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# Peptide Immunoreactivity in Aged Rat Cortex and Hippocampus as a Function of Memory and BDNF Infusion

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CROLL, S. D., C. R. CHESNUTT, N. A. GREENE, R. M. LINDSAY AND S. J. WIEGAND. *Peptide immunoreactivity in aged rat cortex and hippocampus as a function of memory and BDNF infusion.* PHARMACOL BIOCHEM BEHAV **64**(3) 625–635, 1999.—Brain-derived neurotrophic factor (BDNF) modulates neuropeptide levels in hippocampus and cortex of young adult rats. Neuropeptide levels are altered in some age-related disorders, such as Alzheimer's and Parkinson's Disease. BDNF may be able to rectify peptide abnormalities but, because plasticity decreases with age, BDNF may not alter peptide levels as readily in aged animals. To determine if BDNF would regulate peptide levels in aged rats, young, aged memory-impaired, and unimpaired rats were infused with BDNF or vehicle into hippocampus and cortex. Cell profile counts, cell profile areas, fiber counts, and/or fiber terminal densities were measured for sections immunostained for neuropeptide Y (NPY), somatostatin (SOM), cholecystokinin-8 (CCK), and dynorphin A(1–8) (DYN). Results showed that BDNF upregulated cortical NPY-immunoreactivity (ir) and SOM-ir, upregulated hippocampal NPY-ir, and downregulated hippocampal DYN-ir in both aged and young rats. In addition, BDNF significantly and selectively normalized the areas of atrophied deep cortical CCK-ir cell profiles in aged-impaired rats. Finally, decreased CCK-ir fiber density was found in the hippocampal formation of aged memory-impaired rats. © 1999 Elsevier Science Inc.

Cholecystokinin Dynorphin Neuropeptide Y Somatostatin Memory Hippocampus Cortex BDNF Neurotrophin Aged

BRAIN-DERIVED neurotrophic factor (BDNF), a member of the neurotrophin family of neurotrophic factors, has been localized to the adult brain, with especially high levels found in hippocampus (25,39). This pattern of localization suggests that BDNF may have a role in the maintenance or functioning of the adult nervous system. BDNF has been shown to regulate neuropeptide levels in cortical cultures (31), neonatal rat brain (32), and in the adult rat hippocampus and cortex (8). In the adult, BDNF has been demonstrated to increase the levels of neuropeptide Y (NPY), somatostatin (SOM), and cholecystokinin-8 (CCK) in cortex; and NPY and CCK in hippocampus. In addition, it downregulates dynorphin A (DYN) in hippocampus.

Neuropeptide levels are altered in some age-related neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease [for overview see (4,23)]. In Alzheimer's Disease, SOM, and perhaps NPY and CCK, are decreased in cortex (4). In addition, DYN levels are selectively increased in the hippocampus of aged memory-impaired rats (20). Hence, BDNF drives hippocampal and cortical peptide levels in the direction necessary to correct these pathological changes. It is, therefore, possible that BDNF could rectify the peptide abnormalities in Alzheimer's Disease or other neuropathological conditions characterized by peptide abnormalities.

Aged rats may have decreased plasticity with age and, thus, their brain peptide levels might be less likely to undergo inducible changes. In addition, TrkB mRNA levels decrease in the aged cortex (9), so that BDNF may not have as many receptors through which to transduce its signal. This potential reduction in BDNF signaling adds to the possibility that BDNF would not be as effective in modulating neuropeptide levels in aged rats as in young adult rats. In addition, any inability of BDNF to regulate peptides with age could be related to the cognitive ability of the animal, due to altered plas-

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ticity in the animal's hippocampus, cortex, or other structures. Although the density of TrkB mRNA is not related to cognitive performance in the hippocampus and cortex of aged rats (9), it is not clear that TrkB protein levels are equivalent in aged memory-impaired vs. memory-unimpaired rats. In addition, it is possible that aged memory-impaired rats have decreased TrkB receptor functioning, which would interfere with the animal's ability to transduce BDNF's signal. The levels and actions of several neurochemicals have already been shown to be correlated with an aged animal's cognitive capabilities [for discussion see (13)].

To test BDNF's ability to regulate neuropeptides with age, young, aged memory-unimpaired, and aged memory-impaired animals were continuously infused with BDNF or vehicle into the hippocampus and cortex. Brains were prepared histologically and immunostained for NPY, SOM, CCK, and DYN. Cell profiles and fibers were counted by cortical lamina and by hippocampal region. In addition, terminal density was quantified for DYN in the hilus and CA3 of hippocampus. Finally, cell profile areas were measured for NPY, SOM, and CCK. Results show that BDNF is capable of regulating neuropeptide levels in aged rats, including those with learning impairments, to as great an extent as in young animals. In addition, we describe selective decreases in CCK fiber counts in the hippocampal formation of aged memory-impaired rats, as well as selective normalization of atrophied CCK-ir cortical neurons by BDNF.

## METHOD

### *Subjects*

Subjects were 42 male Sprague–Dawley rats. Fourteen were young adult rats, approximately 3–4 months of age. Twenty-eight were aged rats, ranging in age from 22 to 25 months of age. The 28 aged rats had been selected from a larger pool of aged rats. Aged rats were only selected for the experiment if they maintained stable weights, ate and drank normal amounts of food and water, moved around their cages when experimenters entered the colony, explored actively on an open field, groomed, swam competently, visually tracked a moving pen, showed a startle response to a sudden loud hand clap, responded well to being handled, and showed no obvious signs of ill health such as alopecia or dehydration. Subjects were housed one to two per cage in an environmentally controlled animal facility with food and water available ad lib. The colony was maintained on a 12:12-h light:dark cycle with lights on at 0700 h. All experiments were conducted with the approval of the Regeneron Institutional Animal Care and Use Committee, and with great attention to the welfare of the animals.

### *Behavioral Testing*

All rats were tested on a modification of the Morris water maze (29). Rats were given one acquisition trial per day for 7 days. Animals were started at a pseudorandomly determined position in the water maze each day. Each trial ended either when the rat located the submerged, hidden escape platform, or when the rat had been searching for 5 min. After 5 min, the rat was led to the platform by hand. On the eighth day, rats were placed in the pool for 30 s with the platform removed. For this trial, all animals were started in the center of the water maze. The proportion of time that each animal spent in the quadrant that had previously contained the platform was used as a measure of the animal's retention. Aged rats that had retention scores within 1 standard deviation of the young animals were considered aged memory-unimpaired. Aged rats that had scores more than 1 standard deviation below the mean score of the young rats were statistically classified as memory-impaired. Behavioral data from the pretest are shown in Fig. 1A and B (A—acquisition, $F(2, 35) = 4.778$, $p < 0.015$; B—retention, $F(2, 35) = 13.769$, $p < 0.0001$). Mean quadrant-crossing times were also recorded for the animals. There were no significant differences between the groups in mean quadrant crossing time, suggesting that reduced swim speed or fatigue did not contribute to the poorer performance of the aged memory-impaired rats, $F(2, 33) = 2.878$, Fig. 1C).

### *Surgery*

After behavioral testing on the water maze, rats were chronically implanted with a cannula and osmotic minipump for continuous infusion of BDNF (12 μg/day) or phosphate-buffered saline (PBS) vehicle. Animals were grouped such that the BDNF and PBS groups contained animals with equivalent water maze performance. Animals were anesthetized with ketamine/xylazine (54 mg/kg ketamine and 10.8 mg/kg xylazine for young animals; 48 mg/kg ketamine and 9.6 mg/kg xylazine for aged animals, IM). For young animals, a burr hole was drilled in the skull at 3.8 posterior and 2.7 lateral referenced to bregma. For aged animals, the burr hole was located at 4.2 posterior and 2.8 lateral to bregma to achieve the same relative cannula tip location. A cannula 4 mm long for young animals and 4.8 mm long for aged animals (adjusted not only for brain size, but for the greatly increased thickness of the skull) was lowered into the hippocampus unilaterally and secured to the skull with cyanoacrylate glue (Loctite 495 Superbonder Glue, Loctite Corp., Newington, CT). The cannula (Plastics One, Roanoke, VA) was attached to an Alzet osmotic minipump (Model 2002, 14 days, 0.5 μl/h; Alza Corp., Palo Alto, CA) by a medical grade polyvinyl catheter (Bolab, Inc., Lake Havasu City, AZ). The pump was slipped subcutaneously into the interscapular region at the nape of the neck, and the wound was closed with wound clips. The pump with cannula attached was primed in PBS in a 37°C oven overnight before implantation. Infusions were continued for 12 days postsurgery before animals were sacrificed.

### *Histology*

After 12 days of infusion, animals were perfused transcardially with a brief flush of heparinized isotonic saline followed by sequential perfusion with 4% paraformaldehyde in acetate and then borate buffer. After brains were removed, they were postfixed overnight in 4% paraformaldehyde in borate buffer at 4°C and were then transferred to 30% sucrose in borate buffer. After 3–7 days in sucrose, brains were sectioned coronally at 40 μm and stored in cryoprotectant (37) at −20°C.

Sections were immunostained using an avidin–biotin–peroxidase complex reaction (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) as previously described (30). Neuropeptide antibodies to NPY (Incstar, Stillwater, MN; diluted 1:25,000), SOM (Incstar; diluted 1:7500), DYN (to DYN A(1-8); Peninsula Labs, Belmont, CA; diluted 1:15,000), or CCK-8 (Incstar; diluted 1:6,000) were used, and control sections were preabsorbed with an approximately 100-fold excess of homologous peptide to control for specificity of the immunostaining. Sections were also immunostained with a turkey anti-BDNF (Amgen, Thousand Oaks, CA) to verify BDNF

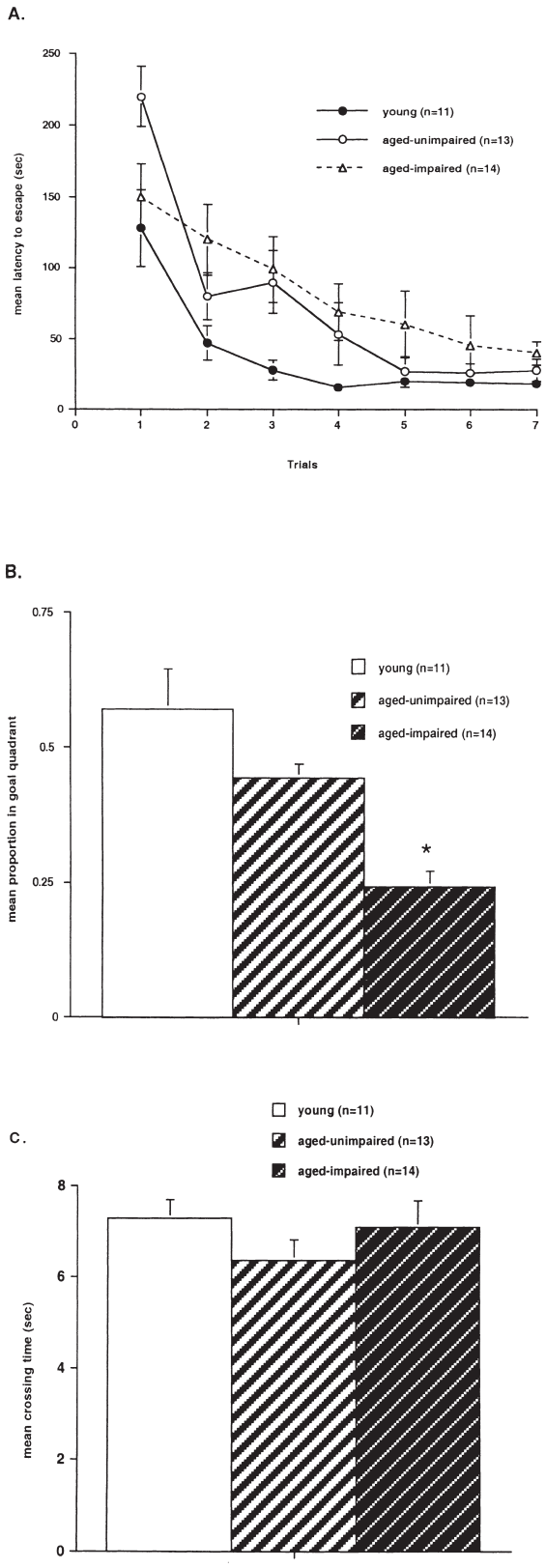


FIG. 1. Water maze data for all three groups. (A) Water maze acquisition represented by mean escape latency across trials. (B) Water maze retention represented by mean proportion of time spent searching the goal quadrant during the probe trial. (C) Mean quadrant crossing time. Error bars represent standard error of the mean. *Significantly different from young animals, $p < 0.05$.

administration. Characterization of this antibody and controls for its specificity have been previously described (30). All stains used diaminobenzidine (DAB) as the chromagen, and all but the NPY stain were nickel sulfate intensified.

*Quantification*

Quantification of the neuropeptides was undertaken on the immunostained sections. Cell profile counts were completed for NPY, SOM, and CCK in the cortex and hippocampal formation. In addition, cell profile areas were measured for these peptides in the cortex and hippocampal formation to look for changes in soma size with BDNF treatment. Cell profile counts were not completed for DYN because all DYN staining was localized in terminals. Instead, the density of DYN terminals was measured in the dentate gyrus and CA3 of the hippocampal formation. DYN was not measured in the cortex because only small amounts were detected. Finally, an additional count was performed for CCK fibers, because more of the CCK immunostaining was found in fibers than in cell profile bodies.

Cell profile counts were performed for each of the six cortical laminae and for four regions of the hippocampal formation. These included the dentate gyrus, CA3, CA1/2, and the subiculum. To avoid losing cell profiles where tissue was missing in the cannula track, cell profiles were counted in the section just before the cannula track and the section just after the cannula track, and the mean of these two counts was used to represent the cell profile count for each animal. Immunopositive cell profiles were counted if they contained nuclear membrane. In the cortex, cell profiles were counted starting just lateral to the cannula tract, and extending about 1.5 mm lateral on a fixed grid. Cell profiles were counted from the surface of the cortex to the white matter using a grid to keep track of the depth of all cell profiles. The grid was then placed on a cresyl violet-stained section from a control rat of the same cortical thickness in the same area of cortex, and the cortical lamina in which each grid was located was determined. This information was used to determine cell profile counts per lamina for each animal. In the hippocampal formation, all cell profiles were counted in the hippocampal formation of the section by region in which they were located. All cell profile counts were corrected for nuclear diameter using Abercrombie's method of correction (1).

Cell profile areas were determined using the JAVA image analysis system (Jandel Scientific, Corte Madera, CA). Using a gridded reticle to verify the laminar location of each cell profile population, 5 (SOM and CCK) or 10 (NPY) cell profiles were pseudorandomly selected per cortical lamina or hippocampal region for measurement in each section. No area measurements were taken in cortical lamina 1, because there were not enough cell profiles to take a substantial or reliable sample. Cell profiles were traced by hand after calibration of the measurement scale, and each cell profile's area was measured in $\mu m^2$. Cell profiles were sampled within the same region described for cell profile counts, and from the same sections. Nuclear diameters were similarly measured.

Cell fiber counts for CCK were also performed in the sections before and after the cannula track and averaged. A sine

wave microscope reticle was used for counting. A fiber was only counted if it followed a clear linear progression across a line on the reticle (that is, it could be traced to both sides of the crossing point). For the cortex, it was difficult to count fibers for each layer accurately because no grid was used. It was possible to count fibers, however, for groups of two layers. Therefore, fibers were counted for lamina 1/2, lamina 3/4, and lamina 5/6. The sine wave pattern was placed in a pseudorandom orientation over an area of these laminae just lateral to the location of the cannula track, and all fibers crossing the lines were counted. In the hippocampal formation, the pattern was pseudorandomly oriented over the dentate gyrus, CA3, CA1/2, and the subiculum, and fibers were counted for each region.

Densitometric measurements of DYN terminal staining in the hippocampus were completed using the Loats image analysis system and Ras-Cal-Plus software (Loats Associates, Westminster, MD). DYN immunostaining was traced by hand in the dentate gyrus and in CA3 for each animal. The optical density of each traced region was automatically calculated.

### *Statistical Analysis*

For cell profile counts and cell profile areas measured in the cortex, 6 (lamina) × 3 (group) × 2 (treatment, mixed factorial ANOVAs were performed for NPY, SOM, and CCK. For cell profile counts and cell profile areas measured in the hippocampal formation, 4 (region) × 3 (group) × 2 (treatment) mixed factorial ANOVAs were performed for NPY, SOM, and CCK. For CCK fiber counts, 3 (lamina pair) × 3 (group) × 2 (treatment) mixed factorial ANOVAs were performed in the cortex and 4 (region) × 3 (group) × 2 (treatment) mixed factorial ANOVAs were performed in the hippocampal formation. Finally, a 3 (group) × 2 (treatment) × 2 (region) mixed factorial ANOVA was performed for DYN densitometry in the hippocampus. The results of statistical analyses are presented as "$F$(degrees of freedom) = $F$-value, $p$-value." Pairwise group comparisons for significant main effects were analyzed using the Tukey HSD post hoc test. For measures that showed significant effects of group, without regard to BDNF treatment, linear regressions were performed to determine if there was a relationship between memory score and peptide levels. For all analyses, $p < 0.05$ was considered statistically significant.

## RESULTS

### *BDNF Immunostaining*

Sections from all brains receiving BDNF infusions, except for one aged-unimpaired rat and two young rats, showed BDNF immunoreactivity (-ir) around the cannula track (data not shown). The staining suggested that BDNF diffused approximately 1.5 mm from the cannula in every direction, and spread up the cannula into the cortex as previously described and illustrated (8). At the rostral–caudal level of the cannula track, the lightest staining was located in layers I and II of cortex, and in the farthest lateral aspects of the hippocampal formation (i.e., the CA3/CA2 transition area). The three brains that did not show BDNF-ir were eliminated from all further analyses.

### *Neuropeptide Y*

The number of NPY-ir cell profiles increased significantly in the cortex of BDNF-treated rats [treatment, $F(1, 33) = 30.648$, $p < 0.0001$; Table 1, Fig. 2], regardless of whether they were aged or memory-impaired [group $F(2, 33) = 0.118$, $p = 0.89$]. The effect of BDNF varied significantly, depending on the cortical lamina [lamina × treatment, $F(5, 165) = 23.009$, $p < 0.0001$], such that NPY-ir cell profile counts increased

TABLE 1
NPY CORTICAL CELL COUNTS AND CELL AREAS

| | Lamina 1 | | Lamina 2 | | Lamina 3 | | Lamina 4 | | Lamina 5 | | Lamina 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell No. | | | | | | | | | | | | |
| Young | | | | | | | | | | | | |
| PBS ($n = 7$) | 0.14 | *0.09* | 3.14 | *1.05* | 10.71 | *3.16* | 6.46 | *2.61* | 5.29 | *1.36* | 12.86 | *2.10* |
| BDNF ($n = 5$) | 0.60 | *0.48* | 7.80 | *1.62* | 26.20* | *4.98* | 20.40* | *5.20* | 34.10* | *11.02* | 27.40* | *3.25* |
| Aged-Unimp. | | | | | | | | | | | | |
| PBS ($n = 7$) | 0.71 | *0.64* | 3.57 | *1.97* | 12.93 | *5.61* | 8.57 | *5.78* | 10.00 | *6.71* | 13.36 | *4.46* |
| BDNF ($n = 6$) | 0.42 | *0.33* | 10.17 | *3.59* | 27.50* | *6.80* | 28.25* | *6.82* | 31.08* | *6.96* | 28.25* | *3.89* |
| Aged-Imp. | | | | | | | | | | | | |
| PBS ($n = 7$) | 0.00 | *0.00* | 1.57 | *0.82* | 7.86 | *1.74* | 3.57 | *0.79* | 3.21 | *0.62* | 9.07 | *1.88* |
| BDNF ($n = 7$) | 0.57 | *0.41* | 9.00 | *2.72* | 31.79* | *5.94* | 31.29* | *3.93* | 38.14* | *5.84* | 35.00* | *6.23* |
| Cell area | | | | | | | | | | | | |
| Young | | | | | | | | | | | | |
| PBS ($n = 7$) | ND | | 71.75 | *5.27* | 107.20 | *6.81* | 99.48 | *5.63* | 107.12 | *11.73* | 103.35 | *1.72* |
| BDNF ($n = 5$) | ND | | 105.85 | *11.81* | 151.24 | *20.04* | 141.19 | *12.84* | 142.61 | *6.90* | 169.90* | *17.82* |
| Aged-Unimp. | | | | | | | | | | | | |
| PBS ($n = 7$) | ND | | 77.79 | *8.70* | 96.78 | *3.28* | 105.32 | *7.04* | 123.52 | *14.66* | 150.37 | *7.74* |
| BDNF ($n = 6$) | ND | | 97.85 | *4.75* | 158.50* | *21.05* | 158.32* | *16.65* | 187.82* | *16.07* | 198.53 | *18.31* |
| Aged-Imp. | | | | | | | | | | | | |
| PBS ($n = 7$) | ND | | 71.27 | *9.75* | 101.14 | *15.18* | 101.14 | *15.18* | 120.57 | *14.25* | 115.42 | *12.27* |
| BDNF ($n = 7$) | ND | | 97.81 | *15.76* | 135.15 | *17.98* | 154.89* | *25.25* | 180.98* | *20.43* | 223.41* | *23.08* |

Standard error of the mean is given in italics after each mean.
ND = not determined—because of the limited number of cells in lamina 1, cell areas could not be properly determined.
*Significantly different from PBS, Tukey HSD $p < 0.05$.

most in laminae 4 and 5. In addition, BDNF significantly hypertrophied NPY-ir cortical cell profiles [treatment, $F(1,29) = 30.139$, $p < 0.0001$; Table 1, Fig. 2] in all animals, regardless of group [group, $F(2, 29) = 0.993$, $p < 0.383$]. Similar to our findings for cell profile counts, the lamina by treatment interaction was significant, $F(4, 116) = 3.722$, $p < 0.007$, for cell profile areas, such that the cell profiles of the deeper layers of cortex were more hypertrophied than in lamina 2.

BDNF also significantly increased the number of NPY-ir cell profiles in the hippocampal formation, $F(1, 32) = 38.373$, $p < 0.0001$ (Table 2, Fig. 2), regardless of the age or memory ability of the animal [group, $F(2, 32) = 0.027$, $p = 0.974$]. There was also a significant treatment by region interaction, $F(3, 96) = 25.817$, $p < 0.0001$, such that the dentate gyrus and CA1/2 were the most affected, and the subiculum was the least affected. Cell profile areas were increased by BDNF treatment as observed in the cortex, $F(1, 30) = 25.807$, $p < 0.0001$ (Table 2, Fig. 2). The treatment by region interaction was significant, $F(3, 90) = 7.641$, $p < 0.0001$ (Table 2) in a similar pattern to that observed for cell counts.

*Somatostatin*

The number of SOM-ir cell profiles increased significantly in the cortex of BDNF-treated rats [treatment, $F(1, 32) = 7.789$, $p < 0.009$; Table 3A], regardless of whether they were aged or memory-impaired [group, $F(2, 32) = 0.240$, $p = 0.788$]. The effect of BDNF varied significantly depending on the cortical lamina [lamina × treatment, $F(5, 160) = 7.678$, $p < 0.0001$], such that SOM-ir cell profile counts increased most in lamina 5. In addition, BDNF significantly hypertrophied SOM-ir cortical cell profiles in the animals [treatment, $F(1, 32) = 15.909$, $p < 0.0004$, Figs. 3 and 4], regardless of group [group, $F(2, 32) = 0.412$, $p < 0.666$]. Again, the lamina by treatment interaction was significant, $F(4, 116) = 3.722$, $p < 0.007$, such that the cell profiles of the deeper layers of cortex were most hypertrophied. Finally, there was a significant group by lamina interaction, $F(8, 128) = 2.411$, $p < 0.019$, such that the aged groups, especially the aged-impaired group, tended to have smaller SOM-ir cell profile areas in layer 2 of the cortex, whereas both aged groups had significantly larger cell profile areas than the young animals in layers 5 and 6 of cortex. In fact, regression analyses revealed a significant predictive value of memory score in estimating SOM-ir cell profile size in lamina 2 of cortex ($r^2 = 0.135$, $p < 0.024$), but not in any other cortical lamina.

In agreement with previous results (8), BDNF had no significant effect on SOM-ir cell profile number in the hippocampal formation, $F(1, 32) = 0.216$, $p > 0.806$, data not shown. In addition, there was no significant change in SOM-ir cell profile area in BDNF-treated hippocampi, $F(1, 32) = 3.130$, $p < 0.087$.

*Cholecystokinin*

The number of CCK-ir cell profiles did not change significantly in the cortex of BDNF-treated rats [treatment, $F(1, 32) = 0.398$, $p > 0.750$; Table 3B]. The effect of BDNF did, however, vary significantly, depending on the cortical lamina [lamina × treatment, $F(5, 160) = 5.539$, $p < 0.005$], such that

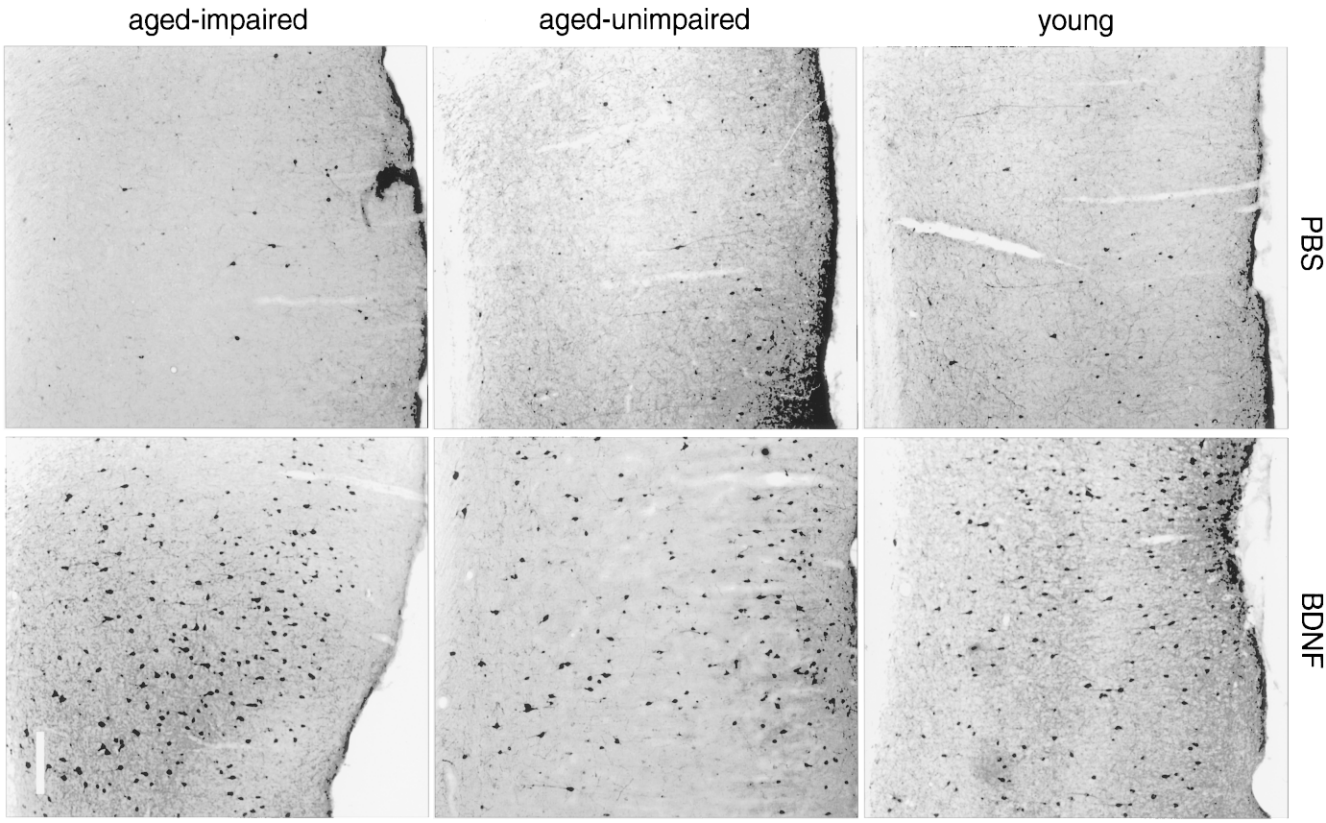


FIG. 2. NPY-ir in cortex for all groups demonstrating BDNF's effect on cell profile number across age groups. Scale bar = 150 μm.

TABLE 2
NPY HIPPOCAMPAL CELL COUNTS AND CELL AREAS

| | Dentate | | CA3 | | CA 1/2 | | Subiculum | |
|---|---|---|---|---|---|---|---|---|
| Cell No. | | | | | | | | |
| Young | | | | | | | | |
| PBS ($n$ = 7) | 43.21 | *7.77* | 18.64 | *3.56* | 84.36 | *16.57* | 14.14 | *2.67* |
| BDNF ($n$ = 5) | 100.70* | *16.07* | 27.70 | *9.25* | 140.00* | *38.40* | 22.60 | *3.86* |
| Aged-Unimp. | | | | | | | | |
| PBS ($n$ = 7) | 34.93 | *6.99* | 13.64 | *4.86* | 61.43 | *11.21* | 12.29 | *3.37* |
| BDNF ($n$ = 6) | 100.83* | *8.34* | 30.75* | *6.56* | 159.00* | *24.34* | 25.83* | *4.14* |
| Aged-Imp. | | | | | | | | |
| PBS ($n$ = 7) | 34.50 | *7.85* | 9.83 | *3.23* | 62.42 | *14.56* | 10.42 | *1.57* |
| BDNF ($n$ = 7) | 120.43* | *10.58* | 25.07* | *2.94* | 149.36* | *10.08* | 27.50* | *4.26* |
| Cell area | | | | | | | | |
| Young | | | | | | | | |
| PBS ($n$ = 7) | 156.88 | *14.30* | 126.52 | *14.01* | 128.32 | *5.08* | 113.03 | *12.28* |
| BDNF ($n$ = 5) | 267.65* | *49.96* | 160.82 | *13.68* | 173.87* | *19.92* | 136.96 | *10.36* |
| Aged-Unimp. | | | | | | | | |
| PBS ($n$ = 7) | 200.41 | *25.10* | 133.79 | *11.73* | 131.72 | *7.15* | 126.84 | *10.64* |
| BDNF ($n$ = 6) | 250.08* | *27.00* | 194.98* | *16.75* | 184.16* | *18.38* | 139.27 | *7.98* |
| Aged-Imp. | | | | | | | | |
| PBS ($n$ = 7) | 128.06 | *14.24* | 144.30 | *23.71* | 138.58 | *20.28* | 134.11 | *10.71* |
| BDNF ($n$ = 7) | 280.19* | *19.03* | 165.45 | *30.74* | 190.44* | *12.16* | 143.38 | *8.98* |

Standard error of the mean is given in italics after each mean.
*Significantly different from PBS, Tukey HSD, $p < 0.05$.

CCK-ir cell profile counts increased with BDNF treatment in laminae 2 and/or 6, but decreased with BDNF treatment in lamina 4. In addition, BDNF significantly hypertrophied CCK-ir cortical cell profiles overall [treatment, $F(1, 31) = 4.253$, $p < 0.048$; Fig. 5]. The treatment by group by lamina interaction was significant, $F(8, 124) = 2.627$, $p < 0.011$, reflecting the specific effect of BDNF on cell profile area in aged memory-impaired rats. Specifically, the cell profile areas of aged-impaired rats was decreased relative to the other groups in laminae 4–6 of the cortex, and BDNF restored these cell

TABLE 3
SOM AND CCK CORTICAL CELL COUNTS

| | Lamina 1 | | Lamina 2 | | Lamina 3 | | Lamina 4 | | Lamina 5 | | Lamina 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SOM | | | | | | | | | | | | |
| Young | | | | | | | | | | | | |
| PBS ($n$ = 7) | 0.00 | *0.00* | 1.00 | *0.43* | 11.92 | *2.96* | 8.33 | *2.76* | 5.08 | *1.62* | 11.25 | *3.21* |
| BDNF ($n$ = 5) | 0.00 | *0.00* | 2.80 | *0.88* | 12.60 | *2.20* | 11.30 | *2.13* | 22.80* | *6.76* | 16.20 | *4.59* |
| Aged-Unimp. | | | | | | | | | | | | |
| PBS ($n$ = 7) | 0.00 | *0.00* | 1.43 | *0.43* | 12.07 | *1.33* | 7.57 | *1.16* | 8.07 | *1.52* | 11.36 | *1.91* |
| BDNF ($n$ = 6) | 0.07 | *0.07* | 4.50 | *2.25* | 15.29 | *2.95* | 15.79 | *3.29* | 24.43* | *5.08* | 19.21 | *4.24* |
| Aged-Imp. | | | | | | | | | | | | |
| PBS ($n$ = 7) | 0.00 | *0.00* | 3.00 | *1.16* | 8.42 | *1.54* | 11.58 | *3.12* | 13.50 | *3.59* | 12.83 | *2.06* |
| BDNF ($n$ = 7) | 0.00 | *0.00* | 3.86 | *1.14* | 13.57 | *2.74* | 13.57 | *4.12* | 23.79* | *6.48* | 18.71 | *5.28* |
| CCK | | | | | | | | | | | | |
| Young | | | | | | | | | | | | |
| PBS ($n$ = 7) | 0.00 | *0.00* | 0.33 | *0.17* | 1.92 | *0.44* | 0.92 | *0.72* | 0.33 | *0.17* | 0.33 | *0.10* |
| BDNF ($n$ = 5) | 0.00 | *0.00* | 0.60 | *0.10* | 2.00 | *1.06* | 0.60 | *0.29* | 0.30 | *0.20* | 1.20 | *0.26* |
| Aged-Unimp. | | | | | | | | | | | | |
| PBS ($n$ = 7) | 0.00 | *0.00* | 0.29 | *0.15* | 2.64 | *0.54* | 1.14 | *0.47* | 0.43 | *0.30* | 1.07 | *0.35* |
| BDNF ($n$ = 6) | 0.07 | *0.07* | 0.86 | *0.09* | 1.21* | *0.29* | 0.21 | *0.10* | 0.71 | *0.21* | 1.36 | *0.45* |
| Aged-Imp. | | | | | | | | | | | | |
| PBS ($n$ = 7) | 0.00 | *0.00* | 0.14 | *0.09* | 1.86 | *0.18* | 0.79 | *0.21* | 0.21 | *0.21* | 0.79 | *0.32* |
| BDNF ($n$ = 7) | 0.08 | *0.08* | 0.75 | *0.31* | 2.17 | *0.31* | 0.42 | *0.15* | 0.33 | *0.10* | 1.75 | *0.34* |

Standard error of the mean is given in italics after each mean.
*Significantly different from PBS, Tukey HSD, $p < 0.05$.

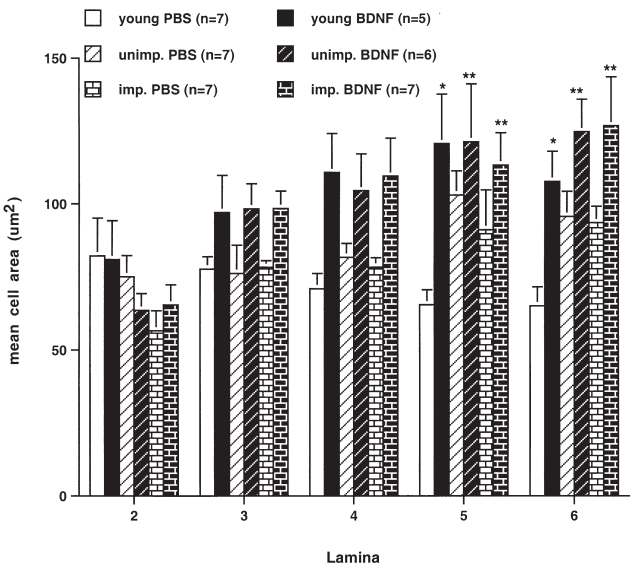


FIG. 3. Bar graph showing mean SOM-ir cell profile areas for all groups in the cortex by lamina. Error bars represent standard error of the mean. *Significantly different from PBS, Tukey HSD post hoc test, $p < 0.05$; **Significantly different from young animals, Tukey HSD post hoc test, $p < 0.05$.

profile areas to greater than or equal to those of the young and aged-unimpaired groups (Fig. 5 and 6). There were no significant effects of BDNF on CCK-ir fiber counts in the cortex [treatment, $F(1, 31) = 1.503$, $p > 0.229$, data not shown].

In the hippocampal formation, cell profile counts failed to reveal any BDNF-induced increase in CCK-ir cell profile counts [treatment, $F(1, 32) = .844$, $p > 0.365$, data not shown], cell profile areas [treatment, $F(1, 31) = 0.734$, $p > 0.398$, data not shown], or fiber [counts treatment, $F(1, 32) = 1.344$, $p > 0.254$, data not shown]. However, a significant effect of group was detected for hippocampal CCK-ir fiber counts [group, $F(2, 32) = 3.924$, $p < 0.030$, Figs. 7 and 8], such that aged-impaired animals had significantly fewer fibers than young animals. The aged-unimpaired animals were not significantly different from either group, reflecting the finding that their fiber counts were between those of the young and aged-impaired rats. Regression analyses revealed that memory score was significantly predictive of CCK-ir fiber count in CA1 ($r^2 = 0.113$, $p < 0.042$), CA2 ($r^2 = 0.114$, $p < 0.044$), and CA3 ($r^2 = 0.127$, $p < 0.031$), but not in the dentate gyrus ($r^2 = 0.004$, $p > 0.707$) or subiculum ($r^2 = 0.056$, $p > 0.157$).

### *Dynorphin*

BDNF induced overall decreases in the density of DYN-ir terminals that approached significance, $F(1, 29) = 4.141$, $p > 0.051$ (Fig. 9), regardless of age or memory impairment [group, $F(2, 29) = .535$, $p > 0.591$]. There was a significant effect of region, $F(1, 29) = 11.189$, $p < 0.003$, reflecting the greater DYN-ir densities in the dentate hilus than CA3 (Fig. 9). The effect of region by treatment was not significant, $F(1, 29) = 3.692$, $p > 0.064$.

## DISCUSSION

### *Summary*

In agreement with previous results (8), BDNF induced an upregulation of cortical NPY and SOM, and hippocampal NPY. In addition, it downregulated hippocampal DYN. The previously reported increases in hippocampal and cortical CCK after BDNF infusions did not achieve significance when quantified. Most of the BDNF-induced peptide regulation oc-

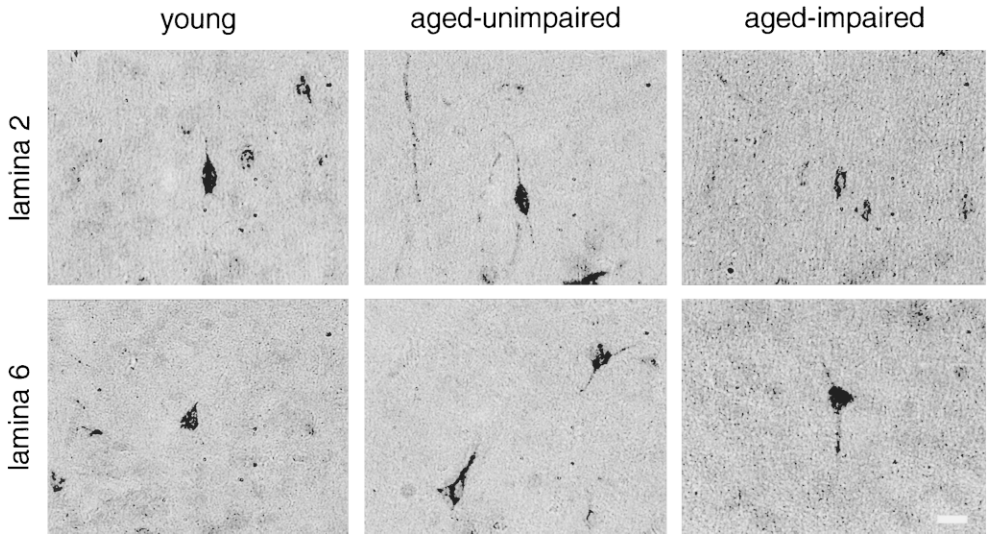


FIG. 4. Enhanced digital images of representative SOM-ir cell profiles in cortex for the three age groups demonstrating age-related differences in cell profile areas. The top panel for each group shows cell profiles in layer 2, in which the aged-impaired animals show decreased cell profile areas. The bottom panel shows cell profiles in layers 5/6, in which both groups of aged animals show increased cell profile areas. Scale bar = 30 μm.

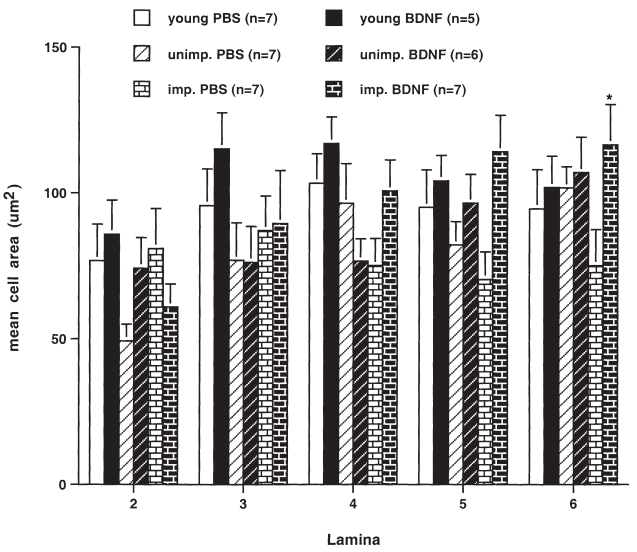


FIG. 5. Bar graph showing mean CCK-ir cell profile areas in the cortex for all groups by lamina. Error bars represent standard error of the mean. *Significantly different from PBS, Tukey HSD post hoc test, $p < 0.05$.

curred in animals regardless of age and memory ability. However, decreases in CCK cell profile area in the cortex were observed in aged-impaired animals, and BDNF selectively increased the areas of these atrophied cell profiles. In addition, the number of CCK-ir fibers in the hippocampal formation was significantly reduced in aged memory-impaired, but not memory-unimpaired, rats. Finally, there was a significant interaction between cortical lamina and age group in cortical SOM-ir cell profiles, such that aged animals tended to have smaller cell profiles in layer 2 and larger cell profiles in layer 6 than the young animals.

*Age-Independent BDNF Effects*

As previously reported, BDNF increased NPY and SOM-ir in the cortex, and NPY-ir in the hippocampus. This increase was characterized by significant changes both in cell profile area and in cell profile number. The increases in these peptides occurred regardless of age or memory status. In fact, aged rats showed no attenuation of any BDNF-related increases in peptide levels. These findings suggest that the aged rat cortex and hippocampal formation retain the ability to experience inducible upregulation of neuropeptide levels. Furthermore, these findings suggest that the decreased trkB mRNA detected in the cortex of aged rats (9) does not lead to a decrease in the biological actions of exogenous BDNF, at least not in modulating the levels of the neuropeptides that we have studied here. It is possible that the BDNF infusions upregulated or increased the sensitivity of the remaining TrkB in the aged brain, hence increasing its responsiveness. Alternatively, it is possible that the normally dense localization of TrkB in the adult cortex is not rate limiting for BDNF's actions. Finally, we cannot rule out the possibility at this point that the decreases found in trkB mRNA do not result in decreases of TrkB protein.

*Regional Differences in BDNF Effects*

In both the cortex and the hippocampal formation, there was a significant regional effect of BDNF treatment, such that BDNF had more or different effects in some regions than others. In the cortex, BDNF tended to produce greater effects in the deeper layers of cortex than in the superficial layers. This differential effect is likely to result, at least in part, from the differential characteristics and connectivity of the cells in these laminae. It is also possible that differential TrkB levels contribute to the laminar differences in responsivity. For example, TrkB immunostaining shows a very dense localization to layer 5 (38), in which BDNF consistently produced strong effects on neuropeptides. In the hippocampal formation, CA1 and the dentate tended to be more affected than CA3 and the subiculum. TrkB is heavily concentrated in the primary cell layers of all of these regions (38), so the differential effects are more likely to be due to the characteristics and connectivity of the cells and the region. The consistently significant interactions between treatment and cortical lamina or hippocampal region suggest that BDNF is causing regionally specific, precisely regulated effects rather than nonspecific increases in neuropeptides across cells. This finding extends our initial report of a differential profile of neuropeptide effects in cortex vs. hippocampus vs. striatum (8).

*Age and Memory-Dependent Effects*

Some of the differences in neuropeptide levels that we report in the current study were dependent on the age or cognitive performance of the animals. The most striking of our age-related effects was a decrease in CCK-ir fiber density in the hippocampal formation of aged rats. The decrease was most striking in the aged-impaired rats, with the aged-unimpaired rats having fiber counts between those of the young and the aged-impaired rats. Wang et al. (36) also reported an age-related decrease in CCK levels extracted from hippocampal homogenates, but this effect was dependent on the time of day the animal was sacrificed. CCK has been shown to improve rat learning in many tasks not requiring food as a motivator (7,10,11,14,16,19,21,24,35). It is, therefore, possible that the decreased number of CCK-ir fibers observed in the hippocampus of aged-impaired rats contributes to their impairment in water maze performance. In addition to our finding of decreased CCK fiber counts in the aged hippocampus, we observed a slightly decreased CCK-ir cell profile area in the deeper layers of cortex of aged animals, particularly in those with memory impairments. BDNF selectively increased these cell profile areas in the aged-impaired animals, such that their cell profile areas approximated those measured in young animals. In contrast to the findings in deep cortex, BDNF did not increase the areas of CCK-ir cell profiles in layers 2 and 3, in which the CCK-ir cell profile areas were normal in aged rats. That is, BDNF selectively normalized cortical CCK-ir cell profile areas in those animals and regions that showed cell profile atrophy. Therefore, one might predict that BDNF could enhance cognitive functioning in aged memory-impaired rats by bolstering CCK cells. Unfortunately, BDNF has not been shown to improve the learning of aged rats and, in fact, can worsen their performance [(12,33); unpublished observations]. BDNF regulates a wide variety of peptides and neurochemicals in the brain (3,8,31,32,34). It is, therefore, possible that any beneficial effects of BDNF treatment on CCK abnormalities are undermined by side effects mediated through

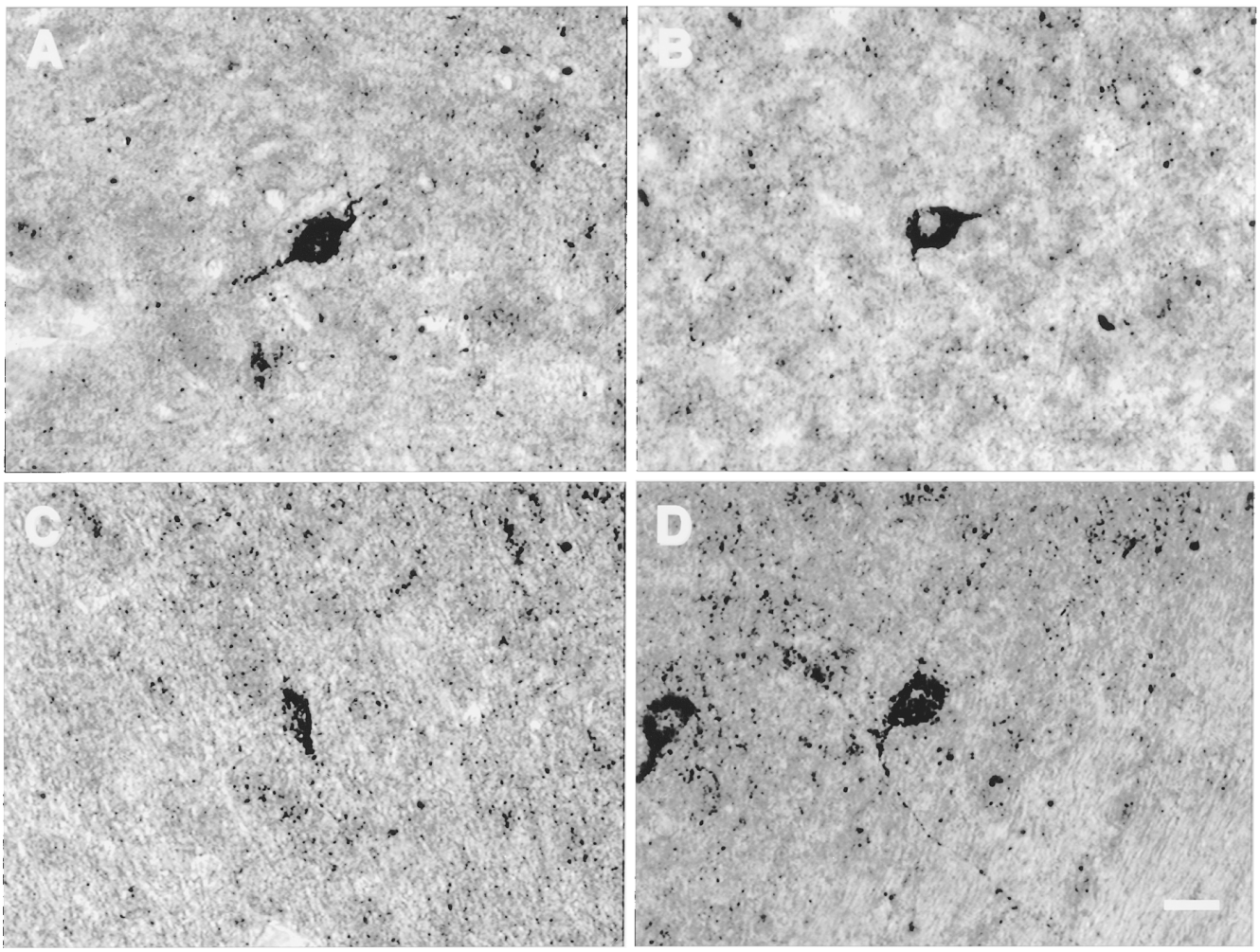


FIG. 6. Enhanced digital images of representative CCK-ir cell profiles in deep cortex for all groups, demonstrating the decreased cell profile areas in aged-impaired rats which is normalized by BDNF treatment. (A) Vehicle-infused young. (B) Vehicle-infused aged unimpaired. (C) Vehicle-infused aged impaired. (D) BDNF-infused aged impaired. Scale bar = 30 μm.

other neurotransmitters or neuromodulators. For example, BDNF could cause problems with attention or locomotion (26), which are crucial elements of successful water maze performance. Further research will be necessary to evaluate these possibilities further.

In addition to the changes in CCK-ir found in aged animals, significant alterations of SOM-ir cell profile area were found in the cortex of aged rats. Specifically, SOM-ir cell profiles were significantly larger in layers 5 and 6 of all aged rats, regardless of learning impairments. In contrast, the areas of SOM-ir cell profiles appeared slightly decreased in layer 2 of aged-impaired rats, illustrating the regional specificity of the effect. Cell profile areas were unaffected by age or memory status in layers 3 and 4. Therefore, the relative size of SOM-ir cell profiles across the age groups varied by lamina. Previous reports have suggested that some cells in the aged rat forebrain are atrophied, while others hypertrophy and become more densely branched as a compensatory mechanism (2,5,6,15,27,40). Although we do not know why there are differential cell profile sizes with age and lamina, it is possible that the cells in layers 5 and 6 are compensating, and that the cells in layer 2 of the aged-impaired rats are either unable to compensate, or have stopped compensating. Slight and regionally specific decreases in SOM-ir cell counts with age have been reported in the cortex (5,28), but we detected no such decrease in our population of aged rats.

Finally, we observed a slight decrease in the density of DYN-ir terminals in the hippocampus of aged-impaired rats. Although not significant, this observation contrasts with the previously reported significant increase in DYN terminal density in aged-impaired rats (20). Two possible explanations for the discrepancy between the two studies are rat strain and method of measurement. We used Sprague–Dawley rats and quantified only the dentate gyrus and CA3 of the dorsal hippocampus immunohistochemically. Jiang et al. (20) used Long–Evans rats, and performed a dynorphin RIA using the entire hippocampus. Further research will be necessary to explain the discrepancy. In addition, we failed to observe any BDNF-induced decrease in the density of DYN-ir fibers in aged-impaired animals. Because we observed low levels of

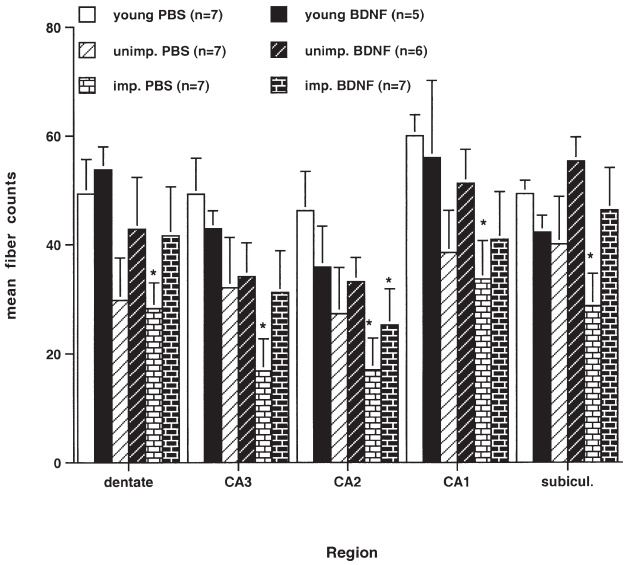


FIG. 7. Bar graph showing mean CCK-ir fibers in the hippocampus for all groups by region. Error bars represent standard error of the mean. *Significantly different from young animals, Tukey HSD post hoc test, $p < 0.05$.

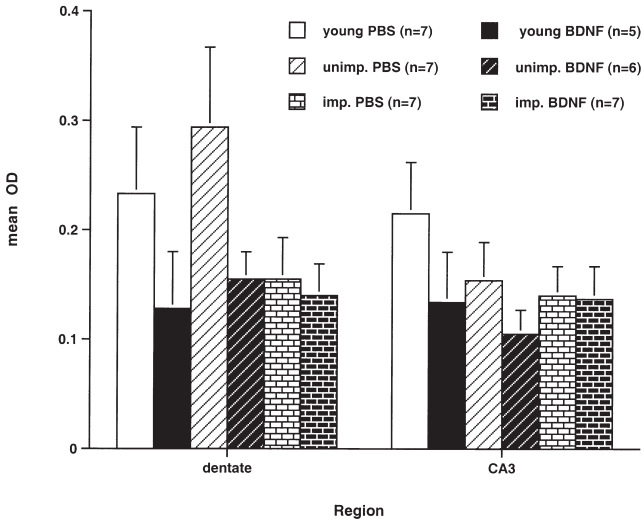


FIG. 9. Bar graph showing mean density of DYN-ir in the hippocampus by region.

dynorphin in vehicle-treated aged-impaired rats, it is possible that BDNF could not reduce DYN levels further.

We found no statistically significant effects of age on NPY-ir, or on BDNF-induced increases in NPY-ir. Previous reports show decreased NPY with age (5,6,17,18,22). Those reports that examined NPY histochemically (5,6,18) suggest a loss of neurons in the cortex and hippocampus. Although we do see a slight decrease in NPY-ir cell profile counts in both the hippocampus and cortex with age, our results in this population of aged male Sprague–Dawley rats do not achieve statistical significance.

## CONCLUSIONS

Our findings have suggested that, as observed for young rats, BDNF is capable of modulating neuropeptides in the aged rat cortex and hippocampal formation. This finding suggests that BDNF could potentially be used to restore normal peptide levels in the cortex and hippocampus of aged patients with neurodegenerative diseases affecting neuropeptide levels. Whether normalizing these peptide levels would result in functional improvements in aged populations remains an issue for future research. Unfortunately, our aged rats did not show the substantial neuropeptide deficits often seen in human disease. Therefore, they may serve as a model of aging, but may not serve as a good model of pathological aging with neuropeptide abnormalities. There was, however, some evidence that BDNF normalized the abnormalities we observed in CCK-ir, suggesting the possibility that BDNF could enhance peptide levels in brains with a pathologically decreased peptide phenotype.

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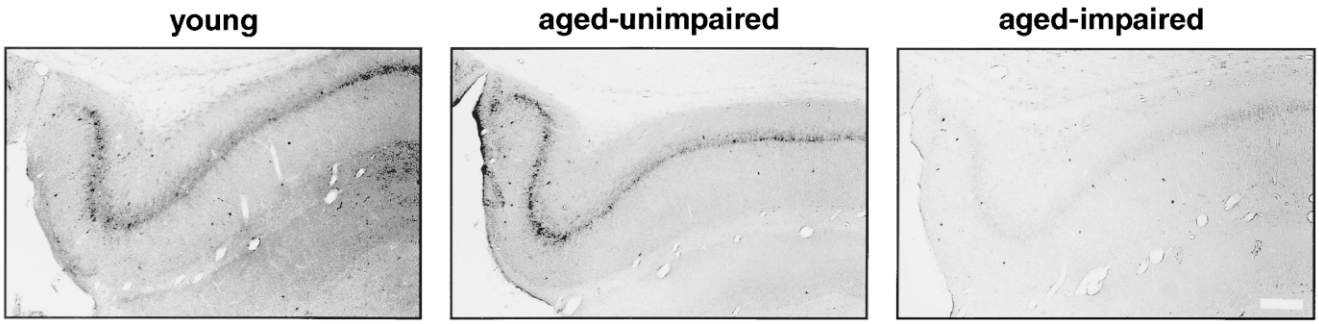


FIG. 8. CCK-ir in CA1 and the subiculum of the hippocampal formation for the three age groups demonstrating age-related differences in fiber density. The aged-impaired animals have significantly reduced fiber density, and the aged-unimpaired animals have a fiber density between that of the young and aged-impaired animals. Scale bar = 200 μm.

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