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Monoamine Neuron Innervation of the Hippocampal Formation: Alteration by Neonatal Irradiation

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Previous studies showed that postnatal irradiation of the hippocampal formation in the rat results in a marked reduction in the granule cell population of the area dentata with an attendant disruption of its normal cytoarchitecture. The present study was carried out to determine the effect of postnatal hippocampal irradiation on the pattern of hippocampal innervation by locus ceruleus norepinephrine neuron axons and midbrain raphe serotonin neuron axons. Irradiated animals exhibit a normal innervation of Ammon's horn by both norepinephrine and serotonin neuron axons. In the area dentata, however, the normal pattern of innervation is markedly altered, with both an apparent decrease in total innervation and a redistribution of the innervation occurring. These effects are interpreted as representing a failure of development of the growth-promoting factors that determine the normal innervation pattern.

INTRODUCTION

The hippocampal formation is innervated by two groups of monoamine neuron axon: norepinephrine-producing fibers arising from the locus ceruleus (10–12) and serotonin-producing axons arising from the midbrain raphe (13). The details of the distribution of this innervation were described fully in the studies noted above, but it is pertinent to the present study to note that there is a dense innervation from both groups to the hilar region of the area dentata, which is particularly evident in the region

Abbreviations: 5-HT—serotonin; NE—norepinephrine.

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immediately adjacent to the granule cell layer (5, 11–13) termed "the limiting subzone" by Ramòn y Cajal (15). This distribution of monoamine neuron innervation of the area dentata is of interest because several studies demonstrated that the granule cell layer of the area dentata is formed, in large part, postnatally in the rodent (1–4, 17) and that postnatal irradiation can greatly reduce the number of dentate granule cells (3, 4). The time course of development of the serotonin (5-HT) innervation of the hippocampal formation in the rat has not been studied, but the norepinephrine (NE) innervation is known to reach the hippocampus prenatally and to innervate the structure concomitant with the development of its neuronal components (14, 15). The purpose of the present study was to determine the effects of postnatal irradiation-induced alteration of area dentata cell organization on the development of the monoamine neuron innervation to that structure in order to provide a better understanding of factors that regulate the normal development and maturation of that innervation.

MATERIALS AND METHODS

The animals used in this study were Purdue–Wistar rats prepared according to the procedure of Bayer and Altman (3, 4). Sixteen control animals and 28 irradiated animals were studied. The irradiated animals received 200 R delivered on postnatal Days 2 and 3 followed by 150 R on Days 5, 7, 9, 11, 13, and 15, as described by Bayer and Altman (4). The animals were then raised in the Purdue animal colony until 30 to 60 days of age, when they were shipped to California. Three studies were carried out. In the first, six control and six irradiated animals were killed by decapitation, their brains were removed rapidly, and the hippocampal formation was dissected from the remaining telencephalon and frozen on dry ice until analyzed for catecholamine content by the enzymatic radioisotopic assay of Coyle and Henry (6). A second group of 8 control and 20 irradiated animals was utilized for histochemical analysis of the NE innervation of the hippocampal formation using either the Vibratome–formaldehyde method (8) or the glyoxylic acid method (10). The distribution of the NE innervation of the hippocampal formation, and particularly the area dentata, was analyzed by fluorescence microscopy. Initially the animals used for the histochemical studies were coded so that control and irradiated animals could not be distinguished except by differences in the histological pattern, but this was discontinued when it became evident that the irradiated animals were easily recognized by the appearance of the area dentata granule cell layer. The 5-HT neuron innervation of the hippocampal formation was also studied in two control and two irradiated animals. Each animal received an injection of tritiated proline into the

midbrain raphe and their brains were processed for autoradiography as previously described (13).

RESULTS

The hippocampal NE content of the control (523 ± 46 ng/g) and the irradiated (571 ± 58 ng/g) animals was nearly identical. The value for the irradiated animals was higher than that for the controls, but the difference was not statistically significant ($P > 0.05$, two-tailed t-test). The dopamine content for both groups was less than 10% the NE content and, as discussed previously (12), within the range to be expected in neurons in which dopamine is a precursor for NE.

The hippocampal innervation by NE fibers arising in the locus ceruleus in the control animals is indistinguishable from that described previously for normal animals (5, 11, 12). In the irradiated animals, the innervation of Ammon's horn also appears very similar to that in normal and control animals. In CA1, the predominant innervation is in the stratum lacunosum-moleculare, with frequent fibers ascending into the stratum radiatum. The

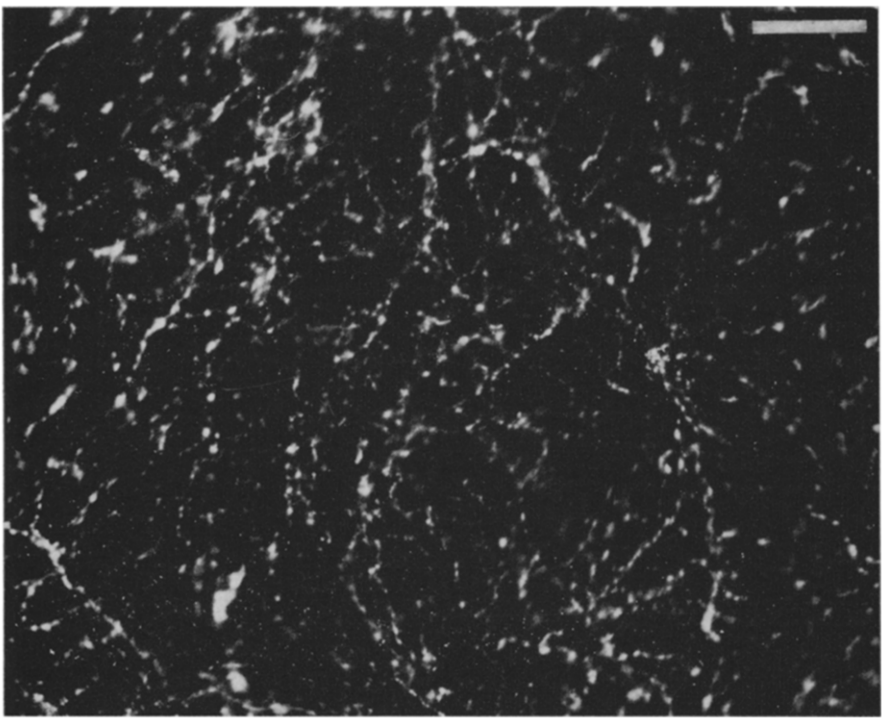

FIG. 1. Stratum radiatum of the CA3 zone of hippocampal formation with a moderately dense innervation by a plexus of norepinephrine-containing axons. Neonatally irradiated animal. Formaldehyde–Vibratome method. Marker bar = 50μm.

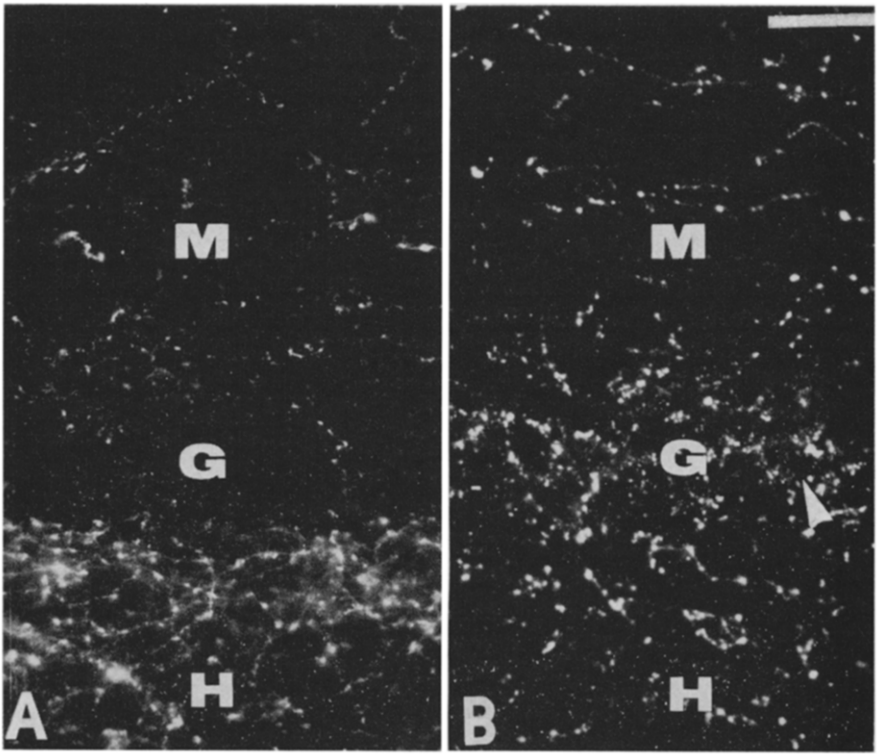

FIG. 2. Norepinephrine axon innervation of the area dentata in control and neonatally irradiated animals. Abbreviations: M—molecular layer; G—granule cell layer; H—hilar zone. A—Control animal exhibiting very few fibers in the granule cell layer and a dense plexus in the infragranular zone. B—Irradiated animal showing very few norepinephrine axons in the infragranular zone. The granule cell layer is thin and exhibits scattered, autofluorescent granules (arrowhead). Glyoxylic acid method. Marker bar = 50 μm.

stratum radiatum of CA3 (Fig. 1) demonstrates a moderate to dense plexus of NE fibers. There is variation from preparation to preparation in the histochemical material, and even small differences in the thickness of section, which are impossible to control in Vibratome-prepared material, may result in apparent differences in innervation. Consequently, although it was noted that the CA3 innervation appeared greater than normal in most of the irradiated animals, the difference is neither sufficiently great nor consistent enough to be interpreted with certainty. The striking change in the irradiated animals is in the area dentata. The granule cell layer, although normal in position and general configuration, is reduced in length and in number of neuronal components and is similar to that shown in previous studies (3, 4). In the fluorescence histochemical material, the NE innervation of the hilar zone is consistently reduced in irradiated animals

compared to controls (Fig. 2), and this is particularly evident in the limiting subzone region. In most of the irradiated brains, there appear to be more fibers passing through the granule cell layer than in the control brains and the innervation of the molecular layer appears more dense than in the controls (Fig. 2).

The 5-HT innervation, as shown by autoradiographic analysis, appears similarly altered. The location of the injection site in the midbrain raphe in the brains prepared for autoradiography does not differ significantly from that described previously (7, 13). There is dense labeling of the nucleus centralis superior with some extension into the adjacent tegmentum and the dorsal raphe. The distribution of label in Ammon's horn (Fig. 3) appears identical in the control and the irradiated animals and identical to that described previously (13). In the area dentata, control brains exhibit a dense band of labeling in the limiting subzone region of the hilus immediately adjacent to the granule cell layer (Fig. 4A). In contrast, the irradiated brains show diffuse, light labeling over the entire hilar zone (Fig. 4B), with no indication of the dense labeling in the limiting subzone evident in control brains. However, the labeling over deeper regions of the hilus is greater in the irradiated brains than in the control brains (Fig.

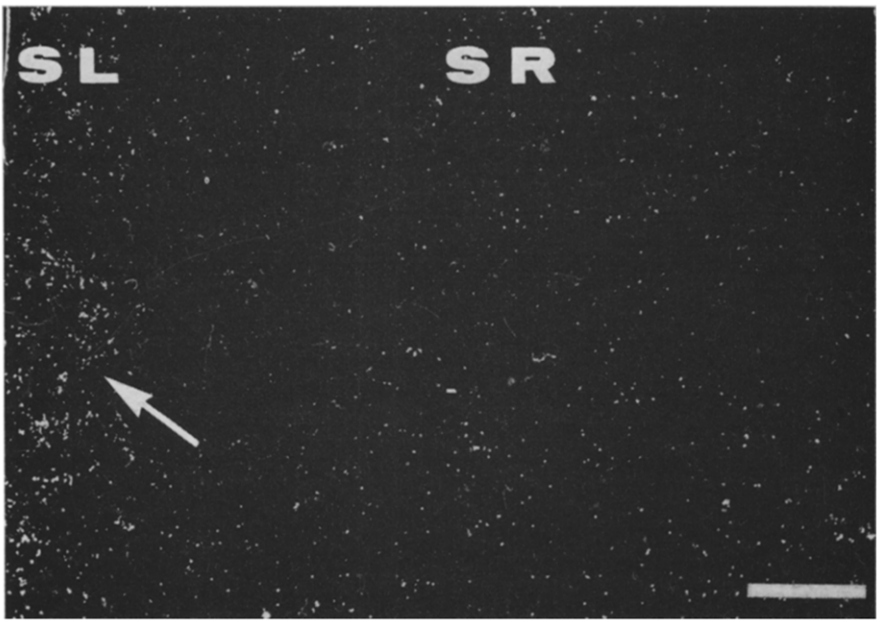

FIG. 3. Stratum lacunosum (SL) and stratum radiatum (SR) from the CA1 zone of a neonatally irradiated animal. The brain was prepared by the autoradiographic tracing method after the injection of tritiated proline into the midbrain raphe. The dense labeling of the SL adjacent to the SR (arrow) and the scattered labeling of the SR are typical of the normal serotonin innervation. Marker bar = 45 μm.

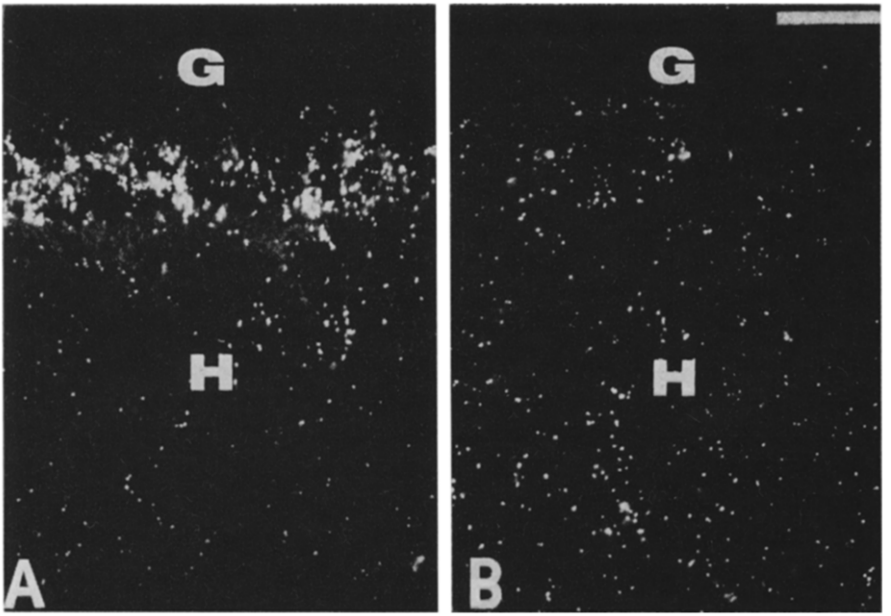

FIG. 4. Area dentata showing the granule cell layer (G) and hilar zone (H) in brains of control and neonatally irradiated animals prepared by the autoradiographic tracing method following the injection of tritiated proline into the midbrain raphe. A—Control animal showing typical dense labeling in the limiting subzone region of the hilus immediately adjacent to the granule cell layer. B—Irradiated animal showing scattered labeling through the hilar zone. Marker bar = 45 μm.

4). This suggests that there is a redistribution of the remaining 5-HT neuron innervation to the area dentata.

DISCUSSION

The observations presented above indicate that the cytoarchitectural changes produced in the area dentata of the rat by postnatal irradiation have significant effects upon the distribution of monoamine neuron axons arising in either the locus ceruleus or the midbrain raphe. In contrast, the innervation of Ammon's horn appears identical in the control and the irradiated animals. These results can be explained by the maturational differences between the cell populations of Ammon's horn and the area dentata in neonate rats. At the time of the initial exposure to X irradiation (postnatal Day 2), the pyramidal cell population in Ammon's horn has completely formed (2), is differentiating, and is already receiving innervation from the locus ceruleus (19). Similarly, in the area dentata, all polymorph and basket cells are present (2) and may possibly receive input from the NE axons of the locus ceruleus (19). Since X irradiation at the dosage used (150 to 200 R) does not destroy differentiating cells (3, 4), these cell populations are not affected by the exposure. On the other hand,

only 15% of the dentate granule cells, 30% of the small hilar cells, and less than 5% of the small neurons in the molecular layer of the area dentata, Ammon's horn strata oriens, radiatum, and lacunosum-moleculare are formed prenatally (3). X irradiation kills both the proliferating precursors of these neurons and the primitive postmitotic cells migrating to their site of differentation into neurons. The hippocampi of the irradiated animals in this report received the same dosage schedule as the 8X group described previously by Bayer and Altman (4), and we can anticipate that about 85% of the dentate granule cell population has been prevented from developing.

This disruption of normal development could result in at least two types of effects on the incoming afferent axon populations. One possibility is that the normal complement of axons invades the hippocampal formation, fails to innervate missing targets in the area dentata, and in response forms a more dense innervation in areas where fewer contacts are normally made. This implies that the predominant determinant of axonal growth and terminal formation resides within the afferent input and not the area to be innervated. Thus, the axons are predetermined to generate a certain density of innervation and will do so despite dramatic changes in their targets. An example of such an effect appears to be the patterns of anomalous axonal growth observed by Schneider (18) in the developing hamster visual system after surgical removal of the optic tectum. That this may be occurring in the irradiated hippocampus is indicated by the following observations. The NE content of the hippocampal formation is not decreased, despite the decrease in area dentata hilar zone innervation. The CA3 stratum radiatum appears to have a greater than normal innervation and there is an extension of an apparently greater than normal plexus of NE axons extending into the area dentata molecular layer.

The second possibility is that the development of the monoamine neuron innervation of the hippocampal formation is determined by the development of hippocampal neurons, which then promote growth and maintain an appropriate pattern of innervation by monoamine axons. Several observations appear to support this hypothesis. First, NE neuron axons appear in the hippocampal formation very early in development, but form their normal pattern of terminal innervation in an orderly time course that parallels the differentiation of the various hippocampal components. Second, in a recent study of the outgrowth of axons from transplants of neurons into the hippocampal formation, Stenevi *et al.* (20) obtained very orderly patterns of growth and terminal plexus formation which are best accounted for by the growth promotion concept. Third, with the exceptions of the apparent increase in NE neuron innervation of the CA3 stratum radiatum and the area dentata molecular layer, and the redistribution of 5-HT innervation in the area dentata hilar zone, anomalous axonal growth does not

occur and the final innervation patterns achieved is appropriate to the postsynaptic sites apparently available. It should be noted that this alteration in innervation pattern is not anomalous in the sense that areas which do not normally receive innervation are invaded but, rather, that there is an increase in innervation density in zones normally innervated by the same fibers. Thus, in each zone in which an increase in density is noted, the monoamine neuron innervation would be considered anomalous only if the terminals were located on abnormal postsynaptic targets. A further consideration is that the 5-HT and NE systems are highly collateralized and diffuse in their distribution and it also is possible that some adjustment in the organization of these systems may occur in other areas, as in the experiments of Sachs and Jonsson (16). The observations of this study contrast with those of Lauerberg and Hjorth-Simonsen (9), who found no alteration in the distribution of entorhinal afferents to the area dentata following neonatal irradiation. The explanation for this difference is not evident but may reflect a greater capacity for plasticity in the highly collateralized and probably less well-specified developing monoamine afferents.

Thus, the data from our study indicate that the development of the innervation of the hippocampal formation by monoamine neuron axons is an orderly process regulated both by intrinsic properties of the monoamine neurons and by the formation and differentiation of hippocampal formation neurons, with the final pattern of innervation determined by the innervated neurons.

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