known, there are at least two possibilities. First, TRH may act on different populations of neurons in the opposing states. Although we have not recorded from hippocampus neurons in the ground squirrel, two main types of neurons have been differentiated, electrophysiologically, in the hippocampus of rats (*13*) which supports this hypothesis. Alternatively, TRH may act on the same population of neurons within the hippocampus in each state. In this case, the variable influences (associated with a change of state) arising from other CNS inputs to TRH-sensitive neuronal networks that project from the hippocampus may alter the final TRH-activated response.

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14. Supported by NSF grant BNS 78-19002 and the A. I. duPont Institute. We thank N. Sartin for histological work, G. Mercer for artwork and photography, and D. Langille for the typescript.

16 June 1981; revised 10 August 1981

Female Sex Pheromone in the Skin and Circulation of a Garter Snake

Abstract. *Serums and extracts of tissues from the female garter snake* (Thamnophis sirtalis parietalis*) each act as a pheromone and elicit male courtship behavior when applied to the back of another male. Since pheromonal activity is present in yolk and liver tissue of untreated females and can be induced with estrogen treatment in the serums and livers of males, the pheromone may be associated with the circulating yolk lipoprotein, vitellogenin.*

In many vertebrates, urine, feces, and vaginal contents, as well as exocrine glandular products, function as sex attractants and serve to facilitate the location and recognition of mates (*1*). We now report an additional source for a vertebrate sex pheromone; the sex attractant pheromone of the female red-sided garter snake (*Thamnophis sirtalis parietalis*) is present in an active form in the liver and in the circulation. We also provide evidence that the pheromone reaches its active site on the skin by passing through the keratinized outer skin cells in an active process associated with courtship.

Male garter snakes use the chemosensory vomeronasal system to locate and recognize potential mates (*2–6*) by detecting an estrogen-dependent, species-specific pheromone on the skin of attractive females (*3, 6–9*). This pheromone can be left as a nonvolatile trail (*8, 10*) or transferred by contact to conspecifics (*4*); males that have come into contact with attractive females will elicit courtship from sexually active males (*3, 6, 7*).

Male *T. s. parietalis* begin courtship with a chemosensory investigation of the female's body; chemical cues are delivered by tongue flicks to the male's vomeronasal system at the Jacobson's organs and are necessary for the release of male courtship (*4, 6, 11*). Chin-rubbing behavior, in which the male rubs his chin forward and backward along the female's back, follows sex recognition and is the first unambiguous behavioral event of courtship. While being courted, females increase their apparent size by hyperventilating (*12*).

In view of the requirement for perception of a pheromone to release male courtship and the lack of any exocrine glandular structures in the dorsal skin of *Thamnophis* (*13*), we reasoned that the pheromone could be carried in the female's circulation and transported via the dermal vascular bed (*14*) to the skin, where it is actively dispersed by the female's hyperventilation during courtship. Two experiments were conducted to test this hypothesis. (i) Serums from estrogen-treated females were applied to the skin of males, and male courtship of these serum-coated males was recorded (*15*); serums from untreated and estrogen-treated males served as controls. (ii) Extracts of the skin of serum donor females were similarly tested.

Serums that elicited behavioral responses were analyzed for lipid. In addition, the source of the pheromone was examined. Since a lipid pheromone could either be released from storage sites (in the fat bodies) or newly synthesized in the liver, homogenates prepared from the liver and fat bodies of intact females were also tested for pheromonal activity by application to the backs of males. Finally, frozen sections of skin from serum donor animals were stained for lipid.

Males receiving an application of 500 μl of serum from estrogen-treated females ($N = 26$) were courted, with 15 of

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26 serums tested eliciting male courtship; the serums of untreated males ($N = 21$) did not elicit courtship ($\chi^2 = 17.8$, $P < .005$) (*16*). Males given an application of serum from estrogen-treated males (*17*) were also courted; seven of nine serums so tested elicited male courtship.

Courtship was also elicited by lipids—but not by proteins—extracted from the skin of estrogen-treated females. Five of six lipid residues extracted from the skin of estrogen-treated females whose serums had elicited courtship were effective when applied to the backs of males (*18*). Soluble and bound proteins extracted from the skin of the same females were not effective when applied in 1-ml concentrates to the backs of males (*19*).

Lipid concentration was significantly lower in male serums (670 ± 40 mg/dl; $N = 19$) than in serums from estrogen-treated females (2990 ± 270 mg/dl; $N = 26$) ($t = 9.81$; $P < .001$) (*20*). Estrogen treatment significantly increased serum lipid in males (1460 ± 204 mg/dl; $N = 10$) ($t = 5.04$; $P < .001$).

To determine whether the behavioral response in these tests was nonspecific for lipid—due solely to quantity of lipid, or a result of estrogen itself—we applied lard (1 g) or estradiol [500 μl of estradiol benzoate in Steroid Suspending Vehicle (1 mg/ml)] to the backs of males. Neither treatment elicited male courtship.

To determine the source of the pheromone, we tested homogenates of fat bodies and livers from females (*21*). None of the fat bodies removed from six untreated but sexually attractive females elicited courtship when applied to males, but two of two liver homogenates from untreated females were effective in eliciting male courtship when applied to males. Two of two liver homogenates from estrogen-treated males were also effective in eliciting courtship.

The finding that serums and livers from estrogen-treated males were positive for pheromonal activity suggested a possible relation of the active pheromone to vitellogenin, the circulating precursor of yolk. Although males do not normally produce vitellogenin, estrogen treatment induces its synthesis (*22*). To determine whether the pheromone is associated with vitellogenin, we tested the yolk from yolking follicles for pheromonal activity. Males receiving an application of yolk ejected from vitellogenic follicles of an untreated gravid female were courted by five of six males.

Histological examination of frozen sections of the skin (*23*) revealed two areas with intense lipid staining. Numerous lipid-filled vesicles were located in the deep dermal striated muscle and connective tissue of estrogen-treated females. These lipid-containing cells were concentrated in the regions between the scales and adjacent to the dermal vascular bed. Untreated males lacked this intense lipid staining; however, estrogen-treated males developed the dense dermal staining pattern observed in treated females. The other skin area with intense lipid staining was within the epidermis of the outer scale surfaces. The lipid within the outer epidermal generation (the skin lost in shedding) was located in the mesos and αo keratin layers. These layers are covered by a thick, densely keratinized lipid-free layer (βo). Since some lipid is present outside the skin under the edges of scales on the dorsal and especially lateral areas, a mechanism that allows lipid material to pass through the skin must be present in *Thamnophis* females.

In several species of the related genera *Natrix* and *Macropisthodon*, as well as in the gekkonid lizard *Diplodactylus*, there are ductless dermal poison glands (*24*). In those species, muscular contractions force the exudate through ruptures in the skin in the hinge region between the scales, where the keratinization is only one cell thick. Dermal lipid staining in *Thamnophis* females is concentrated in the hinge regions, and examination of the inner surface of fresh skin revealed paired thinnings of the skin in the hinge regions of anterior dorsal scales.

The female attractiveness pheromone of *Thamnophis*, then, is produced in the liver under the control of estrogen and is present there and in the circulation in an active form. In reptiles, both the liver and the fat bodies contain estrogen receptors (*25*), and estrogen treatment of *Thamnophis* females leads to a rapid increase in liver weight and a slower decrease in fat body weight (*26*).

Since the female attractiveness pheromone of *Thamnophis* is present in the liver, but not in the fat bodies, of untreated females, and since estrogen treatment can induce the pheromone in the liver and serums of males, we suggest that the pheromone is either the lipoprotein vitellogenin or a lipid-rich part of that large molecule. The finding that yolk elicits male courtship when applied to males further supports this conclusion.

Because of the findings that (i) there is no sex or treatment difference in lipid staining within the epidermis, (ii) the epidermal lipid is trapped under a heavily keratinized layer, and (iii) lipid is present on the outside of the skin, we suggest that the sequestering of the pheromone in *Thamnophis* is a consequence of an active process analogous to the ejection of poison in certain related snakes. Hyperventilation by the female during courtship, by moving adjacent scales apart and stretching the skin, forces dermal lipid material through the thin skin in the hinge region and serves to potentiate male courtship.

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16. Serum donor animals were killed with an overdose of Brevital sodium (Eli Lilly & Co.); they were exsanguinated from the heart, and the skin was removed. Blood was allowed to clot at 5°C for 24 hours and then was centrifuged for 15 minutes at 2000 rev/min; the serum was removed and stored at −20°C. A small sample of body skin was fixed in 10 percent neutral buffered Formalin for histology, and the rest was stored at −20°C.
17. Estrogen-treated males and females received the same treatment. Estradiol benzoate (40 μg per 75 g of body weight) was given intraperitoneally in Steroid Suspending Vehicle (National Cancer Institute) daily for 7 days. Treated females were used as stimulus females beginning on day 5 of the regimen and were tested until 3 days after the last injection. They were then killed.
18. Portions of the body skin (2.5 g each) were homogenized with a Polytron (Brinkmann) in 50 ml of a mixture of chloroform and methanol (2:1) and extracted twice overnight at room temperature. After centrifugation, the paired extracts were pooled, and the solvent was removed with a rotary evaporator (Buchler) at

room temperature. Samples were stored at 5°C in a desiccator until they were used. Residues were applied with cotton swabs directly to the backs of males.

19. Soluble proteins were extracted overnight at 5°C with 100-ml portions of 0.05*M* phosphate-buffered saline and 1*M* NaCl in 0.05*M* phosphate from 5-g portions of body skin that had been homogenized with a Polytron. After centrifugation for 15 minutes at 2000 rev/min, the supernatants were dialyzed (Spectrapor, MW cutoff 3500) against five changes of distilled water at 5°C and lyophilized. Lyophilized samples were stored at −20°C until they were used; a sample was dissolved in 1 ml of distilled water immediately before it was used in behavioral testing. The entire extracts were applied to the backs of males.
20. Total serum lipid was measured by the sulfuric acid–vanillin reaction, and calculation of concentration was based on an olive oil standard [N. Tietz, *Fundamentals of Clinical Chemistry* (Saunders, Philadelphia, 1970)].
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26. Female *T. s. parietalis* were treated with estradiol benzoate (40 μg per gram of body weight). Liver weight increased from 2.9 ± 0.2 percent of the body weight in the control group to 4.1 ± 0.2 percent by 3 days and 5.9 ± 0.6 percent after 7 days of treatment and 3 days without (*17*). Fat body weight was 4.8 ± 0.7 percent of the body weight in the control group.
27. We thank R. Tokarz for critical comments and suggestions, and K. Garstka for help in preparation of the manuscript. Supported by NIH grant HD 12709 and by NIMH Research Scientist Development Award 1 KOZ MH 00135 (to D.C.).

3 February 1981; revised 6 July 1981

Increased Intracranial Self-Stimulation in Rats After Long-Term Administration of Desipramine

Abstract. *The effects of long- and short-term administration of the tricyclic antidepressant desipramine on intracranial self-stimulation in rats were studied with electrodes in the A10 region of the dopamine-containing cell bodies of the ventromedial tegmentum. Long-term desipramine administration resulted in a significant shift to the left in the ascending portion of the rate–current intensity function, indicating that the activity of the mesolimbic dopamine system was enhanced. These findings point to a possible dopaminergic mechanism of action of antidepressants and support speculations concerning the role of dopamine-containing neurons in the pathophysiology of depression.*

The catecholamine hypothesis of affective illness posits that mania and depression are related to abnormal increases and decreases, respectively, in the function of central noradrenergic systems (*1*). More specifically, it has been proposed that depression may result from a pathological hypoactivity of a reward system in the brain which uses a catecholamine as its neurotransmitter (*2*). The observation that clinically effective antidepressants, such as desipramine and related tricyclic compounds, block neuronal uptake and increase synaptic concentrations of norepinephrine is consistent with this hypothesis (*3*). However, not all clinically effective antidepressants share this property (*4*), and many recent studies have failed to provide evidence that central norepinephrinergic systems are involved in the mediation of reward (*5*). Instead, both basic and clinical studies have indicated that central dopamine (DA)-containing neurons—specifically, the mesolimbic system—may be important neuronal substrates for some forms of reward. For example, intracranial self-stimulation (ICS) obtained from electrodes in the origin or the pathway of the mesolimbic DA system has been shown to be dependent on the integrity of the ascending projections of this system (*6*). Furthermore, the reinforcing properties of cocaine and amphetamine are blocked by DA receptor antagonists and by selective lesions of DA terminals in the nucleus accumbens, a major area of projection of the mesolimbic DA system (*5, 7*).

These considerations prompted us to examine the effect of desipramine on a DA-mediated behavior, namely, ICS obtained from electrodes in the ventromedial tegmentum (*6*). Because the clinical effects of tricyclic antidepressants typically require several weeks to become manifest, we administered desipramine on long- and short-term bases. Twenty-four male Wistar rats weighing 300 to 330 g were anesthetized and implanted with electrodes in the A10 DA region (*8*). For 5 days following surgery all the animals were given a 30-minute test once each day to screen them for ICS (*9*). On days 6 and 7 ICS rate–current intensity functions were obtained by using an ascending and descending method of limits (*10*). Beginning on day 8, half the animals received daily injections of desipramine HCl (10 mg/kg) for 14 days and half received an equal volume of vehicle (*11*). On days 8 and 9 the effect of short-term desipramine administration on the rate-intensity functions was determined 30 minutes after the injection. The effect of long-term desipramine treatment on the rate-intensity functions was determined on days 15 and 16, 24 and 48 hours after the last injection. The data were analyzed by repeated-measures analysis of variance and appropriate post hoc tests (*12*). After completion of the behavioral experiments the electrode placements were confirmed histologically (*13*).

Difference scores were obtained for statistical analyses by subtracting the individual baseline rates from those obtained after treatment. Scores were obtained for the control group and both desipramine-treated groups under current presentation modes in which intensity was increased or decreased at regular intervals. Analysis of variance yielded a significant three-way interaction ($P < .05$) which indicates that, at suprathreshold current intensities, the ICS rates for rats receiving long-term desipramine treatment were significantly higher than those for the vehicle control and short-term desipramine groups (Fig. 1). This interaction was found only for data obtained during current presentation in the ascending mode. No significant differences were observed between the baseline ICS rates and those following short-term desipramine treatment, and the associated data are omitted from Fig. 1.

The effect of desipramine was also analyzed by determining the amount of current necessary to increase ICS to half the maximal rate before and after drug treatment (*14*). Vehicle treatment did not affect this value (Table 1). In contrast, long-term but not short-term desipramine treatment significantly reduced the current necessary to produce half-maximal ICS rates during presentation of ascending intensities. Before drug treatment the ICS rates obtained at the middle current intensities were higher during presentation in the ascending order than in the descending condition. These higher rates can be attributed to positive contrast effects. In previous work with the ICS paradigm, positive contrast was found under similar circumstances (*15*).

These results suggest that the function of the mesolimbic DA system is facilitated by long-term but not short-term desi-

0036-8075/81/1106-0683$01.00/0