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6. "The anti-anxiety agents share a similar central depressant action: the ability to produce mild sedation in doses that are generally unlikely to cause soporific effects or to adversely affect the quality of consciousness and the quality of psychomotor performance" [American Medical Association Department of Drugs, *American Medical Association Drug Evaluations* (Publishing Sciences Group, Acton, Mass., 1973), p. 317].
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8. Conflicting reports of the effects of tranquilizers on detection have been made, but these have been based on rather informal observations: M. A. Berman, *J. Am. Polygraph Assoc.* **4**, 329 (1975); C. Hess, *ibid.*, p. 314; A. B. Kristofferson and R. H. Cormack, in *Drugs and Behavior*, L. Uhr and J. G. Miller, Eds. (Wiley, New York, 1960).
9. E. L. Kelly *et al.*, *Arch. Neurol. Psychiatry* **80**, 247 (1958); V. G. Laties, *J. Abnor. Soc. Psychol.* **59**, 156 (1959); G. A. Leinert and W. Traxel, *J. Psychol.* **48**, 329 (1959).
10. In field polygraph testing the actual guilt or innocence of subjects usually does not become established for a long time, if at all. More serious for the purpose of scientific investigation is that the ultimate disposition of the case cannot be considered to be independent of the polygraph test outcome: suspects who appear truthful may not be examined as thoroughly as those who appear deceptive. Finally, it is not typically feasible to introduce experimental treatments, such as tranquilizers, into field polygraph tests. Consequently, we must depend upon laboratory experiments. The experimental subject cannot be expected to suffer the same degree of apprehension as the field subject. Although this must be kept in mind, it would be an obstacle to experimental research only if the detection of deception failed under such conditions. Many studies document the effectiveness of the psychophysiological detection of deception in the laboratory, particularly with the guilty knowledge test.
11. The subjects were male college students, 18 to 24 years of age, recruited through advertisements and paid $2 an hour.
12. "Guilty" subjects were told that the polygrapher would do his best to obtain a confession but that it was possible to "beat the polygraph" by controlling one's emotions. "Innocent" subjects were told that the polygraph examiner would suspect them and that it was often very difficult to prove one's innocence in a lie-detection test. As a test of their "ability to perform under stress," the innocent subjects completed the same timed, interpolated tasks as did the guilty ones, but without learning words.
13. Meprobamate taken orally reaches its peak plasma concentration in 1 to 2 hours [B. J. Ludwig and J. R. Potterfield, in *Advances in Pharmacology and Chemotherapy*, S. Garattini, A. Goldin, F. Hawking, I. J. Kopin, Eds. (Academic Press, New York, 1971), vol. 9].
14. Experimenter 2 was a male medical student. Sensors were attached as in field exams, a blood-pressure cuff on the left arm, EDR electrodes on the first phalange of the second and third fingers of the right hand, and a respiration belt around the chest; recordings were made on a Stoelting polygraph.
15. D. T. Lykken, *J. Appl. Psychol.* **43**, 385 (1959); *ibid.* **44**, 258 (1960); *Am. Psychol.* **29**, 725 (1974). Lykken has outlined the logic underlying the technique of detecting guilty knowledge, in contrast to the controversial lie test or control-question test more widely used in the field. Field polygraphers assume that the conditions for the guilty knowledge test, the existence of information known only to the guilty person, can rarely be met, but Reid and Inbau (*5*) report many ingenious uses in the field of a peak-of-tension test, a field variant of guilty-knowledge detection. The guilty-knowledge technique may come to be used more widely in light of the relatively low accuracy of the physiological data generated by the lie test as widely used in the field [F. S. Horvath, *J. Appl. Psychol.* **62**, 127 (1977)].
16. Finally, experimenter 3 conducted a postexperimental interview and debriefing and answered the subject's questions about the experiment.
17. Amplitude of the EDR, suppression of breath amplitude, and amplitude of the cardiovascular response for each stimulus were measured as in Thackray and Orne (*20*). Detections were scored separately for each channel. A detection was counted if the critical stimulus in a set of four words evoked a larger physiological response than any of the three other items. For innocent subjects one word in each category was randomly designated to be the critical item for purposes of analysis.
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19. The three meprobamate subjects who were classified as deceptive, however, were detected as frequently as the most detectable placebo or no-pill subjects. Thus, although there were statistically significant differences between the groups in the number of deceivers detected, there was no such difference in the mean number of lies detected.
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25. Supported in part by a grant from the Institute for Experimental Psychiatry. We thank David F. Dinges, Kevin M. McConkey, Frederick J. Evans, and William H. Putnam for their substantive comments during the preparation of the manuscript.

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Pheromonal Control of Dealation and Oogenesis in Virgin Queen Fire Ants

Abstract. *In the fire ant* Solenopsis invicta, *sexually mature virgin females are prevented from shedding their wings and becoming functional egg layers by the presence of the mated queen. Experimental data suggest that this inhibitory effect results from the action of a relatively nonvolatile primer pheromone (or pheromones) produced by the mated queen and distributed by the workers. Target ants are both virgin queens and workers.*

In most species of social insects individual colonies have only one queen (*1*). Virgin queens are reared seasonally, but these do not become reproductively active until they have either left the parental nest to mate and found colonies of their own, as in ants, wasps, and termites, or until the old queen has left with a swarm, as in honey bees. It has been generally assumed that, among the ants, either the act of shedding the wings (dealation) after a mating flight, or flight itself, initiates the complex physiological and behavioral changes in newly mated queens that permit them to found new colonies (*2*). One of the most significant of these changes is the development of eggs in the ovaries (oogenesis) with materials from the now defunct flight muscles and from the fat body. In the fire ant, *Solenopsis invicta* Buren, the fundamental processes of dealation and oogenesis are usually prevented from occurring while virgin queens are still in the parental nest by the presence of one or more primer pheromones secreted by the mother queen (*3*).

During 1979 we collected 151 colonies of *S. invicta* in the state of Georgia, for laboratory study. About 2 weeks after collection, when the ants had moved from the soil into artificial nests (*4*), we found a single mated queen in each of 58.3 percent of the colonies. Among the remaining colonies were many that appeared to be polygynous, but dissection of all the queens present showed that none was inseminated, although some had many mature oocytes in their ovarioles. Since these colonies also contained worker pupae, we assumed that they had been orphaned during collection, and we hypothesized that the presence of the mother queen of a colony prevents dealation and oogenesis among sexually mature virgin females (*5*).

To test the hypothesis, we orphaned colonies containing sexually mature virgin females. Dealates began to appear within 24 hours, and a few days later the workers started to execute some of them (*6*). Dealation ceased while numerous female alates were still present, but execution of the dealates continued for up to 3 weeks until very few remained. These few had enlarged ovaries containing numerous oocytes. It seemed, therefore, that they had taken over the egg-laying function of the mated queen and that they too could inhibit dealation among sexually mature virgin females. It was also evident that the workers would tol-

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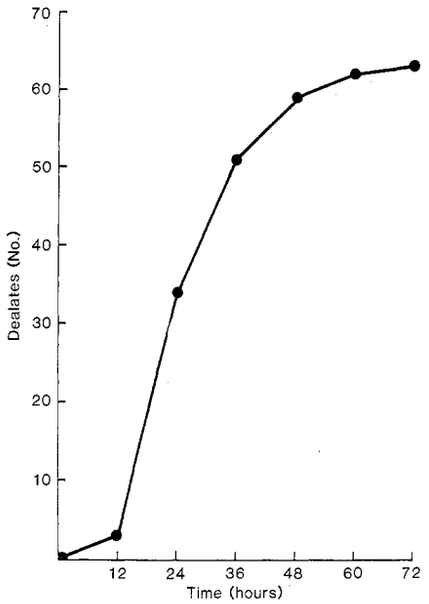

Fig. 1. Rate of dealation among virgin females in queenless units. Cumulative totals for 15 units each containing five females have been plotted.

erate very few such virgin replacement queens in the nest. We used these results to design the main test of the hypothesis.

We divided each of five colonies, consisting of a mated queen, approximately 20,000 workers, 40 to 50 cm^3 of worker larvae and pupae, and several hundred virgin females, into four equal parts: three queenless units and a queenright control. We left only five virgin females in each unit to prevent a protracted series of executions by the workers, thereby providing all units with an equal opportunity to produce functional virgin queens by the end of the experiment.

One week after subdivision, no virgin females had dealated in the five control units, but a mean of 4.2 ± 0.8 (standard deviation) had dealated in each of the 15 queenless units (Fig. 1). The workers executed 12 of these dealates. At the end of the 7-day period, the surviving virgins in the queenless units, as well as all the alates in the controls, were subjected to an oviposition test. This consisted of isolating them on a damp surface for 5 hours and afterward examining the surface for eggs. At least one dealate in every queenless unit oviposited (the mean number per unit was 2.5, standard deviation = 1.0), although none of the remaining alates in these units, and none of those from the controls, laid eggs.

Immediately after these tests we dissected all the virgins. None had sperm in the spermatheca, but the functional egg layers nevertheless had moderately well developed ovaries. On an arbitrary scale from 0 to 6, in which "0" denoted no oocytes in the ovarioles and "6" indicated the maximally developed ovaries of physogastric queens containing many hundreds of oocytes, these functional virgins had ovaries that rated 2 and 3. The alates in both the queenright and queenless units rated 1 (*7*).

These results confirmed that the mated queen of a fire ant colony inhibits dealation and oogenesis among sexually mature virgin queens. We anticipated that one or more pheromones would be involved, and therefore investigated the mode of action experimentally as follows.

We divided small plastic nests into two compartments of equal size with a single wire screen through which ants could make contact with each other, but through which they could not pass, and we divided other similar nests with two screens 1.5 mm apart to prevent physical contact. We placed workers and brood in each compartment of a nest, together with five sexually mature virgin females, but a mated queen was added to one compartment only. With this arrangement dealation would be prevented in both compartments whether a nest was divided by a single or double screen if the queen produced an inhibitory pheromone that was fairly volatile, because the screens would not be a barrier to its passage. On the other hand, if a pheromone had to be passed either orally or by bodily contact from one compartment to the other, dealation would occur in the queenless compartment of the double screen nests, but not necessarily in the single screen nests. The results were assessed after 48 hours, since most of the dealation could be expected to occur in that time (Fig. 1).

Some virgin females dealated in the queenless compartment of every double screen nest, and some, but significantly fewer ($P = < .02$, t-test), also dealated in queenless compartments of the single screen nests (Table 1). No dealation occurred in any queenright control compartment. These results are consistent with the hypothesis that the queen produces an inhibitory pheromone that is relatively nonvolatile and is distributed by the workers either trophallactically or through physical contact to target individuals (*8*). It appears that in the single screen test the amount of this pheromone transferred to the queenless side was inadequate to prevent dealation completely.

The target ants appear to be both workers and virgin queens. Young virgins (1 to 2 months old) separated from the parent colony and kept either singly or in small groups for 72 hours, dealated rapidly if accompanied by workers, but in very small numbers only in the absence of workers. Hence, the inhibitory pheromone acted mainly indirectly on the virgins by influencing the behavior of the workers toward them (*9*). On the other hand, older virgin queens (more than 4 months) dealated readily in isolation or in small groups even when unaccompanied by workers, and must therefore have been influenced directly by the pheromone while in the parental nest. We will report more fully on this aspect, and on the manner in which disinhibited workers cause young virgins to dealate, in a separate paper.

Indirect evidence for the existence of reproductive inhibition by queen fire ants over other queens in the nest has been described (*10*), but this appears to be the first report of a social insect pheromone that prevents dealation among virgin queens. Pheromones serving this function may perhaps be common among the very large number of ant species that found colonies in a similar manner to that of *S. invicta*.

The delay that occurs between orphaning a colony and the responses of dealation and oogenesis requires that the inhibitory principle produced by the mother queen be classified as a primer pheromone, of which there are few known examples among insects compared with the large number of releaser

Table 1. Effects of separating sexually mature virgin females from the queen by means of double screens (DS) or single screens (SS). Twelve plastic nests (8 by 7 cm and 3.5 cm deep) with close-fitting lids were divided in half with a single wire screen (11 mesh per centimeter), and 12 similar nests were divided with two screens 1.5 mm apart. A sieve of the same wire mesh was used to remove from the experimental colonies the few small ants that could pass through the mesh. Into each compartment of a nest were placed about 300 workers, 1 cm^3 of worker brood, and five virgin female alates. A mated queen was added to one compartment only. The ants on both sides of a screen were fed. The number of virgins that dealated were recorded after 48 hours.

Nest type	Queenless side		Queenright side: nests with dealates (No.)
	Nests with dealates (No.)	Dealates per nest	
DS	12	3.3 ± 1.6*	0
SS	10	1.8 ± 1.4	0

*Mean ± standard deviation.

pheromones that have been identified (*11*). In the social insects primer pheromones inhibit oogenesis among workers, and are probably also involved in caste determination (*12*), although little information is available on the latter because of the difficulties of bioassay.

If we should be able to isolate the fire ant primer pheromone using inhibition of dealation as a bioassay, it will then be possible to determine whether this same pheromone inhibits oogenesis in sexually mature virgin queens, either directly or indirectly, and whether it is also involved in the execution of dealate virgins by workers.

Our results may be useful for the future control of fire ants. The execution of supernumerary dealates once they become functional egg layers suggests that the workers will tolerate only a certain level of a particular queen pheromone, or complex of pheromones, in the colony, and hence, that artificial elevation of the amount present above the critical level may result in the execution of the mother queen by her own workers. The data also raise important questions as to why pheromone levels are critical and how many discrete queen pheromones exist in *S. invicta* (*13*). Further investigation of these and other aspects indicated above should provide new information on factors controlling the organization of insect societies.

DAVID J. C. FLETCHER
MURRAY S. BLUM
Department of Entomology, University of Georgia, Athens 30602

References and Notes

1. Some species of social insects, particularly among the ants, have colonies with multiple queens (polygyny). The significance of the number of queens in the evolution of ants is discussed by B. Hölldobler and E. O. Wilson [*Naturwissenschaften* **64**, 8 (1977)].
2. See, for example, W. M. Wheeler, *Ants Their Structure Development and Behavior* (1910; reprinted by Columbia Univ. Press, New York, 1965), p. 184; J. H. Sudd, *An Introduction to the Behaviour of Ants* (Arnold, London, 1967), p. 143.
3. Since they are uninseminated, the virgin queens that become functional are able to lay only unfertilized eggs and cannot, therefore, establish new colonies or even maintain the worker population of the parent colony.
4. The nests were circular (15 cm in diameter) and made of plastic. They contained damp Castone (Ransom and Randolph, Toledo, Ohio).
5. Sexual maturity is attained 7 to 10 days after pupal eclosion according to C. S. Lofgren, W. A. Banks, B. M. Glancey [*Annu. Rev. Entomol.* **20**, 1 (1975)]. The terms, virgin queen and virgin female, are used here as synonyms.
6. The manner of execution was similar to that described by E. O. Wilson [in *Symp. R. Entomol. Soc. London* **3**, 81 (1966)] after supernumerary queens had been introduced into *S. invicta* colonies.
7. Sexually mature virgin queens from queenright field and laboratory colonies have up to about 20 maturing oocytes in the bases of their ovarioles. Newly eclosed virgins have none.
8. There is good evidence that in the honey bee, *Apis mellifera* L., queen pheromones are distributed throughout a colony by physical contact among the bees rather than by trophallaxis. Queen attendants acquire the pheromones mainly on the anterior part of the body while grooming the queen. When they leave the queen they move actively about the combs and are attractive to other bees for a short time. They are termed substitute queens by H. H. W. Velthuis [*Behaviour* **41**, 105 (1972)] and messenger bees by T. D. Seeley [*Behav. Ecol. Sociobiol.* **5**, 391 (1979)]. A similar mode of pheromone distribution might exist in *S. invicta* and other ants, but the possible involvement of trophallaxis has not been ruled out.
9. When a colony of honey bees is orphaned, the workers respond by rearing replacement queens. A useful view of the response is that disinhibited workers behave in such a manner as to replace the lost source of queen pheromones. A similar view may be taken of the behavior of disinhibited fire ant workers in causing some of the alates present in an orphaned colony to dealate and become functional queens, even though they lay only unfertilized eggs.
10. W. R. Tschinkel and D. F. Howard [*Behav. Ecol. Sociobiol.* **3**, 297 (1978)] orphaned colonies in the field and subsequently found egg-laying replacement queens in many of them. A few of these were mated but most were uninseminated, which suggested that both categories of queens were reproductively inhibited by the mated queen prior to orphaning.
11. For a review of the chemistry of insect pheromones see J. M. Brand, J. Chr. Young, R. M. Silverstein, *Progress in the Chemistry of Organic Natural Products* (Springer-Verlag, New York, 1979), vol. 37, pp. 1–190.
12. E. O. Wilson, *The Insect Societies* [Belknap (Harvard Univ. Press), Cambridge, Mass., 1971], pp. 136–196 and 233–305.
13. At present, the minimum number of chemically distinct queen pheromones that may be postulated for *S. invicta* is two: the relatively nonvolatile inhibitory primer pheromone and a queen attractant reported by D. P. Jouvenaz, W. A. Banks, C. S. Lofgren [*Ann. Entomol. Soc. Am.* **67**, 442 (1974)] which by the definition of an attractant, must be a volatile releaser pheromone.
14. Supported in part by a cooperative agreement with the U.S. Department of Agriculture and a grant from the American Farm Bureau.

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Morphiceptin (NH_4-Tyr-Pro-Phe-Pro-$CONH_2$): A Potent and Specific Agonist for Morphine (μ) Receptors

Abstract. *The synthetic peptide NH_2-Tyr-Pro-Phe-Pro-$CONH_2$ (morphiceptin), which is the amide of a fragment of the milk protein β-casein, has morphinelike activities and is highly specific for morphine (μ) receptors but not for enkephalin (δ) receptors. It is as active as morphine in the guinea pig ileum but much less active in the mouse and rat vas deferens. The discovery of this specific morphine receptor ligand substantiates the hypothesis of multiple opiate receptors. The ligand, which may be of physiological significance since a very similar, or identical, activity can be detected in enzymatic digests of β-casein, may prove useful for further investigation of the functions of opiate receptor subtypes.*

Recent binding studies have indicated that there are at least two subtypes of opiate receptors, morphine (μ) and enkephalin (δ) receptors, in brain (*1, 2*). Morphine binds to μ receptors in the absence of Na^+ with an affinity about 100 times greater than that for δ receptors (*2–4*). In contrast, methionine-, leucine-, [D-Ala^2, Leu^5]-, and [D-Ala^2, D-Leu^5]enkephalin bind to δ receptors with about five to ten times better affinity than to μ receptors (*1–4*). Naloxone binds to μ receptors with 20- to 30-fold greater affinity than to δ receptors (*1–4*), while diprenorphine binds about equally well to both receptor sites (*4, 5*). Morphine receptors can be studied by measuring the specific binding of low concentrations of ^{3}H-labeled naloxone (*1–7*), naltrexone (*1*), and dihydromorphine (*1–5*), and ^{125}I-labeled Sandoz FK33824 (*3, 4*) or [D-Ala^2, N^{α}Me-Phe^4,Met(*O*)^{5}ol]enkephalin. Enkephalin receptors can be characterized by using ^{3}H-labeled enkephalins (*1, 5, 6*) or their metabolically stable analogs (*8*) and ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin (*2–4*).

No opioid alkaloid selective for δ receptors has yet been found with these two binding assays. The binding affinity of the enkephalin analog Sandoz FK33824 was found to be similar to that of morphine, its affinity for μ receptors being about 20 times greater than its affinity for δ receptors (*3*). However, benzomorphan drugs such as ketocyclazocine, ethylketocyclazocine, and *N*-allylnormetazocine can bind to δ receptors with affinities greater than morphine-like alkaloids, and the difference in their affinities for the two receptor subtypes is much reduced compared to morphine (*9*). Sodium ion (*10*) and guanosine triphosphate (GTP) (*11*) preferentially decrease the affinity of opiate agonists and enkephalins for both μ and δ receptors. On the basis of the relative affinities to these two subtypes of opiate receptors and the differential effects of Na^+ and GTP, a subclassification of opioid alkaloids and peptides into seven separate classes was suggested recently (*9*); (i) μ agonists; (ii) δ agonists; (iii) mixed agonist-antagonists; (iv) κ agonists; (v) σ agonists; (vi) nalorphine-type antagonist; and (vii) opiate antagonists.

We now report that a synthetic tetrapeptide fragment, NH_2-Tyr-Pro-Phe-Pro-$CONH_2$, of the milk protein β-casein has potent opioid activity both in vivo and in vitro (*12*). It binds to opiate receptors in a highly selective manner, having

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