for the septal and entorhinal projections to the dentate gyrus. Seemingly, both the septal and entorhinal inputs to the dentate gyrus relate to the behavioral significance of sensory stimuli. The functional characteristics of these two inputs make it possible for the granule cells to respond differentially to such stimuli. The nature of this response depends on the position of the sensory stimulus along an abstract continuum anchored at one end by "unexpectedness" or lack of behavioral significance and at the other by features that provoke maximum expectation of biologically significant events through associative learning. In essence, such a system would be capable of discriminating the degree of familiarity of any sensory stimulus, a function previously suggested to explain many of the similarities in memory disruption in humans and animals resulting from hippocampal damage (*17*).

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Imipramine: Effect of Ovarian Steroids on Modifications in Serotonin Receptor Binding

Abstract. *Long-term administration of imipramine caused a decrease in serotonin$_2$ receptor binding in rat brain cerebral cortex, an effect that was abolished by ovariectomy. In contrast, ovariectomy had no effect on imipramine-induced decreases in hippocampal serotonin or in cerebral cortical and hippocampal β-adrenergic receptor binding. Administration of estradiol or progesterone separately or in combination reestablished the effect of imipramine treatment on cortical serotonin$_2$ receptors. These results suggest that ovarian steroids may play an important, but subtle, role in the neurochemical and perhaps clinical response to this drug.*

Affective illness is thought to be related to a dysfunction in monoamine activity in the central nervous system (*1*). This hypothesis is supported by the finding that clinically effective antidepressants, such as imipramine, modify monoaminergic transmission (*1*, *2*). Recent studies suggest that antidepressants share a common action in that long-term administration leads to a dose- and time-dependent decrease in the number of β-noradrenergic and serotoninergic receptor sites in brain (*3*). Of particular interest is the fact that these changes in neurotransmitter receptor binding and function correspond, in a temporal fashion, with the delay observed in the onset of the therapeutic response to these drugs. Thus, antidepressant-induced neurotransmitter receptor alterations may be a useful index for measuring the therapeutic efficacy of this drug class.

Steroid hormones may also be involved in some types of affective disorders (*4*). Ovarian hormones may be important in this regard since depression is often associated with physiological states related to ovarian hormone secretion such as menstruation, parturition, and menopause (*4-6*). In addition, abnormalities in hormone secretion and activity have been noted in depressed patients (*7*).

We have examined whether there is a relation between ovarian hormones and the neurochemical effects of imipramine. The results indicate that the imipramine-induced decrease in serotonin, but not β-adrenergic, receptor binding depends on the presence of these steroids and suggest that the response to imipramine may be altered in patients suffering from ovarian hormone deficiencies.

Sprague-Dawley rats (125 g) were ovariectomized bilaterally and 7 days after surgery imipramine treatment was initiated. Imipramine was administered intraperitoneally at 10 mg/kg once daily for 21 days. In replacement experiments, groups of animals received daily subcutaneous injections of 17β-estradiol (40 μg/kg), progesterone (4 mg/kg), the combination of estradiol and progesterone, or vehicle (ethanol and safflower oil) only, beginning at 2 days after surgery and continuing for 26 days. On day 6 of hormone replacement treatment, imipramine administration was initiated and continued for 21 days. All the animals were killed 48 hours after the last injection, which was 30 days after ovariectomy.

The animals were killed by decapitation and their brains were rapidly removed, dissected, and stored at −20°C. Cerebral cortical and hippocampal serotonin$_2$ (5-HT$_2$) receptor binding was analyzed by using [^{3}H]spiroperidol (New England Nuclear; 23 Ci/mmole) as a ligand (*8*); β-adrenergic receptor binding was assayed by using [^{3}H]dihydroalprenolol ([^{3}H]HDHA; New England Nuclear; 49 Ci/mmole) as a ligand (*9*). For these assays, brain membranes were incubated with either 0.3 n*M* [^{3}H]spiroperidol or 0.5 n*M* [^{3}H]DHA in the presence or absence of unlabeled serotonin (10^{-1}*M*) for the former or *dl*-propranolol (10^{-5}*M*) for the latter. Binding assays were terminated by rapid filtration under vacuum over Whatman glass fiber filters with three 5-ml rinses in cold buffer. The filters were counted by liquid scintillation spectrometry. Protein concentrations were determined by the method of Lowry (*10*). Specific receptor binding is de-

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Table 1. Effect of ovariectomy on imipramine-induced alterations in 5-HT_2 and β-adrenergic receptor binding in rat brain cerebral cortex and hippocampus. Treated animals received imipramine (10 mg/kg) once daily for 21 days and were decapitated 48 hours after the last injection. Imipramine treatment was initiated in ovariectomized animals 7 days after surgery. Neurotransmitter receptors were analyzed by ligand binding assays as described in the text. Each value is the mean ± standard error of 6 to 11 separate experiments, each performed in triplicate. Levels of significance were determined by a two-tailed Student's t-test.

Animal	Treatment	Specific receptor binding (fmole/mg protein)			
		5-HT_2		β-Adrenergic	
		Cerebral cortex	Hippocampus	Cerebral cortex	Hippocampus
Intact	Saline	31 ± 4	20 ± 0.5	52 ± 4	52 ± 5
Intact	Imipramine	16 ± 4*	13 ± 3*	42 ± 3*	27 ± 2*
Ovariectomized	Saline	33 ± 3	18 ± 1	62 ± 6	55 ± 6
Ovariectomized	Imipramine	36 ± 4	14 ± 1†	46 ± 3†	23 ± 2†

*$P < .05$, compared to intact, saline-treated controls. †$P < .05$, compared to ovariectomized rats receiving saline.

fined as the difference in amount of radioactive ligand bound in the presence and absence of displacer.

Long-term (21 days) administration of imipramine caused a significant reduction in both β-adrenergic and 5-HT_2 receptor binding in rat brain cerebral cortex and hippocampus (Table 1). Ovariectomy itself had no significant effect on either β-adrenergic or 5-HT_2 receptor binding in either brain area (Table 1) nor did it affect cerebral cortical γ-aminobutyric acid (GABA), serotonin$_1$, cholinergic muscarinic, benzodiazepine, or α-adrenergic receptor binding (data not shown). However, ovariectomy did prevent the imipramine-induced change in 5-HT_2 receptor binding in cerebral cortex, though this drug was still capable of causing a decrease in this receptor site in hippocampus and in β-adrenergic receptor binding in both brain regions (Table 1). Thus, in intact animals, imipramine reduced 5-HT_2 receptor binding in the cerebral cortex by 50 percent, an effect that was totally abolished by ovariectomy.

Studies of receptor binding site saturation revealed that, as reported by others (*3*), the decrease in 5-HT_2 and β-adrenergic receptor binding was due entirely to a change in the concentration of binding sites (B_{max}) with no alteration in the receptor affinity (K_d) for the ligand. Furthermore, the B_{max} and K_d for 5-HT_2 receptor binding was identical in imipramine-treated ovariectomized and control animals, indicating that imipramine treatment had no effect on this receptor site after ovariectomy.

The effect of estrogen and progesterone on the response to imipramine in ovariectomized animals was also studied (Table 2). Continuous treatment with either steroid or the combination of hormones reversed the effect of ovariectomy on the 5-HT_2 receptor response to imipramine (Table 2). Thus, imipramine treatment caused a 30 percent reduction in 5-HT_2 receptor binding in ovariectomized animals receiving estradiol alone, progesterone alone, or a combination of the two steroids. Neither steroid, alone or together, had any effect on the β-adrenergic receptor response to imipramine.

The time course of the change in β-adrenergic receptor binding was unaltered by ovariectomy, and at no time during the imipramine treatment was there any significant reduction in cerebral cortical 5-HT_2 receptor binding in ovariectomized animals.

Table 2. Effect of hormone replacement on the response of neurotransmitter receptor binding to imipramine in ovariectomized animals. Once-daily hormone treatments were initiated 2 days after ovariectomy and continued throughout the course of the experiment. Imipramine treatment commenced at 7 days after ovariectomy and continued for 21 days. All animals were decapitated 48 hours after the last imipramine injection. Both progesterone (4 mg/kg) and 17β-estradiol (40 μg/kg) were administered subcutaneously. Neurotransmitter receptors were analyzed by ligand binding assays as described in the text. Each value is the mean ± standard error of seven separate experiments, each performed in triplicate. Levels of significance were determined by a two-tailed Student's t-test.

Treatment	Cerebral cortical specific receptor binding (fmole/mg protein)	
	5-HT_2	β-Adrenergic
Saline	26 ± 2	65 ± 4
Imipramine	22 ± 1	47 ± 4*
Estradiol + imipramine	17 ± 2*	44 ± 3*
Progesterone + imipramine	18 ± 2*	43 ± 3*
Estradiol + progesterone + imipramine	18 ± 2*	50 ± 2*

*$P < .02$, compared to saline-treated controls.

These findings suggest that ovarian steroids play a permissive role with regard to the imipramine-induced alteration in cerebral cortical 5-HT_2 binding in rat brain. This effect does not appear to be due simply to a change in the pharmacokinetic properties of imipramine in ovariectomized animals since no effect was noted on the imipramine-induced change in hippocampal 5-HT_2 or in cortical and hippocampal β-adrenergic receptor binding. Rather, the specificity of this action would seem to suggest that ovarian hormones modify some aspect of serotoninergic activity which is crucial for the development of a decrease in cerebral cortical 5-HT_2 receptor binding. Since both estrogen and progesterone are capable of reversing the effect it is conceivable that the response is due primarily to a change in the circulating concentrations of gonadotropins or gonadotropin-releasing factors, rather than to the steroids themselves. It is also possible that the effect is solely an estrogen-mediated phenomenon, since progesterone can be converted to estrogen after systemic administration (*11*). Support for this hypothesis was provided by our finding that the imipramine-induced decrease in cerebral cortical 5-HT_2 receptor binding in male rats can also be prevented by castration. Since it is known that testosterone can be converted to estrogen-like steroids in the central nervous system (*12*), this too may suggest that the effect is mediated primarily by estrogen.

Another possibility might be that ovarian hormones have a direct effect on cerebral cortical serotonin neurons or receptors that is essential for the action of imipramine. Such an effect would not be surprising since specific estrogen receptor sites have been found in various mammalian brain areas, including cerebral cortex (*13*). In addition, administration of ovarian hormones causes an increase in monoamine turnover in selected areas of the rat brain (*14*). Since imipramine acts indirectly to alter neurotransmitter activity by inhibiting transmitter uptake, the neurochemical, and possibly behavioral, effects of imipramine are related to the basal activity of the appropriate monoaminergic system, an activity that is modified by these hormones. However, other interpretations are possible.

Although the mechanism of this hormone-drug interaction is unknown, the phenomenon itself may be important in understanding the pharmacology of antidepressants and the etiology of affective disorders. A significant number of patients fail to respond to treatment with

antidepressant drugs (*15*). Our results suggest that some of these clinical failures may be the result of an alteration in ovarian hormone release or response. Support for this is provided by the recent report indicating that estrogen administration is effective in treating depressed women who have failed to respond to more conventional therapies (*16*). Further studies will be necessary to determine whether this interaction is peculiar for imipramine or also influences the action of other antidepressant drugs.

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Parasitoids as Selective Agents in the Symbiosis Between Lycaenid Butterfly Larvae and Ants

Abstract. *The larvae of* Glaucopsyche lygdamus *(Lepidoptera: Lycaenidae) secrete substances that attract ants. In two field sites in Colorado, tending ants protect caterpillars of* G. lygdamus *from attack by braconid and tachinid parasitoids. This protection may have been an important feature in the evolution of the association between lycaenid larvae and ants.*

The caterpillars of many species in the butterfly family Lycaenidae secrete substances that attract ants. Previous accounts of interactions between these taxa include descriptions of the associations and of the histology of the glands (*1-3*). There have been few experimental inquiries into the behavioral and ecological mechanisms underlying the evolution of this symbiosis (*4, 5*).

We present evidence that tending ants protect the caterpillars of *Glaucopsyche lygdamus* Doubleday from attack by parasitoid insects. This protection may act as a potent selective force in maintaining the symbiosis between these lycaenid larvae and ants.

Typically, a cryptic, grublike larva feeding on flowers is surrounded by a retinue of ants that groom it and palpate it with their antennae (Fig. 1). The seventh abdominal segment of the larva bears a dorsal gland, Newcomer's organ, that oozes a honeydew which the ants harvest (*6, 7*). Recent study suggests that regions of the caterpillar eliciting palpation are covered with epidermal glands which secrete substances that attract and appease ants (*6*). Occasionally, the larva everts a pair of hairlike tentacles from the eighth segment, and these might act as defensive structures when the honeydew gland has been depleted or if the larva is alarmed (*6, 8*).

Parasitoids such as tachinid flies and braconid wasps can attack a caterpillar of *G. lygdamus* during any stage of its development. Once infected, a larva survives until just before or immediately after pupation, at which point the parasitoids emerge and kill their host.

Controversy exists concerning the nature of the advantage of being tended by ants. Some argue that tending ants might ward off potential parasitoids (*1, 2*), while others assert that the larvae produce appeasement substances and honeydew simply to escape from ant predation (*6*). Our observations indicate that, by producing attractive substances, *G. lygdamus* caterpillars secure ant defense against parasitoid attack.

Experiments were performed in two habitats in Gunnison County, Colorado, where *G. lygdamus* utilizes different larval food plants and interacts with different species of ants. The "Gold Basin" site, at 2300 m, about 16 km southwest of Gunnison, is a dry region where the chief woody vegetation is sagebrush with scattered aspen groves. Here, *G. lygdamus* feeds primarily on the developing inflorescences of *Lupinus floribundus* Greene, and is tended primarily by the ant *Formica altipetens* Wheeler. The "Naked Hills" site, at 2900 m in Gothic, is a wet alpine meadow where *G. lygdamus* feeds on the flowers, seed pods, and leaves of *Lupinus bakeri* Greene, and is tended largely by workers of *Formica fusca* Linnaeus (*9*).

In both places, ants began tending the larvae at the third instar, and almost all larvae were tended. At this stage, we excluded ants from an experimental group of caterpillars by coating the lupine stems with a viscous barricade of bird or tree Tanglefoot (Tanglefoot Co., Grand Rapids, Michigan) to prevent ants from ascending to the upper leaves, seed pods, and flowers where the caterpillars were feeding. A halo, 0.5 m in radius, was clipped around the base of each plant to eliminate grass bridges that might provide access to the larvae. Controls were treated in the same manner, except that the Tanglefoot was applied on only one side of the stem so that ants could still reach the larvae. Only infested lupines were used in each area, and plants were designated alternatively control or experimental. A total of 106 lupines at Gold Basin and 46 lupines at Naked Hills were monitored during the experiment. Larval densities were not manipulated. Many larger plants with multiple inflorescences contained more than one larva, but seldom more than three or four (*10*).

Experimental and control caterpillars were censused every third day until they reached the final instar, whereupon they were collected. Only one larva was collected from any single inflorescence. The

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