DEVELOPMENT OF DYNORPHIN-IMMUNOREACTIVITY IN THE CHICK COCHLEAR NUCLEUS

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Date

To the Associate Vice President for Research and Dean of the Graduate School:

I am submitting herewith a thesis written by Amanda E. McDaniel entitled "Development of Dynorphin-immunoreactivity in the Chick Cochlear Nucleus." I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biology.

Major Professor

We have read this thesis and recommend its acceptance:

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ABSTRACT

DEVELOPMENT OF DYNORPHIN-IMMUNOREACTIVITY IN THE CHICK COCHLEAR NUCLEUS.

AMANDA E. MCDANIEL, MAY 1998

The purpose of the present study was to investigate the development of dynorphin-immunoreactivity (DYN-I) in the chick cochlear nucleus magnocellularis (NM) from embryos through young adults using an antibody to dynorphin-B (DYN-B). Auditory brainstem tissue from White Leghorn chicks from embryonic day 13 (E13) to posthatch day 22 (P22) was examined under the light microscope after using standard immunohistochemical procedures. DYN-I first appeared in NM at E16 as short flat structures surrounding NM neurons. The density of DYN-I terminals appeared to increase during development, reaching a peak at P6, and then declining until few DYN-I terminals were seen at P22. Thus, DYN-I appears to be expressed during a restricted time period. Because DYN-I terminals appear after the auditory end-bulbs of Held have already formed around NM neurons, it is unlikely that DYN is involved in their development. Additionally, DYN-I terminals appeared to be randomly distributed with no spatial gradient within NM at any of the ages examined. Thus, DYN-B is unlikely to be related to the tonotopic gradient that exists in NM. Although DYN-B appears to be contained within the auditory end-bulbs of Held, its function remains unknown.

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CHAPTER 1

INTRODUCTION

Dynorphin (DYN), along with the enkephalins, belongs to a group of opioid peptides endogenous to the central nervous system (CNS). These endogenous opioids are known for their association with the processing and/or modulation of nociception in the spinal cord (Khachaturian et al., 1993). The presence of these opioid peptides at other sites in the CNS suggests that they may have other neuromodulatory functions. For example, DYN influences glutamate release in cerebrocortical and hippocampal areas (Nicol et al., 1996; Faden, 1992).

In the mammalian auditory system, DYN and enkephalin have been found in neurons of the lateral olivocochlear efferent system (for review see Altschuler and Fex, 1986; Roberts and Meredith, 1992; Eybalin, 1993) whose axons exit the brainstem and project back to the base of the inner hair cells in the cochlea (see Warr, 1992 for review). DYN also has been reported in the dorsal and ventral cochlear nucleus in the mammalian brainstem (Sahley et al., 1995). In contrast, avian cochlear efferent neurons do not appear to contain dynorphin-immunoreactivity (DYN-I) (Zidanic and Fuchs, 1995, 1996). Instead, DYN-I can be observed in auditory nerve terminals in the chick cochlear nucleus magnocellularis (NM) (Code, 1996).

There are two cochlear nuclei in chicks, the nucleus magnocellularis (NM) and the nucleus angularis (NA). The auditory nerve bifurcates and synapses on the cells of both NA and NM (Ramon y Cajal, 1908; Parks and Rubel, 1978). In the NM, which consists almost exclusively of a single cell type, the auditory nerve synapses on the round nerve cell

bodies via specialized endings called end-bulbs of Held (Jhaveri and Morest, 1982a). Since the auditory nerve provides the only excitatory input to the ipsilateral NM (Parks and Rubel, 1978), it is believed to release an excitatory neurotransmitter, either glutamate or aspartate (Nemeth et al., 1983). Other inputs to NM are provided by nerve terminals that release GABA (Carr et al., 1989; Code et al., 1989; Code et al., 1990; Muller, 1987) or glycine (Code and Rubel, 1989).

Among these three types of nerve endings in NM, the GABAergic, the glycinergic, and the excitatory auditory nerve terminals, DYN-I is suspected to be in the end-bulbs of Held. Evidence for this comes from a previous study of DYN-I in NM after unilateral cochlea removal. Cochlea removal results in the degeneration of the auditory nerve terminals in the ipsilateral NM, while those on the contralateral, unoperated side remain unaffected. Five days after unilateral cochlea removal, DYN-I terminals in the ipsilateral NM were no longer seen while those in NM on the unoperated side of the same animal were still present (Code, 1996). These results strongly suggest that the auditory end-bulbs of Held contain DYN-I.

The following objectives evolved as a consequence of the discovery of DYN-I terminals in the chick NM. The first objective was to determine the normal time-course of DYN-I expression in NM. Since DYN-I appears to be contained within auditory nerve terminals, one might expect its development to follow the pattern of auditory nerve terminal development. For example, immature end-bulbs of Held first appear in NM around embryonic day 11 (E11)-E13. At E16, three or four immature end-bulbs of Held coalesce to form one to two mature end-bulbs in NM which reach their final form by E19 (Jhaveri and Morest, 1982b). Thus, we wished to determine if the development of DYN-I is correlated with known developmental events in the auditory nerve terminals.

The second objective was to examine regional differences in the expression of DYN-I within NM. This objective is significant for two reasons: (1) NM is tonotopically organized such that neurons in medial and rostral portions of the nucleus respond best to high frequency sounds, while nerve cells in the lateral and caudal portions respond best to lower frequencies, and neurons in the middle regions of NM respond best to middle-frequency sounds (Rubel and Parks, 1975). We wished to determine whether there were spatial differences in the distribution of DYN-I terminals that might be correlated with the tonotopic organization within NM. Such an investigation of regional differences of DYN-I may lead to clues as to the possible role of DYN in the processing or modulation of auditory information. (2) The second reason to study the spatial distribution of DYN-I in NM was to determine if it could be correlated with the known morphological gradient of dendritic length in NM. Throughout their lives, nerve cells in the rostromedial regions of NM have no dendrites, whereas cells in the middle regions of NM have stubby dendrites and those in the caudolateral NM have some short dendrites (Rubel and Parks, 1988). Since DYN may influence the development of cytoskeleton proteins that are needed in growing dendrites (Mangoura and Leung, 1996), we might expect levels of DYN-I to be higher in the caudolateral regions and lower in the rostromedial regions of NM. For the purposes of this study, DYN-B antisera was utilized because of previous success in the chick model and for reasons discussed later.

CHAPTER 2

MATERIALS AND METHODS

White Leghorn chicks of either sex from embryonic day 13 (E13) to posthatch day 22 (P22) were used for this study. At the desired age, the chicks were overdosed with sodium pentobarbital and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1M phosphate buffer (PB). The brains were then removed from the skull and blocked in the coronal plane and sunk in a 30% sucrose/4% paraformaldehyde solution. Frozen sections, 30 μm thick, were cut through NM and collected in 0.1 M phosphate buffered saline (PBS). After four rinses of PBS (15 minutes each), the sections were pre-incubated for 1 hr in 8% normal goat serum (NGS) with 0.4% Triton-X in PBS (PBS-X) at room temperature. They were then rinsed again as above and incubated in a rabbit polyclonal antibody raised against DYN-B (provided by Dr. Stanley J. Watson, Jr., U. of Michigan School of Medicine), diluted 1:200 in NGS/PBS-X for 48-72 hrs at 4° C. This antibody has been used in other areas of the avian brain and is blocked specifically by DYN-B peptide (Anderson and Reiner, 1990). In addition, some sections were processed using a dynorphin-A (DYN-A) antibody to support the specificity of the antibody to DYN-B. The sections were then processed with standard immunohistochemical procedures using the avidin-biotin-peroxidase complex (ABC) method (Hsu et al., 1981) and reagents from Vectastain kits (Vector Labs, Inc.). Following four more 15 min rinses in PBS, sections were incubated in a biotinylated goat anti-rabbit secondary antibody, diluted 1:200 in PBS-X, then rinsed again 4 × 15 min in PBS. They were finally incubated in ABC for 1

hr, rinsed 2 × 10 min in PBS, and rinsed 2 × 10 min in Tris-imidazole buffer. Sections were then developed in 0.04% diaminobenzidine and 0.003% hydrogen peroxide for 1 to 5 min, then rinsed 2 × 5 min in Tris-Imidazole buffer, and 2 × 15 min in PBS. The sections were mounted onto gelatin-coated slides, dehydrated, and coverslipped for viewing under the light microscope.

Data Analysis. The number of DYN-I terminals in NM were counted on one side of the brainstem. The criteria for defining a labeled terminal were strictly adhered to and were as follows: (1) the labeled structure must have been stained above background; (2) only labeled structures surrounding the large cell bodies of NM were counted; (3) labeled structures had well-defined borders rather than jagged edges. If there was any question of whether the structure observed was a terminal or artifact, it was not included in the analysis.

DYN-I terminals were counted with the aid of an Olympus microscope using an OS/2 Image Analysis System (Imaging Research, Corp.). NM was observed under a light microscope utilizing a 40X objective lens. An image of NM was displayed onto the video screen. A 5,625 sq. μm sample box (75 μm on a side) was drawn onto an acetate sheet taped onto the video screen over the image of NM. The following counting criteria were used: (1) any DYN-I terminal that fell within the sample box (5625 sq. μm) either entirely or partially, was counted; (2) all DYN-I terminals within a single focal plane were counted; (3) two separate terminals were counted if the labeled structures were separated by a space; (4) the sample boxes used in NM were non-overlapping and fit within the boundaries of

NM in that section; (5) the location of the sampling boxes was kept in approximately the same mediolateral regions of NM in each animal.

Quantification procedures were similar to those used in a previous study (Code et al., 1990). The regions of NM to be analyzed were predetermined in order to rule out any bias in selection of the tissue. Briefly, tissue sections within each animal were arbitrarily chosen at 25%, 50%, and 75% of the total posterior-anterior (P-A) length of NM. If a section was not available at a particular level due to damage during sectioning or tissue processing, the next closest section was used. Within each section, the density of DYN-I terminals was determined in the medial, central, and lateral regions of NM (see Fig.1). The medial region of NM is defined as that zone closest to the midline of the animal, and the lateral region is that zone farthest from the midline. The central region is the zone halfway between the medial and lateral zones. Within each of these regions, three sampling boxes, 75μm on each side (or 5625 sq. μm), were used to count the number of DYN-I terminals. Due to the small size of NM in some rostral regions (75%) of most animals, in these areas only two sampling boxes were used in the medial, central, and lateral regions. An average density of DYN-I terminals was obtained from the three sampling boxes in each of the medial, central, and lateral regions of NM and at the 25%, 50%, and 75% P-A levels for each animal such that a total of nine areas were sampled throughout NM in each animal.

Tissue sections from two embryos during the week before hatching at age E16 and posthatch animals at ages P1-2, P6, P13 (N=2 at each age) and P22 (N=1) were used to estimate the average density of DYN-I terminals in NM.

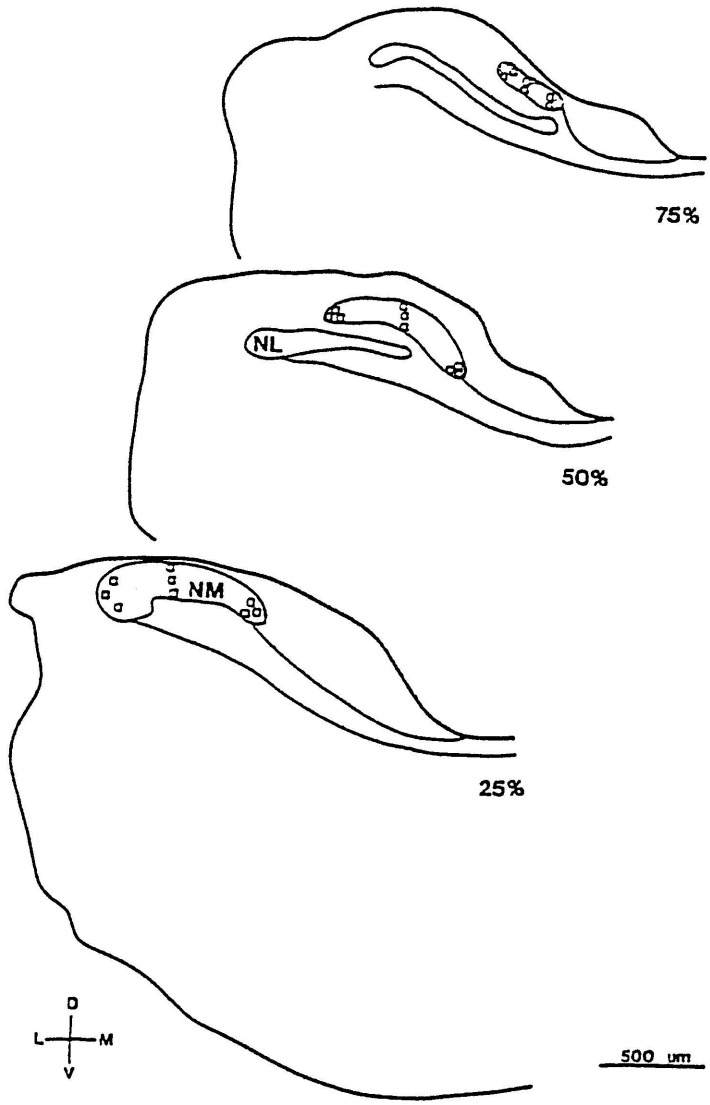


Fig. 1 Approximate locations of sampling boxes in NM. Tissue sections within each animal were arbitrarily chosen at 25%, 50%, and 75% of the total posterior-anterior (P-A) length of NM. Within each section, the density of DYN-I terminals was determined in the medial, central, and lateral regions of NM. Within each of these regions, three sampling boxes, 75 μm on each side (or 5625 sq. μm) were used to count the number of DYN-I terminals. Nucleus laminaris, NL, is shown for reference.

CHAPTER 3

RESULTS

The development of DYN-I terminals in the chick NM was examined in chicks from ages E13 to P22 using an antibody to DYN-B. In order to determine specificity of the antibody to DYN-B, antisera to another structually similar opioid peptide, DYN-A, was also used. This antibody to DYN-A resulted in a different pattern of staining than that of DYN-B. DYN-A antibody resulted in labeling of the crossed dorsal cochlear tract (XDCT) fibers but there was no terminal labeling in NM, in contrast to the antibody to DYN-B (Fig. 2).

DYN-B immunoreactive terminals will be described qualitatively and then a more quantitative analysis of DYN-labeled terminal density will be provided.

Qualitative observations. No DYN-I terminals were present in NM at E13 (Fig. 3) but were observed at E16. At this age, DYN-I appeared as short, flat terminals surrounding some cells in NM (Fig. 4A). At ages P1-P2, there appeared to be slightly more DYN-I terminals in NM than there were at E16. Around this age, the DYN-I terminals appear to be somewhat larger and more rounded than those at E16 (data not shown). DYN-I terminals at age P6 appear to surround more NM neurons and are more darkly stained than at E16 (Fig. 4B). The terminals at this age form large chalice-shaped structures surrounding the nerve cell bodies of NM. Compare figures 4A and 4B to see the differences in DYN-I terminal morphology at E16 versus P6. Terminal morphology from age P6-P22 did not appear to change.

Quantitative analysis. At each of the ages examined, DYN-I terminals appeared to

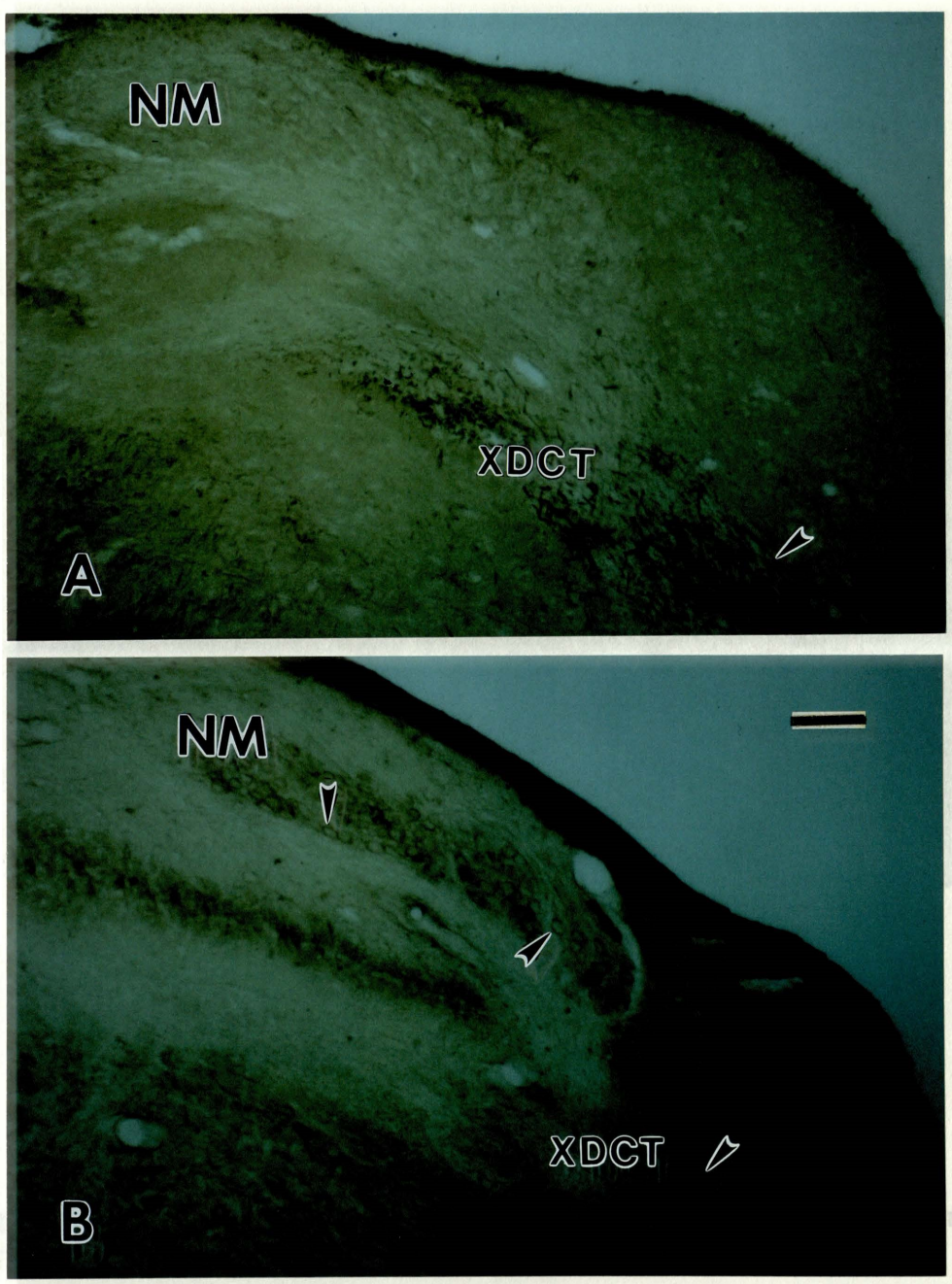


Fig. 2 Differences in immunostaining patterns between DYN antisera.
A) DYN-A antibody labels fibers (arrow) in crossed dorsal cochlear tract (XDCT) but not in NM.
B) DYN-B antibody labels terminals (arrows) in the cochlear nucleus magnocellularis (NM) but not fibers in the XDCT. (Scale bar, 50 μm, applies to both panels.)

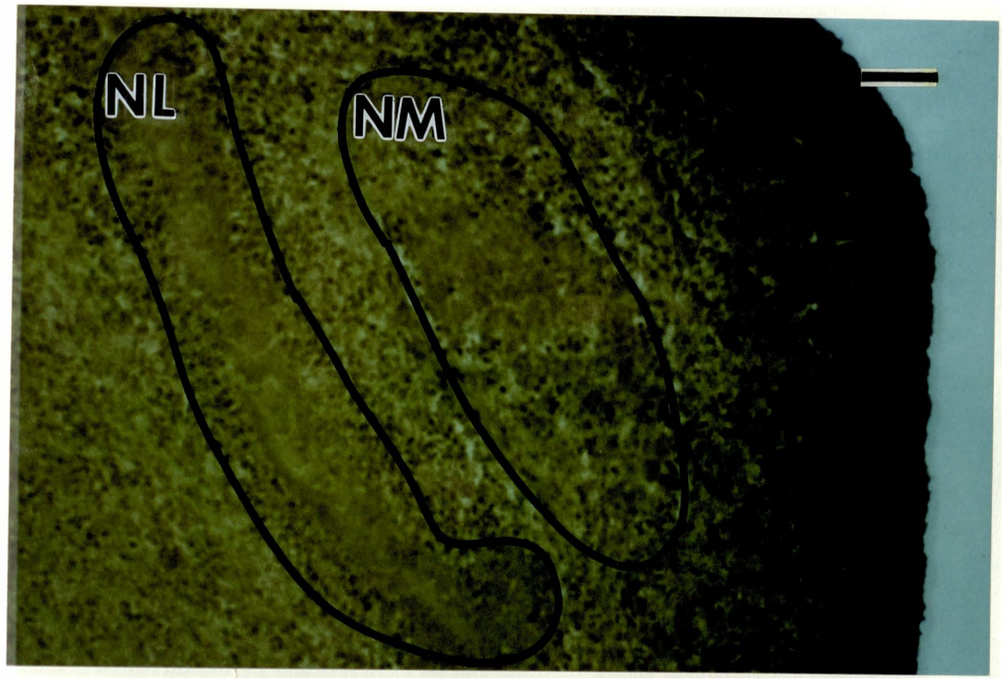


Fig. 3 Transverse section through NM at E13 using DYN-B antisera. No DYN-I is seen in NM at age E13. NL, nucleus laminaris. (Scale bar, 50 μm)

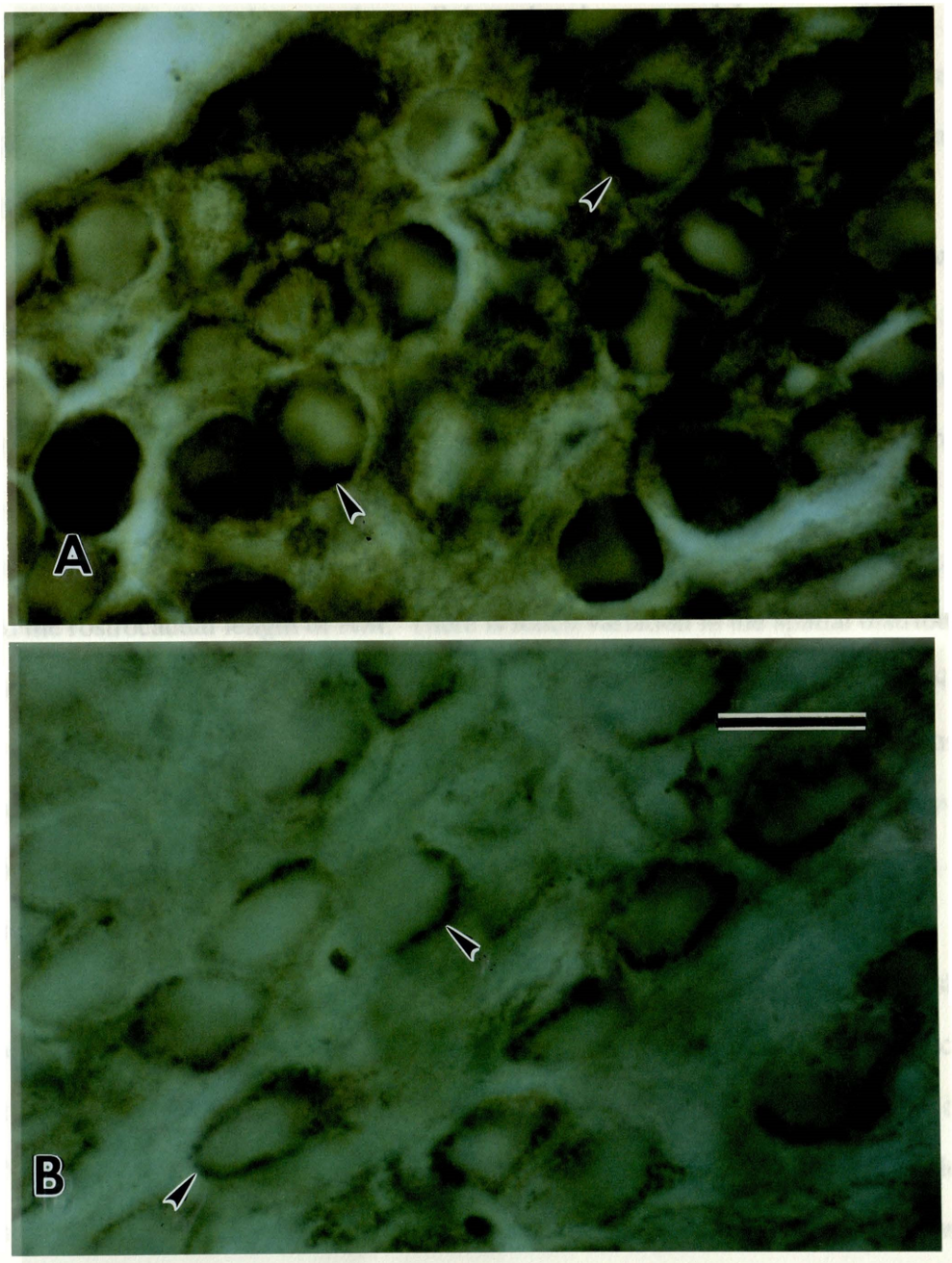


Fig. 4 High power view of DYN-I in NM terminals at E16 vs. P6.
A) DYN-I first appears at E16 as short, flat terminals (arrow points to individual terminal) around some NM neurons.
B) At P6, DYN-I terminals appear as larger, more prominent, chalice-shaped structures (arrows point to individual terminals). (Scale bar, 20 μm, applies to both panels.)

be randomly distributed across the mediolateral and rostrocaudal extents of NM. Figure 5 (A, B, C, D) shows the pattern of DYN-immunolabeling in NM from one representative animal at ages E16, P6, P13 and P22, respectively. DYN-I terminals do not appear to be concentrated in any one particular medial-to-lateral region of NM.

To support our qualitative observations, the density of DYN-I terminals was quantitatively analyzed within nine sampling regions across NM at age E16, P1-2, P6, P13, and P22. Figures 6, 7, 8, 9, and 10 show the density of DYN-I terminals in NM at each of the ages indicated across the medial-to-lateral extent of NM at three different posterior-to-anterior (P-A) levels of NM. Within each animal, there seems to be considerable variation in the density of DYN-I terminals across both the mediolateral extent and the rostrocaudal length of NM. There is much variation in the spatial distribution of DYN-I terminals between animals as well. The number of DYN-I terminals in NM, however, appears to change with age. An increase in the number of terminals surrounding neurons in NM is observed from age E16 to P6 (compare Figs. 5A and 5B). Around P13, DYN-I terminals appear to decline in number compared to P6 (Fig. 5C), with an even further decline at P22 (Fig. 5D).

These observations were supported by quantitative analysis. Figure 11 shows the average density of DYN-I terminals across the nine sampling areas in NM for each individual animal as a function of age. Figure 12 shows the changes in average density of DYN-I terminals combined from the animals within each age group during development. The density of DYN-I terminals in NM appears to increase during development with a peak at P6, followed by a decline.

Summary. DYN-I is first detectable in NM at age E16 as small, flat terminal endings. DYN-I terminal density appears to gradually increase until the age of P6. At this

age, DYN-I terminals appear as large calyceal-shaped structures surrounding the neurons in NM. By age P13, the density of DYN-I terminals appears to decrease and continues to do so until very few DYN-I terminals are see in NM at age P22. Across all animals examined, there was no obvious spatial gradient of DYN-I terminals across the mediolateral or rostrocaudal extent of NM.

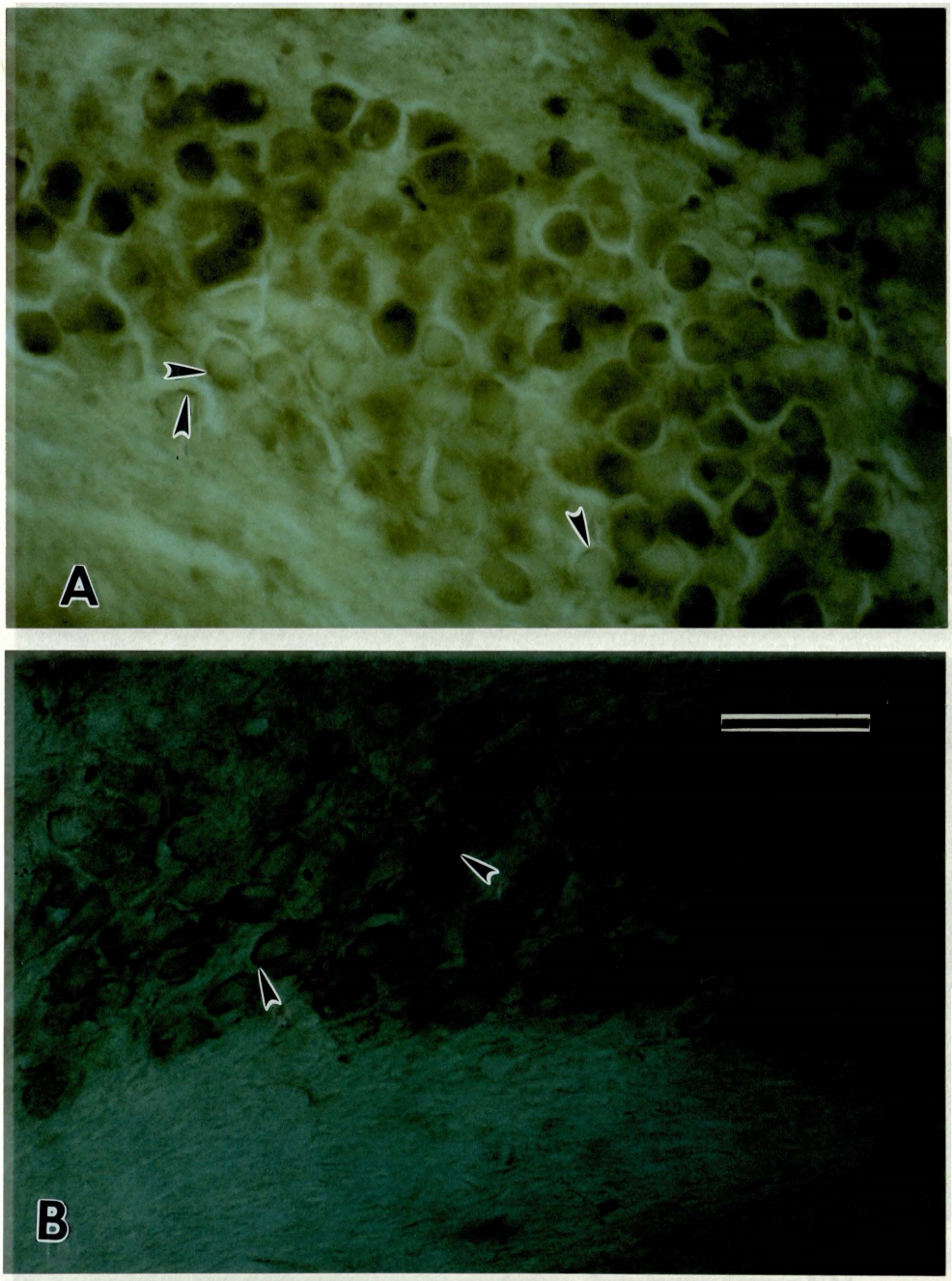


Fig. 5 Random distribution of DYN-I terminals in NM at E16 and P6.
A) At E16, a few short, flat DYN-I terminals (arrows) are scattered across NM.
B) At P6, more DYN-I terminals (arrows) are seen but still appear to be randomly distributed throughout the mediolateral extent of NM. (Scale bar, 50 μm, applies to both panels.)

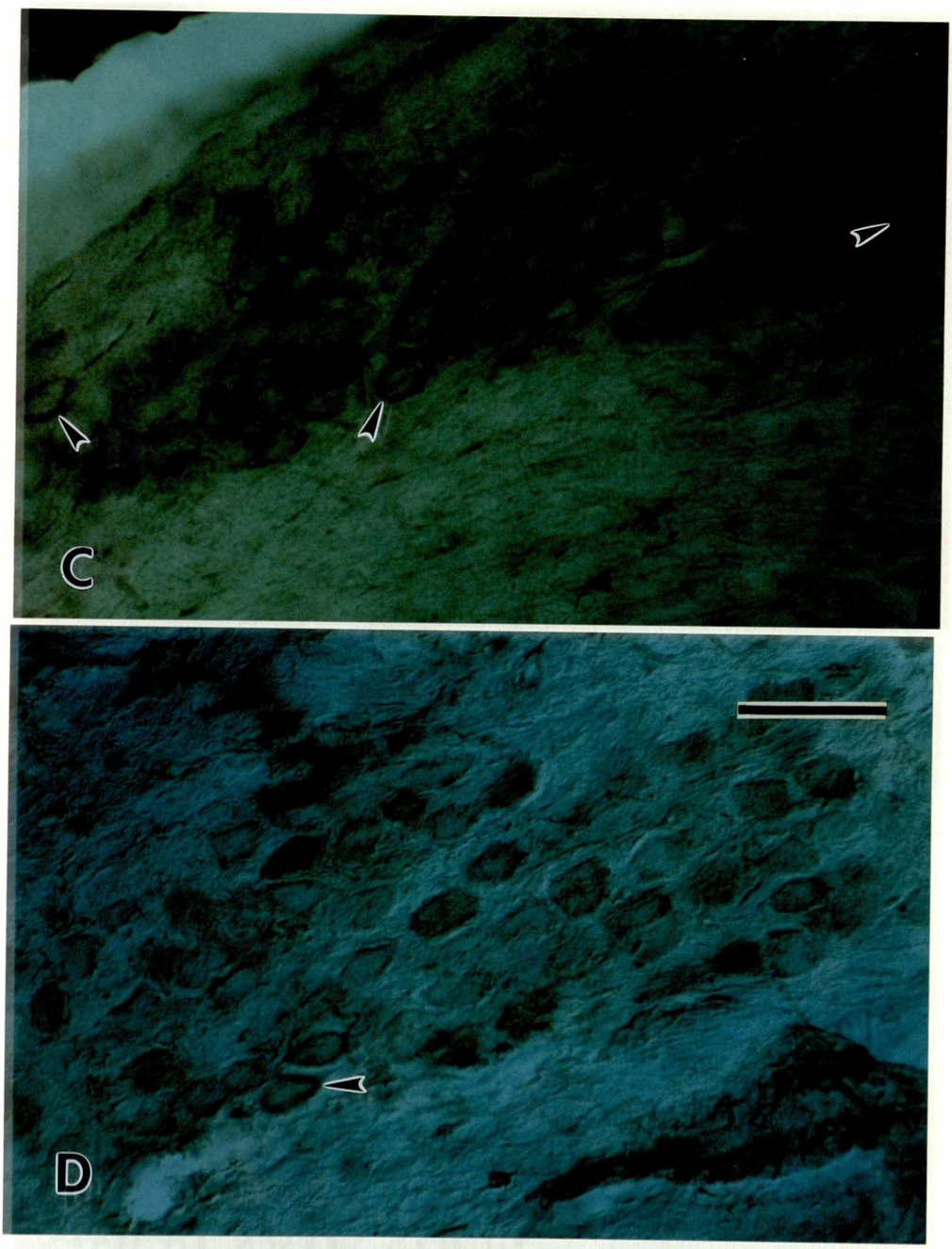


Fig. 5 Random distribution of DYN-I terminals in NM at P13 and P22. DYN-I terminals (arrows) appear to be randomly distributed across NM.
C) age P13.
D) age P22. (Scale bar, 50 μm, applies to both panels.)

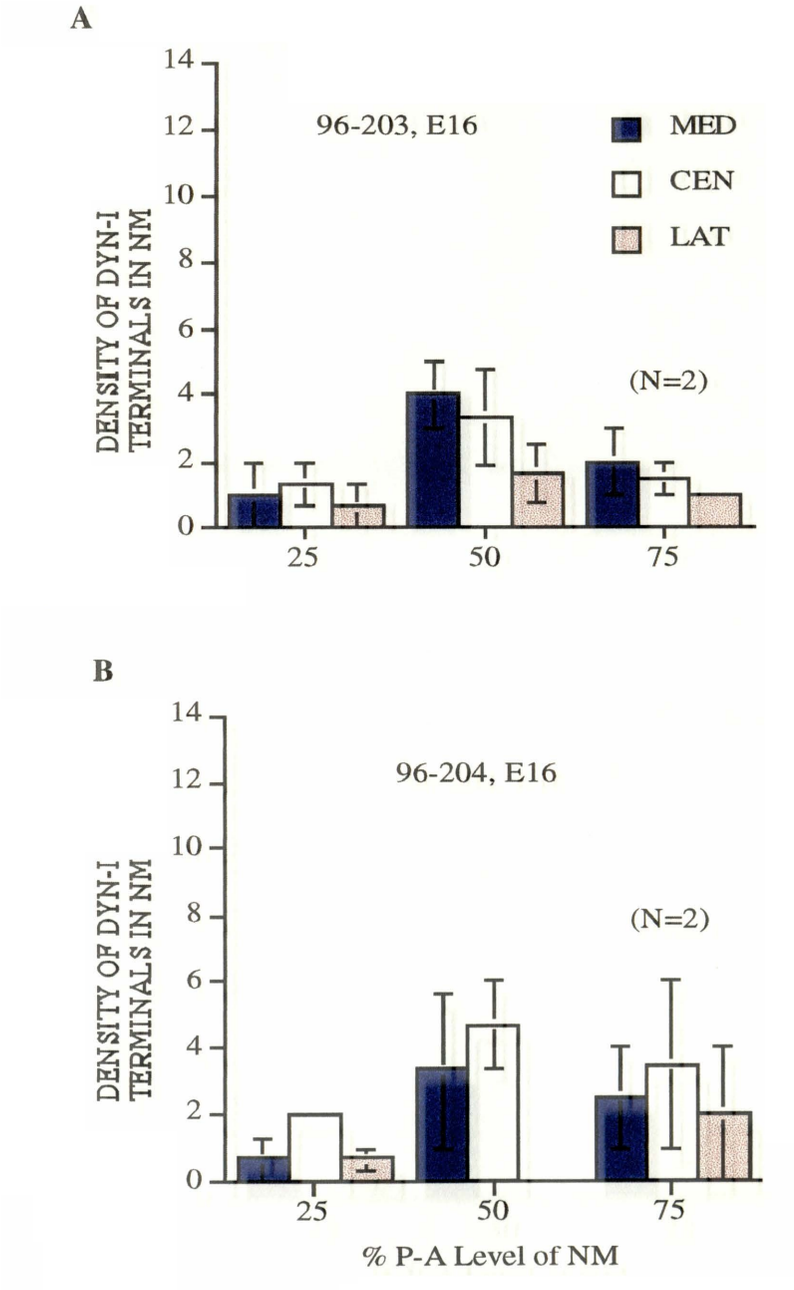


Fig. 6 Average density of DYN-I terminals in NM of two E16 animals. The average density of DYN-I terminals within the medial (MED), central (CEN), and lateral (LAT), regions in NM. Three posterior-to-anterior (P-A) levels of the nucleus are shown for two E16 animals, 96-203 in (A) and 96-204 in (B). The number in parenthesis refers to the number of sample boxes. Error bars indicate the standard error of the mean.

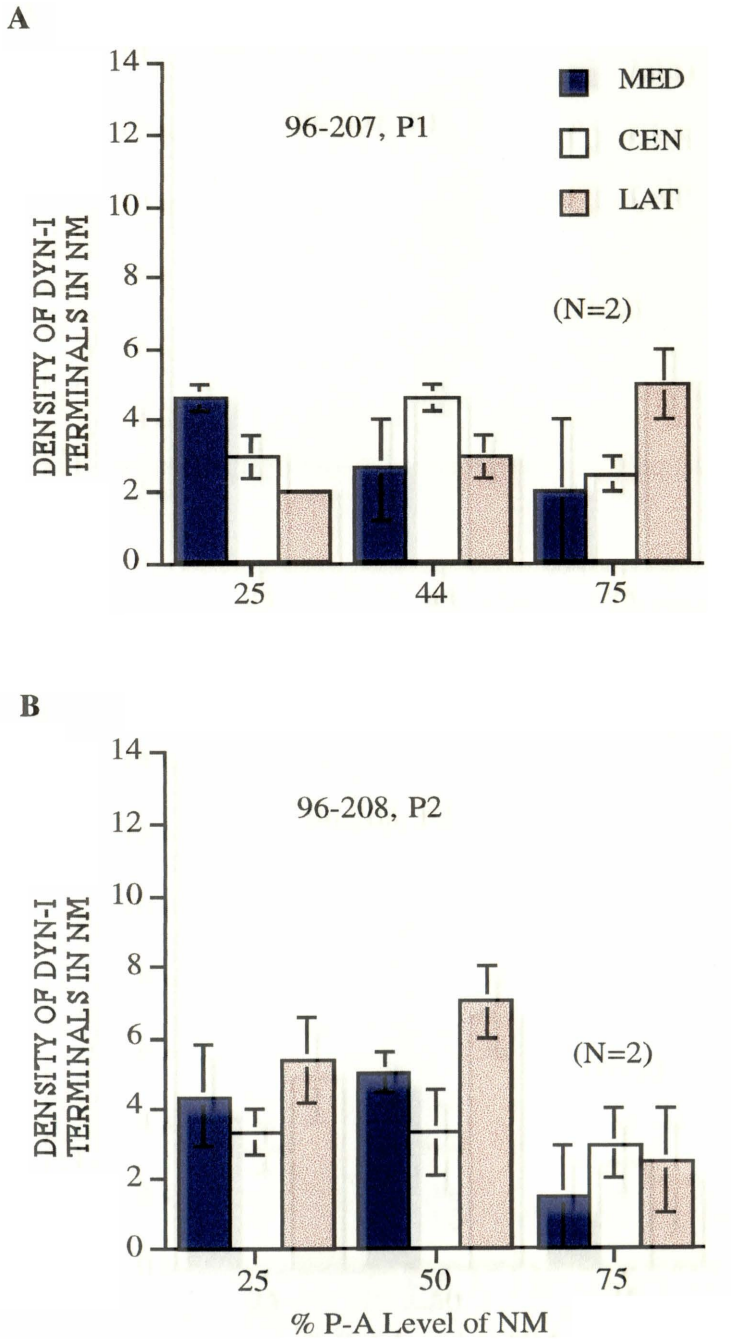
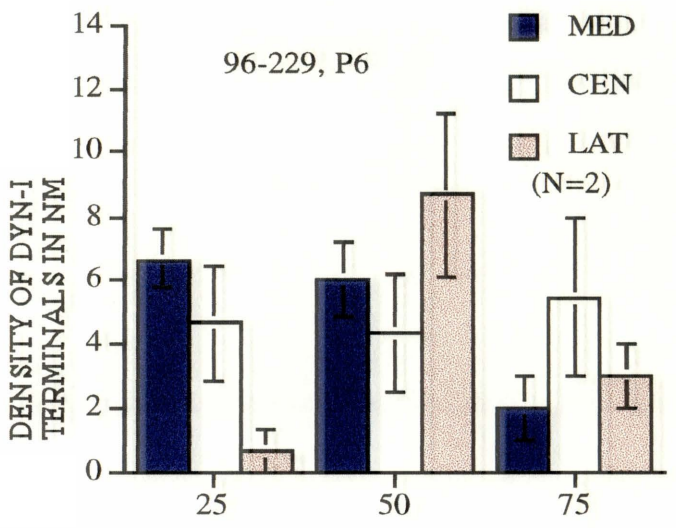


Fig. 7 Average density of DYN-I terminals in NM in two animals, ages P1 and P2. The average density of DYN-I terminals in NM in two animals, 96-207 in (A) and 96-208 in (B), ages P1 and P2. MED, medial; CEN, central; LAT lateral. P-A, posterior-to-anterior. The number in parenthesis refers to the number of sampling boxes. Error bars indicate the standard error of the mean.

A



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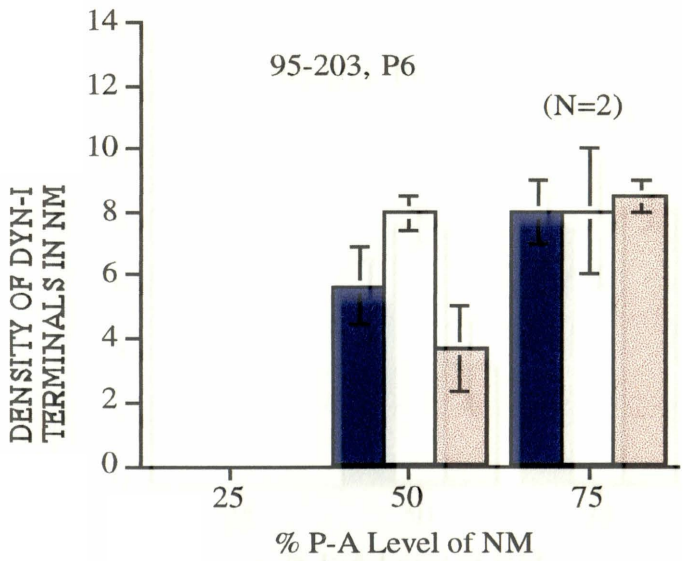

Fig. 8 Average density of DYN-I terminals is shown for two P6 animals. For two animals, 96-203 (A) and 96-229 (B), the density of terminals appears to be randomly distributed across NM. MED, medial; CEN, central; LAT, lateral. P-A, posterior-to-anterior. The number in parenthesis refers to the number of sampling boxes. Error bar indicates the standard error of the mean. Due to damage during processing or sectioning, the tissue section at the 25% level of NM in 95-203 was not available for analysis.

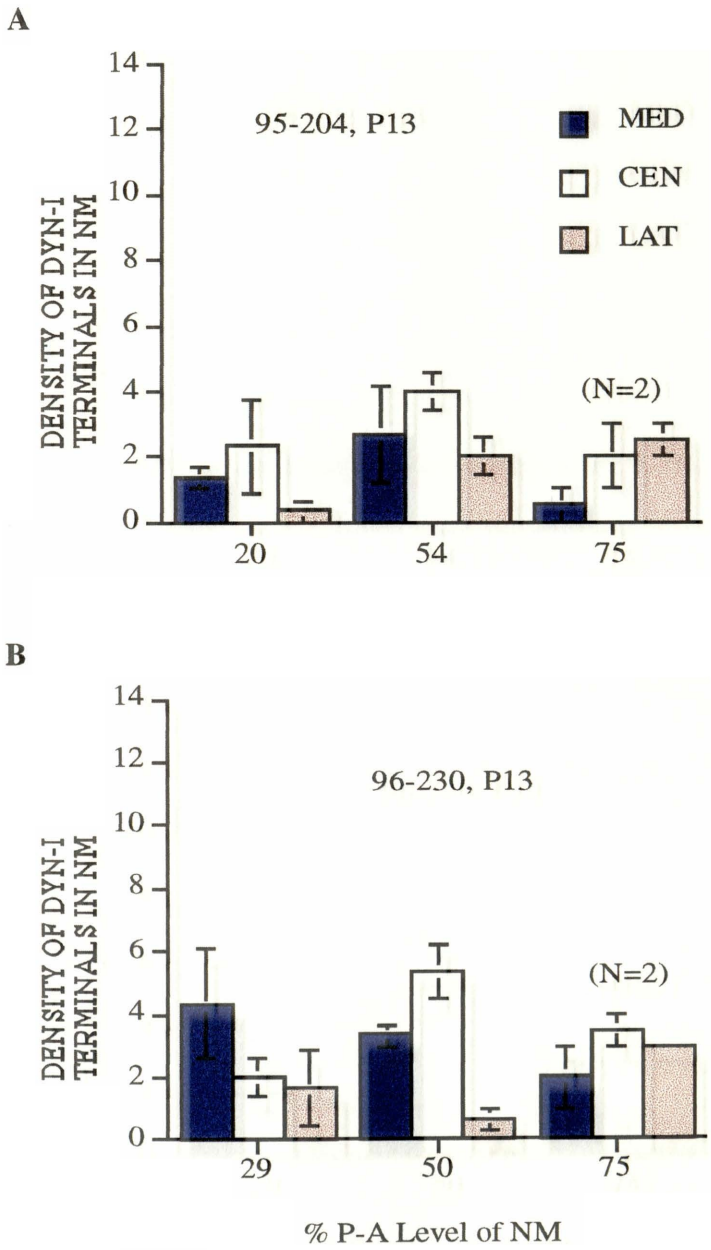

Fig. 9 Average density of DYN-I terminals in NM of two animals at age P13. The average density of DYN-I terminals within the nine sampling regions of NM for two animals, 95-204 (A) and 96-230 (B), at age P13. MED, medial; CEN, central; LAT, lateral. P-A, posterior-to-anterior. The number in parenthesis refers to the number of sampling boxes. Error bar indicates the standard error of the mean.

A

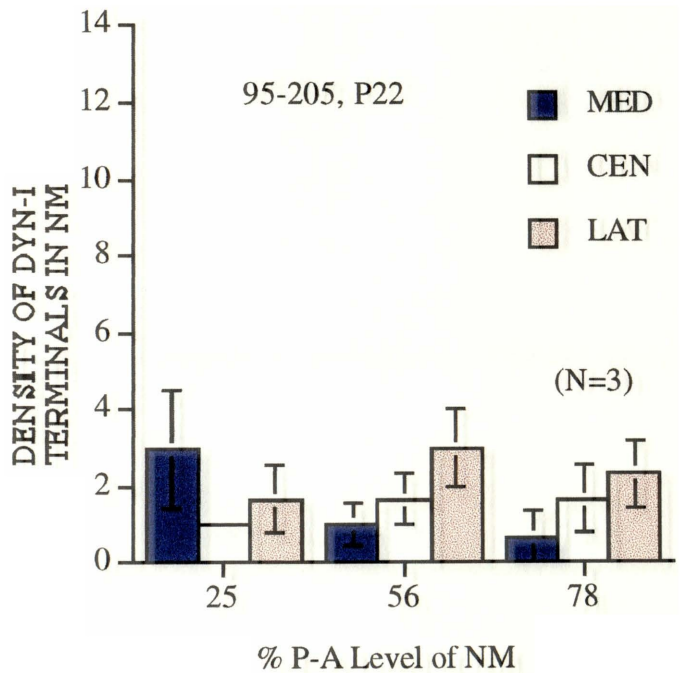

Fig. 10 The average density of DYN-I terminals in NM for one animal at age P22. MED, medial; CEN, central; LAT, lateral. P-A, posterior-to anterior. The number in parenthesis refers to the number of sampling boxes. Error bar indicates the standard error of the mean.

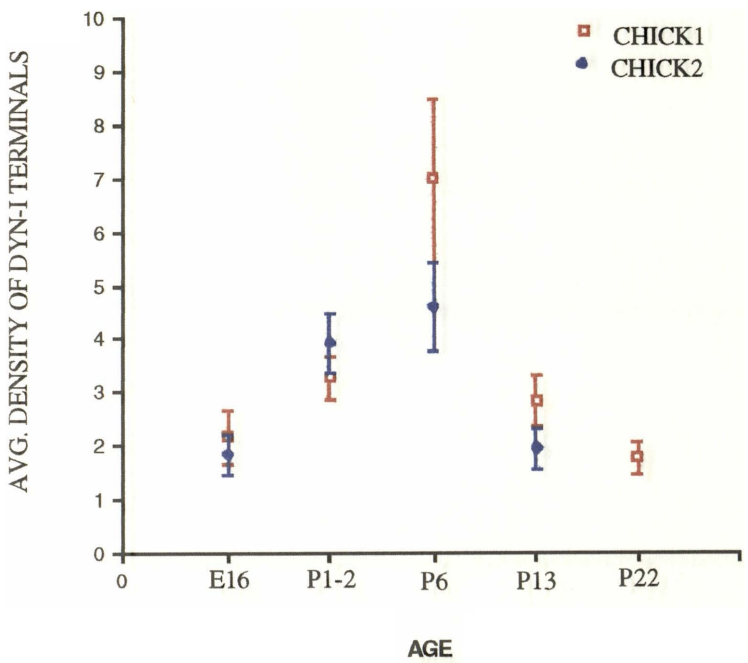

Fig. 11 The average density of DYN-I terminals from nine sampling regions in NM for each animal. There appears to be a general trend of increasing density of DYN-I terminals during development until a peak at the age of P6 followed by a decrease. Error bars indicate the standard error of the mean.

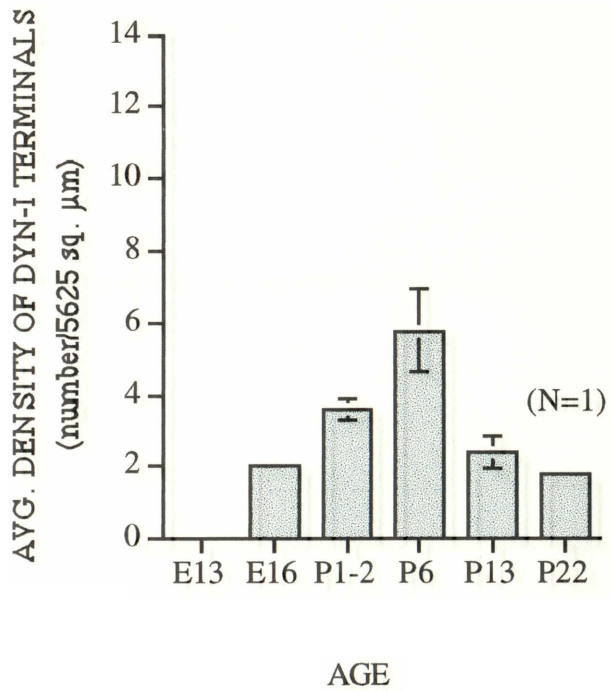

Fig. 12 The average density of DYN-I terminals combined from the two animals within each age group. There was only one animal at P22. The average terminal density appears to change during development with a peak at P6. Error bars indicate the standar error of the mean.

CHAPTER 4

DISCUSSION

In this study, we examined DYN-I terminals in NM at ages E13, E16, P1-2, P6, P13, and P22. DYN-I first appeared in NM at E16. The density of DYN-I terminals appeared to increase during the first posthatch week, reaching a peak at P6, then declining until little DYN-I was seen at age P22. Thus, DYN-I terminals appear to be evident in NM during a restricted time in development.

In this section, I will discuss the specificity of the DYN-B antibody compared with antibodies to other structurally similar opioid peptides. The development of DYN-I terminals in NM in relation to the development of the end-bulbs of Held, and the spatial distribution of DYN-I in relation to known morphological and functional gradients in NM will then be discussed. Finally, I will compare the distribution and morphology of DYN-I terminals to that of other known terminals in NM.

Specificity of the DYN-B antibody. It is likely that the immunostaining seen in NM in this study was specific to DYN-B and not to other structurally similar opioid peptides such as DYN-A or leu- or met-enkephalin for the following reasons: (1) Additional tissue sections that were processed with an antibody to DYN-A gave a different labeling pattern than that using the DYN-B antibody. Using the antibody to DYN-A, there was no terminal labeling in NM. Instead, DYN-A labeled fibers were seen in the crossed dorsal cochlear tract (XDCT) which contains axons that originate in NM and terminate in the contralateral NL (see Fig. 2). In contrast, terminals surrounding the nerve cell bodies in NM were labeled with the DYN-B antibody. (2) In a previous study to test for specificity (Anderson

and Reiner, 1990), DYN-B antibody was pre-adsorbed with the dynorphin-B peptide. This resulted in no staining. Also, the DYN-B antibody was tested for cross-reactivity by pre-adsorption with other structurally similar opioids such as DYN-A, met- and leu-enkephalin (Anderson and Reiner. 1990). Incubation of the tissue with this pre-adsorbed antibody, however, did result in immunostaining. Thus, the DYN-B antibody appears to be specific for DYN-B and to have no cross-reactivity with these other opioid peptides (Anderson and Reiner, 1990). (3) Finally, Code and Carr (1995) demonstrated the absence of enkephalin-immunoreactive terminals in NM in chicks aged P7-P17 (Code and Carr, 1995). Collectively, these results support that the terminal immunostaining seen in NM in this study is specific for DYN-B and not for other opioid peptides.

DYN-I in relation to end-bulb development. The DYN-I seen in NM appears to be located in the auditory end-bulbs of Held because after cochlea removal, DYN-I terminals in NM disappear on the operated side of the brain but remain in NM on the unoperated side (Code, 1996). This discovery led us to ask whether the development of DYN-I follows a similar time-course of development as that of the end-bulbs of Held and thus, perhaps plays a role in their development.

The auditory end-bulbs develop as the terminal branches of cochlear nerve axons first make contact with NM neurons around E10-E12 (Jhaveri and Morest, 1982b). By E16-E17, the auditory terminals form a calycine ending in NM characteristic of the mature end-bulb of Held (Jhaveri and Morest, 1982b). Finally, the number of auditory end-bulbs that contact NM cells remains the same throughout embryonic development and during the posthatch life of the animal (Jackson and Parks, 1982). The time-course of development of DYN-I in NM, however, differs from that of the end-bulbs in two respects: (1) DYN-I

first appears in NM at E16, which is later than the first appearance of end-bulbs. (2) The density of DYN-I terminals in NM appears to decline after the first posthatch week. Thus, it appears that the role of DYN-B is likely to be one other than that involved in auditory end-bulb development.

The distribution of DYN-I terminals in relation to known gradients in NM. A tonotopic gradient exists in NM such that neurons in the more rostromedial regions of the nucleus respond best to higher frequency sounds, neurons in the caudolateral region respond best to lower frequency sounds, and neurons in the middle region respond best to middle frequency sounds (Rubel and Parks, 1975). This gradient exists because regions in the cochlea that respond best to a particular frequency of sound project to corresponding regions in NM. DYN-I terminals, however, appear to be randomly distributed throughout the length and width of NM and are not concentrated in any "best frequency" region of NM. This random distribution suggests that DYN is not playing a role in the processing of frequency information in NM.

In addition to a tonotopic gradient, there is also a spatial gradient in the number of dendrites of NM. From E8 to E12, NM neurons are multipolar cells with many dendrites emerging from the cell body (Jhaveri and Morest, 1982b). However, from about E13 to E15, dendritic retraction occurs leaving some of these neurons in NM without dendrites. This retraction occurs first in the rostromedial region such that by E14-E15, neurons in this region are essentially adrendritic, whereas neurons in more caudolateral regions retain several short dendrites that remain throughout life (Jhaveri and Morest, 1982b). Since this occurs before DYN-I first appears at E16, then it is unlikely that DYN is involved with the retraction of the dendrites on NM neurons. As noted earlier, DYN-I terminals are

randomly distributed and are not concentrated in any particular region of NM. Thus, the density of DYN-I terminals does not appear to be related to the number of dendrites on NM neurons.

GABAergic and glycinergic terminals in NM. The only excitatory input to NM is from the ipsilateral auditory nerve which ends in chalice-shaped terminals, the end-bulbs of Held, that contact NM neurons (Parks and Rubel, 1978). There are other terminals in NM, however, that do not originate from the auditory nerve, those being immunoreactive for the inhibitory neurotransmitter, GABA (Muller, 1987; Code et al., 1989, 1990) and those immunostained for glycine (Code and Rubel, 1989). GABA-I terminals in NM are small, round, or oval structures surrounding NM neurons, whereas DYN-I terminals are larger and more chalice-shaped. GABAergic terminals make their first appearance in NM around E15 (Code et al., 1989). Additionally, around E17-E19, GABA-I terminals begin to become distributed in a spatial gradient such that there is a higher density of GABA-I terminals in caudolateral regions and a lower density in mediolateral regions of NM. This spatial gradient of GABAergic terminals in NM appears to remain throughout the life of the animal (Code et al., 1989). In contrast, DYN-I terminals in NM appear to be randomly distributed in NM and their density appears to decline after P6 until few terminals can be seen at P22. Thus, due to differences in their morphology and spatial organization, DYN-I terminals in NM can be easily distinguished from GABAergic terminals.

Another non-primary ending containing glycine (GLY) has also been localized in NM. These terminals have been described as small, round punctate-shaped endings surrounding neurons in NM (Code and Rubel, 1989). Like DYN-I terminals, GLY-I terminals are also randomly distributed throughout NM but can be distinguished from

DYN-I terminals based on their shape, density, and source. DYN-I terminals are chalice-shaped whereas glycinergic terminals are smaller and more punctate. Also, very few glycinergic terminals are present in NM compared to the number of DYN-I terminals. Finally, although the source of the glycinergic terminals is presently unknown, it does not appear to come from the auditory nerve (Code and Rubel 1989), whereas DYN-I terminals have been determined to originate from the auditory nerve (Code, 1996). Thus, DYN-I terminals can be distinguished from glycinergic terminals located in NM.

Possible function of DYN in NM. The present results show that DYN-I terminals are found in NM within a limited period during development. Thus, it appears that dynorphin may be involved in something other than end-bulb development, tonotopic or gradient development, or dendritic retraction in NM. One possibility is that dynorphin may be acting as a neuromodulator at the auditory terminal/NM synapse. Further studies would have to be performed to support this hypothesis. For example, one such study would be to utilize immunocytochemistry and electron microscopy to tell us whether DYN-B is actually packaged in synaptic vesicles in the end-bulbs. Another study that would support the hypothesis that DYN-B is acting as a neuromodulator at this synapse would be to determine if DYN-B is being released along with glutamate when the auditory nerve is stimulated. Finally, another piece of supporting evidence would be to demonstrate the presence of kappa opioid receptors in NM which are the type of receptors to which DYN-B binds. Until we know more about the specific effects of DYN-B on NM neurons, however, we can only speculate on its possible function.

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