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65. SPIDER GLANDS AND PSYCHOTROPICS

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There exists evidence that the silk glands of the spider *Araneus diadematus* Cl. work in close connection with the central nervous system (CNS). The CNS appears to send signals to the glands, "telling" them to produce more or less silk; it seems also "informed" of the amount of silk available in the ampullate glands at a given time. Silk being so important in the spider's life — for trap-building, moving around, and communication — such two-way mechanisms appears of great survival value: enough material is made available, but no energy is wasted on excess. The close interrelationship between CNS and glands explains that drugs which affect CNS function, the psychotropics, influence silk gland activity and that webs built after these drugs reflect their effects in web-weight and -pattern. The evidence will be reviewed.

To establish the amount of silk which a spider uses for one web, the whole structure is cut off from its supports, rolled up on a piece of relatively nitrogen-free filter paper, and digested in selenium-sulfuric acid. The amount of nitrogen determined with an optical micromethod in the web digest is a measure of the total amount of polypeptide in the web. Fig. 1 shows the results of an experiment with 23 spiders which were treated with 1 mg/kg physostigmine by mouth in sugar water and 23 control animals. The webs were digested daily and on Tuesday both groups showed the same mean of 39 microgram N per web. 36 hours after the drug had been given, on Wednesday morning, the physostigmine-treated animals had built webs containing a mean of 48.9 $\pm$ 4.4 microgram N per web, while controls built significantly lighter webs with 20-30 micrograms N. The following day, Friday, all animals, controls and drugged spiders, built webs with the same mean of 34.5 microgram N per web. It was concluded that webs after physostigmine are heavier or contain more polypeptide thread.

In order to test the hypothesis that the cholinergic drug stimulates silk production rather than promotes a more thorough squeezing out of the ampullate silk glands, Peakall performed experiments using 3 kinds of methods (1, 2):

A: Glands were pulled empty of thread at regular intervals, f.e. every 6 hours, and the quantity thread pulled was determined in the selenium-sulfuric acid digest. Such procedure takes advantage of the experience that a spider which sits on a rough surface lets out thread onto the rotating axle of a motor until the glands are empty. Peakall's results with physostigmine and carbachol, 2 cholinergic drugs, and atropine, an anticholinergic drug, show that with increasing dose of the drug, silk quantity increased after the cholinergic and decreased after the anticholinergic drug.

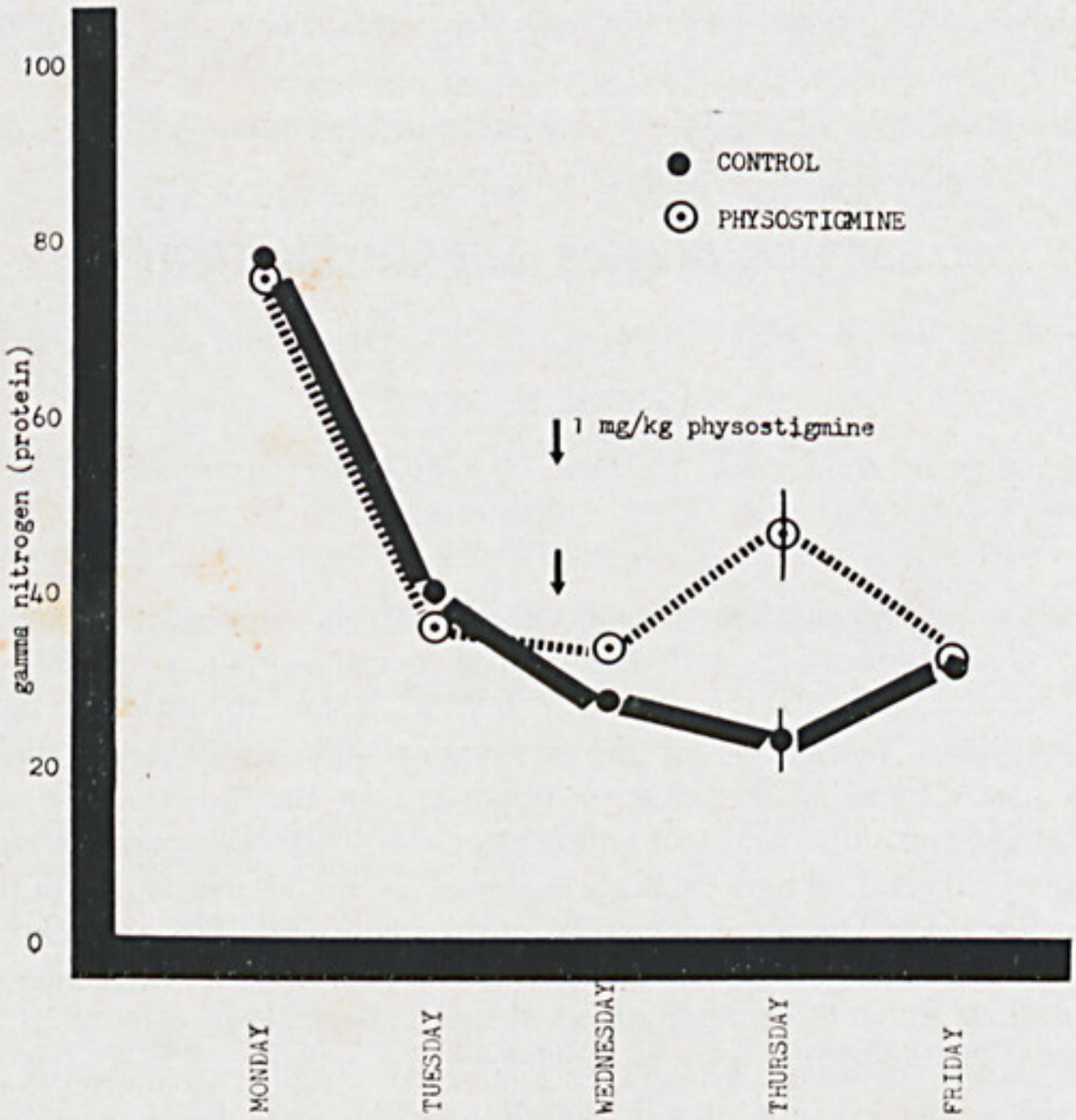

Fig. 1 — Nitrogen values in the digest of webs of 23 spiders treated with 1 mg/kg physostigmine on Tuesday night (12 hours before the Wednesday web-building time) and 23 controls. Each point represents the mean of all measurements, and the vertical line the standard error of the mean. The two Thursday values are significantly different below the 1% probability level. Note the increase of web nitrogen 36 hours after the drug.

B: Another procedure uses labelling of a silk precursor, alanine, with C14, injecting it into the spiders' abdomen, and measuring the speed with which the label turns up in the silk. 6 hours after alanine was given, significantly more label appeared in the silk after physostigmine, carbachol and paraoxon than in untreated spiders (Table I). It was also found that just emptying the glands promoted incorporation of the label, and that emptying plus drug treatment was not additive, but rather made the glands behave like after one treatment alone. We can therefore assume that two mechanisms regulate speed of silk production: 1) a feed-back from the lumen of the gland via the inner epithelial membrane and 2) a possibly neurohumoral mechanism via the outer epithelial membrane.

C: A third way of measuring changes in silk production is the use of radio-autography: the labelled silk precursor, again C14 alanine, leaves black dots on a photographic film which is spread thinly over the sliced tissue. By using this method at different times after alanine injection, its progress from the body fluids into the gland epithelium and from there into the gland lumen can be followed. Table II shows that 4 hours after injection of C14 alanine, most of the label had left the intestine and blood of the spiders and appeared in the gland lumen in pulled or physostigmine treated animals, while atropine treated

TABLE I — AMOUNT OF INCORPORATION OF C-14-ALANINE INTO WEB PROTEIN DURING 6 hr.

The labelled alanine was given at the start of the experiment. Thread was pulled for determination 6 hr afterwards. (From D. B. PEAKALL, *Comp. Biochem. Physiol.*, **12**, 467, 1964).

TREATMENT	No. of spiders	Activity counts/min	Standard error of the mean	Micrograms nitrogen/ 100 mg b.wt.	Standard error of the mean
Unpulled, no drug	15	123	11.1	—	—
Unpulled, physostigmine	18	290	27.4	—	—
Unpulled, carbachol	14	301	25.4	—	—
Unpulled, paraoxon	6	329	60.7	—	—
Pulled, no drug	18	314	13.5	15.0	1.4
Pulled, physostigmine	20	346	17.9	16.2	1.6

TABLE II — COUNTS FROM 7-DAY AUTORADIOGRAPHS OF INTESTINE, BLOOD AND SILK GLAND EPITHELIUM AND LUMEN IN THE INTACT SPIDER

Counts are per mm^2 at magnification of 60. Each figure represents the average, with standard deviation, from 50 counts (10 counts from each of five spiders). Background count (Bkd) were 1-4/mm^2. (From D. B. PEAKALL, *Comp. Biochem. Physiol.*, **15**, 511, 1965).

Time in hr	Treatment	Hind intestine	Blood	Epithelium of silk gland	Lumen of silk gland
2	None	61.0 ± 8.0	29.7 ± 4.3	4.4 ± 1.9	Bkd
	Re-pulled	55.1 ± 6.9	26.3 ± 5.1	8.8 ± 3.7	Bkd
	Physostigmine	54.9 ± 7.9	29.0 ± 2.9	6.1 ± 1.9	Bkd
	Atropine	64.1 ± 9.1	27.1 ± 5.0	Bkd	Bkd
4	None	15.1 ± 2.5	17.8 ± 3.0	25.5 ± 4.1	4.9 ± 0.8
	Re-pulled	10.3 ± 1.8	13.0 ± 5.2	54.3 ± 8.3	18.0 ± 4.2
	Physostigmine	8.4 ± 2.1	8.9 ± 3.1	48.0 ± 7.5	12.5 ± 4.3
	Atropine	16.7 ± 3.0	22.1 ± 4.6	25.5 ± 4.8	6.1 ± 2.1
8	None	Bkd	31.8 ± 1.9	33.8 ± 4.8	38.4 ± 4.0
	Re-pulled	Bkd	4.7 ± 1.9	15.3 ± 2.8	55.6 ± 4.2
	Physostigmine	Bkd	5.6 ± 2.7	16.2 ± 3.5	62.8 ± 9.8
	Atropine	Bkd	14.0 ± 7.4	44.4 ± 9.6	22.4 ± 7.5

or resting glands had not yet taken up the bulk of the label. In histological slides the size and shapes of the ampullate glands and the position of the label can be identified.

If we assume that acetylcholine is the neurotransmitter substance which is responsible for carrying the signal for silk production from the nerve to the gland tissue, we must look for places on the gland which could bind acetylcholine. Peakall's work shows autoradiographic proof that labelled acetylcholine is accumulated on the gland epithelium. This is possibly the area for reception of the neurohumoral signal.

Let us now take a look at the whole animal and see the geographical location and possible interrelationships of silk glands and CNS in *Araneus diadematus* Cl.

F. Meier (personal communication) has identified nerves leading from the big subesophageal ganglion in the cephalothorax to the silk glands. Such nerves could carry signals in both directions, coordinating leg movements of the web-building spider with silk supply in the gland.

Behavioral experiments with web-building spiders have shown that there are 3 possible ways which can lead to webs built with a shorter thread:

1. Atropine sulfate, 1, 2 or 4 mg/kg, was given by mouth to 19, 19 or 39 spiders 12 hours before web-building time. The two lower doses caused webs which showed no change in size or regularity but were built with wider meshes, covering the same area with less thread. The highest dose caused significantly smaller and less regular webs built with less silk. This latter change lasted through the second day after drug application. The interpretation assumes that atropine shows its effect on polypeptide synthesis in the spider's glands as well as interferes with centrally regulated exactness of movements. It is interesting to note that not the size of the catching area in the web was decreased when less thread was available, but the shorter thread was wider spaced so that the trap was full size and only lost the smallest insects.

2. Spiders also built webs with shorter thread after a weight had been attached to their backs. These experiments (3) were undertaken to test the hypothesis that psilocybin, the hallucinatory mushroom poison, caused in spiders similar effects as an increase in body weight. This substance is known to change in man perception of one's body. Does a spider after psilocybin "feel" heavier and therefore builds a weight-web? It could be shown that 150 mg/kg psilocybin given to 9 and 23 spiders in two independent experiments 12 hours before web-building time, as well as a 30% increase in body weight of 15 spiders, decreased average thread length significantly by about 30%. However, when the webs were digested and N determined, a significant difference in the amount of silk was found between the psilocybin and weight-webs: the webs after psilocybin were built with less silk, the shorter thread was as thin as before; the heavier spiders, in contrast, built webs with thicker thread, using equal amounts or even more protein than before. The interpretation for the results of the experiment with heavier spiders assumes that they built a thicker thread to hold themselves up; as they had no more material than usual in their glands, the thicker thread had to be shorter. The psilocybin webs must be interpreted in a different way, as will be seen later. If the interpretation is correct, the spiders' CNS must integrate information on thread length as well as silk quantity stored in the glands during web construction.

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3. Experiments performed under the influence of the tranquilizer Valium (diazepam) may help to interpret psilocybin effects (4). When 100 mg/kg Valium were given to 40 spiders 36 hours before web-building time, all animals built smaller webs with a shorter thread and less material. This could be the result of a decrease in silk production in the glands through the tranquilizer, or the glands were not completely emptied at the end of web construction. Experiments with thread pulling after Valium answered the question in favour of the second interpretation: when silk was pulled from 13 spiders one and two days after 100 mg/kg Valium and from 9 control spiders, no difference in quantity could be found. The effect of Valium is therefore interpreted as possibly affecting the spiders' "drive" so that they build smaller webs using only part of the material. The glands stay partly filled at the end of the construction period. This, in time, would slow down new silk synthesis by the feed-back mechanism which was shown by Peakall.

Thus, the three ways in which webs with shorter thread were produced through drugs and weight changes, provide some evidence for the close interconnection between silk glands and CNS. If the tranquilized CNS "instructs" the legs to build a smaller web, less silk is pulled from the glands. Fig. 2

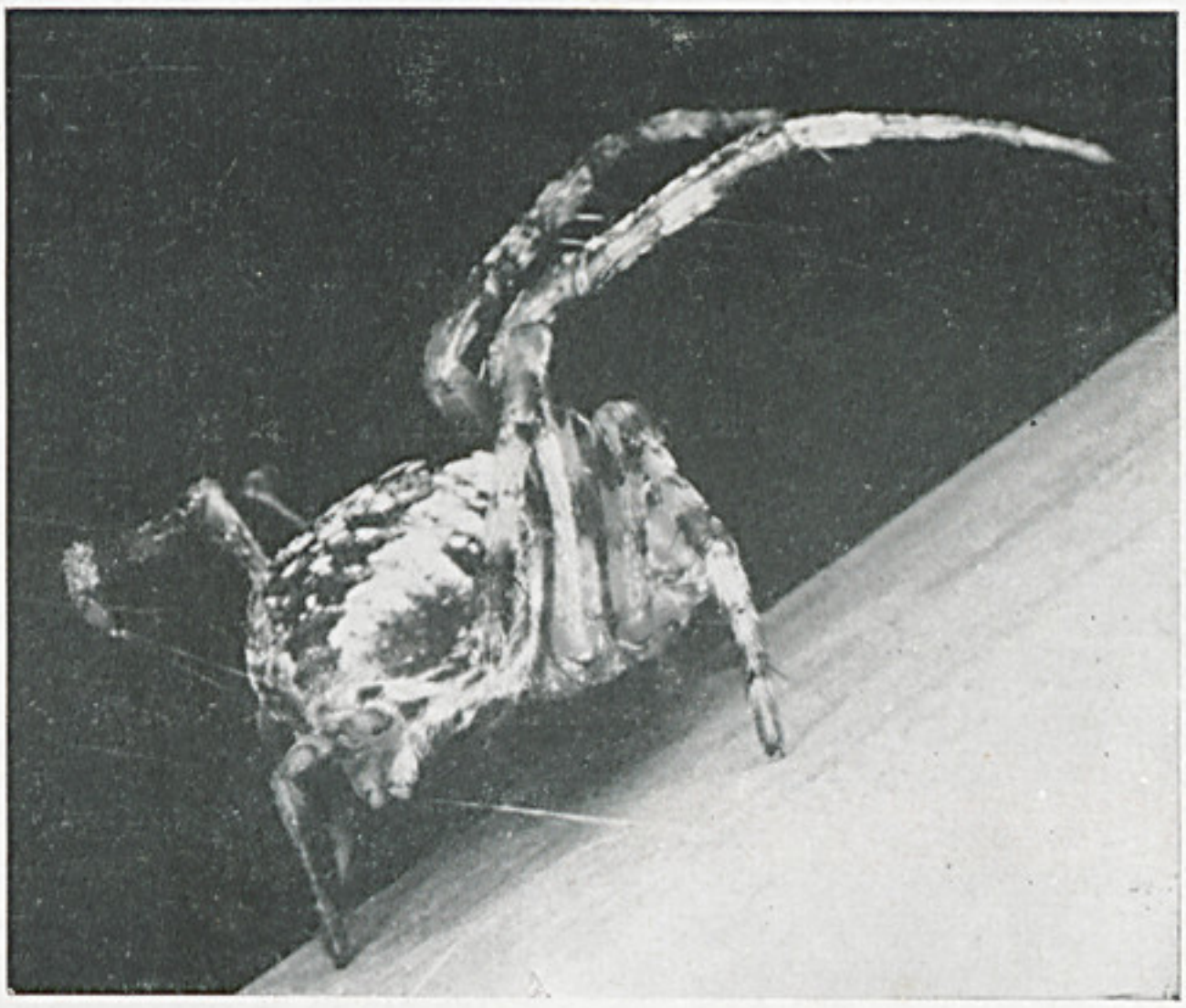

Fig. 2 — *Araneus diadematus* Cl. sitting on a rough surface and facing away from the camera. Note the posterior spinnerets from which the 8th leg pulls two threads, and the anterior spinnerets from which a thread runs to the ground.

illustrates how the spider pulls silk from the spinnerets by means of its hind leg. It can also lower itself on the thread through its weight and regulates speed and possibly thickness with the eighth leg. The funcition of Wilson's control valve (5,6) in this process and its interrelationship with CNS and glands is a matter for future investigations which will we hope further clarify psychotropic drug effects on web-building.

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