hence release of catecholamines, adrenergic receptor responses are compensatorily increased, resulting in a higher level of spontaneous behavioral activity. Our observation of a positive correlation between spontaneous motor activity and the responses of the norepinephrine-sensitive cyclic AMP–generating system in midbrain-striatum is consistent with the hypothesis that the relevant adrenergic receptor response is a norepinephrine-elicited accumulation of cyclic AMP in midbrain-striatum. The negative correlation obtained between spontaneous behavioral activity and norepinephrine-induced accumulation of cyclic AMP in cerebral cortex would at first appear to be inconsistent with the above hypothesis. However, stimulation of adrenergic "receptors" in the cerebral cortex, manifested as an increased accumulation of cyclic AMP, might in this brain region be associated with inhibitory (*7*) rather than excitatory pathways. A high "receptor" activity could then result in a lowered level of spontaneous behavioral activity.

Our observation that relatively high accumulations of cyclic AMP elicited in midbrain-striatum by norepinephrine are associated with low levels of tyrosine hydroxylase in this brain region supports the proposal (*4*) that low levels of tyrosine hydroxylase are associated with a relatively high functional response of adrenergic receptors. At present, it is uncertain whether a similar correlation obtains between tyrosine hydroxylase activity and the magnitude of norepinephrine-elicited accumulation of cyclic AMP in cerebral cortex. Comparison of tyrosine hydroxylase activity in midbrain and striatum with responses of the cortical norepinephrine-sensitive cyclic AMP–generating system reveals, however, a negative correlation. One possible explanation is that the enhanced activity of midbrain and striatal receptor systems results in compensatory reductions in responses of cortical systems. Certainly, the magnitude of norepinephrine-elicited accumulations of cyclic AMP in cortical and midbrain and striatal slices have a negative correlation. These interrelations between the activity of cyclic AMP–generating systems in different brain regions and adrenergic function in brain require further investigation.

PHIL SKOLNICK, JOHN W. DALY
National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Maryland 20014

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Dopamine Nerve Terminals in the Rat Limbic Cortex: Aspects of the Dopamine Hypothesis of Schizophrenia

Abstract. *The existence of cortical dopamine nerve terminals is demonstrated with a highly sensitive modification of the Falck-Hillarp fluorescence technique. This confirms previous biochemical reports of high dopamine levels in the cortex. The histochemistry reveals that the distribution is regional and confined to the limbic cortex.*

Increasing interest has been focused on central catecholamines (CA) as a possible biochemical correlate of schizophrenia (*1, 2*). This hypothesis is mainly based on clinical experience with and basic research on certain types of adrenergic drugs (*3*). Thus, amphetamine, which is known to release endogenous CA (*4*), in small doses aggravates the symptoms of schizophrenic patients and in large doses evokes a psychosis clinically indistinguishable from acute paranoid schizophrenia [see references cited by Snyder (*3*)]. It has been proposed by Kety (*5*) that this state may be considered a "model" schizophrenia. On the other hand, a good correlation between the beneficial effects of certain phenothiazines in the treatment of schizophrenia and the effects of these drugs on monoamines in the central nervous system has been observed (*2*). As originally suggested by Carlsson and Lindqvist (*6*), phenothiazines may cause a blockade of central CA, especially dopamine (DA), receptors. Thus, two groups of drugs known to affect transmission at CA synapses in opposite ways seem to have mental effects in man which may be interpreted as antagonistic.

The best correlation, as far as monoamines are concerned, seem to be between DA and schizophrenia (*3*). However, as judged from fluorescence histochemical studies (*7*) and also biochemistry (*8*), DA was until recently assumed to be localized almost exclusively in the basal ganglia, the subcortical limbic system, and the hypothalamus, areas which do not immediately seem related to some of the classical symptoms in schizophrenia. The elegant combined biochemical and lesion experiments of Thierry *et al.* (*9*) demonstrate comparatively high levels of DA in the rat cortex and give convincing evidence that this DA is present in nerves probably not identical with the cortical noradrenaline (NA) neurons described in previous histochemical studies (*10*). These results have prompted us to reinvestigate the existence of cortical DA neurons with some recent, highly sensitive modifications of the Falck-Hillarp technique (*11*) in combination with drug models designed to visualize selectively DA neurons (*12*).

Different cortical brain areas of three groups of male albino rats (Sprague-Dawley, 150 to 200 g) were studied. In the treated groups drugs were administered intraperitoneally, at the specified times before the animals were

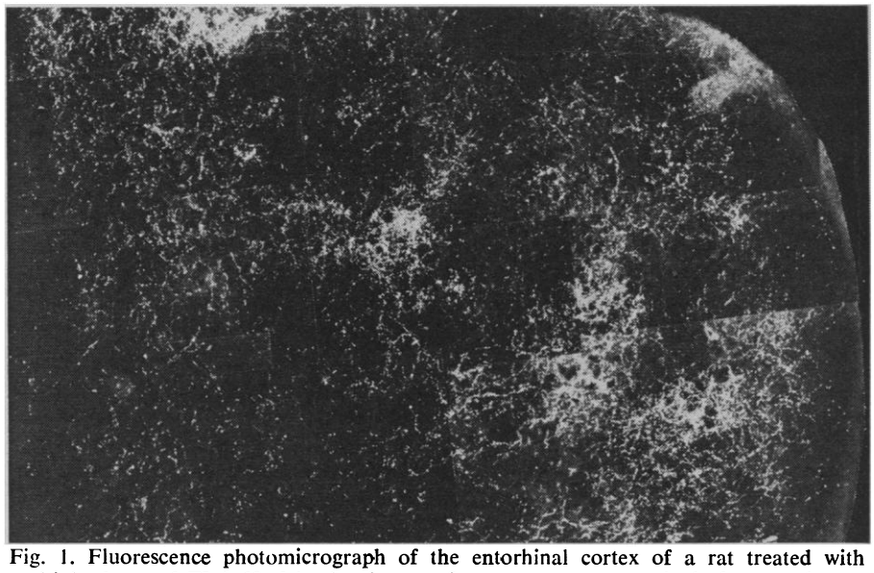

Fig. 1. Fluorescence photomicrograph of the entorhinal cortex of a rat treated with multiple doses of FLA-63. Islands of green fluorescent nerve terminals form a characteristic patchy innervation pattern. It is assumed that the majority of these terminals belong to DA neurons. However, since FLA-63 does not totally deplete NA stores; some NA terminals may also be present (× 30).

killed. The groups were (i) untreated rats; (ii) rats treated with multiple doses of FLA-63 [bis(4-methyl-1-homopiperazinyl-thiocarbonyl)disulfide], a potent dopamine-β-hydroxylase inhibitor (*13*) (25 mg/kg at 24, 12, 8, 4, and 2 hours); and (iii) rats treated with reserpine (10 mg/kg at 18 hours), followed by MK-486 [1-α-hydrazino-β-(3,4-dihydroxyphenyl)propionic acid] (*14*) (100 mg/kg at 90 minutes) and L-dopa (200 mg/kg at 60 minutes), which leads to selective demonstration of DA nerve terminals (*12*). The rats were perfused with 2 percent glyoxylic acid; the brains were dissected out; and sections 20 to 30 μm thick were cut on a Vibratome, dried, exposed to formaldehyde vapors, mounted, and examined in a fluorescence microscope (*11*).

In agreement with earlier work based on the routine Falck-Hillarp technique, extensive networks of green fluorescent nerve terminals of varying density were found in all cortical areas of untreated rats (*10*). These terminals are assumed to belong to NA systems. With the present technique, in addition, hitherto unknown green fluorescent nerve terminal plexuses were observed in widespread areas of the limbic cortex, but not of the neocortex of untreated rats: the gyrus cinguli; the entorhinal cortex; to a minor extent in the prepyriform cortex; the hippocampus; the claustrum; the amygdaloid cortex, especially in the lateral posterior and in the basal lateral nucleus; and, finally, in the most basal layers of the dorsal frontal cortex. Each area had its own characteristic pattern, such as the patchy innervation of the entorhinal cortex (Fig. 1), and this will be described in detail elsewhere (*15*). No green fluorescent cell bodies were observed in any cortical area studied.

The new types of terminals were also observed in the rats treated with reserpine, MK-486, and L-dopa and in those given multiple doses of FLA-63. The terminals found in all cortical regions—that is, those originally described as NA nerve terminals —were not present after the treatment with reserpine, MK-486, and L-dopa and were absent or only weakly fluorescent after the FLA-63 treatment.

The pharmacohistochemical results reported here are interpreted as strongly supporting the existence of DA nerve terminals in the rat cortex, as originally suggested by Thierry *et al.* (*9*). In addition, our findings demonstrate a characteristic regional distribution with the DA plexus confined to the limbic cortex, and show a peculiar patchy innervation pattern in the entorhinal cortex. At least in this strain of rats and with the present sensitivity of the methods, there was no evidence for DA nerve terminals in the neocortex, with the exception of the basal parts of the frontal cortex. Since no CA cell bodies were found in the cortical areas, these DA fibers probably have an extracortical, probably mesencephalic, origin.

The evidence that the newly discovered green fluorescent cortical nerve terminal plexuses indeed represent DA fibers may be summarized as follows. (i) The present technique is more sensitive for CA in general and there is less diffusion, especially of DA, than with the original Falck-Hillarp technique. Thus, the green fluorescent nerve terminals in areas like the neostriatum, which are known to contain high concentrations of DA, appear as distinct dots with the present technique, whereas with the routine Falck-Hillarp technique the fluorescence is diffuse. (ii) The new putative DA terminal plexus displays itself with the same fluorescence intensity and same distribution in rats treated with FLA-63 as in untreated rats. This comparatively potent dopamine-β-hydroxylase inhibitor is known to reduce NA levels below 5 percent of normal levels after repeated doses (*16*). (iii) After the treatment with reserpine, MK-486, and L-dopa, only the putative DA network shows up. This treatment is believed to selectively demonstrate DA neurons (*12*), possibly because there is a reserpine-resistant accumulating mechanism which is much more efficient in the DA granules than in the NA granules (*17*).

The existence of cortical DA nerve terminals mainly confined to the limbic region further supports current hypotheses of a correlation between dopaminergic mechanisms and mood and thought disorders like schizophrenia (*1–3, 5*). Thus, it may be assumed that several symptoms in schizophrenia, such as thought disorder, hallucinations, and impairment of emotional relations and goal-directed behavior, are more likely related to disorders of the cortical areas than of the basal ganglia. In this connection, however, the importance of subcortical DA innervation of the limbic system (tuberculum olfactorium, nucleus accumbens, and nucleus septalis lateralis) should not be underestimated in view of the connections of these areas with the limbic cortex.

The most simple and general explanation for the effects of adrenergic drugs on schizophrenic patients seems to be an increased dopaminergic influence in this disorder. The exact nature of the dopaminergic involvement may be searched for at different levels, for example, as abnormalities in the DA transmission process ranging from presynaptic events to changes in receptor sensitivity (*18*). However, ab-

normalities in DA innervation patterns, such as hyperinnervation of areas already receiving a dopaminergic input or growth of DA terminals into areas previously lacking this type of nerve, should also be considered. In this case, the changes in DA innervation may be secondary to a primary lesion in other pathways leading to plasticity changes, as described by Raisman (*19*).

It is desirable to investigate possible cortical DA innervation in other species, especially man. Nyström *et al.* (*20*) and Olson *et al.* (*21*) have shown that surgical biopsies as well as postmortem brains may be used to study central CA with histochemical techniques. Such studies, with the improved methods we used, might indicate whether tentative cortical DA networks have a similar extent and distribution in normal and schizophrenic humans.

T. HÖKFELT, Å. LJUNGDAHL
K. FUXE, O. JOHANSSON
Department of Histology,
Karolinska Institutet,
104 01 Stockholm, Sweden

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Cleaning Symbiosis Provides a Positive Reinforcer for Fish

Abstract. Chaetodon auriga, *a common marine fish in Hawaii, can be conditioned by presentation of a moving model of a cleaner fish as a positive reinforcement on an instrumental schedule. Reinforcement is probably through tactile stimulation and might help to shape the response of fish to cleaners. Tactile stimulation might serve as a valuable reinforcer in studies of fish learning.*

Cleaning behavior is a symbiotic relationship that is particularly common in marine fish (*1*, *2*). Cleaning organisms (cleaners) remove ectoparasites and other material from host animals. In fish, communication between cleaners and hosts includes recognition and signal movements by both symbionts that favor initiation and maintenance of cleaning interactions (*2*). Cleaners inspect a host by swimming close and occasionally contacting the host's body. Hosts pose for cleaning by assuming an unusual posture or swimming pattern, erecting fins, or changing coloration. Many hosts pose in this manner for artificial models that resemble cleaners such as the wrasses *Labroides dimidiatus* or *L. phthirophagus* (*2*, *3*).

The pose response appears subject to modification through experience. The most compelling evidence is that fish pose for previously neutral stimuli, such as unrealistic fish models and pieces of wire, after presentation of these stimuli has been paired with tactile stimulation (*2*, *4*). Our study indicates that the major mode of modification may be through tactile reinforcement of the host by the cleaner.

Marine butterfly fish (*Chaetodon auriga*) were trapped in Kaneohe Bay, Oahu, and groups of three were placed in 450-liter aquariums. These fish had undoubtedly encountered the cleaner *L. phthirophagus* before capture. A motorized model of *L. phthirophagus* on the end of a thin piece of wire was moved to the center of the aquarium during the reinforcement procedure (Fig. 1), which consisted of moving the model back and forth over a distance of about 5 cm at about 2 hertz for 25 seconds and then returning it to the side of the tank. During an initial screening period, the groups of *C. auriga* were given 37 reinforcements on a 1.2-minute variable interval schedule. Fish that posed for the model during the screening period were isolated and retained for conditioning. During conditioning, the cleaner model was partially concealed in an enclosure at the surface of the water, and a photocell was placed behind and 15 cm below the enclosure (Fig. 1). Behavior was shaped so that the fish would occlude the photo-

Table 1. Results of a three-way analysis of variance of the number of responses per session during instrumental conditioning. Variables tested for differences were individual fish, training versus extinction reinforcement schedules, and the day of the session (two each for training and extinction); interaction terms were also tested. Probability values are not corrected for multiple testing (*5*).

Source of variance	Degrees of freedom	*F*	*P*
Variables			
Training-extinction	1	75.58	$\ll .0005$
Individuals	8	4.95	$< .0005$
Day	1	0.21	.25
Interaction terms			
Day and training-extinction	1	8.37	$< .005$
Individuals and training-extinction	8	2.18	$< .05$
Individuals and day	8	1.00	.5
Individuals and day and training-extinction	8	1.21	.5