days of age and 0.2 mg/kg b.wt from 53 to 75 days of age. All rats were rapidly killed at 75 days of age, in darkness under dim red light. Testes, seminal vesicles and ventral prostates were dissected and weighed. Pineals were dissected within 5 min, weighed and stored in a Petri dish on solid CO_2. NAT activity was determined within 48 h by a modification[18] of the method of Deguchi and Axelrod[19]. Differences between means were analyzed for significance using Student's t-test.

Results and discussion. As previously reported[3], neonatal TP-treatment decreased the weights of testes, seminal vesicles and prostate (fig. 1). Pinealectomy (PINX) did not affect the weights of reproductive organs in TP-treated rats kept under LD 12:12. Isoproterenol administrations lowered the weights of seminal vesicles and prostate in both intact and PINX TP-treated rats, but the testis weights only in intact animals. Isoproterenol treatment decreased the weights of reproductive organs significantly more in intact than in pinealectomized TP-treated rats. Part of the antigonadal effect of isoproterenol is thus mediated by the pineal gland.

Isoproterenol induced precocious increase of NAT activity, but it did not affect the time of spontaneous morning decline (fig. 2). The NAT activity was thus elevated above 3 nmoles $\cdot$ $mg^{-1} \cdot h^{-1}$, i.e. above the value which already gives almost maximal melatonin production[10] for 7.5 h in controls and for 9.6 h in isoproterenol treated rats. Isoproterenol treatment thus depressed the weights of reproductive organs in TP-treated rats partly via the pineal and concomitantly prolonged the period of high NAT activity. This observation supports the idea that extension of the period of high melatonin production may inhibit the growth of reproductive organs in rats treated neonatally with testosterone propionate.

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Chemical stimuli eliciting courtship by males in *Drosophila melanogaster*[1]

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Summary. By progressive removal of various sense organs, and testing for inter-male courtship in the absence or presence of females, it is demonstrated that *D. melanogaster* males require close-range, probably contact, chemical stimuli for the initiation of courtship.

Volatile chemicals have been implicated in the initiation of courtship by males in *Drosophila melanogaster*[3-6]. Removal of the antennae, which have long been considered to be the major olfactory receptors, does not, however, prevent males from courting[7,8]. Similarly, although contact chemicals received by the male when tapping the female with his foretarsi appear to be important[8,9], foretarsiless males court readily[8]. Other organs which may receive volatile and/or contact chemicals are the proboscis and the palps[9], and the aristae[10]. To resolve the relative importance of these stimuli in the initiation of courtship, and which organs receive them, males in which various combinations of these organs had been removed were tested for their propensity to court females or other males. Also, propensity for intermale courtship was tested in the absence or presence of physically distanced females to test the importance of volatile chemicals.

Materials and methods. 4-day-old Canton-S males were collected after 24 h from standard cultures, kept isosexually 10 per vial, and then singly for 20–24 h before testing. Virgin females were collected after 12 h, kept 10 per vial, and used after a further 12–24 h. The males were operated on under ether anesthesia 2 h before testing. Aristae, foretarsi (plus or minus the basitarsi which bear the sex-combs) and proboscides were snipped off with fine scissors, and palps and antennae were plucked off with forceps. Only the 3rd segment of the antenna, the funiculus, which bears the arista and the olfactory receptors[10], was removed. Some observations were conducted in red light in a photographic darkroom to remove visual stimuli. Single pairs were observed for 10 min with a binocular dissecting microscope in either side of a standard perspex observation chamber (22 mm diameter; 8 mm deep) with a double-layer nylon gauze partition. This allowed simultaneous observation of 2 pairs, and the gauze provided better purchase for the foretarsiless males. Whether the male courted, as indicated by wing extension and vibration, was noted. Although courtship is not all or none[11], quantitative

Table 1. Effect of various amputations on the propensity of *D. melanogaster* males to court females*

Group	Light	Aristae	Antennae	Palps	Proboscis	Foretarsi	Basitarsi	n_1	n_2
1	+	−	+	+	+	+	+	5	5
2	+	+	+	−	−	+	+	5	5
3	+	+	+	+	+	−	−	5	5
4	+	−	−	+	+	+	+	10	10
5	−	−	−	+	+	+	+	10	10
6	−	+	+	+	+	−	−	10	10
7	+	−	−	−	−	+	+	5	4
8	+	+	+	−	−	−	−	5	5
9	+	−	+	+	+	−	−	10	9
10	+	−	−	+	+	−	+	5	4
11	+	−	+	−	−	−	−	10	10
12	+	−	−	−	−	−	+	10	9
13	+	−	−	+	+	−	−	15	2
14	+	−	−	−	+	−	−	10	1
15	+	−	−	+	−	−	−	10	0
16	+	−	−	−	−	−	−	10	0

*n_1, number of pairs observed; n_2, number in which male courted; +, intact; −, amputated.

measures would be unrealistic when comparing these surgically mutilated males.

In the 2nd experiment, test males (normal or foretarsiless) were placed with wingless target males in one half of the chamber, first without and later with 10 virgin females in the other half. The amount of orientation and wing vibration by the test males was measured simultaneously with stopwatches for 5 min in each condition.

Results and discussion. Initial results confirmed that amputation of either the aristae (1), the palps and proboscis (2), the entire foretarsi (3) or the antennae (4) does not prevent males from courting (table 1). Although visual stimuli are used in the initiation of courtship, they are not essential in *D. melanogaster*[12,13]. Even when removed together with the antennae (5) or the foretarsi (6), courtship was not prevented. Progressive removal of all these organs (7-10) suggested that the foretarsi or antennae alone are sufficient for the males to court. Indeed, males with only antennae (11) or only basitarsi (12) courted fairly readily and continuously. Some males with only proboscis and palps (13) or only a proboscis (14) courted, but males with only palps (15) or all the organs removed (16) never courted. These last males (16) suffered minimally more operative trauma than those with only basitarsi (12), yet the latter courted readily, so it can be concluded that lack of courtship was not simply due to operative trauma. Also, the males were not simply courting in the absence of stimuli.

Males in every category usually tapped the female (for example, 6 of the 10 males with all the organs removed tapped), so volatile chemicals, unless received by still another organ, are unnecessary for tapping. In all the tests with considerable amputation the males were also tested with 3-day-old males, either immediately before or after testing with the female. Most tapped the male, but almost all either did not court, or, especially after courting the female, started courting but stopped within the 10-min observation period after tapping vigorously. The only exception was one of the 2 antennaeless and foretarsiless males which courted the female (13), and then did not stop courting the male within 10 min. Thus tapping is not sex-specific, and males apparently either do not release the stimuli which normally elicit courtship of females, or release different stimuli in the same modalities.

That males with only antennae (11) or only basitarsi (12) courted females but not males suggests that both volatile and contact chemicals are normally used, but either is sufficient to release courtship. However, the males with only antennae, after initially tapping the female or male with their foretibiae, then pressed their antennae against the other fly. Those with both antennae and foretarsi removed (13, 14) sometimes licked the female with their proboscides. Males with only the basitarsi remaining (10, 12) did not do this, which suggests that it was not simply the result of closer contact of the male with the female. Perhaps these foretarsiless males were using their antennae or even their proboscides to obtain the chemicals normally received via the foretarsi. This is not too improbable because the anterior edges of the antennae bear contact chemosensory hairs similar to those on the foretarsi (personal observations). Moreover, in another *D. melanogaster*-group species, *D. malerkotliana*, the males apply their antennae to the females' genitalia in an action analogous to normal *D. melanogaster* licking prior to attempted copulation[14], and presumably gain contact chemicals thereby. I therefore suggest that only a close-range, probably contact, chemical stimulus is required for the initiation of courtship.

Table 2. Amount of courtship directed by normal of foretarsiless males at wingless target males in 5-min tests in the absence or presence of 10 females

Parameter	Test male	n	Control	Females	t*	p
Orientation	Normal	15	7.9±3.0	9.0±2.9	1.68	NS
	Foretarsiless	10	10.3±3.8	10.2±3.4	-0.12	NS
Vibration	Normal	15	0.5±0.6	0.7±0.7	1.10	NS
	Foretarsiless	10	0.9±1.0	1.0±0.9	0.43	NS

*Student's t-test for related samples.

Most previous experiments examining the importance of relatively long-range olfactory perception in the initiation of courtship did not exclude the possibility of perception of close-range or contact chemicals, because the males had the opportunity to touch the extracts from the females or males[5,6,15]. Only 1 experiment by Tompkins et al.[6] involved physical separation of the males from the extract. In a similar experiment I did not find a significant increase in courtship of wingless target males by test males (even when foretarsiless) in the presence of 10 females in the other half of the chamber (table 2). Since Tompkins et al.[6] were unable to repeat the earlier olfactometry experiments[3,4], the evidence for the involvement of long-range volatile chemicals in the initiation of courtship remains slender, and the present results further question their importance.

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Immunohistochemical localization of S-100 protein in human cerebral and cerebellar cortices

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Summary. S-100 protein, a highly acidic protein specific to the nervous system, is immunohistochemically localized exclusively in glial cells, but not in any type of neuron in human cerebral and cerebellar cortices.

The biological role of S-100 protein, an extremely acidic protein unique to the nervous system, is still speculative, although physicochemical properties are well known[1,2]. The presence of S-100 protein in glial cells is generally accepted, but its presence in or absence from neurons currently remains controversial. Although a considerable number of studies exist regarding the immunohistochemical localization of S-100 protein in mammalian brains[3-8], no report has described the cellular distribution of S-100 protein in human brain. The present communication is concerned with the immunohistochemical localization of S-100 protein in human cerebral and cerebellar cortices.

Materials and methods. For light microscopy, 5 adult human cerebral and cerebellar cortices obtained at autopsy were fixed with 10% neutral formalin, dehydrated and embedded in paraffin. 8-µm-thick paraffin sections were processed for the immunohistochemical staining of S-100 protein by Sternberger's PAP method[9] using monospecific rabbit antiserum against bovine S-100 protein which had been shown to be antigenically identical to human S-100 protein[10]. As a control, the sections were treated with normal rabbit serum instead of the antiserum against S-100 protein. For immunoelectron microscopy, 5 adult human cerebral cortices (frontal lobes) were obtained at lobectomy for internal decompression to inaccessible tumors. Blocks of fresh cerebral tissue (approximately 50 mm^3) were fixed by immersion in PLP solution[11] for 4 h at 4 °C immediately after removal, washed with 3 changes of 10% sucrose in phosphate buffered saline overnight, and quickly frozen after embedding in O.C.T. (Ames Co.). 6-µm-thick sections cut on a cryostat were processed for immunoelectron microscopy by the direct immunoperoxidase method using either rabbit Fab' conjugate against bovine S-100 protein or normal rabbit Fab' conjugate as detailed elsewhere[12]. The ultrathin sections cut on a ultramicrotome were examined and photographed in a Hitachi HS-8 electron microscope without any counterstain.

Results and discussion. By light microscopy, the positive immunohistochemical staining for S-100 protein, a dark accumulation of diaminobenzidine reaction precipitates, is localized exclusively in both the astrocytes and oligodendrocytes, but not in any type of neuron in human cerebral cortex (fig. 1,a and b). The precipitates from enzymic reaction are evenly distributed through the glial cells and are occasionally faintly seen in the nucleus (fig. 1b). The degree of staining intensity for S-100 protein in glial nuclei varies from cell to cell. The nuclear localization of S-100 protein suggests this highly acidic protein to be an integral constituent of the chromatin acidic proteins[13] involved in the genomic regulation of glial cell differentiation, because the appearance of S-100 protein in developing mammalian brains is a relatively late ontogenic event[14,15]. In the cerebellar cortex, the positive immunoperoxidase staining for S-100 protein is observed in the Bergman's glial cells which

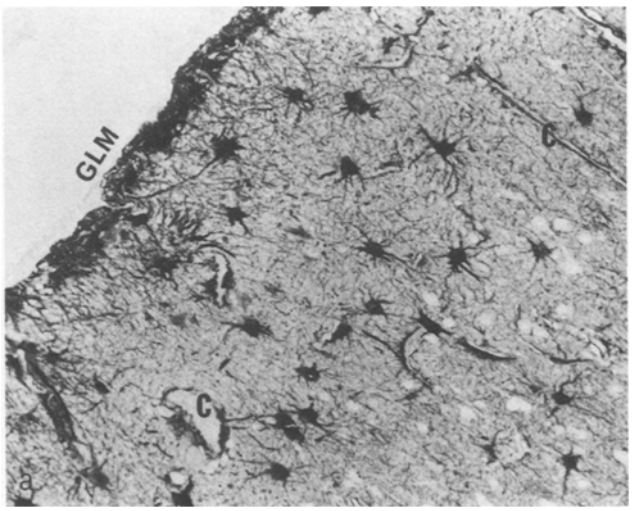

Figure 1. *a* By light microscopy, the positive immunoperoxidase staining for S-100 protein is seen as dark accumulation of diaminobenzidine reaction precipitates in both the astrocytes and oligodendrocytes, but not in any type of neuron in the human cerebral cortex. The glial limiting membrane (GLM) and the glial cytoplasmic processes surrounding capillaries (C) are also stained intensely. × 82.