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# Acetylcholinesterase and Catecholamine Distribution in the Locus Ceruleus of the Rat[1]

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ALBANESE, A. AND L. L. BUTCHER. *Acetylcholinesterase and catecholamine distribution in the locus ceruleus of the rat.* BRAIN RES. BULL. 5(2) 127–134, 1980.—In an attempt to characterize more extensively the distribution and morphologies of acetylcholinesterase- (AChE, EC 3.1.1.7) and norepinephrine-containing neurons in the rat locus ceruleus, we histochemically examined the two neurochemicals either separately or on the same tissue section. Some animals were pretreated with *bis*(1-methylethyl)phosphorofluoridate, an irreversible cholinesterase inhibitor, in order to reduce background AChE staining and, consequently, to enhance visualization of neurons containing the cholinergic degradative enzyme. Neuronal somata containing AChE were round, oval, fusiform, or pyramidal. On the basis of cell body size, three different populations of cerulear neurons were discerned: small (maximum soma extent: 10–20 μm), medium (maximum soma extent: 20–30 μm), and large (maximum soma extent: >30 μm). The proportion of large dimension cells increased, whereas the percentage of small neurons decreased, from rostral to caudal levels of locus ceruleus. The proportion of medium-sized cells was roughly constant at all levels of the nucleus. The morphologies and distribution of norepinephrine-containing neurons appeared identical to those of AChE cells. In tissue sections stained both for AChE and catecholamines, it was found that all cerulear neurons containing norepinephrine also appeared to possess AChE. The significance of AChE's association with norepinephrine somata and proximal processes in locus ceruleus is unknown, but one possibility is that the cholinergic degradative enzyme inactivates acetylcholine released from cholinergic afferents, the origin of which are currently unknown.

Locus ceruleus  Acetylcholinesterase  Norepinephrine  Histochemistry

COMPOSED of approximately 1650 neuronal somata (Nissl stain: [31]), the rat locus ceruleus has been implicated in a variety of functions ranging from cardiovascular and respiratory regulation to intracranial self-stimulation and learning (for review, see [2]). Locus ceruleus exhibits the highest concentration of cupric ions in the mammalian brain [32], and this neurochemical feature of the nucleus probably accounts for its appellation "blue-black streak," a term coined by Reil in 1809 (see [2]). In addition to copper, locus ceruleus contains extensive amounts of norepinephrine, a putative neurotransmitter and/or neuromodulator [8], and it is the noradrenergic character of the somata aggregation that has attracted most attention since the landmark histochemical studies of Dahlström and Fuxe [12]. Locus ceruleus also contains appreciable quantities of acetylcholinesterase (AChE, EC 3.1.1.7), and in previous reports it has been demonstrated that this cholinergic degradative enzyme is associated with cerulear somata and proximal processes also possessing norepinephrine [1,4]. Little information currently exists, however, concerning the detailed comparative distribution in locus ceruleus of norepinephrine and AChE; it is to this topic that the present study is addressed.

## METHOD

### *Experimental Animals*

Male and female Sprague-Dawley rats (Simonsen Laboratories; Gilroy, CA; U.S.A.) were used. The animals weighed 200–330 g at the time of experimentation and were housed under conditions of constant temperature (22°C) and relative humidity (50%). They were maintained on a 12 hr light-dark schedule of illumination; injections and animal euthanasia were performed during the dark phase of the cycle (6:00–18:00 hr).

### *AChE Histochemistry*

Some rats were killed by decapitation. Most animals, however, were given an anesthetic dose of sodium methohexital, 50 mg/kg IP, and, subsequently, were sacrificed by perfusion with 120 ml cold (3–6°C) 0.9% saline followed by 120 ml cold 10% buffered formalin (pH=7). The brains were then rapidly removed from the cranial cavity and placed into cold buffered neutral formalin for 16–48 hr at 3–6°C. They were subsequently transferred to a cold 30% sucrose solution for

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an additional 24–48 hr. The tissue was then cut into 4–6 mm slabs, frozen in 2-methylbutane cooled by solid $CO_2$, and mounted on a brass specimen holder. Frozen sections in transverse, horizontal, or saggital planes were cut at 8, 10, 20, or 40 $\mu$m intervals either on a sliding microtome or in a cryostat. The resulting brain sections, mounted on glass slides or free-floating, were then stained for AChE according to the direct-coloring protocol of Karnovsky and Roots [17] as modified by Butcher, Eastgate, and Hodge [6]. The reagents in a 10 ml volume of incubation medium were 5 mg acetylthiocholine iodide, 6.5 ml of 0.2 M Tris-maleate buffer (pH=5.7), 0.5 ml of 0.1 M sodium citrate, 1.0 ml of 0.03 M cupric sulfate, 1.0 ml of 0.005 M potassium ferricyanide, and 1.0 ml of distilled, de-ionized water. Brain sections were incubated with agitation at 22°C or 38°C for 1 or 2 hr.

The time of formalin fixation we used was adopted after extensive parametric experimentation. In our hands, little or no histochemically detectable difference in intensity of AChE staining could be detected among sections fixed for 16–48 hr as described previously *(vide supra)*, brain sections fixed only by perfusion with 120 ml cold 10% buffered formalin (pH=7), and cryostat sections of unfixed brain tissue.

The specificity of the reaction of AChE was tested in several ways. (1) Acetylthiocholine iodide was omitted from the Karnovsky-Roots incubation medium. (2) Butyrylthiocholine iodide was substituted for acetylthiocholine iodide in the reaction mixture. (3) To inhibit butyrylcholinesterase, brain sections were preincubated in 1 $\mu$M, N, N$^1$-*bis*(1-methylethyl)pyrophosphorodiamidic anhydride (iso-OMPA; K and K Laboratories; Plainview, NY; U.S.A.) for 45 min followed by incubation in the Karnovsky-Roots medium having either butyrylthiocholine iodide or acetylthiocholine iodide as substrate and containing 1 $\mu$M iso-OMPA. (4) AChE was inhibited by placing brain sections in 50 $\mu$M 1:5-*bis*(4-allyldimethylammoniumphenyl)pentan-3-one dibromide (BW284C51; Burroughs Wellcome Co.; Research Triangle Park, NC; U.S.A.) for 45 min prior to incubation in a Karnovsky-Roots reaction mixture having either butyrylthiocholine iodide or acetylthiocholine iodide as substrate and containing 50 $\mu$M BW284C51. (5) To inhibit both butyrylcholinesterase and AChE, brains sections were preincubated with 30 $\mu$M eserine sulfate; the sections were then incubated in a Karnovsky-Roots medium containing 30 $\mu$M eserine sulfate and either butyrylthiocholine iodide or acetylthiocholine iodide as substrate.

### *AChE Histochemistry after Irreversible Enzyme Inhibition*

It is very difficult to ascertain the detailed morphology of AChE-containing neurons in locus ceruleus in rats not given DFP, or a similar irreversible inhibitor, presumably because those cell bodies lie close to one another, are numerous, and have extensively overlapping proximal processes [4]. Observation of individual somata, axons, and dendrites is thereby obscured [4]. Regeneration of AChE following DFP administration is apparently due to *de novo* synthesis of the enzyme [23]. Since the sequence of AChE recovery preferentially occurs proximo-distally from the cell body, this pharmaco-histochemical regimen enables visualization of AChE-containing somata and proximal processes in brain regions such as locus ceruleus where those subcellular constituents are ordinarily obscured by intense neuropil staining. The details and rationale for this procedure, based ultimately on the observations of Nichols and Koelle [23], have been described extensively in Butcher [5].

Rats were injected intramuscularly with a sublethal dose, 0.8 mg/kg, of *bis*(1-methylethyl)phosphorofluoridate (DFP), dissolved in arachis oil, at varying times before they were sacrificed and their brain processed subsequently for AChE. An injection-sacrifice interval of 4 hr was found optimal to demonstrate neuronal somata in locus ceruleus. Slightly longer intervals, 6 or 8 hr, were necessary to observe neuronal processes.

### *Catecholamine Histochemistry*

Rats were killed by decapitation. Their brains were removed within 1.5 min from the cranium, cut into 4–6 mm slabs, mounted on brass specimen holders, frozen in a cryostat maintained at −25°C, and cut at 6, 8, or 10 $\mu$m intervals. The brain sections were mounted on glass slides and reacted with glyoxylic acid according to the procedure of de la Torre and Surgeon [14]. The slides were coverslipped under mineral oil and subsequently examined in a Zeiss RA fluorescence microscope.

### *AChE and Catecholamine Histochemistry on the Same Brain Section*

Rats were injected intramuscularly with 0.8 mg/kg DFP; 4, 6, or 8 hr thereafter they were sacrificed by decapitation. Some animals were given 100 mg/kg nialamide (Pfizer Inc.; Brooklyn, NY; U.S.A) IP 6 hr before euthanasia to enhance catecholamine fluorescence; nialamide pretreatment did not affect AChE staining. Their brains were then processed for catecholamine histochemistry as detailed previously in this report. DFP treatment did not affect catecholamine fluorescence, at least as assessed histochemically [7].

Following the recording of the location of the slide on the microscope stage and subsequent photography of catecholamines, the coverslip was removed manually. The slide was then immersed in xylene for 1 min in order to remove the mineral oil coverslipping medium, blotted on absorbent paper, air-dried at 22°C for 10 min, dipped in 0.9% saline, and placed in a Coplin jar containing the AChE reaction mixture described elsewhere in this paper. The time of incubation ranged from 4 to 12 hr. Following this incubation period and subsequent rinsing of the slide in 0.9% saline and dehydration, the brain sections were coverslipped under mineral oil if further thionin or cresyl violet staining was to be done or under Permount® (Fisher Scientific Co.; Fair Lawn, NJ; U.S.A.) if no further processing was deemed necessary. The exact areas of the brain photographed after catecholamie histochemistry were then photographed for AChE.

### *Counterstaining with Thionin or Cresyl Violet*

Some brain sections processed for catecholamines or AChE alone or for catecholamines and AChE on the same brain section were additionally stained for Nissl substance with either thionin or cresyl violet. All tissue to be counterstained with thionin or cresyl violet was coverslipped under mineral oil. Following manual removal of the glass coverslip, the mounted brain sections were immersed in xylene, blotted, air-dried at 22°C, and stained according to the thionin procedure described in Skinner [29] or according to the following cresyl violet protocol: slides were placed into 25% ethanol for 5 min and were then stained in a solution containing 0.75 g cresylecht violet, 69.37 ml 1 M acetic acid, 5.63 ml 1 M sodium acetate, 150 ml distilled deionized water, and 75 ml ethanol. The mounted sections were sub-

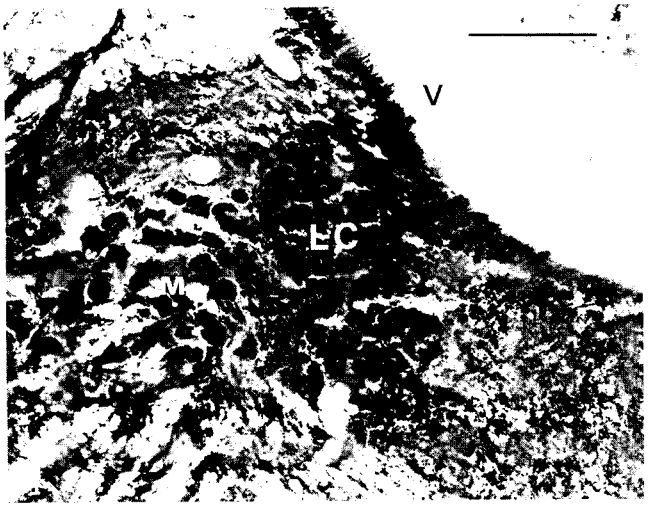


FIG. 1. Acetylcholinesterase in transverse section of the brainstem of a rat at the level of the locus ceruleus (LC). AChE staining performed without previous DFP treatment. M=mesencephalic nucleus of the trigeminal nerve. V=ventricle. Calibration=200 $\mu$m.

sequently placed into 0.50% acetic acid for 2 min followed by 95% ethanol for 2 min and then a solution of 225 ml absolute ethanol and 75 ml chloroform for 2 min. The slides were then immersed in toluene before being coverslipped under Permount®.

## RESULTS

### *Histochemical Controls*

Omission of acetylthiocholine iodide from the incubation medium abolished staining for AChE in locus ceruleus and other neural regions. Brain sections incubated with butyrylthiocholine iodide showed a weak reaction that roughly paralleled the sites of cholinesterase activity demonstrated when acetylthiocholine iodide was used as a substrate. Specifically in the region of the locus ceruleus, however, the staining after butyrylthiocholine iodide incubation was associated with blood vessels and not neurons, a finding consistent with observations summarized by Friede [15].

Incubation of brain sections with iso-OMPA abolished or greatly diminished cholinesterase staining when butyrylthiocholine iodide was used as a substrate; the reaction with acetylthiocholine iodide as substrate was minimally affected or unaltered. AChE in brain sections incubated with BW284C51 and acetylthiocholine iodide was absent or markedly decreased in areas normally staining for the enzyme, including locus ceruleus; the intensity of cholinesterase staining after butyrylthiocholine iodide incubation was unaffected or only slightly reduced. Eserine sulfate completely or greatly inhibited cholinesterase staining regardless of the thiocholine substrate used. We conclude from these experiments that the histochemical reaction we observed in locus ceruleus neurons, using acetylthiocholine iodide as substrate, was selective for AChE.

Controls for the specificity of the glyoxylic acid procedure for catecholamines that we used have been published previously [14] and were not repeated in the current experiments.

### *AChE in Locus Ceruleus*

In AChE-stained brain sections from rats not injected with DFP, locus ceruleus displays intense enzyme activity (Fig. 1). The russet-colored reaction product appears uniformly distributed throughout the neural region, and individual neuronal somata are difficult, if not impossible, to discern (Fig. 1), probably for reasons discussed elsewhere in this paper.

In AChE material from DFP-treated animals, morphologic detail in locus ceruleus is markedly improved, as is the morphology of the large AChE-containing cells in the mesencephalic nucleus of the trigeminal nerve (Fig. 2; compare with Fig. 1). On the basis of soma dimensions, two major divisions of locus ceruleus appear to emerge, a dorsal portion consisting of generally smaller cells and a ventral region of typically larger neurons (Fig. 2). These two divisions are extant at all levels of the approximately 900 $\mu$m rostro-caudal extent of the nucleus (Fig. 2). Dorsal locus ceruleus contains 7–8 times more neurons than does ventral locus ceruleus. At caudal levels of locus ceruleus and ventrolateral to it, an additional division of the nucleus manifests itself that has been termed the subcerulear area or nucleus subceruleus (Fig. 2, F; see [2]). Subcerulear neurons are very similar to those in the ventral locus ceruleus, however, and may not represent a category of cerulear neurons that are clearly separable on morphologic bases from those in the ventral aspect of the nuclear mass (Fig. 2, F; see also [2]). In material counterstained with thionin or cresyl violet, all cell bodies in locus ceruleus and the subcerulear area appeared to contain AChE.

Somata demonstrating AChE in locus ceruleus and nucleus subceruleus are round (Figs. 2, 4), oval (Figs. 2–4), fusiform (Figs. 2–4), or pyramidal (Fig. 3). The vast majority of these AChE-containing neurons have maximum soma extents ranging from 10 to 40 $\mu$m. Slightly over one-half of the total number of cerulear neurons have dimensions in the range of 20–30 $\mu$m; these we term medium-size cells. Small neurons are those measuring less than 20 $\mu$m in maximum soma extent whereas those somata over 30 $\mu$m are designated large.

Oval, multipolar neurons represent the majority of cerulear neurons. They are abundant in the dorsal locus ceruleus, where they are densely packed and have their processes and long axes primarily oriented rostrocaudally and their oblique axes from dorsolateral to ventromedial aspects of the nucleus (Fig. 2). As evidenced in horizontal sections, the axons of oval neurons appear to exit the nucleus from its rostral aspect (see also [4]). Oval somata in the dorsal locus ceruleus tend to be smaller and to stain heavily for AChE; those in the ventral division are generally larger and display a greater variation in staining intensity ranging from moderate to intense.

Fusiform neurons possess two AChE-containing processes (Fig. 3) and typically stain heavily for AChE. They are medium- or large-sized and appear to be preferentially distributed within the dorsal portion of the nucleus, although fusiform neurons are also observed in the ventral aspect of locus ceruleus.

Round neurons are multipolar and either small or medium-sized, whereas the infrequently observed multipolar pyramidal somata, 4.5% of the total, are preferentially located in the ventral locus ceruleus and range in size from small to large.

At all rostrocaudal levels of the locus ceruleus studied (Fig. 2), medium-sized neurons represent approximately 53%

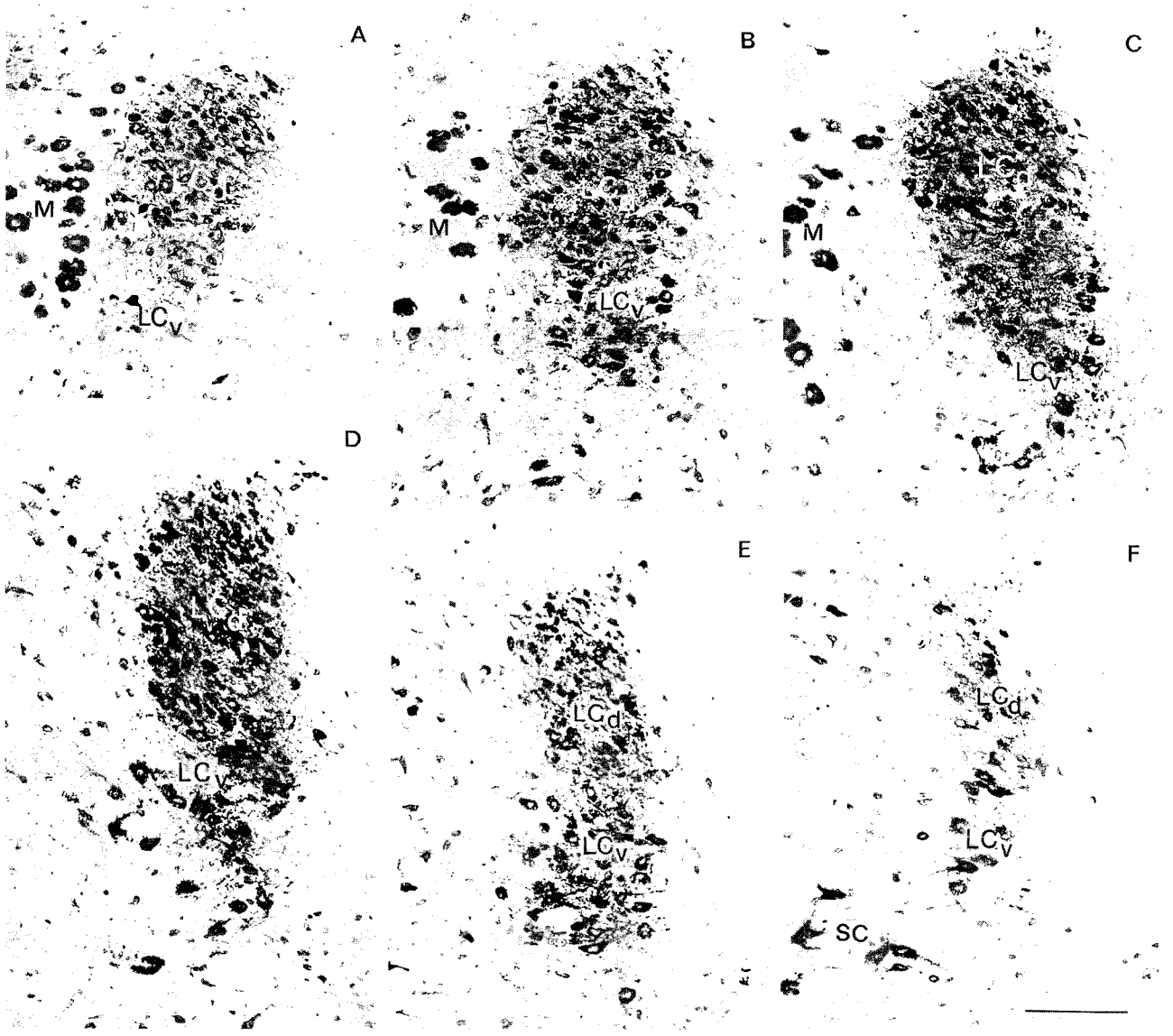


FIG. 2. Acetylcholinesterase in transverse sections of the locus ceruleus (LC) of a DFP-treated rat. Individual frames are displayed rostro-caudally from A to F at 150 $\mu$m intervals. The dorsal (LCd) and the ventral (LCv) divisions are unevenly represented throughout the anterior-posterior extent of the nucleus. Sections A-C include cells of the mesencephalic nucleus of the trigeminal nerve (M). Figure 2F includes a few cells located in the subcerulear area (SC). Calibration=150 $\mu$m.

(SD=4.6%) of the total number of cells. An inverse correlation exists between the percentage of small and large neurons at these same levels (Pearson r= −0.91). Large neurons increase from 6% of the total at rostral levels to 30% caudally; the proportion of small cells decreases from 45% anteriorly to 23% posteriorly.

In addition to the previously described variations in the representation of large and small neurons at different levels of locus ceruleus, the dorsal and ventral divisions of the nuclear mass are themselves differentially represented, the dorsal portion being larger at rostral levels than posteriorly and the ventral aspect being more evident caudally but considerably less prevalent anteriorly (Fig. 2).

Approximately one-third of the total number of cerulear somata, regardless of their shapes or sizes, stain lightly for AChE whereas the remaining cells stain heavily. Weakly staining neurons are preferentially situated in the ventral locus ceruleus but are also present in the dorsal aspect, scattered among more heavily stained cells.

### *AChE and Norepinephrine in Locus Ceruleus: Demonstration of the Two Substances on the Same Tissue Section*

All neuronal elements in locus ceruleus containing norepinephrine appeared to possess AChE (Fig. 4); the converse was true also. In some instances, both AChE and norepinephrine were found in proximal processes of cerulear cells (uppermost arrow; Fig. 4, C and D), and in many neurons, the regions of their somata staining most intensely

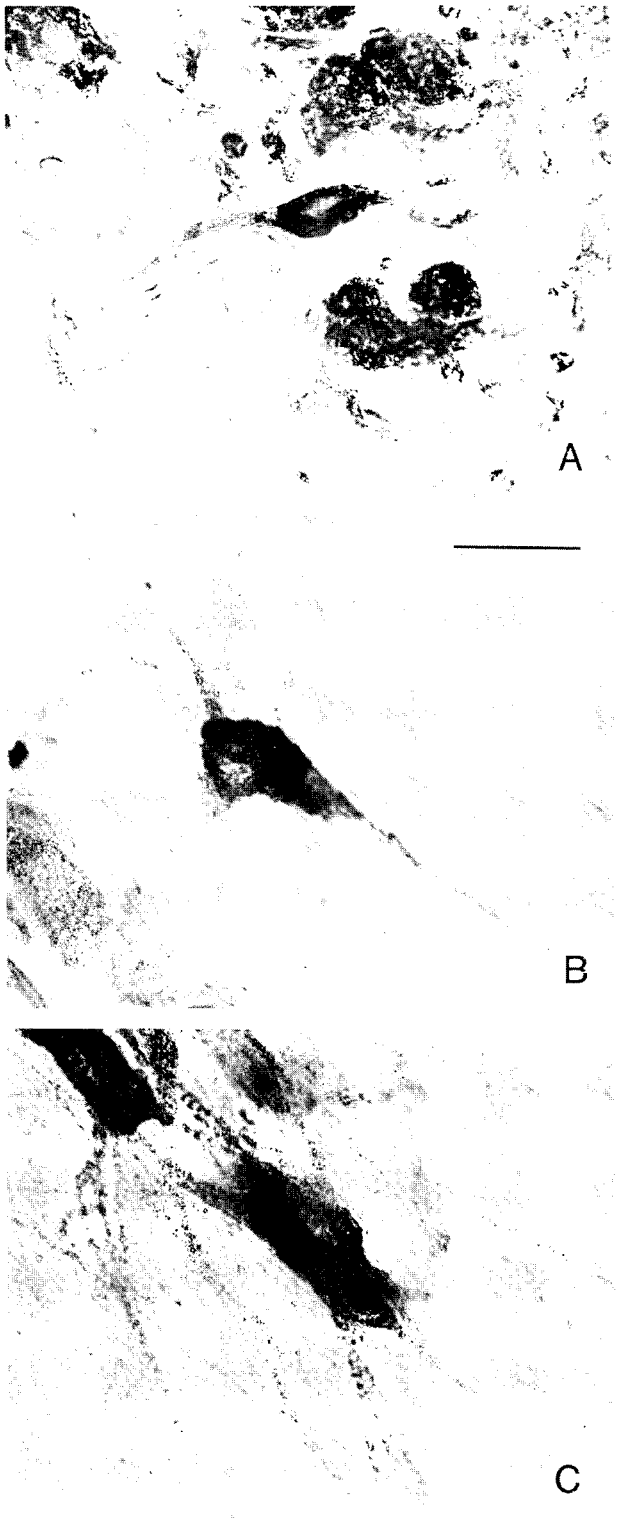


FIG. 3. Examples of three AChE-containing soma types in the locus ceruleus of the rat. A fusiform cell, displaying a spindle-shaped soma from which two processes originate, is shown in A. In B is a pyramidal-shaped cell, and C shows an oval multipolar neuron. Calibration=30 $\mu$m.

for norepinephrine also appeared to possess greatest amounts of AChE reaction product (arrows; Fig. 4, A–F). This last-mentioned pattern of correspondence did not always obtain, however.

In sections processed for catecholamines alone and then counterstained with thionin or cresyl violet, it was observed that all cerulear somata demonstrating Nissl substance also appeared to possess norepinephrine.

## DISCUSSION

### *Use of DFP in Combination with AChE Histochemistry*

The AChE in locus ceruleus and adjacent peribrachial structures, visualized after intramuscular administration of DFP, is associated to a large extent with cell bodies and their proximal processes within those neural regions (see also [4]). Discrete AChE-containing somata are not observed reliably or consistently, if at all, in those brain areas in animals not given DFP (e.g., see [25]). Irreversible inhibition of AChE by DFP requires that the neurons synthesize new stores of the enzyme before cholinesterase activity recovers to normal levels [13]. Such resynthesis appears to occur preferentially in cell bodies at short intervals following a DFP challenge [10]; neuronal processes are seen best at longer intervals after administration of the organophosphorous compound [10]. Histochemical staining for these new enzyme stores at appropriate times after DFP enables the enhanced visualization of discrete neuronal somata and proximal enzyme-containing processes. The use of DFP in conjunction with AChE histochemistry emerges, therefore, as a valuable procedure for morphologic studies on the organization of cholinesterase systems in the brain (see also [5]).

### *Organization of AChE and Norepinephrine in Locus Ceruleus: Relationship to the Total Population of Cerulear Neurons*

Although the present results represent, to our knowledge, the first detailed report of the distribution of AChE-containing somata in locus ceruleus and their relationship to norepinephrine, our observations on the morphologies and organization of those neurons are highly compatible with data derived from previous Golgi, immunohistochemical, monoamine histochemical, and Nissl studies [16, 27, 28, 31]. The division of the nucleus into dorsal and ventral divisions, as well as the orientation of the component cell bodies, as emphasized by Swanson [31], is demonstrated well in AChE stained material. The sizes and shapes of AChE-containing cerulear somata that we observed also appear consistent with previous reports in the literature in which other histologic and histochemical techniques were used [16, 27, 28, 31].

In neural material stained first for either AChE or catecholamines and then counterstained with thionin or cresyl violet, we found that all cerulear somata we examined possessed AChE or norepinephrine. Similarly, all norepinephrine neurons in locus ceruleus appeared to contain some amount of AChE, and, in many cells, the staining patterns of the two neurochemicals followed each other precisely. On the basis of these observations, the conclusion seems warranted that all neurons in locus ceruleus contain both AChE and norepinephrine. Although Swanson [31] reported that only 88% of cerulear somata demonstrated dopamine-$\beta$-hydroxylase, a marker for norepinephrine-containing neurons, he also pointed out that his estimation of the number of

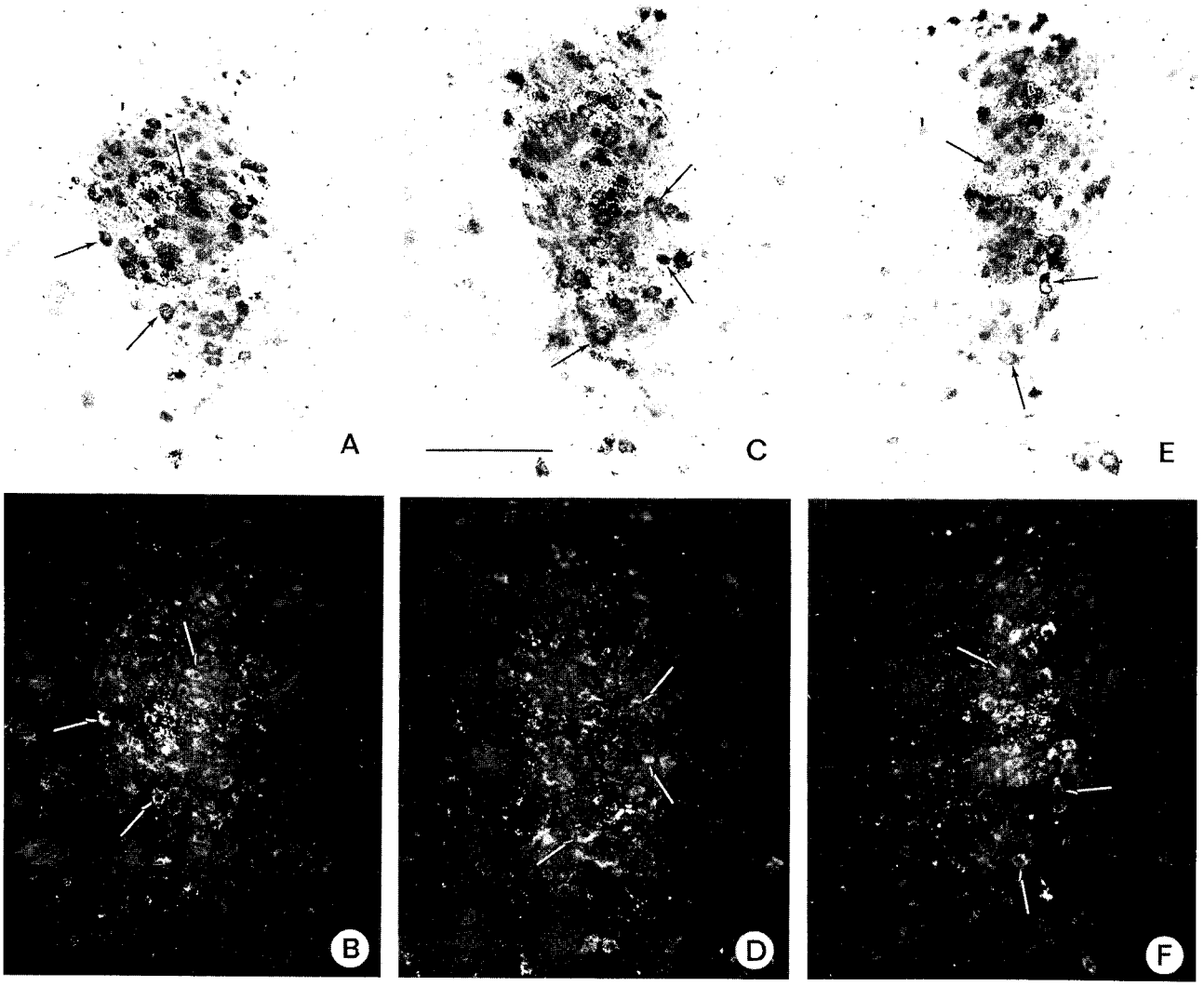


FIG. 4. Acetylcholinesterase (A,C,E) and norepinephrine (B,D,F) in transverse sections of the locus ceruleus at three different anterior-posterior levels: rostral (A,B), intermediate (C,D) and caudal (E,F). The same tissue sections are displayed in A and B, in C and D, and in E and F. Arrows point to the same neuronal somata in the appropriate dyad (i.e., A-B, C-D, or E-F). Calibration=200 $\mu$m.

cerulear neurons based on immunohistochemistry was less reliable, owing to methodologic difficulties, than his estimate of the total number of neurons in locus ceruleus determined in Nissl-stained paraffin sections.

Also somewhat at variance with our conclusion that all neurons in locus ceruleus contain both AChE and norepinephrine are the observations of Sladek and Walker [30] that a few serotonin-containing somata are found in the locus ceruleus of the juvenile macaque brain. These authors [30] did not find 5-hydroxytryptamine-containing neurons in the adult monkey, however, and we did not find evidence for such cells in the adult rat [1].

No unqualified exceptions currently exist in the literature, therefore, concerning our contention that all cerulear somata contain AChE as well as norepinephrine.

### *Is There a Physiologic Role for AChE Contained Within and/or on Norepinephrine Neurons in Locus Ceruleus?*

Controversy exists concerning the significance of the association of AChE with norepinephrine-containing neurons in locus ceruleus in terms of what such a neurochemical configuration means for cholinergic-noradrenergic interactions in that structure. Mason and Fibiger [22], for example, state that "It is known that the locus coeruleus receives cholinergic input from adjacent cell groups . . . (p. 521)" and that their behavioral results ". . . demonstrate for the first time a clear behavioural role of the biochemically indicated noradrenaline-acetylcholine interaction in the *brain* . . . (p. 523)." In another report from the same laboratory in the same year, Lehmann and Fibiger [19], taking into account the work of Lewis and Schon [20], emphasize that "On a cellular level, AChE is found in fairly high activity on (sic) some neurons which are known *not* to be cholinergic and furthermore are not thought to be cholinoceptive . . . the noradrenergic neurons of the locus coeruleus . . . (p. 1939)."

Careful examination of the relevant literature reveals that a plausible case can be made for the existence of cholinergic mechanisms in locus ceruleus, as well as in certain of the

target structures of those neurons. Some of the possible mechanisms and the loci at which they may operate are considered in the following discourse.

Since the soma of AChE-containing neurons is a primary site of cholinesterase synthesis [26], it is possible that AChE in cerulear cell bodies is enzyme earmarked solely for somatofugal transport along dendrites and axons to assume functions at various loci along those processes or at their terminals (see [4]). In this functional capacity, AChE would have no physiologic role per se in neuronal somata in locus ceruleus.

Alternatively, however, it is possible that cholinergic neurons project to locus ceruleus, and AChE is postsynaptically localized in and/or on norepinephrine cell bodies and/or dendrites there to inactivate released acetylcholine. In support of this conjecture are the observations of Kuhar and his associates [18] that iontophoretic administration of acetylcholine increases the rate of unit electrical discharges of cerulear neurons that can be potentiated by the co-application of physostigmine. In addition, Cheney *et al.* [11] have reported that locus ceruleus contains both acetylcholine and choline acetyltransferase. On the basis of electron microscopic information, however, Lewis and Schon [20] suggested that there was no evidence for cholinergic mechanisms operating in locus ceruleus because AChE could not be detected in synaptic clefts or associated with presynaptic membranes. Although this conclusion does not obviate the possibility that cerulear AChE has cholinergic functions at loci other than the cell bodies of the nucleus (see previous discussion), Kuhar *et al.* [18] have argued cogently that the negative data of Lewis and Schon [20] should be interpreted with caution due to potential methodologic difficulties with Lewis and Schon's tissue preparation procedures. Furthermore, there is evidence that behaviorally significant cholinergic-noradrenergic interactions do exist in locus ceruleus [3] and that long-term synthesis of tyrosine hydroxylase in the nucleus may be mediated by muscarinic receptors [24].

Finally, it is conceivable that the AChE synthesized in the norepinephrine-containing somata of locus ceruleus is transported somatofugally (see [4]), possibly to terminal projection areas of those cerulear noradrenergic neurons (e.g., septum, hippocampus; see [21]). But what would be the function of AChE in axon terminals containing norepinephrine? One possibility is that neurons containing both AChE and norepinephrine and originating in locus ceruleus make axonal synpatic contact with cholinergic cells in some cerulear target structures (e.g., septum; see [18]). Acetylcholine released from those target neurons might act dendro-axonally or somato-axonally to modify subsequent liberation of norepinephrine operating in an axo-dendritic or axo-somatic direction; AChE in the afferent noradrenergic axons would function to catabolize the "post-synaptically" released acetylcholine (see [8]). This type of neuromodulatory role for acetylcholine has been proposed to account for the localization of AChE within axons of nigro-striatal neurons containing dopamine, and considerable experimental evidence supports the existence of such a mechanism (see [9]).

Perhaps none of the interactive schema proposed in this report will prove valid, or even heuristically useful, when additional data are collected. Nonetheless, it seems reasonable to suggest that the nature of cholinergic-noradrenergic interactions in locus ceruleus and its target structures is a topic worthy of further investigation.

ACKNOWLEDGEMENTS

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