and tobacco smoke were observed<sup>2</sup>. The female of *Cyrtophora cicatrosa* most probably emits a sexual pheromone on its web to attract conspecific males<sup>3</sup>. The receptors transmitting such odorous stimuli in spiders are still unknown. Earlier authors<sup>2,4,5</sup> mistook the slit sense organs for olfactory receptors; in fact the slit sense organs are mechanoreceptors<sup>6</sup> and not chemoreceptors<sup>7</sup> as erroneously stated by Keller<sup>2</sup>. The so-called tarsal organ is widely regarded as a possible chemoreceptor<sup>8</sup>. It consists of

a cuticular cup lying beneath the cuticular surface in a capsule open to the outside. According to electron microscopical investigations, this organ consists of 6–7 sensilla in the web spider *Araneus diadematus*<sup>9</sup>. These are innervated by 3 (rarely 4) sensory cells, each with several inbranched dendrites. The presence of cuticular pores<sup>9</sup> and behavioral experiments<sup>8</sup> suggest an olfactory or also hygroreceptive function of the tarsal organ. Electrophysiological proof for this suggestion is so far lacking. It is given in the present study. Contrary to statements in the literature<sup>2</sup>, *Cupiennius salei* is provided with tarsal organs. As in other spiders, one of

Reactions to 8 different stimuli in 20 recordings from the tarsal organ of *Cupiennius salei* which are representative for the total of 200 recordings

	Recordings from females								Recordings from males										
Formic acid	+	0	0	0	0	+	0	0	+	0	0	+	0	+	0	0	0	+	0
Valerian acid	+	0	0	0	0	+	0	0	+	0	0	+	0	0	0	0	0	+	0
Caproic acid	+	0	0	0	0	+	0	0	+	0	0	+	0	0	0	0	0	+	0
t-2-Hexenale	+	0	0	0	0	+	0	0	+	0	0	+	0	0	0	0	0	+	0
Hexanone	+	0	0	0	0	+	0	0	+	0	0	+	0	0	0	0	0	+	0
Tobacco smoke	0	+	+	+	0	0	0	+	+	+	+	0	0	+	0	+	+	0	+
<i>Cupiennius</i> female	0	0	0	0	0	0	0	0	0	0	0	+	0	+	0	0	0	+	0
Propylamine	—	0	0	0	+	—	+	0	—	0	0	—	0	+	0	+	+	—	0

+, Increase of the spontaneous cell activity of at least 10 imp/sec; -, decrease of the spontaneous cell activity to 0 imp/sec; 0, no reaction.

them is found on every leg on the dorsal tarsus near its distal end (figure 1). Despite the similarity with the sockets of pulled-out trichobothria, a tarsal organ can be identified unequivocally, on account of its constant location. Only on 15 out of 200 investigated legs was the tarsal organ missing. Experimental animals were 5 subadult and 15 adult females and 10 adult males raised in our laboratory. After light anaesthesia with CO<sub>2</sub>, they were waxed on a brass holder

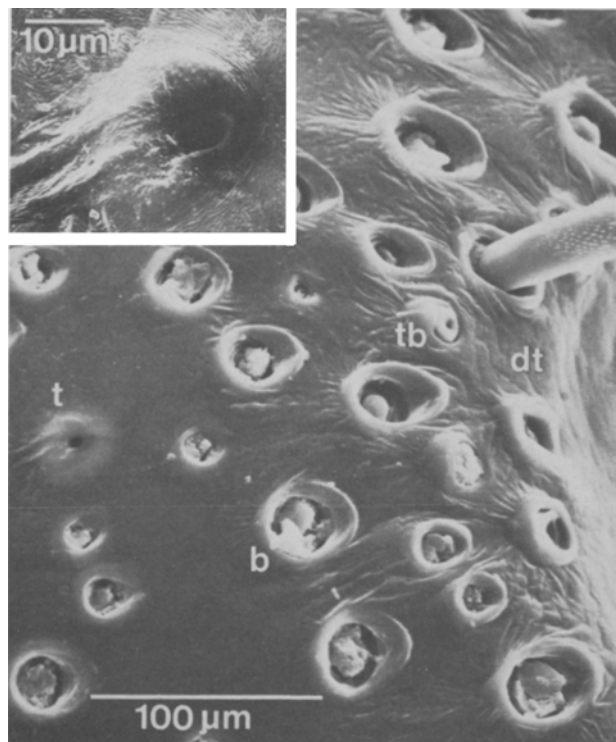


Fig. 1. Scanning electron micrograph of the tarsal organ (t) on the first right walking leg of *Cupiennius salei* ♀ surrounded by remains of ablated bristles (b) and trichobothria (tb) near the distal end (dt) of the tarsus. Inset: Tarsal organ in greater magnification.

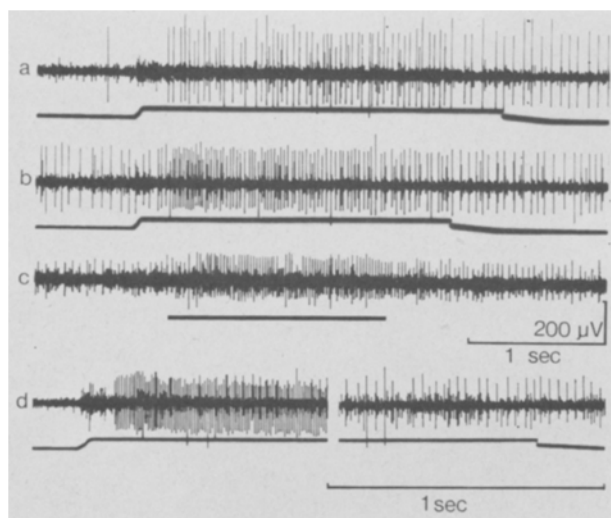


Fig. 2. Extracellular recordings of 3 different tarsal organs of *Cupiennius salei* stimulated with various substances. Lower beam indicates duration of stimulus. a and b ♀, valerian acid (a) and t-2-hexenale (b); c ♂, odor of an adult female of *Cupiennius salei*; d ♀, tobacco smoke (1.3 sec of the registration were left out).

by their legs. Electrolytically sharpened tungsten electrodes were placed into the organ or its immediate vicinity to record spike potentials. The electrode formed an angle of about 60° with the long axis of the tarsus and were tilted towards the distal end of the leg as is also the cuticular capsule containing the tarsal organ. The indifferent electrode was grounded and placed into the opisthosoma of the spider. The following stimuli were applied: Synthetic odorous substances (see below), tobacco smoke, humid air (out of the gas room of a sprayer half filled with water of 25–35 °C), the odor of conspecific living adult males and females, the odor of living or squashed prey (*Calliphora* and *Periplaneta*), and the odor of threads produced by male or female *Cupiennius salei*. An air current (speed circa 60 cm/sec) carried the stimuli to the organs. It was led through a glass tube filled with filter paper which was either soaked with the odorous substances or the squashed prey or filled with spider threads. For stimulation with the odor of living spiders and prey, these animals were encaged in small boxes and placed immediately beneath the preparation. During pauses of stimulation, a current of fresh air removed the odorous substances from the gasroom surrounding the preparation.

Stimulation with formic acid, valerian acid, caproic acid, t-2-hexenale, hexanone, and tobacco smoke raised the spontaneous impulse frequency of 1–5 imp/sec to up to 140 imp/sec (figure 2). Only cells in the tarsal organ of males reacted to the odor of adult *Cupiennius* females with an increase of the spike frequency. Neither males nor females showed any response when stimulated with the odor of living males. Propylamine in some recordings led to an increase, in others to a decrease of the spontaneous cell activity. All other stimuli (benzochinone, geraniol, humid air, odor of living and squashed flies and cockroaches, and threads of male and female spiders) did not cause any reaction in about 200 recordings.

Cells responding to valerian acid in all cases reacted to formic acid, caproic acid, t-2-hexenale, and hexanone. They were inhibited by propylamine. In some recordings only these substances were effective stimuli. In other cases, only tobacco smoke or the odor of living females or only propylamine showed an effect (table). In some recordings fatty acids and also tobacco smoke or the odor of living females were answered, but from different cells with different spike amplitudes. Accordingly we can distinguish at least 4 different cell types. Cells with overlapping spectra of odorous substances were not found. Their existence cannot be excluded, however, on account of the small number of test substances. Differences in the responses of the organs of the different legs were not observed.

One of these cell types in the tarsal organs of *Cupiennius* males and females is excited by fatty acids and also by t-2-hexenale and inhibited by propylamine. In the reaction spectrum it is quite similar to the grass odor receptor of *Locusta*<sup>10,11</sup>. While this locust receptor serves to perceive forage crops, the biological significance of the corresponding receptor remains unknown.

The results show that the tarsal organ is an olfactory chemoreceptor. A function of this organ as a hygroreceptor for the walking legs is unlikely. The tarsal organs of the pedipalps were not examined. Possibly they differ in function from those of the walking legs.

The reaction of sensory cells in the tarsal organ of *Cupiennius* males to the odor of conspecific females is of particular interest with respect to the role of this organ played in behavior. The present study suggests a participation of olfactory stimuli in the males' recognition of the females. According to Homann<sup>12</sup>, *Cupiennius salei* belongs to the wolf spiders (*Lycosidae*). For the *Lycosidae* it was observed several times that taste receptive, and not olfactory stimula-

tion, of the females is important during copulation behavior<sup>13-20</sup>. Only in the wolf spider *Trochosa singoriensis* there is evidence in favour of olfactory stimuli guiding the males towards the females<sup>21</sup>. There is no reaction of tarsal organs to odor of prey. Olfactory recognition of the prey is therefore unlikely as also shown in behavioral experiments<sup>22</sup>. In these experiments blinded *Cupiennius salei* did not react to motionless prey (*Calliphora*), even when walking past it with a tarsus as close as 1 cm.

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## Effects of brain monoamine depletion on thermoregulation, active avoidance, and food and water intake in rats<sup>1</sup>

M.T. Lin, W.Y. Chia, C.T. Tsai and T.H. Yin

Department of Biophysics, National Defense Medical Center, Taipei (Taiwan), 10 October 1977

**Summary.** Intraventricular administration of 6-OHDA or 5,6-DHT suppressed food intake, whereas their effect on active avoidance produced a suppression with the former and an enhancement with the latter. The increased water intake was specifically associated with 5,6-DHT treatment in rats.

The use of neurotoxins including 6-hydroxydopamine (6-OHDA) and 5,6- or 5,7-dihydroxytryptamine (5,6- or 5,7-DHT), to destroy either catecholamine (CA) or 5-hydroxytryptamine (5-HT) nerve terminals and axons more or less specifically, provides a unique opportunity for examining the role of the brain monoaminergic systems played in physiology and behavior<sup>2-4</sup>. However, the observations made by various authors were not always consistent. For example, Myers<sup>5</sup> and Waller et al.<sup>6</sup> claimed that monkeys and rats treated with intrahypothalamic injections of 5,6-DHT showed acute increases in body temperature when exposed to warm or cold environment. In contrast, Lin and Stitt<sup>7</sup> and Lin<sup>4</sup> demonstrated that rabbits treated with intraventricular injections of 5,7-DHT were capable of maintaining their body temperature within normal limits. Similarly, the role of brain 5-HT in mediating avoidance behavior remains to be ascertained. Intracisternal injections

of 5,6-DHT was found to facilitate learning an active avoidance task in rats<sup>8</sup>, yet intrabrain stem injections of 5,7-DHT had no effect<sup>9</sup>.

This study was a concerted effort to detect any changes in thermoregulation, shock avoidance and food and water intake in rats following destruction of the CA or 5-HT pathways within the CNS with 6-OHDA or 5,6-DHT respectively.

43 male rats of Sprague-Dawley strain ranging between 250 and 350 g at the time of surgery, served as subjects. The rats being housed individually in wire-mesh cages in a room at 25±1°C with natural light-dark cycles were given free access to tap water and granular young chicken feed. Food and water intake and b.wt were measured daily at 10.00 h.

3 separate experiments were carried out using 3 groups of rats for each experiment. The 3-groups consisted of a) sham-treated control rats, b) rats with intraventricular administration of an aliquot of 100 µl containing 100 µg of 5,6-DHT, resulting in lasting depletion of 5-HT in the CNS, and c) rats with intraventricular administration of an aliquot of 100 µl containing 200 µg of 6-OHDA, resulting in lasting depletion of CA in the CNS. Each of the rats had been implanted with an intraventricular cannula under general anesthesia according to the methods described previously<sup>10</sup>. Instead of the drugs, the sham-treated controls were injected with 100 µl of saline vehicle.

The 3 separate experiments for the 3 groups of rats were: a) food and water intake, b) temperature responses to thermal stress, and c) active shock avoidance.

The table summarizes the results of the 1st experiments, in which food and water intake before and after injection of saline vehicle, 6-OHDA or 5,6-DHT for the 3 groups of animals, consisting of 5 each, was measured. The sham-treated control animals had a temporary decrease of food intake during the first 6 days following the injection of saline vehicle, but it returned to the pre-injection level during the subsequent 6-day period. Their water/food ratio and weight gain were unaffected. The 6-OHDA treated

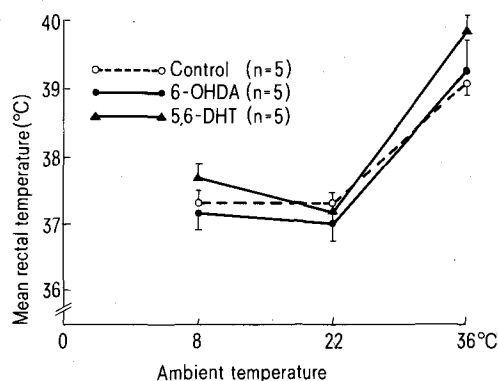


Fig. 1. The maximal changes in rectal temperature of groups of control, 6-OHDA treated and 5,6-DHT treated rats, 5 for each group, after 1 h of heat (36°C) or cold (8°C) exposure. Both amine-depleted rats maintained their rectal temperatures within normal limits.