DEPLETION OF BIOGENIC AMINES AND ENHANCEMENT OF CHOLINERGIC ACTIVITY IN THE OLFACTORY BULB AND CENTRAL OLFACTORY CONNECTIONS WITH CHRONIC METHEDRINE INTOXICATION

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Macrosmatic animals under the influence of chronic doses of amphetamine develop stereotyped patterns of sniffing, looking, gnawing and grooming. The most prominent stereotopy in cast is repetitive sniffing. The purpose of this report is to present evidence of histochemical changes in the olfactory system of cats, which had developed a marked sniffing stereotopy with prolonged methedrine treatment.

MATERIAL AND METHODS

A group of 12 cats weighing 2 to 3 kg. was studied. The animals were injected twice daily with increasing doses of methedrine 7.5-40 mg/kg for 10 days. The whole brain was dissected from the animal under a light pentobarbital anesthesia. Selected blocks of fresh tissue were immediately frozen in liquid nitrogen. The tissue was then processed histochemically to develop biogenic amine fluorescence and the cholinergic reaction, using procedures of Falck and Hillarp [6] and Karnovsky and Roots [7] as well as our modification of both histochemical techniques (Duarte-Escalante et al. [3, 4]).

RESULTS

In the normal adult cat, the cytoarchitectony of the olfactory bulb resembles the pattern of other mammals such as the rabbit and guinea pig. A few differences are observed in the increased number of afferent fibers ending at the olfactory glomeruli and in the fibers connecting the mitral, tufted and granular cells which appear to be multibranched in the cat. Using the fluorescent technique, adrenergic varicosities of medium to large size (1-2 microns) are observed around

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the glomeruli while small varicosities (0.5 to 1 micron) are observed around the mitral and granular cells. Small varicosities are observed in the tuberculum olfactorium as well. Mitral and granular cells are generally non-fluorescent, but we observed a group of a few mitral cells in the vicinity of the cribiform plate which had developed a weak to medium intensity of greenish-yellow fluorescence (Fig. 1). Small varicosities course along the short fibers directed to and from the glomeruli and the mitral cells, as well as to and from the mitral and granular cells. No long adrenergic fibers have been observed within the olfactory bulb. The acetylcholinesterase reaction is more intense in the tufted cells than the granular cells. The mitral cells appear to be less cholinergic than either the granular or the tufted cells. Both short and long cholinergic peripheral fibers appear to end around the glomeruli (Fig. 3). Within the bulb, short varicose fibers form a plexiform structure connecting the glomeruli to the mitral and granular cells. A long type of fiber extends from the tufted and mitral cells to course along the glial layer where they give off collaterals to the granular cells (Fig. 4). These long fibers were noted to be a continuation of the lateral and medial olfactory tracts.

In the cats given a single dose 7.5 mg/kg of methedrine, most of the fluorescent varicosities around the olfactory glomeruli, as well as those around the mitral and granular cells, disappear; no significant changes are observed in the fluorescence of mitral cells. In a similar manner, most of the fluorescent varicosities at the ventral and medial areas of amygdaloid complex and the ventral area of the septum disappear with the single large dose of methedrine. The cholinergic activity does not show any significant change either in the olfactory bulb or in the central olfactory structures.

In the material from chronically intoxicated animals, on the other hand, all the fluorescent varicosities within the olfactory bulb and the tuberculum olfactorium disappear. The fluorescence from the mitral cells disappears completely as well. In the same way, the fluorescence of all the adrenergic varicosities in the ventromedial areas of the amygdaloid complex and septal area disappear completely. In 5 animals, the appearance of small fairly intense yellowish varicosities (serotonin content) around the glial layer, as well as around the internal granular layer, was observed (Fig. 2). Only very faint yellowish fluorescence was noted in the glial layer in normal controls. In the material from chronic intoxicated cats there was a marked increase in cholinergic activity within the olfactory bulb and other central nervous structures. The cholinergic activity appears especially intense at the short branched plexus extending between the granular, mitral and tufted cells, as well as in the mitral cells themselves (Fig. 4). In addition, preliminary observations reveal that the long cholinergic fibers coursing the lateral and medial olfactory tracts also develop a more active cholinergic activity, including the group of fibers directed to the amygdaloid complex and the septum. Although following chronic intoxication some neurons from the reticular system of medulla oblongata underwent pronounced chromatolysis these changes were not noted in neurons in the anterior olfactory forebrain.

DISCUSSION AND CONCLUSION

From our results, as well as the reports of Salmoiraghi et al.[8] and Dahlström et al.[2], it is evident that monoamines and acetylcholine are present within the olfactory bulb. The reports of Von Bumgarten et al.[9], Salmoiraghi et al.[8] and Bloom et al.[1] have demonstrated the functional significance of the olfactory bulb neurons to the locally applied serotonin, acetylcholine (facilitory) and noradrenaline (inhibitory). Other studies suggest that the majority of the fluorescent (adrenergic) varicosities within the tuberculum olfactorium and olfactory bulb belong to the efferent group

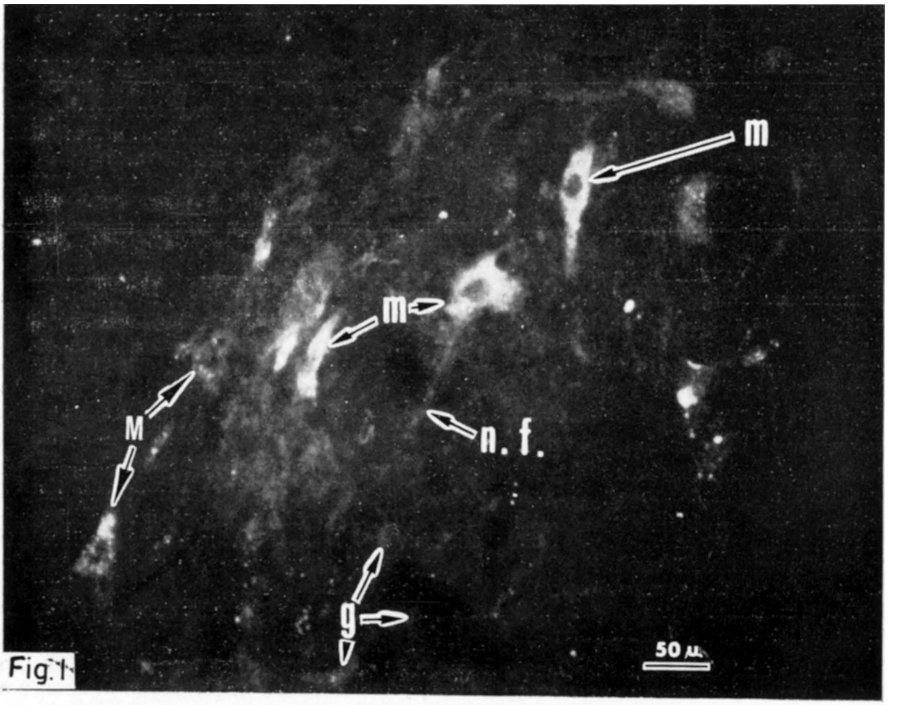

Fig. 1

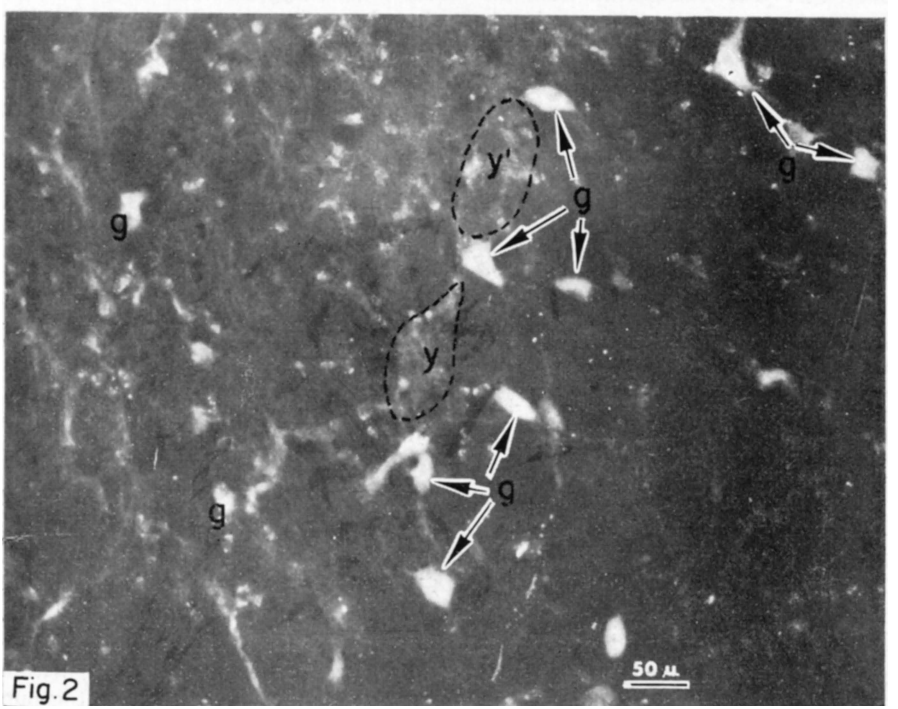

Fig. 2

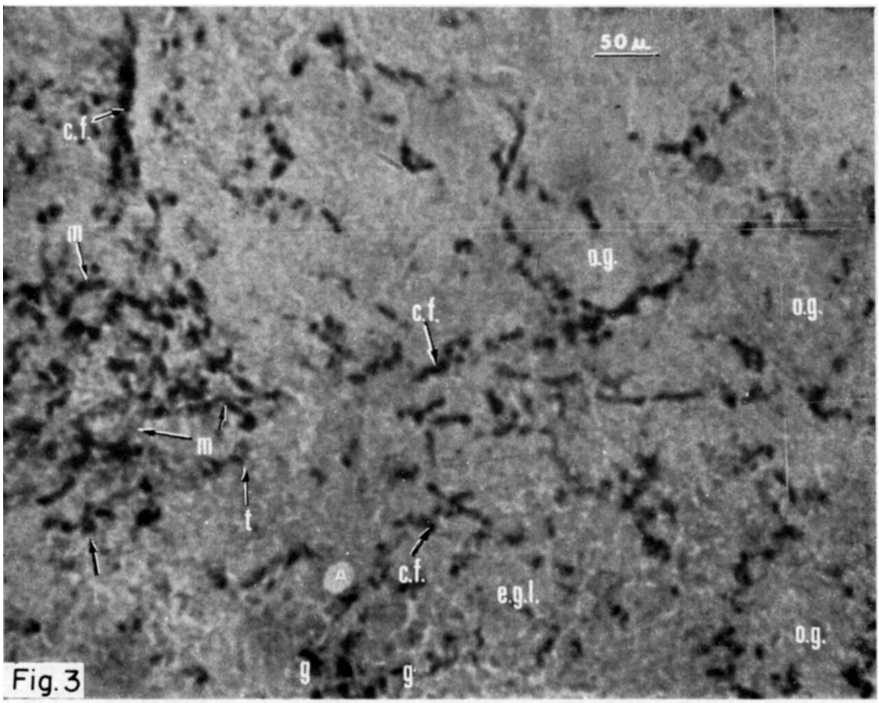

Fig. 3

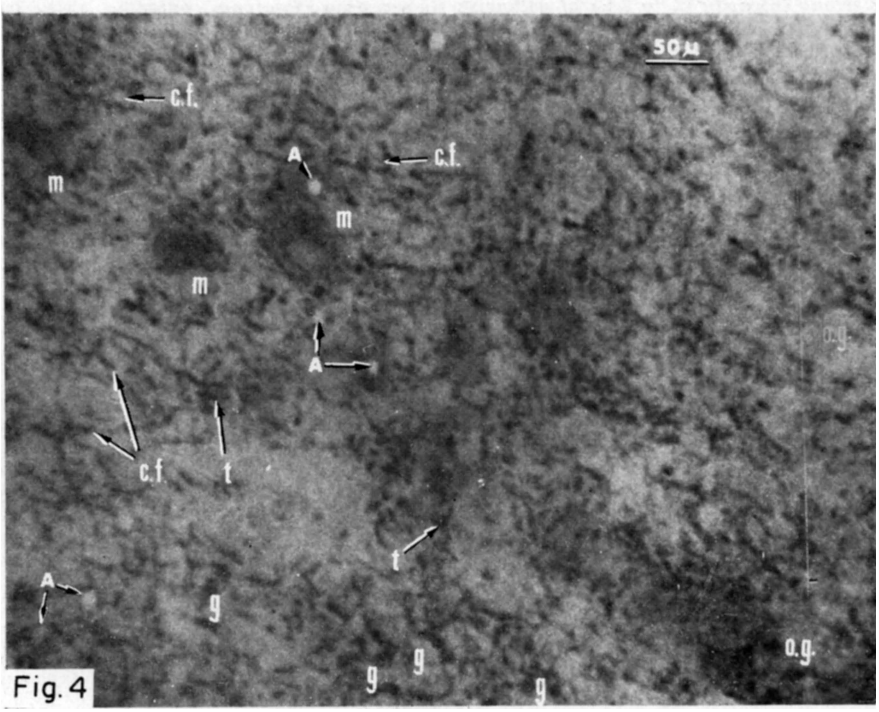

Fig. 4

of fibers originating in the diencephalon and brain stem (Dahlström et al. [2]). Our studies indicate the existence of another group of short multibranched adrenergic fibers within the olfactory bulb which appear to have connections with fluorescent neurons in the olfactory bulb (e.g., mitral and granular cells). These multibranched fibers appear to be extended to the isthmus of the olfactory bulb where the connections might be established with the

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Fig. 1 — Olfactory bulb. Normal adult cat. A frozen horizontal section treated with paraformaldehyde gas (70% relative humidity). Fluorescent microscopy, 160 ×. It is demonstrated the group of fluorescent mitral (m) cells located in the side of the bulb facing the cribiform plate. The mitral cells developed a medium to strong (2+ to 3+) intensity of greenish-yellow fluorescence, while the adjacent granular cells (g) are devoided of fluorescence or with a weak (less than 1+) intensity of the specific monoamine fluorescence. Several varicosities of large size (2 μ) are observed around the mitral cells; other varicosities of small size (1 μ) are scattered in the granular cell layer. Short segments of nerve fibers (n.f.) with a medium size varicosities (1-2 μ) are observed to be extended in between the mitral and granular cells and in between the mitral cells itself. Photographs were taken with the high speed color film Ektachrome-X (EX-135), exposure 10 sec.

Fig. 2 — Olfactory bulb. Adult cat treated with methedrine during 2 months. The section is approximately at the same level of fig. 1. Paraformaldehyde gas treatment (70% relative humidity). Fluorescent microscopy, 160 ×. The greenish-yellow fluorescence was not observed in the mitral or in the granular cells; no fluorescent varicosities were evidenced at these levels. The picture shows the internal granular layer closer to the glial layer. Some of the small granular cells developed a strong intensity (4 to 5+) of yellowish fluorescence (serotonin content). Yellowish varicosities of small size (less than 1 μ) are observed around the fluorescent cells. A "packing" of a large number of these small varicosities makes a type of area with diffused fluorescence or a "yellowish dotted-area" (y-y'). Film Ektachrome-X (EX-135), exposure 20 sec.

Fig. 3 — Olfactory bulb. Normal adult cat. An alternate frozen section of fig. 1 was developed for specific cholinesterase enzyme activity (acetylcholinesterase). Incubation with iso-OMPA (tetraisopropylpyrophosphosphoramide). Phase microscopy, 160 ×. The mitral cells (m) develop a very weak cholinergic activity. Thick granules (x) of cholinergic reddish-brown deposit are observed around the mitral cells. Short and long cholinergic fibers (c.f.) appear to be branching around the mitral cells and directed to and from the olfactory glomeruli (o.g.) and the external granular layer (e.g.l.). The granular cells (g) developed a more intense cholinergic activity than the mitral and tufted cells (t). An artifact of frozen preparation is observed in (A). Film Ektachrome-X (EX-135), exposure 1.5 min.

Fig. 4 — Olfactory bulb. Adult cat treated with methedrine during 2 months. An alternate frozen section of fig. 2 was developed for specific cholinesterase enzymes activity. Incubation with iso-OMPA. Phase microscopy, 160 ×. The picture illustrates the field of the group of mitral cells (m) located approximately at the same level of picture 1 and 3. The mitral cells developed a medium to strong intensity of cholinergic activity. It is observed some increase in the enzymatic deposit of granular cells (g), but the cholinergic activity appears especially intense at the short multibranched plexus extending between the granular, mitral and the olfactory glomeruli (o.g.). The short fibers appear to be branched profusely around the mitral, granular and tufted cells (t). Some of the long cholinergic fibers (c.f.) are observed to course along the mitral cell layer. The majority of the long fibers (e.f.) course along the glial layer to continue with the lateral and medial olfactory tracts. Artifacts of frozen section (A). Film Ektachrome-X (EX-135), exposure 1.5 min.

neurons of the septum through the medial olfactory tract and the neurons of the amygdaloid complex through the lateral olfactory tract. The present evidence is not in opposition to the reported long ascending fibers from the brain stem, but rather is evidence of the existence of adrenergic neurons within the olfactory bulb. These neurons are sensitive to the reserpine and amphetamine effect. These pharmacological facts represent a strong evidence of the neurons being another of the units of synthesis and metabolism of biogenic amines.

In our material from animals without amphetamine treatment, the results (to be published) from another pharmacological preparations (e.g., monoamine enzyme inhibitors) are suggesting that noradrenaline is highly concentrated around the olfactory glomeruli. On the other hand, dopamine appears to be mainly localized around the mitral and external granular cells. The smaller varicosities around the internal granular cells and closer to the glial layer appear to have a small amount of serotonin. The content of this compound appears to increase (++ to +++ of yellowish fluorescence intensity) in the material from animals with a prolonged period of methedrine treatment.

There is no doubt that amphetamine incites a release of catecholamines from the nerve terminals and from the neurons itself when the amphetamine is used in higher dose and for a prolonged period of time (Ellinwood and Duarte-Escalante [5]). But, what is moot is the observation of the increase of serotonin fluorescence in the animals under chronic methedrine intoxication. We speculate the possibility that a complete depletion of noradrenaline and dopamine from neurons and nerve terminals by methedrine allows a clear appearance of serotonin fluorescence in the small varicosities of some special nerve fibers. Or, it is possible that the amphetamine itself provokes a direct action on the molecule of serotonin. Recently is has been reported a biochemical increase of brain serotonin in mice under amphetamine treatment (Welch and Welch [10]).

Only in the animals chronically intoxicated with methedrine we have observed an increase of acetylcholinesterase enzymes in some of the olfactory bulb neurons (e.g., mitral and granular cells) and in some long cholinergic fibers coursing the olfactory tracts directed toward the septum and amygdaloid complex. A same type of cholinergic activity enhancement was observed in the neurons from the medial and ventral areas of both septum and amygdaloid complex.

An interesting question is to distinguish how the physiological enhancement of the cholinergic system is the result of the depletion of the adrenergic compounds, or if the cholinergic system is overstimulated by the excess of released adrenergic agents under the amphetamine effect. Histochemically the increase of acetylcholinesterase can be understood by assuming that more enzyme is needed to destroy the excess of acetylcholine released. This may be an indirect effect of amphetamine on the cholinergic system.

R E S U M O

Depleção de aminas biogênicas e aumento da atividade colinérgica no bulbo olfatório e nas conexões olfatórias centrais mediante intoxicação pela metedrina.

Em seqüência a estudos anteriores os autores visam, neste relato, a apresentar as alterações histoquímicas que ocorrem no sistema olfatório de gatos nos quais se desenvolveu nítida estereotipia de fungação (sniffing) após administração prolongada de metedrina. Foram intoxicados 12 gatos mediante injeções diárias, durante 10 dias, de doses progressivas de metedrina. Os tecidos a examinar (bulbos olfatórios e suas conexões centrais) foram preparados histoquìmicamente para demonstrar a fluorescência das aminas biogênicas e reações colinérgicas. Mediante algumas modificações à metodologia recomendada por outros pesquisadores, os autores puderam demonstrar a presença de monoaminas e de acetilcolina no bulbo olfatório e de grupos de fibras adrenérgicas curtas e multi-ramificadas que parecem ser conectadas com os neurônios fluorescentes do bulbo olfatório, a partir de onde estabelecem conexões, pelo tracto olfatório medial, com os neurônios do septum e, pelo tracto olfatório lateral, com os neurônios do complexo amigdalóide.

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