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EFFECTS OF AMPHETAMINE AND CHLORPROMAZINE ON SINGLE CELL ACTIVITY IN THE LOCUS COERULEUS

ALLAN W. GRAHAM

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EFFECTS OF AMPHETAMINE AND CHLORPROMAZINE ON SINGLE CELL ACTIVITY IN THE LOCUS COERULEUS

by Allan W. Graham

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To my wife Joan for her patience and support

and

To George Aghajanian for his stimulating guidance

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INTRODUCTION

Most studies of amphetamine's effects on the central nervous system have relied upon relatively indirect observations derived from techniques of biochemical assays (12,24), histochemistry (12,21), and behavioral observations (7,10,38,37). By contrast, this experiment has been designed to investigate amphetamine's action on neuronal activity in a specific pontine nucleus, the locus coeruleus. There were two reasons for selecting the locus coeruleus. First, amphetamine's action on the central nervous system is related to its ability to release and to inhibit the reuptake of dopamine (DA) and norepinephrine (NE) at presynaptic terminals (12,24,25,27). Second, abundant catecholamine (CA) stores have been demonstrated in the cells of the locus coeruleus, group A6 in Dahlström and Fuxe's studies (17,22,34). Coupling these two observations, it seems reasonable to postulate that amphetamine administration will cause the release and decreased reuptake of CA from locus coeruleus cells. Amphetamine, by causing the accumulation of CA postsynaptically, might thereby initiate a neuronal feedback inhibition of locus coeruleus units. A similar type of compensatory feedback mechanism has been postulated by Scandanavian investigators to explain their biochemical observations of the effects of tranquilizers

on central monoamine neurons (3,11,14,39) and by Aghajanian et al (1,2) to explain their observations of LSD's effects on the inhibition of raphe neurons.

To evaluate these hypotheses of amphetamine action, extracellular microelectrode recordings were made from single neurons in the rat locus coeruleus, before and after amphetamine administration.

Following these recordings, chlorpromazine was given to evaluate its anti-amphetamine effects; these effects have been previously described in both clinical (18) and behavioral settings (10,38,37).

METHODS

All investigations were performed using male, Charles River rats weighing 225 - 300 grams.

Fluorescence histochemical studies of the brain were undertaken using the techniques described by Falck et al (6,19), Dahlström and Fuxe (17), and modified by Van Orden (personal communication). Animals were generally pre-treated with pargyline (100 mg./kg. intraperitoneally 24 hours before sacrifice) to enhance fluorescence of mono-amine structures. The impressive fluorescence of parakarya in the locus coeruleus in untreated animals were additionally enhanced by pargyline pre-treatment; however, pargyline was used primarily because it improved visualization of monoamine terminals. Rats were decapitated without anesthesia using a guillotine technique. Brains were removed, sectioned in 1 - 2 mm. slices, mounted on gel foam strips, and quenched in liquid propane-propylene mixture (ratio of 20:1 respectively, melting point -180°C.). Tissue samples, adhering to the gel foam, were transferred to a vacuum descicator jar, precooled to -45°C. and sealed using a rubber ring gasket and Lab Spray^{tm} lubricant. Samples rested on a fine wire screen suspended over 20 grams of powdered phosphorus pentoxide. Vacuum pressures of less than $5x10^{-5}$ torr, monitored by an NRC 851 vacuum ionization

gauge, were produced by a Consolidated Vacuum Corporation diffusion pump (model VMF 10) in tandem with a mechanical vacuum pump. Six days of vacuum drying in the freezer at -45°C. and one day at room temperature were adequate to achieve thorough drying. After releasing the vacuum, the tissue, still adherent to the gel foam strips, was placed in a liter jar with 6 grams paraformaldehyde powder, equilibrated to a relative humidity of 60% (15). The jar was then heated at 80°C. for 1 hour to allow tissue penetration by paraformaldehyde gas. Samples were then vacuum embedded in paraffin, sectioned at 10µ and mounted on slides coated with low fluorescence immersion oil. Viewing was performed through a Zeiss universal microscope with an HBO 200 W mercury lamp (emission peaks 365, 405, and 435 mµ), 2 Zeiss exciter filters BG38 (transmission 300-550 mu) and BG 12 (transmission 330-500mu with peak at 400), and 2 Zeiss barrier filters -65 (transmission 350-650 mu) and 50 (transmission greater than 500 mµ). DA and NE have a characteristic greenish-yellow fluorescence (peak activation 395-405 mµ and peak emission 480-490 mµ). Serotonin (5-HT) has a distinctive yellow appearance (activation 395-405 mµ, emission 510-520 mµ).

Extracellular microelectrode recordings were performed using the techniques described by Aghajanian et al (1). Animals were anesthetized with chloral hydrate (400 mg/kg. intraperitoneally) and mounted in a stereotaxic apparatus.

Stereotaxic coordinates were based on reference to the intersection of the sagittal and occipital sutures as lateral 0 µ and anterior 150 µ. A 3 mm. burr hole was made at coordinates within the range of L. 1000-1300 µ and P. 1700-2200 µ. These coordinates, representing the outer dimensions of the locus coeruleus, were estimated by direct measurement from serial pontine sections of the brain from a Charles River rat, sectioned along the axes of Konig and Klippel (30). Tungsten microelectrodes with tip diameters of 1 µ were lowered through the burr hole using a Kopf hydraulic microdrive. Signals were passed through a negative-capacitance amplifier and displayed on an oscilloscope. Unit rates were followed on an electronic counter whose analog output was traced graphically by a potentiometric recorder. Signals from the oscilloscope (700-200 hz.) also drove an audio monitor.

The locus coeruleus lies 5.8-6.3 mm. below the outer survace of the skull and has a flattened, teardrop shape. Its small size sometimes necessitated as many at 5 penetrations of the brain before the nucleus was entered. Nonetheless, due to the small electrode tip diameter, negligible tissue disruption appeared histologically within the immediate vicinity of the nucleus even after multiple penetrations. Once a locus coeruleus or other unit was located, spontaneous activity was observed for 5-10 minutes. Prerequisites for a unit's inclusion in the study were a stable rate

and a uniform spike amplitude and waveform. Drugs were administered intravenously via the tail in 0.05-0.2 ml. volumes given slowly over 5-10 seconds. d-Amphetamine sulfate and chlorpromazine hydrochloride were given in solution with distilled water in concentrations of 0.25 mg./ml. or 2.5 mg./ml. At the completion of an experiment, the electrode's location was marked by making an anodal electrolytic lesion (2 µAmps for 60 seconds). Animals were perfused through the left ventricle of the heart using a solution of 5% gluteraldehyde in saline. Serial frozen sections were cut at 50 µ and stained with cresyl violet. The location of each recording electrode was precisely identified in this manner for every experimental animal.

RESULTS

Extracellular, single unit recordings were made from 35 rats. Of 10 recordings verified histologically to be in the locus coeruleus, all 10 units showed a marked decrease in spontaneous activity (decreasing to less than 1/3 of the original rate) following small intravenous doses of d-amphetamine. The greatest response to amphetamine occurred with mean doses of 0.2 mg./kg. Higher doses yielded further depression with maximum effect being achieved at total amphetamine levels of 0.5-1.5 mg./kg. (figures 1-3).

25 cells were recorded from regions outside the locus coeruleus; these cells were in the ventral lateral central grey, pontine and mesencephalic reticular formations, and the ventral folia of the cerebellum. 14 cells (56%) showed marked increase in rate (greater than twice the original rate); 4 cells (16%) showed significant slowing (depressed rate 1/2 to 1/3 of original), and 7 cells (28%) demonstrated no significant change in rate. The dramatic slowing response seen in locus coeruleus units following amphetamine administration was unique among the units studied.

Of 7 locus cells treated with chlorpromazine (0.2-1.5 mg./kg.) following amphetamine pre-treatment, 4 showed a prompt return to two-thirds normal activity one to two minutes after intravenous drug administration (total chlorpromaz

0.2-1.0 mg./kg.) and 3 showed small or transient increases in activity (total doses 0.8-1.5 mg./kg.). The rate increasing effects of chlorpromazine were found to be partially reversable by additional amphetamine treatment. However, the dose-response to amphetamine was poorer if chlorpromazine had been given and the depression of firing rate was only transient. (fig. 3).

Neurons of the locus coeruleus, in chloral hydrate anesthetized rats, were found to have a slow rate of 1 to 2.5 spikes per second with a regular rhythm. In the immediate vicinity of the locus (within 500 μ), 3 additional cell types were commonly observed. The first type responded to touch. Some of these cells were sensitive to deep pressure and showed a 2-3 fold increase in spontaneous rate post-stimulus; others were sensitive to light touch and showed transient bursts of discharges immediately after a stimulus. The second cell type was noted to have discharges synchronous with respiration. Several of these cells were found showing bursts of 8-10 spikes preceding each inspiratory movement. Cells of the third type had firing rates which increased and decreased in very regular cyclic patterns (1-0.3 cycles per minute). These cycles were not influenced by tactile, aural, or visual stimuli; but they were markedly disrupted by small doses of amphetamine. These three cell types were a marked contrast to locus coeruleus units, which had no synchronization with respiration

and rarely responded to tactile stimuli (2 units were noted) to show transient post-stimulus suppression following deep pressure applied to the hind toes).

Cresyl violet stained sections were used to identify the location of every cell from which a recording was made. Such identification depended upon microscopic visualization of anodal lesions made through the recording electrodes (fig.4).

Fluorescence histology was performed on the brains of normal and pargyline treated rats; the findings were consistent with the descriptions by Dahlström and Fuxe of their group A6 cells (17). The histological appearance of group A6 is identical to the appearance of the locus coeruleus as visualized on routine cresyl violet stained sections (figures 4 and 5). In the rat, this tightly packed nucleus appears to be composed of homogenous cells characterized as medium sized, oval cells with a fine granular fluorescence evenly distributed throughout the cytoplasm. At the rostral, ventrolateral periphery of the locus, large multipolar cells were found adjacent to the central densely packed core of the nucleus. Fiber projections from the locus coeruleus appear to extend rostrally following a dorso-lateral course in close proximity to the ventral and dorsal surfaces of the superior cerebellar peduncle. There is also a suggestion of medially oriented axonal projections; these are far less striking than their rostral counterparts and are lacking prominent vericosities..

DISCUSSION

From the preceding results it can be concluded that amphetamine in small doses (0.2-0.5 mg./kg.) produces a dramatic decrease in the spontaneous rate of firing in locus coeruleus cells. A definite response to amphetamine is achieved with a dose of 0.2 mg./kg. With this dose spontaneous activity decreases to ½ to ¼ of the original rate; some units even stop firing with this small a dose (fig.2). Additional amphetamine usually produces additional depression of activity with a maximum effect being achieved at levels of 0.5-1.5 mg./kg. Of cells not in the locus coeruleus, 21 (84%) showed either an increase in rate or no change at all following amphetamine treatment. The remaining 4 cells (16%) outside the locus showed a decrease in spontaneous activity even in doses greater than 1.0 mg./kg. However, this decrease never exceeded 2/3 the original activity; in contrast, locus units could be depressed beyond this level. Previous studies in other regions of the brain (9,31) have shown generally excitatory effects of parenterally administered amphetamine. The current results are of interest in that they represent a direct observation of the effects of amphetamine on cellular activity in a CA nucleus. They are of particular interest when compared to a previous study from this laboratory of amphetamine's effects on the serotonin-containing cells of the midbrain raphe nuclei (20). That study demonstrated that amphetamine

(0.5-1.0 mg./kg.) either increased the activity above the spontaneous rate or had no effect at all on raphe cells.

The actual mechanism by which amphetamine causes slowing in cells of the locus coeruleus might be related to direct action on the locus cells or to action at some other central or peripheral site. The work of Glowinski and Axelrod (24,25) suggests that in the central nervous system amphetamine is able to release presynaptically bound NE and to block its reuptake. Carr and Moore's work (13) is consistent with this hypothesis; using the push-pull canula technique, they showed that NE and normetanephrine levels in cerebroventricular effluents increased following amphetamine administration (50 µg/ml). In the locus coeruleus system, following amphetamine treatment, the accumulation of post-synaptic NE might be expected to stimulate post-synaptic activity and produce a neuronal feedback inhibition of the locus units. This concept of neuronal feedback is similar to that suggested by Carlsson and Lindqvist (11). They formulated the concept to explain their observation that chlorpromazine enhances the accumulation of o-methylated CA metabolites after monoamine oxidase inhibition, without affecting the CA levels themselves. They felt that chlorpromazine, by inhibiting the effects of NE, set off a compensatory increase in presynaptic CA activity. Anden et al (3) and Roos (39) proposed the same hypothesis that chlorpromazine produces a feedback

stimulation of CA cells in order to explain their observations that phenothiazine tranquilizers increase levels of dopamine metabolites in the corpus striatum.

The effects of amphetamine on the locus coeruleus certainly could be attributed to a direct release of CA and a compensatory negative feedback. However, several other hypotheses explaining amphetamine's action should be considered. Smith (42) and Bradley (8) have suggested that amphetamine may function in the central nervous system as a directly acting sypathomimetic amine. If such an effect is at work, the results from this experiment would suggest that slowing in locus cells results from either a direct inhibitory response to sympathetic input or an indirect feedback inhibition from a post-synaptic neuronal circuit. α-methyl tyrosine pretreatment may be of use in distinguishing between the direct and indirect sympathomimetic actions. Another mode of amphetamine action might be related to an induced alteration of neuronal input to the locus coeruleus. Changes in sensory input flow might very well result in alterations in the depolarization threshold of locus units. One type of altered input might be related to the peripheral adrenergic effects of amphetamine; however, Foote et al (20) found that these effects after low doses of amphetamine (0.2-1.0 mg./kg.) were negligible. Finally, some unique action of amphetamine on locus coeruleus cells is a possibility which cannot be entirely ruled out.

The responses to chlorpromazine administration were less uniform than those to amphetamine. In 4 of 7 locus coeruleus cells in which chlorpromazine (0.2-1.0 mg./kg.) was given, a prompt and nearly complete reversal of amphetamine slowing was observed. This response was achieved at a dose of 0.2-0.4 mg./kg. Additional increases in rate were not observed with total doses exceeding 1.0 mg,/kg. It is noteworthy that these responses to chlorpromazine were transiently and only partially reversable with additional doses of amphetamine. The remaining 3 of 7 locus cells showed only small or transient increases in rates after chlorpromazine (0.5-2.0 mg./kg.). It is not clear why there was a variable response to chlorpromazine; further studies are needed to elucidate this matter. It also remains to be seen what effect chlorpromazine has on spontaneous activity of locus cells in the absence of amphetamine.

The doses of amphetamine and chlorpromazine used in these experiements were considerably lower than those used by most other investigators. In fact, the usual dose of amphetamine (0.2 mg./kg.) has been shown to be too small to produce the peripheral sympathetic effect of increasing blood pressure (20). Other investigators, most of them using behavioral models, have given amphetamine in doses from 1.0-4.0 or 8.5-15.0 mg./kg. (10,27,32,37,38,42); they all stated that their largest doses of amphetamine

produced more pronounced behavioral changes than their smaller doses. The anti-amphetamine effects of chlorpromazine reported by these investigators have been observed following doses ranging from 0.5-28.8 mg/kg. The current experiment showed that at doses less than 1.0 mg/kg, chlorpromazine had definite anti-amphetamine effects on 4 of 7 locus cells and that doses exceeding 1.0 mg/kg produced no greater change in rate. It is not known whether the CA systems that might be affected in behavioral experiments are involved with the locus coeruleus.

It is obvious that data collected on cellular responses to pharmacologic agents is only meaningful in so far as histological identification can be performed on the cells under investigation. It was found that electro-lytic lesions 5 μA for 60 seconds) made through the recording electrodes assured definitive localizations. As seen in figure 4, very precise localizations can be determined in this manner. The results revealed that cells of the locus coeruleus have a characteristic and distinctive rhythm which is slow and regular at 1-2 spikes/second. This rhythm was not affected by a variety of ourside stimuli (touch, noise, or light) nor by intrinsic muscular movements. One exception to this autonomy of rhythm was observed in two locus cells; both were sensitive to toe pinch and showed an initial post-stimulus burst of 304 spikes, followed by a transicnt suppression of

locus coeruleus activity lasting 2-3 seconds. Both of these cells were found in the caudal tip of the nucleus. By contrast, neighboring cells (within 100-500 μ of the nucleus) often showed a variety of responses associated with tactile stimulations, respiratory movements, or internal cyclic rhythms of unknown etiology. These neighboring cells had characteristic locations relative to the locus coeruleus which made them useful indicators of electrode orientation; this information made it possible to reorient the recording electrodes intelligently if the initial penetration failed to pass into the nucleus.

The results of fluorescence histology confirmed that Group A6 (17) correlated precisely with the locus coeruleus as visualized on cresyl violet stained sections. These observations were consistent with Dahlström's descriptions of cellular morphology. With regard to projections from the nucleus, the fluorescence observations on transverse and sagittal sections of normal brains were perhaps as misleading as they were informative. Microscopic visualizations of structures using fluorescence technique requires relatively high concentrations of localized monoamines. Generally, only cell bodies and synaptic terminals possess adequate concentrations of monoamine to be visualized. Therefore, fiber bundles will tend to be visualized only in regions where the mass of fibers or concentration of amines in the bundle is particularly great. Bearing these conditions

in mind, the current observations suggested that the locus had abundant rostral projections which lay in close proximity to the superior cerebellar peduncle. Numerous fluorescent varicosities as well as fluorescent fibers were easily visualized. These observations were consistent with descriptions by Anden et al (4) and Loizou (33) of an ascending CA fiber tract from cell bodies in the medulla oblongata and pons (A1,A5,A6,A7). Loizou (34) also suggested that fibers from the locus coeruleus extend toward the dorsal motor nucleus of the vagus nerve and the commissure tractus solitaris. The current work revealed a limited number of cells which had caudal projections; these cells were located along the caudal, ventral edge of the locus and appeared to direct their axons in a medial-ventral-caudal orientation. There were no prominent fiber bundles or terminal varicosities associated with these projections. Loizou's conclusions concerning caudal projections were based on observations following electrolytic lesions in the vicinity of the locus coeruleus; unfortunately, he failed to include diagrams or photographs of these lesions.

Ultimately, the effects of amphetamine on the locus coeruleus depend upon the patterns of neuronal connections within which the locus participates. Analysis of this pattern will lead to a clearer definition of the function of this neuronal system and of the effects of amphetamine upon it.

APPENDIX I. INPUTS AND PROJECTIONS

Classical studies have suggested a variety of afferent inputs to the locus coeruleus and to areas adjacent to the nucleus. Russell (41) reviewed the literature concerning those inputs in 1955. He noted that Cajal (1909) had described an "extensive, diffuse interstitial plexus" of afferent fibers lying in the periventricular grey and adjacent to the tegmental reticular formation, extending from the mesencephalic trigeminal nucleus to the vagal nuclei. Willems (1911) described colaterals from the commissure of the lateral lemniscus going to the locus coeruleus. Lorente de No' (1922) felt that the locus received afferents from the ipsilateral mesencephalic and superior sensory nuclei of the trigeminal. Crosby and Woodburne (1951) described afferents from the hypothalmus extending to the locus coeruleus via the dorsal longitudinal fasciculi. At the electron microscopic level Mizumo and Nakamura (36) demonstrated that modest amounts of axodendritic presynaptic terminal degeneration occurs following electrolytic lesions in the supramamillary area or posterior hypothalmus. Additional inputs to the area neighboring the locus have been observed coming from the lateral spinothalamic tract (pain and temperature), the anterior spinothalamic tract (light touch), and the ascending spinoreticular fibers (described by O'Leary (1958)).

My findings regarding afferent input suggest that many cells neighboring the locus are involved with sensory and respiratory functions.

In classical degeneration studies two different projections from the locus coeruleus have been suggested. The bundle of Probst is described by Truex and Carpenter (43) as the major efferent projection from the locus. Its course descends through the dorsolateral reticular formation and terminates on the nucleus intercalatus and dorsal motor nucleus of the vagus. Russell (41),however, did not find convincing evidence that the bundle of Probst always degenerates following electrolytic lesions in the locus coeruleus. He asserted that the principal efferent projection from the locus is the "lateral tegmento-reticular tract" (29) which is a lateral or ventrolateral tract terminating in the ipsilateral ventromedial medullary reticular formation, just above the inferior olivary nucleus.

Recent degeneration studies using fluorescence histochemical techniques,instead of classical staining (eg. Marchi or Nauta), may be more reliable for evaluating the efferent projections from monoamine nuclei. In these studies changes in fluorescence occur if monamine nuclei or fiber tracts are damaged by the experimental lesions.

Anden et al (4) demonstrated the existence of an ascending CA fiber tract beginning with cell bodies in the medulla oblongata and pons (groups A1,A5,A6,A7) and extending uncrossed via the medial forebrain bundle to the

limbic forebrain, the neocortex, and the hypothalmus. Their conclusions were based on degenerative changes observed following electrolytic lesions in the caudal, lateral mesencephalic tegmentum. They observed retrograde swelling, atrophy, and chromatolysis in the cells of groups A1, A5-7. They also measured a 75% decrease in NE levels in the diencephalon and telencephalon following the mesencephalic lesions. Loizou (33) confirmed the findings of Anden et al and stated that the tract traversed the dorsal reticular formation of the medulla and pons, shifted dorsomedially through the mesencephalic tegmentum so as to lie dorsal and ventral to the superior cerebellar peduncle (fig. 6), emerged dorsal to Forel's Field H1, and subsequently divided in two, so as to send some fibers ventromedially to the medial hypothalmic nuclei and other fibers ventrolaterally to the medial forebrain bundle. In further studies, Loizou (34) suggested that lytic lesions in the locus coeruleus decrease the number and intensity of fluorescent terminals in the mesencephalic central grey, the dorsal raphe nucleus, and the nucleus of Edinger-Westphal and in the dorsal motor nucleus of the vagus and the commissure tractus solitaris. Unfortunately, Loizou included no photographs nor detailed descriptions of the precise extent of his lesions. My findings were consistent with his descriptions of the rostral projections of the nucleus, but I was unable to appreciate prominent caudal projections.

Recent studies by Arbuthnott et al (5) suggest that the locus coeruleus contributes heavily to the ascending CA fiber bundle passing just lateral to the dorsal central grey of the mesencephalon. They propose that this bundle terminates in the ipsilateral cortex cerebri and hippocampal formation.

APPENDIX II. MONOAMINE CONTENT

There is some controversy as to the amine content of the cells in the locus coeruleus. Using conventional fluorescence microscopy, one cannot distinguish between the fluorescence of NE and DA in cellular structures. Dahlstrom and Fuxe (17) suggested that to circumvent this limitation, the differential depletion effects of α-methyl meta-tyrosine might be useful for studying CA terminals. They explicitly described the failure of this technique to differentiate the CA's in cell bodies. Bjorkland et al (6) described a relatively complicated microspectroflorometric technique for distinguishing NE from DA. Recently, Corrodi et al (15,16) described a new technique utilizing a dopamine-β-hydroxylase inhibitor (FLA 63). They showed that FLA 63 produced a marked depletion in the fluorescence of the locus coeruleus and concluded that NE must be primarily responsible for the florescence in locus cells.

Another technique for attempting to distinguish DA from NE in the locus coeruleus is based on biochemical assays. These assays are generally performed on regional homogenates of brain tissue (17,35). The assays have shown very low dopamine levels in the medulla and pons, higher levels in the mesencephalon, and very high levels in selected areas of the diencephalon (substantia nigra and neostriatum). Obviously, standard biochemical assays

are too gross a method for differentiating catecholamines in specific nuclei. Gerardy et al (22) devised a more discrete technique for studying monoamine content of small regions of the brain. By using thin layer chromatography, they were able to demonstrate substantially higher levels of dihydroxyphenylacetic acid and DA than of NE in the locus coeruleus of cow and rabbit. Certainly, their results are suggestive that the locus is principally a DA containing nucleus. Such a result is plausable since one might well expect a significant biochemical similarity between the two pigmented nuclei of the human brain, the locus coeruleus and the substantia nigra (a DA containing nucleus).

APPENDIX III. FUNCTION

The function of the locus coeruleus is still very much a matter of controversy. Johnson and Russell (29) originally hypothesized that the nucleus was part of the pneumotaxic center; later Russell (41) suggested that it might be a general regulator of vegetative functions. Their conclusions were based on observations following large electrolytic lesions involving many structures surrounding and including the locus. As the current study indicates, there are numerous cells in close proximity to the locus that are clearly associated with repiratory functions but which are not part of the locus coeruleus. Any lesions presumed to involve only the locus must be very precise and well defined in order to be of significance.

Roussel et al (40) hypothesize that the nucleus functions as a trigger mechanism for paradoxical sleep. Their conclusions are based on observations of paradoxical sleep and NE levels in the telecephalon and mesencephalon following lytic lesions in the locus or in neighboring structures. They report a significant reduction in paradoxical sleep and in brain NE levels only when bilateral lesions were made in the locus coeruleus. Their published results include schematic diagrams rather than photographs or precise descriptions of the extent of their lesions.

Another hypothesis of function has been raised indirectly by neuropathologists who have long associated Parkinson's disease with pathologic changes in both the locus coeruleus and the substantia nigra. Hartog Jager (28) stated that intracytoplasmic structures, Lewy bodies, are present in the substantia nigra and/or the locus coeruleus in over 90% of patients with idiopathic Parkinson's disease; Lewy bodies are rarely found in patients with postencephalitic Parkinsonism. The composition of these bodies is poorly understood and their significance is unknown.

My results offer no substantial contribution to the controversy of the locus coeruleus' function; however, the results do show that distinct pharmacological and functional differences exist between cells of the locus coeruleus and its neighboring neurons.

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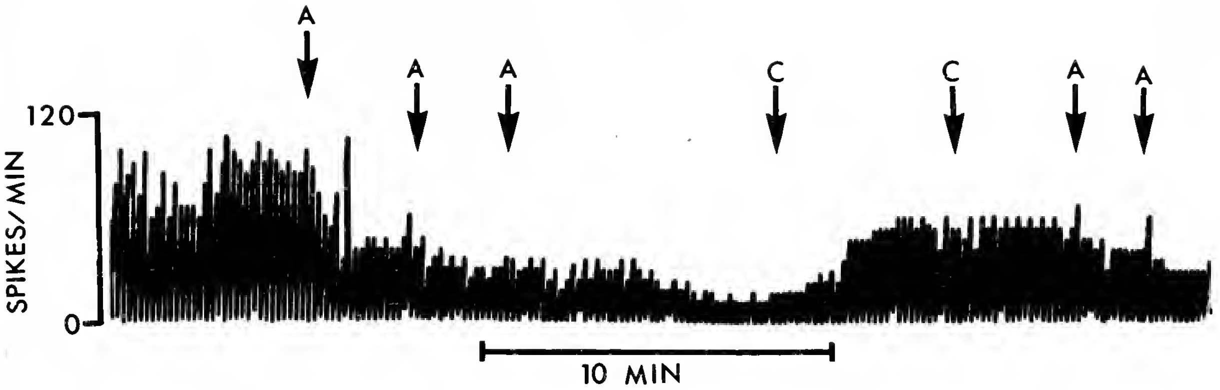

Fig. 1 Typical response of a locus coeruleus unit to amphetamine (A) and chlorpromazine (C). Spontaneous rate was 90 spikes/minute. The record consists of the number of spikes counted over consecutive 10 second intervals. Within 30 seconds after the i.v. injection of amphetamine (0.2 mg./kg.), a marked decrease in rate was observed. Two subsequent doses of amphetamine (0.4,1.0) produced additional slowing (to 12 spikes/min.). A recovery of rate to 2/3 the original was observed within 1 min. after i.v. chlorpromazine (1.0 mg./kg.). Additional chlorpromazine (1.0) had no additional effect. Subsequent doses of amphetamine (0.5,1.0) were less effective in decreasing the rate than in the untreated animal.

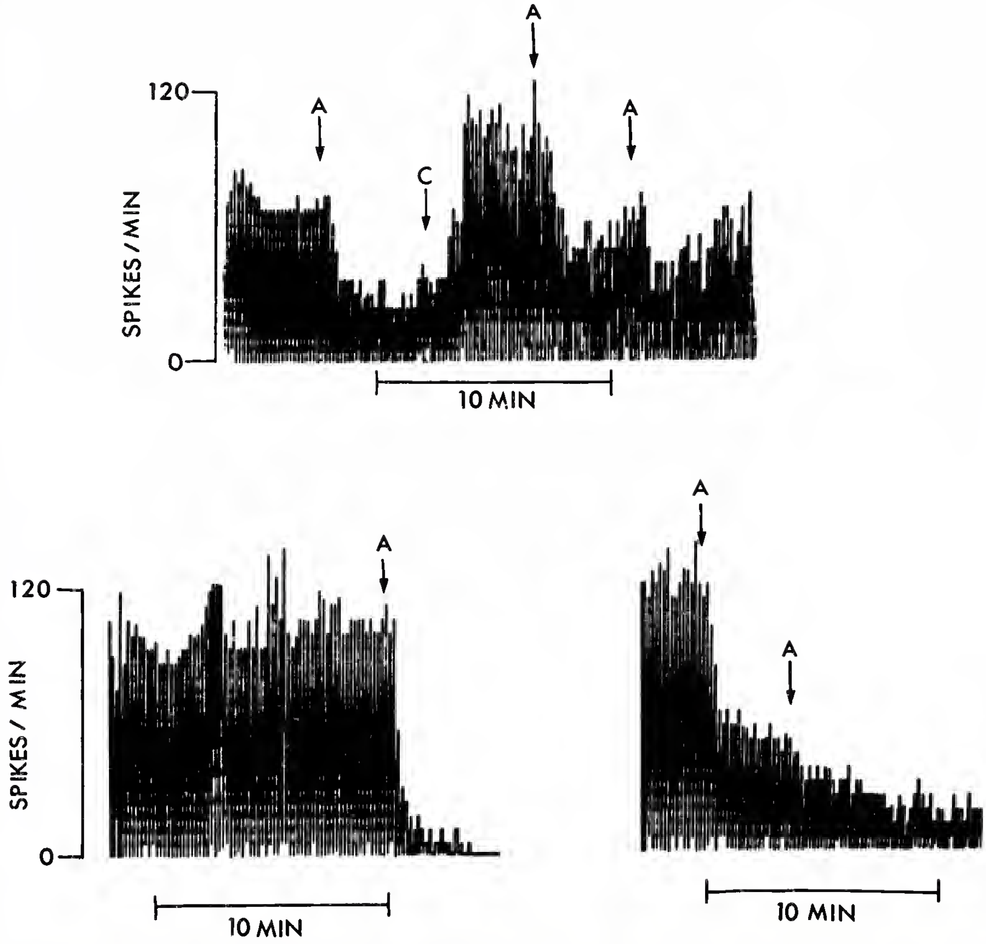

Fig. 2 Responses to amphetamine and chlorpromazine in three different locus coeruleus units.

Top. Spontaneous rate 70/min. The marked decrease in rate following 0.2 mg./kg. of amphetamine was promptly reversed by chlorpromazine (0.2 mg./kg.). The increase of rate to greater than the original was a consequence of chlorpromazine's activating a second locus unit which was not previously being recorded. Subsequent amphetamine (0.2,0.2) was less effective in suppressing the rate , and its effects were only transient.

Bottom. Two locus coeruleus units showing dramatic decreases in their rates following small doses of amphetamine (left, 0.2 mg./kg.; right, 0.2, 0.2 mg./kg.). This response was unique to cells in the locus.

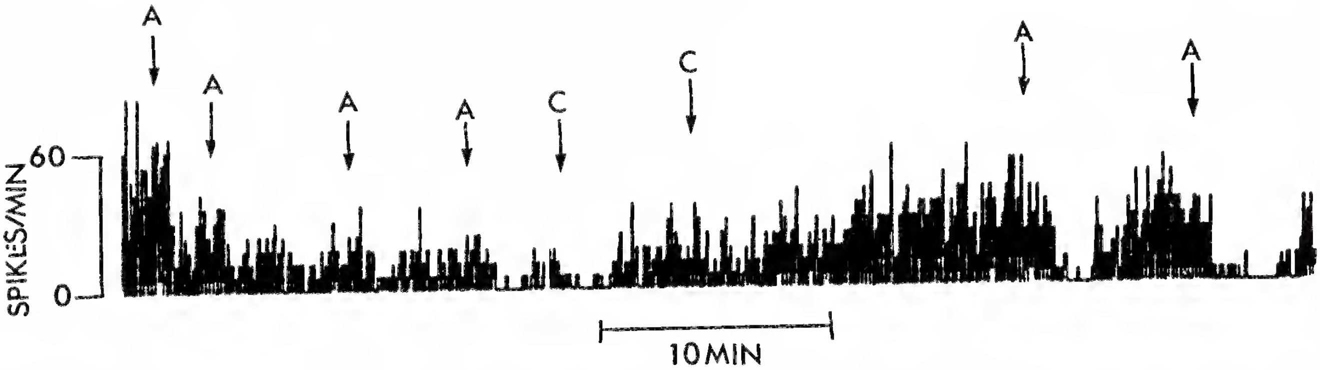

Fig. 3 Locus coeruleus unit showing decreased activity following amphetamine (0.1, 0.2, 0.2, 0.5 mg./kg.), partial reversal of amphetamine suppression following chlorpromazine (0.25, 0.5), and finally transient suppression after further doses of amphetamine (1.0, 1.0).

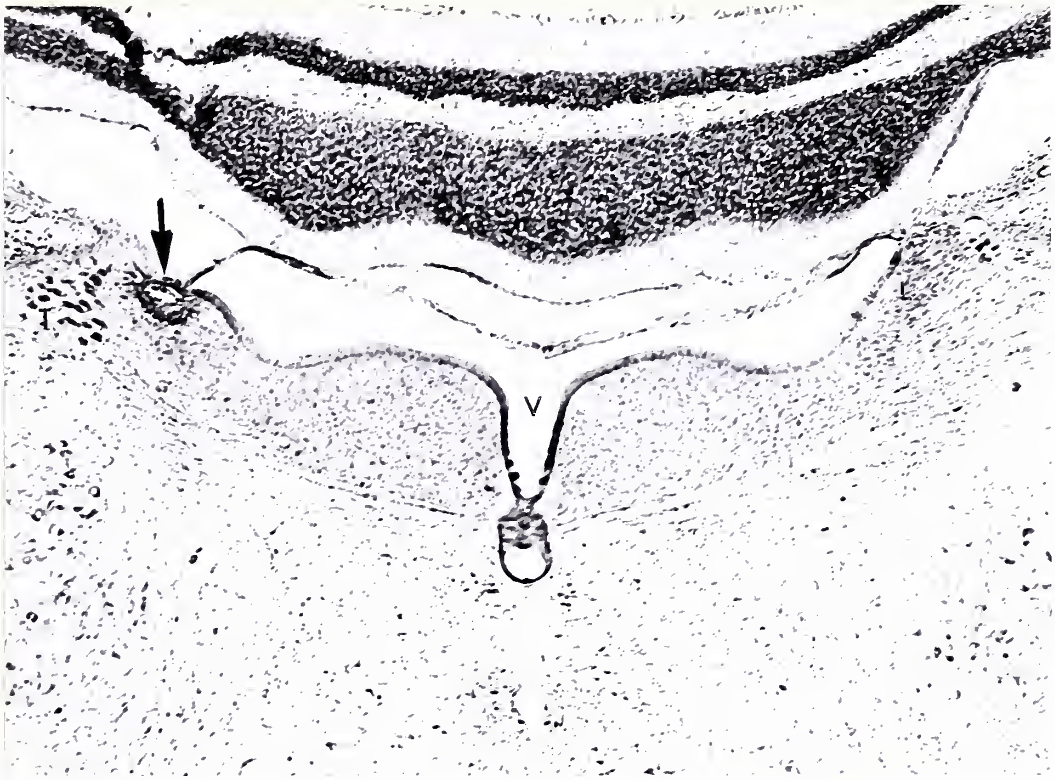

Fig. 4 Transverse histologic section showing an electrolytic lesion (5 uA, 60 sec.) in the locus coeruleus on the left (arrow) and a normal locus on the right (L). V, fourth ventricle; T, mesencephalic nucleus of the trigeminal nerve. Cresyl violet staining. X35.

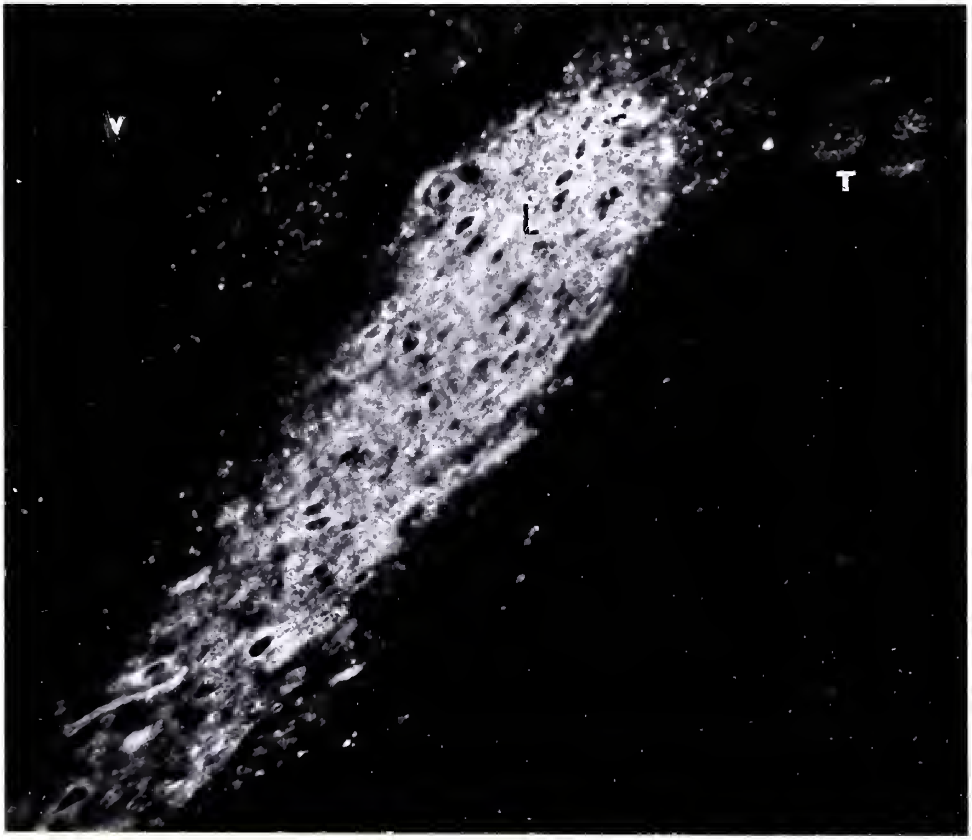

Fig. 5 Fluorescence micrograph of locus coeruleus (L) in normal rat brain. The locus parallels the floor of the fourth ventricle (V). Three autofluorescent cells of the mesencephalic nucleus of V (T) are seen adjoining the nucleus along its dorsal lateral border. X250.

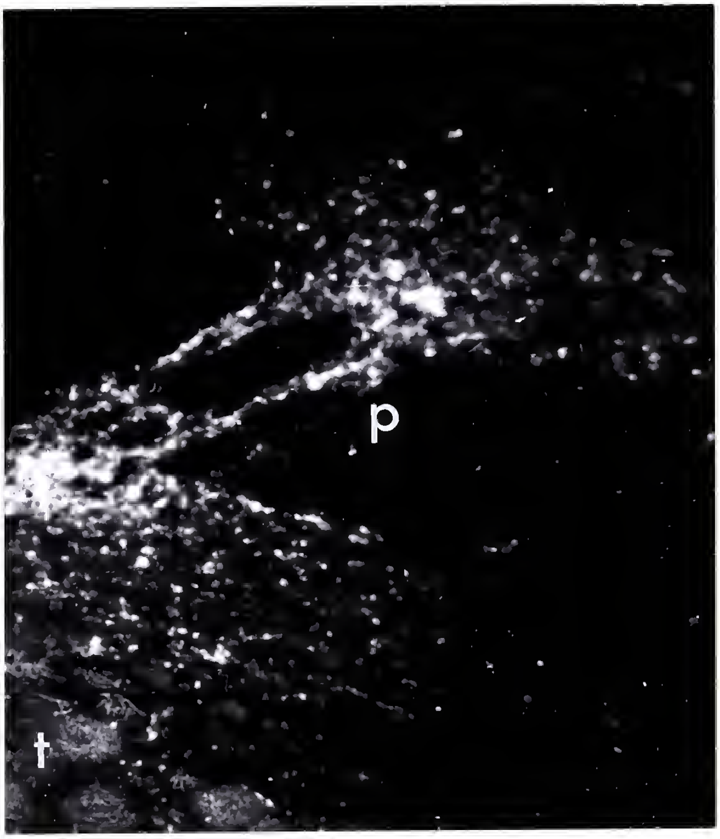

Fig. 6 Fluorescent fibers and varicosities from the locus coeruleus passing both dorsal and ventral to the superior cerebellar peduncle (P). Autofluorescent cells of the mesencephalic nucleus of V (T) are seen to lie ventral to the peduncle.

Fig. 7, 8, and 9 Fluorescent photomicrographs of transverse sections through the locus coeruleus arranged in anterior to posterior sequence. X250.

Fig. 10, 11, 12, and 13 Fluorescent photomicrographs of sagittal sections through the locus coeruleus arranged in medial to lateral sequence. X250.

Fig. 7

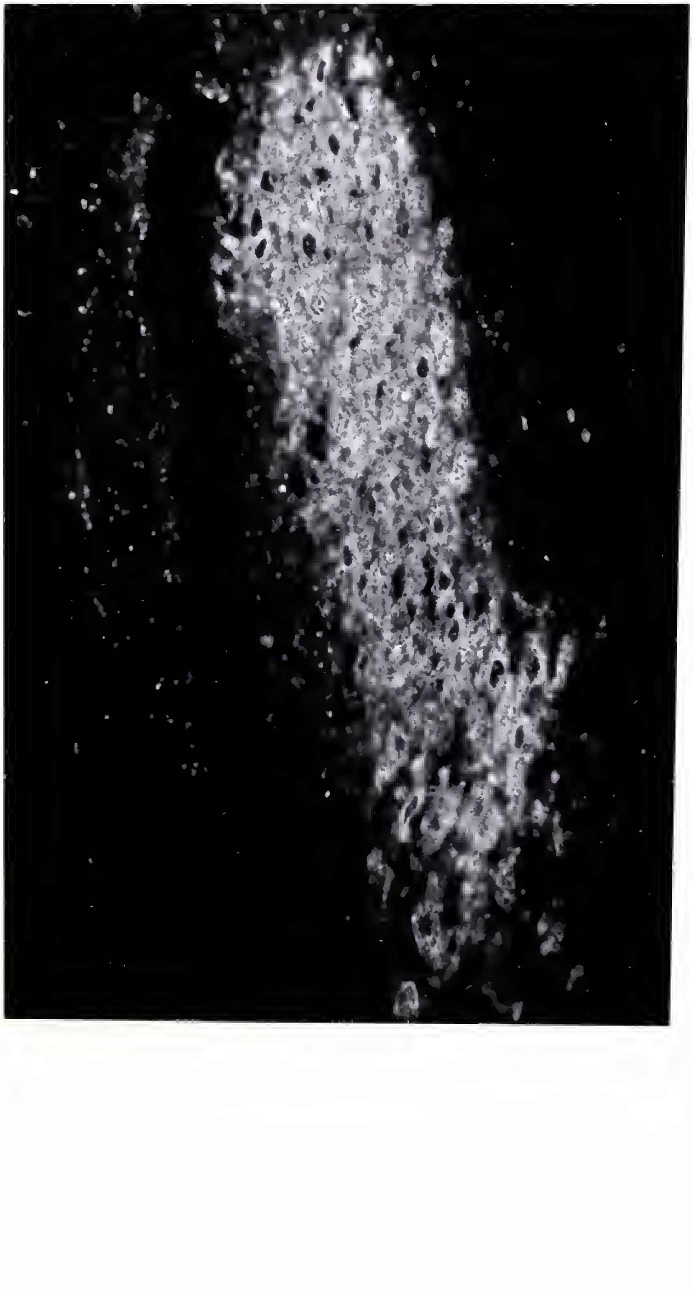

Fig. 8

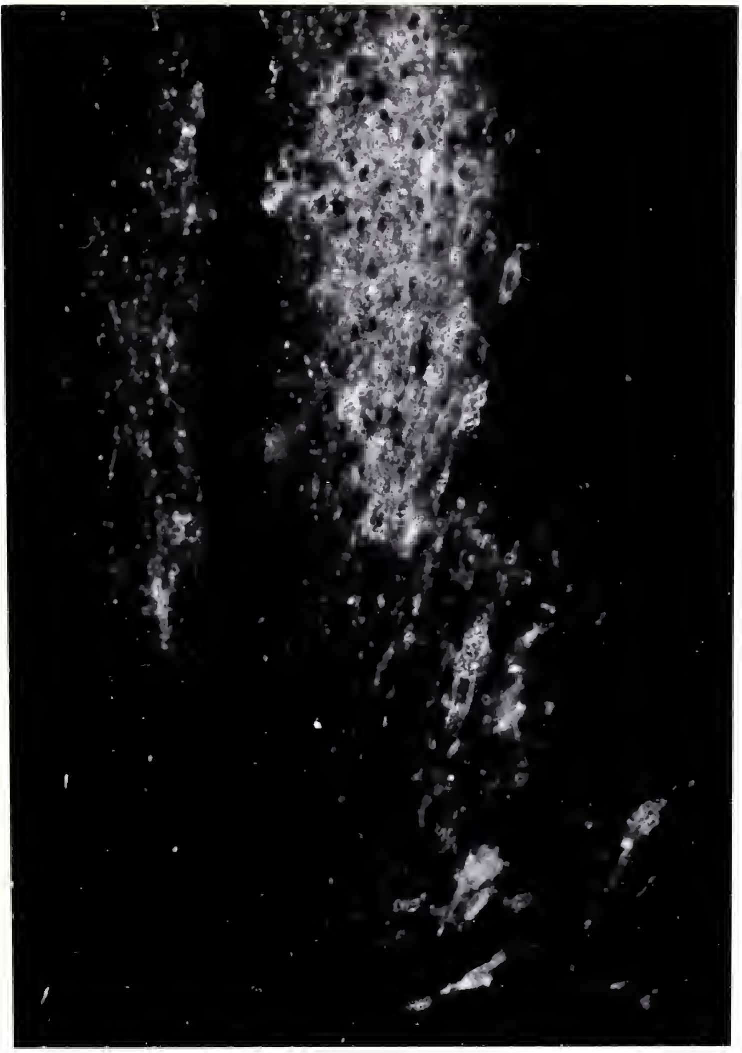

Fig. 9

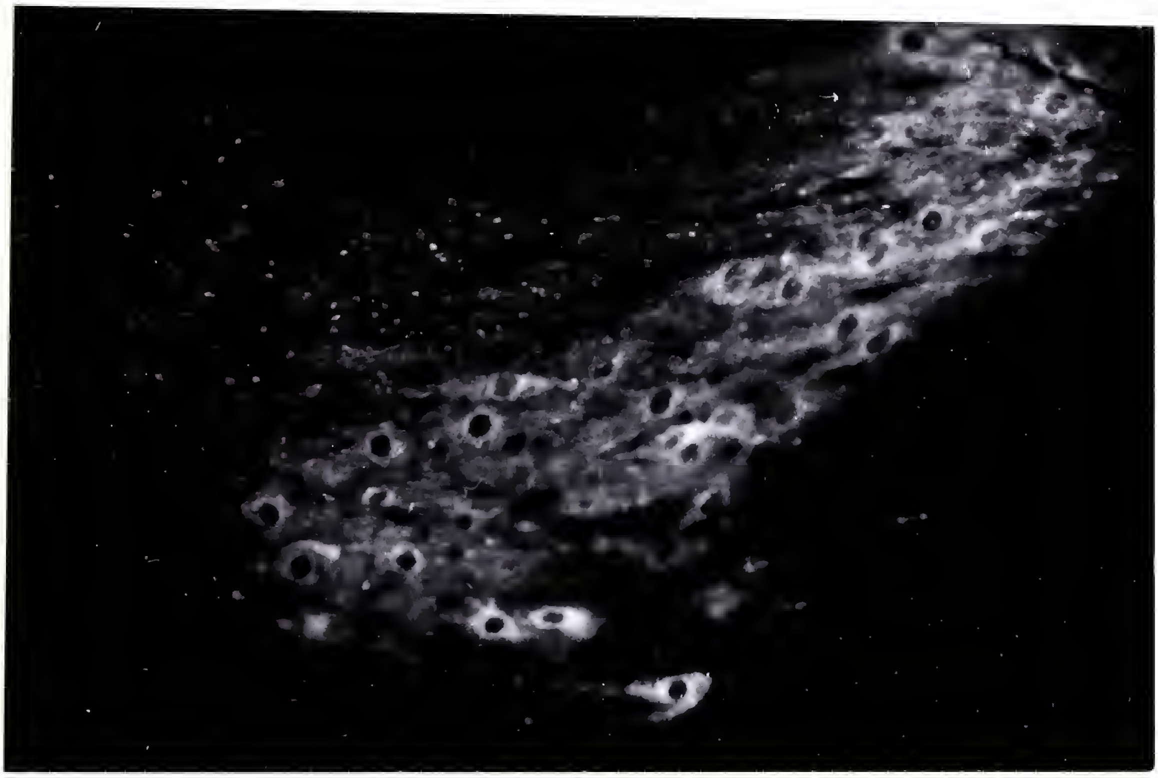

Fig. 10

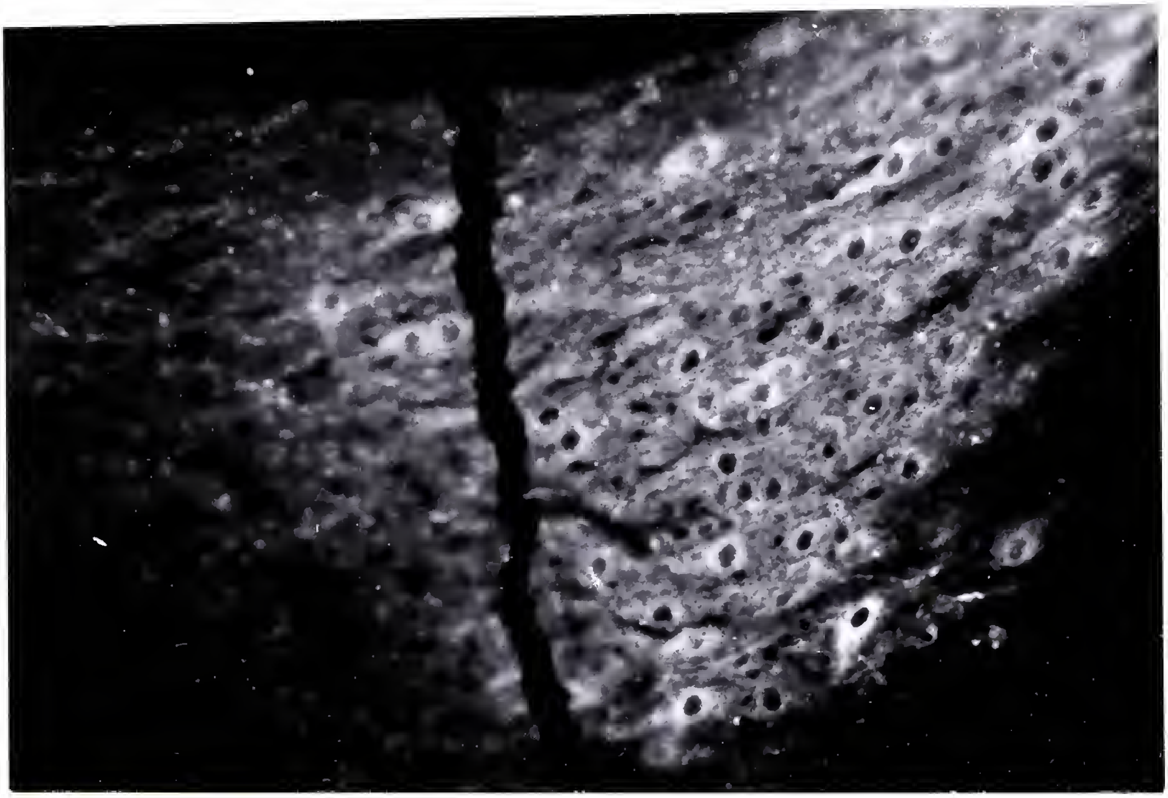

Fig. 11

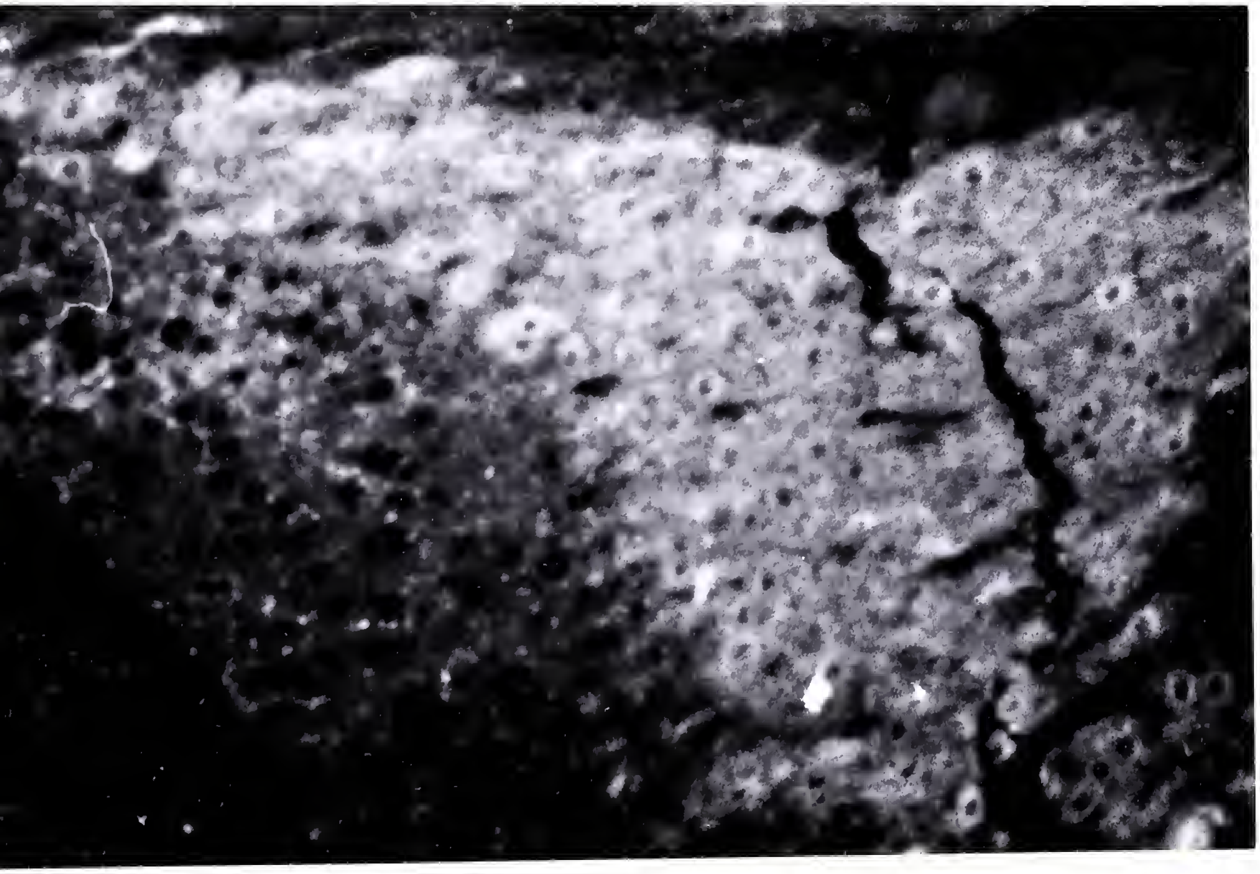

Fig. 12

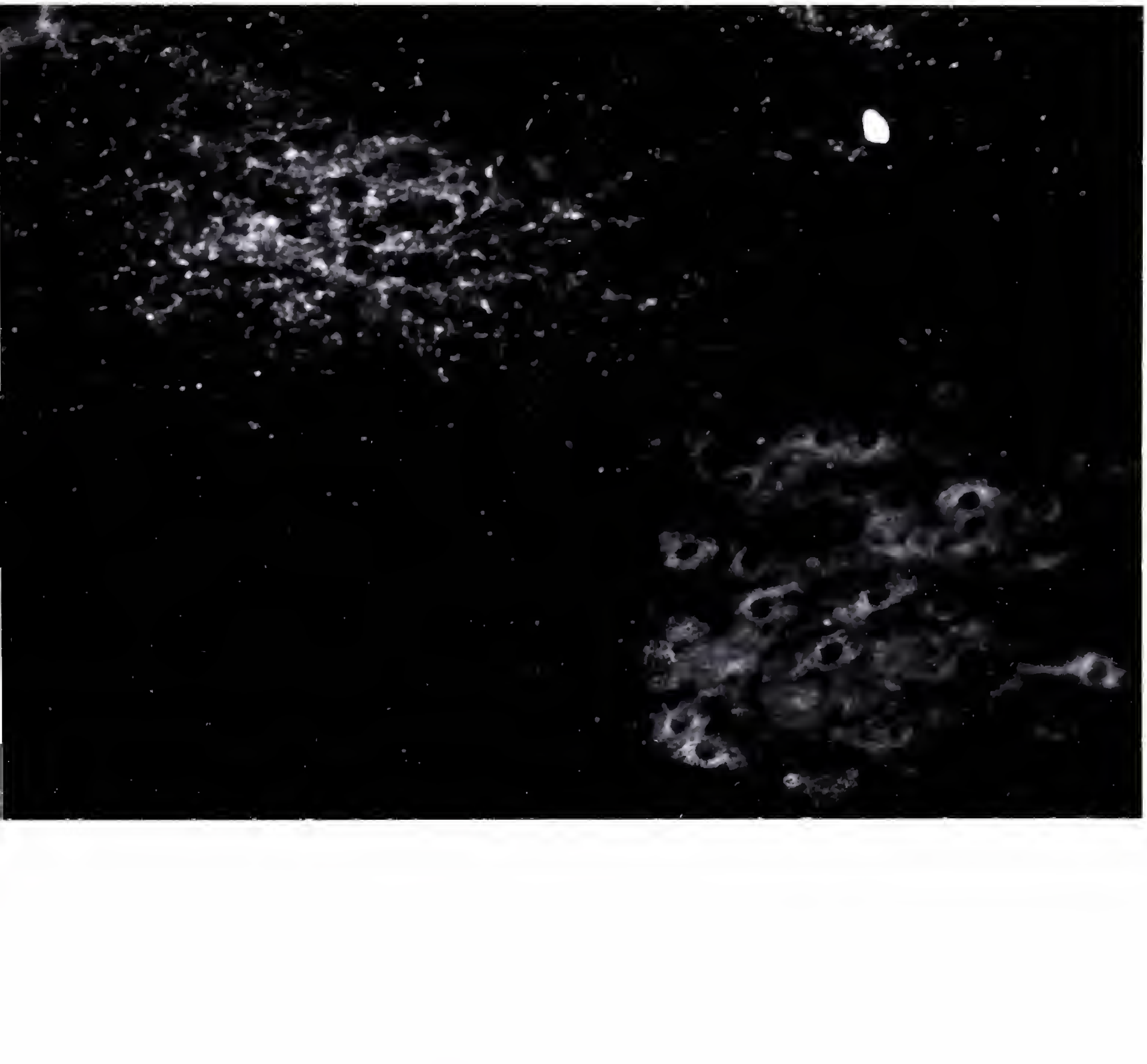

Fig. 13