DISTRIBUTION OF MONOAMINE-CONTAINING CELLS IN THE CENTRAL NERVOUS SYSTEM OF THE CHICKEN

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A large number of papers has revealed the existence of catecholamines (CA) and 5-hydroxytryptamine (5-HT) in the central nervous system of mammals (1-5). These amines were extensively distributed in the brain, especially in the subcortical areas. Moreover, many investigators have made considerable contributions to studies on the distribution of CA and 5-HT in the central nervous system of vertebrates phylogenetically below Mammal (6-13). Of them, Brodie, Bogdanski and Bonomi (7) reported the ratio of 5-HT to CA in the brain of all vertebrate classes. This ratio is about 1:1 in the rat, whereas it is 2 or more in birds, reptiles and amphibians. Significant interplay of these monoamines concerning functions of the central nervous system might be conclusive, even though definite evidences have been awaiting.

Scandinavian group (14-16) has developed histo-chemical method for the detection of monoamines by using the emission of fluorescence from CA and 5-HT. This method made it possible to observe the cellular localization of monoamines not only in the peripheral organs but also in the central nervous system. In the central nervous system of mammals (mainly in rats), the distribution of CA and 5-HT in different nerve cells and in a variety of nerve terminals and fibers have been demonstrated (17-21). On the other hand, such investigations in the brain of vertebrates phylogenically below Mammal have been carried out only in the limited animal species and the limited region of the brain. As far as we know, there were such reports with regard to the upper brain stem of the pigeon (22), the median eminence of the chicken (23, 24), and the brain of fish (25). Therefore, the present study was undertaken to establish the topographical distribution of monoamine-containing cells in the central nervous system of the chicken and to observe their responses to various drugs interfering with the monoamine metabolism. The distribution of monoamine-containing terminals and fibers in the central nervous system of the chicken will be described in the coming paper.

MATERIALS AND METHODS

Experiments were carried out by using 71 male White Leghorn chickens 2 days to 15 weeks of age, weighing from 30 to 1500 g. Of them, 43 animals had been treated with drugs shown in Table 1. The solutions were prepared as described by Dahlström and Fuxe (17)
and injected intraperitoneally immediately after preparation. The specimens from animals treated with drugs were always handled in identically the same way as the corresponding specimens from normal animals.

The birds were killed by decapitation. The central nervous system was divided between the brain and spinal cord at the level of the first cervical spinal cord. The whole brain and various portions of the spinal cords were secured. They were rapidly frozen in isopentane cooled by liquid nitrogen and dried in vacuo at -35°C under phosphorus pentoxide for the first five days. At the end of the fifth day the temperature was raised to -20°C. At the end of the sixth day a cooling-apparatus of compressor type was turned off, so that the specimens reached room temperature at the end of the seventh day. Thereafter, they were placed in a glass jar fulfilled with formaldehyde gas for 1 hour at 80°C in oven (15). Relative humidity of formaldehyde gas was regulated approximate 70% by storing in a vessel containing a solution of sulphuric acid for 5 to 7 days (26).

After histochemical treatment, the specimens were immersed into soft paraffin (m.p. 52-54°C) for 30 minutes in vacuo. Then, they were treated similarly in hard paraffin (m.p. 56-58°C) for 2 hours and finally embedded. Sections, 10 μ in thickness, were obtained every 100 μ of each specimen and streched on a slide-glass maintained at 60-65°C. Thereafter, they were mounted with Entellan-xylene mixture (1 : 1) for microscopic analysis. Fluorescence microscopy was performed with a Nikon fluorescence microscope at an illumination of 200 high pressure mercury lamp. The sections stained with cresyl-violet were used for histological identifications of the fluorescence-emitting structures. Microphotographs were taken with Kodak Tri-X film at times of exposure ranging between 40 and 90 seconds.

Intensity of fluorescence was arbitrarily classified as none (0), very weak (1+), weak (+), medium (2+), strong (3+), and very strong (4+).

The terminology of the central nervous system used in this text is mainly followed a description of Jungherr (27).

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**Table 1. Time before killing the animals after the first injection.**

| Drug          | Dose mg/kg body wt. | Hours |
|---------------|---------------------|-------|
| Reserpine i.m.| 3                   | 1(2)  |
| Nialamide i.p.| 500                 | 2.5(5)|
| Reserpine i.m.+ | 3                 | 18    |
| Nialamide i.p. | 500                 | 4-5(5)|
| Nialamide i.p. | 500                 | 2.5   |
| L-dopa i.p.   | 100                 | 0.5(4)|
| Nialamide i.p.+ | 500              | 2.5   |
| DL-5-HTP i.p. | 100                 | 0.5(2)|

Number of animals in parentheses
RESULTS

*Morphology and distribution of monoamine-containing cells in the central nervous system*

1. **Spinal cord**

   At the various levels of the spinal cord examined, none of monoamine-containing cells were observed, although it was observed that these regions contained very fine, CA and 5-HT containing terminals.

2. **Cerebellum**

   There were no monoamine-containing cells in this area. Although it was known that a relatively small amount of CA or 5-HT was present (9), none of monoamine-containing nerve terminals were also observed.

3. **Medulla oblongata**

   **Groups of CA containing cells**

   Group A1. This group was situated immediately dorso-lateral and dorso-medial to the nucleus vagus motorius ventralis (nucleus ambiguus) in frontal sections at the level of the nucleus vagus motorius dorsalis (Text-figs. 1, 2) and was consisted of small to medium-sized nerve cells, which emitted green fluorescence at medium to strong intensity (Figs. 1, 2). The cells were round or oval and often clearly multipolar. They were found to accompany with medium green fluorescent varicose fibers or terminals.

   Group A2. Small-sized cells belonged to this group lay scatteringly in a fairly broad area. These cells were round to oval and weakly fluorescent materials in the cytoplasm. Most of cells were present just lateral and dorso-lateral to the nucleus vagus motorius dorsalis (Text-fig. 1, Figs. 3, 4). At the level of the nucleus commissurae infimae, some of cells were also localized in the dorso-lateral part of the nucleus commissurae infimae and just under the dorsal surface of the brain. These nerve cells were surrounded with strongly to very strongly green fluorescent, varicose fibers and terminals, and seemed to distribute within the fasciculus solitarius. The non-fluorescent cells of the nucleus vagus motorius dorsalis were surrounded with fine, weakly green-fluorescent terminals. It seems that most of cells in this group have axons which form descending fibers toward the spinal cord, and which terminate in the gray matter.

   Group A3. At the level slightly caudal to the nucleus abducens major, a few small-sized, very weakly to weakly fluorescent, round to oval cells were situated just dorso-lateral to the nucleus olivaris superior pars dorsalis (Text-fig. 2, Fig. 5). This localization was maintained also in the rostral level of the nucleus abducens major. These cells were observed together with strongly green-fluorescent fibers and terminals.

   Group A4. This group was situated dorso-lateral to the nucleus facialis motorius ventralis, probably within the reticular formation (Text-fig. 2). However, these nerve cells were entirely different from the nucleus reticularis superior described by Jungherr (27), for the former was situated somewhat laterally than the latter and was smaller. The cells were round or oval-shaped and small to medium-sized, and had weakly to medium green fluorescence.
Group A5. At the level slightly caudal to the nucleus trochlearis, a small group of small-sized, round to oval nerve cells of very strongly green fluorescence were present about 2 mm lateral to the medial longitudinal fasciculus (Text-fig. 3, Figs. 6, 9). This group was localization mainly within the nucleus tegmentalis dorsalis. In the ventro-lateral direction, a fairly large number of green-fluorescent terminals emitting very strong fluorescence was located (Fig. 6). The processes from the cells which belong to this group seem to run in the ventro-lateral direction and to continue to the terminals described above. The number of cells in this group dwindles in the rostral direction, necessarily in the caudal level of the mesencephalon, where the cells had a somewhat weaker fluorescence intensity than in the medulla oblongata (Text-fig. 4, Figs. 10, 11). This cell group is considered to be homologous with the locus coeruleus in the rat brain (group A6 according to Dahlström and Fuxe, 17) in view of topography.

Groups of 5-HT containing cells

Group B1. This group is of small size and its medium cells are situated between the medial longitudinal fasciculus, perhaps in the nucleus raphis, from the level of the nucleus commissurae infimae up to the level of the caudal third of the nucleus vagus motorius dorsalis (Text-fig. 1). The round to oval nerve cells were closely packed (Fig. 7) and had very weak to weak fluorescence. At the level of the caudal and middle third of the nucleus vagus motorius, B2 group (see below) appeared ventral to this group, where no distinct borderline existed between group B1 and B2.

Group B2. This group was observed in the fairly broad area from the level of the caudal and middle third of the nucleus vagus motorius dorsalis up to the level of the caudal of the nucleus trochlearis (Text-figs. 2, 3). Most of the cells were present within the nucleus raphis along the midline of the medulla oblongata. The cells were round to oval-shaped, medium to large-sized and had a coarse, weak to medium yellow-fluorescence (Fig. 8). It seems that this group consists in reality of at least two groups. One group comprised the cells which located in the dorsal part of the nucleus raphis and the short axons of the cell bodies was often parallel to the raphe, while the other group included the cells in the ventral part of the nucleus raphis and their axons were often parallel to the ventral surface of the brain (Fig. 8). No such division was made, however, since these cell groups could not be clearly separated from each other. Fine, green fluorescent, varicose fibers and terminals were observed within the raphe and encircled the 5-HT cells.

Group B3. A few medium-sized, round to oval-shaped cells were also seen just ventro-lateral to the nucleus olivaris superior pars dorsalis in transverse sections (Text-fig. 2). The cells emitted a very weakly to weakly yellow fluorescence.

Group B4. At the level of the nucleus abducens major, a few medium to large-sized, very weakly to weakly fluorescent, round to oval-shaped cells were present immediately dorsal to the nucleus olivaris superior pars ventralis which located below the nucleus facialis motorius ventralis (Text-fig. 2).

4. Mesencephalon

Groups of CA containing cells
Group A5. This group has already been described above.

Group A6. At the level through the caudal part of the nucleus oculomotorius and the nucleus interpeduncularis, this group appears ventro-lateral to the nucleus isthmo-opticus (Text-figs. 5, 6). This localization was maintained also in the rostral direction, where A6 cells were observed mainly within the nucleus mesencephalicus profundus pars lateralis in the reticular formation (Text-fig. 6). The cells all indicate the same general appearance, that is, they are round to oval-shaped, medium-sized and show a medium to strong green fluorescence (Figs. 12, 14). Since this group was surrounded with a few number of fine, green fluorescent terminals, A6 group could be separated from A9 group which was observed together with a large number of fine, varicose fibers and terminals (see below).

Group A7. A small group of medium-sized, round to oval nerve cells of medium to strongly green fluorescence were situated just ventro-caudal to the nucleus ruber (Text-fig. 6). A7 cells were often seen together with 5-HT cells which belonged to B7 group. It must be pointed out that there is no distinct border-line laterally between A7 and A9.

Group A8. This small group of medium to strongly green-fluorescent cells were situated immediately lateral to the nervus oculomotorius (Text-fig. 6). The cells were round to oval-shaped, medium sized and were present together with a few, fine varicose terminals (Figs. 12, 13).

Group A9. A large cell group of medium to strongly green-fluorescent cells were observed within the ventro-lateral part of the mesencephalon. The area containing these cells extendedcranially and ventrally from a position ventro-lateral to the caudal part of the nucleus oculomotorius, passed between the nucleus ruber and the nucleus ectomammillaris, and terminated lateral to the nervus oculomotorius (Text-fig. 6). They lay in a fairly compact band, together with a large number of fine, varicose green-fluorescent fibers and terminals (Fig. 12). The cells were medium-sized, round to oval and often clearly multipolar. Some of these cells seem at least to correspond to the nucleus mesencephalicus profundus pars ventralis. In the sagittal sections, a large tract of the nerve bundles containing very weakly green-fluorescent fibers was observed just cranial to this group. It appears to a great extent to arise from this group and to run in a rostral direction within the medial forebrain bundle in the dorso-lateral part of the hypothalamus.

Groups of 5-HT containing cells

Group B5. At the level of the nucleus trochlearis, a fairly large group of cells which emitted a medium to strongly yellow fluorescence were situated mainly within the nucleus annularis, which formed a ring-shaped arrangement around the medial longitudinal fasciculus (Text-figs. 3, 4, Figs. 10, 11). It seems that this group consists in reality of at least three groups. One comprises the cells which locate in the lateral part of the nucleus annularis, that is, just ventro-medial to the nucleus tegmentalis dorsalis in which A5 group is present, and some of them are also observed within the reticular formation ventro-lateral to the nucleus annularis. Another group consists of the cells which locate immediately ventral to the medial longitudinal fasciculus. The third group includes the cells lying on the medial and dorsal sides of this fasciculus. The cells on the lateral and ventral parts of this group
present the same general appearance. Because of this, no distinct border-line was observed between these groups. These cells were round to oval-shaped and mainly medium sized, and showed a medium yellow fluorescence. On the other hand, the cells lying on the medial and dorsal sides indicated the different appearance from the cells of two groups described above, for the cells in the former were clearly smaller and had a somewhat stronger fluorescence intensity than in the latter (Fig. 10). After reserpine-nialamide treatment, very weakly yellow-fluorescent fibers that seemed to arise from the cells of these groups were seen to run ventro-cranially. These fibers appear to join the medial forebrain bundle.

Group B6. A large group of cells which present a same general appearance as those of B5 group were observed in transverse sections within the nucleus linearis caudalis (Text-figs. 3, 4). These cells were arranged as two parallel rows just lateral to the midline. Scattered cells of this group were also observed at the level of the rostral part of the medulla oblongata. It must be pointed out that there is a distinct border-line between the caudal part of B6 group and the rostral part of B2 group, but no distinct border-line between the rostral part of B6 group and caudal part of B7 group.

Group B7. At the level of the caudal third of the nucleus oculomotorius, a very large group of medium sized, round to oval nerve cells were observed within the nucleus decussationis cerebellaris superior, which formed a triangle (with the apex ventrally oriented) in the midline (Text-fig. 5, Fig. 15). The cells showed a medium to strongly yellow fluorescence. In the frontal sections, yellow-fluorescent axons that arose from the cells of this group were seen to run laterally, whereas these axons were also observed to run rostrally in the sagittal sections. Consequently, most of these axons penetrated into the medial forebrain bundle and joined the green and yellow fluorescent bundles of the other groups described above. Some of them seemed to terminate the hypothalamus, while the rest ran more cranially and appeared to end with the septal region or the corpus striatum.

Group B8. This group is of small size and is observed just lateral to the nucleus interpeduncularis (Text-fig. 5). The cells were round to oval-shaped and were often clearly multipolar, and showed a medium to strongly yellow fluorescence (Fig. 16).

5. Diencephalon

The "modified ependyma" in the hypothalamus was an only structure consisting of monoamine-containing cells throughout the diencephalon. This is a richly vascularized structure (organum vasculosum) lying within the ependyma of the ventricle.

Group A10 and group B9. The modified ependyma was found to consist of CA and 5-HT containing cells. It was seen to lie in a row from the level of the corpus mamillaris up to the approximately middle level of the hypothalamus (Text-figs. 6, 7). The cells were small-sized, round to oval-shaped and had a strong green and a very strongly yellow fluorescence respectively (Figs. 17, 18). They were observed to send processes toward the ventricle and lateral or dorsal parts of the hypothalamus (Fig. 18). The processes which directed toward the ventricle were short and thick, while the other were long and somewhat less thick.

In contrast to the modified ependyma, the rest part of hypothalamus and the all parts of the thalamus contained no fluorescent cells, although these parts contained a few to many
fluorescent terminals.

6. Telencephalon

There were no monoamine-containing cells in all parts throughout the telencephalon. However, it was observed that some regions were abundant in monoamine-containing terminals.

Effects of drugs interfering with the metabolism of the monoamines

Various drugs interfering with metabolism of the monoamines were used to observe how the fluorescence of the monoamine-containing cells were changed, and to compare the natures of these neurons in the chicken with those in the pigeon or mammals. Drug used, dosage and method of administration of them are given in Table 1.

Reserpine treatment. A large dose of reserpine (10 mg/kg) induced a severely sedative state in the animals and resultant death in some of the chickens. A dose of 3 mg/kg used

| Groups | Normal fluorescence intensity | After administration of reserpine (3 mg/kg i.m.) | Reserpine (3 mg/kg 18 hr) + Nialamide (500 mg/kg 5 hr) |
|--------|-----------------------------|-----------------------------------------------|-------------------------------------------------|
|        | 1 hr | 2 hr | 4 hr | 6 hr | 8 hr | 12 hr | 18 hr | 24 hr |                |
| A1     | +---# | (+)-+ | (+)-+ | (+)-+ | (+)-+ | 0-(+) | (+)  | +    | +    |
| A2     | +    | 0-(+) | 0-(+) | (+)  | 0 (+) | 0-(+) | +    | +    |
| A3     | +---# | 0    | 0    | 0    | 0    | 0    | +    | +    |
| A4     | +---# | (+)-+ | (+)-+ | (+)-+ | (+)-+ | (+)-+ | +    | +    |
| A5     | +---# | (+)-+ | (+)-+ | (+)-+ | (+)-+ | (+)-+ | +    | +    |
| A6     | +---# | 0    | 0    | 0    | 0    | 0    | +    | +    |
| A7     | +---# | 0    | 0    | 0    | 0    | 0    | +    | +    |
| A8     | +---# | 0    | 0    | 0    | 0    | 0    | +    | +    |
| A9     | +---# | 0    | 0    | 0    | 0    | 0    | +    | +    |
| A10    | +---# | 0    | 0    | 0    | 0    | 0    | +    | +    |

Fluorescence intensity: none 0, very weak (-), weak +, medium +, strong ++, very strong +++.

Table 3. Changes in yellow fluorescence intensity after reserpine and reserpine-nialamide treatment.

| Groups | Normal fluorescence intensity | After administration of reserpine (3 mg/kg i.m.) | Reserpine (3 mg/kg 18 hr) + Nialamide (500 mg/kg 5 hr) |
|--------|-----------------------------|-----------------------------------------------|-------------------------------------------------|
|        | 1 hr | 2 hr | 4 hr | 6 hr | 8 hr | 12 hr | 18 hr | 24 hr |                |
| B1     | (+)-+ | 0    | 0    | 0    | 0    | 0    | 0-(+) | (+)  | +    |
| B2     | +---# | 0    | 0    | 0    | 0-(+) | 0-(+) | (+)-+ | ++   | +    |
| B3     | (+)-+ | 0    | 0    | 0    | 0    | 0    | (+)  | +    | +    |
| B4     | +---# | 0    | 0    | 0    | 0    | 0    | (+)  | +    | +    |
| B5     | +---# | 0-(+) | 0    | (+)  | (+)-+ | (+)-+ | ++   | ++   |
| B6     | +---# | 0-(+) | 0    | (+)  | (+)  | (+)  | ++   | ++   |
| B7     | +---# | 0    | 0    | 0    | (+)  | (+)-+ | ++   | ++   |
| B8     | +---# | 0-(+) | 0    | (+)  | (+)  | (+)  | ++   | ++   |
| B9     | +---# | 0-(+) | 0    | (+)  | (+)  | (+)  | ++   | ++   |

Fluorescence intensity: none 0, very weak (+), weak +, medium ++, strong +++, very strong +++.
in the present experiment induces a moderately sedative state but leads to disappearance of
the fluorescence of CA in the digestive tract of the chicken (28). Tables 2 and 3 show the
depletion and recovery of the fluorescence of the monoamine-containing cells following the
administration of the reserpine. The specific fluorescence of both of nerve cells was markedly
reduced as early as 1 hour after administration. At 2 and 4 hours later, their fluorescences
were strongly reduced and were almost completely disappeared, except those in groups A1,
A2 and A5 of CA containing cells. In groups A1 and A2, the disappearance of the fluores-
cence was somewhat retarded than in the other groups, when the fluorescence in A5
group was still very to fairly weak. It must be pointed that the green-fluorescent
terminals and fibers in the medulla oblongata almost completely disappears at 2 and 4 hours
later, while those in the mesencephalon, diencephalon and telencephalon did not disappear
completely even at 8 hours later. Recovery in general was quicker in the 5-HT containing
cells than in the CA containing cells. The former showed a visible fluorescence at 12 hours
after administration of reserpine, while the latter was still in states of reduction, except cells
of group A5, which showed a medium green fluorescence. Both of monoamine-containing
cells recovered their normal fluorescence intensity between 24 and 48 hours later. It was
also observed that the recovery of the fluorescence in the terminals and fibers was fairly slower
than in the cells.

Naalamide treatment. A large dose of this potent monoamine-oxidase inhibitor (500
mg/kg, 2.5 and 5 hours before killing) caused a slight to moderate increase in the fluorescence
intensity of 5-HT cells. It is compatible with findings in the pigeon (22) and the rat (17).
The increase in intensity in general was significantly marked in the groups showing a relatively
weak fluorescence before treatment (e.g. groups B1, B2, B3 and B4). On the other hand,
nialamide failed to increase in intensity of fluorescence of 5-HT cells of the modified
ependyma, due to strong emission without any treatment. The yellow-fluorescent fibers
and terminals also showed a slight increase in intensity, while the green-fluorescent cells,
fibers and terminals were unaffected. It must be pointed that the background also showed
a weakly yellow-fluorescence, probably due to the accumulation of 5-HT in the tissue.

Reserpine-nialamide treatment. The green-fluorescent cells in animals treated with
reserpine plus nialamide showed the same appearance as in those treated with nialamide alone.
Moreover, the fibers and terminals of the CA type did not recover any green fluorescence.
In contrast to the cells of the CA type, the cells of 5-HT type showed the strong increase in
the yellow fluorescence intensity (Table 3). The fibers and terminals belonging to 5-HT
neuron also showed the moderate increase in the fluorescence intensity. These findings agree
with those in the rat and pigeon (17, 19, 22).

Nialamide-dopa treatment. The background fluorescence was remarkably increased,
probably due to the presence of dopa in the brain tissue. This phenomenon was considerably
marked in the regions of the posterior hypothalamus and the fasciculus solitarius, or the
preoptic region. It suggests strongly that these regions take up and accumulate higher
amounts of dopa. The endothelium of the brain capillaries showed no fluorescence but an
unidentified fluorescence was observed in the lumen of the capillaries. This is in marked
contrast to the finding in mammals, where a strong green fluorescence was observed in the endothelium (29, 30). Neither CA neurons nor 5-HT neurons showed any detectible changes in fluorescence intensity. This reason might be attributed to the fact that the specific fluorescence of these cells have been masked by the strong background fluorescence.

**Nialamide-5-hydroxytryptophan treatment.** The background showed a moderately yellowish fluorescence, especially in the hypothalamus. There was no fluorescence in the endothelium of the brain capillaries, while there was an unknown fluorescence in the lumen of the capillaries. No definite changes were observed either in the green or in the yellow-fluorescent neurons.

**DISCUSSION**

It has already been reported that the mammalian brains contain the monoamine neuron systems of two distinctly different types, one being a primary catecholamine type and the other 5-HT type (17–21). These monoamine neurons specifically synthesize and store CA (dopamine or noradrenaline) and 5-HT respectively and seem to function by release of these amines from their terminals. Fuxe and Ljunggren (22) also reported that the same neuron systems as in mammals existed in the upper brain stem of the pigeon. The present investigation shows that neurons of the same general appearance as in the pigeon and mammals are present in the central nervous system of the chicken. Moreover, it was demonstrated that the same systems existed in fish (25), amphibian and reptile (unpublished data), respectively.

It is probable that cells of groups A1 and A2, and of groups B1 and B2 in the medulla oblongata send their axons to the spinal cord, where they end with the corresponding terminals in the gray matter. Conveniently, a few, fine CA terminals were observed in the lateral parts of the dorsal horn at almost all levels or around the centra canal of the thoracic spinal cord, probably in columna preganglionica Terni, while a weak, but diffused, yellow fluorescence, which appears to indicate the existence of 5-HT terminals, was observed mainly in the columna lateralis of the ventral horn. In the rat brain, there is good evidence that most of the fluorescent nerve cells present in the caudal parts of the medulla oblongata (groups A1, B1 and B2 according to Dahlström and Fuxe, 17) have axons that form descending fibers toward the spinal cord, and which end with the corresponding terminals in the gray matter (18, 31). However, it must be pointed that the monoamine-containing terminals in the spinal cord of the chicken are less in number and weaker in intensity than those in the mammalian spinal cord. This appears to reflect the fact that the monoamine-containing terminals present in the chicken spinal cord are much finer as compared with mammals.

The topographical distributions of monoamine-containing cells in the mesencephalon agree with those in the pigeon (22). Many of the monoamine-containing cells in the chicken brain are situated within the mesencephalon. In general, 5-HT cells are distributed along the midline at the caudal and middle parts of the mesencephalon, whereas CA cells are found more laterally at the rostral parts of the mesencephalon (Text-figs. 4, 5 and 6). These cells probably give rise to most of CA and 5-HT terminals situated rostral to the mesencephalon as in the pigeon or mammalian brains (22, 32–36). Actually, a number of weakly green-
fluorescent fiber bundles were observed in sagittal sections from these cell groups in the mesencephalon up to the middle part of the hypothalamus. This fiber tract may correspond to the medial forebrain bundle in mammals (Fig. 66 in Fuxe, 19). Outline of the fibers is rather smooth and somewhat strongly fluorescent as compared with the corresponding fibers in mammals. Some of the CA containing fibers seem to end with the hypothalamus, thalamus and preoptic area, while others appear to terminate within the septal area or to go into the corpus striatum as reported in the pigeon by Bertler, Falck, Gottfries, Ljunggren and Rosengren (37). Most of 5-HT containing fiber bundles run rostrally a more medial position of the medial forebrain bundle, and then seem to terminate within the hypothalamus, thalamus, septal area and corpus striatum. Some of them are separated at the level of the mamillary body and probably end with the mamillary complex or median eminence. 5-HT containing fiber bundles in the mesencephalon and diencephalon of the chicken can be seen fairly distinctly even in untreated animals.

In the chicken hypothalamus, the modified ependyma is only a structure which contains the monoamine-containing cells. It consists of two different cells which contain CA and 5-HT respectively as in the pigeon brain (22). Falck, Ljunggren and Nordgren (38) reported that CA present in this structure was noradrenaline. In fish, amphibian and reptile, cells of the modified ependyma were also observed to contain CA and 5-HT (25, unpublished data).

The nucleus arcuatus in the rat hypothalamus consists of dopamine-containing cells (group A12 in Dahlström and Fuxe, 17) and sends axons to the external layer of the median eminence (19). However, the corresponding nucleus in the chicken hypothalamus was not observed. This fact may explain why the fluorescence of the external layer of the chicken median eminence is not such strong as in mammals (23, 24).

In contrast to the findings in the medulla oblongata, mesencephalon and diencephalon, there were no monoamine-containing cells in the spinal cord, cerebellum and telencephalon, although CA and 5-HT containing terminals were present in two regions except the cerebellum.

A dose of reserpine caused a very marked decrease in the fluorescence of the cells described above as early as 1 hour later but a small decrease was produced in yellow fluorescence of the modified ependyma, probably due to contain higher amounts of 5-HT than in the other 5-HT containing cells. At 2 and 4 hours later, the fluorescence of all monoamine-containing cells disappeared almost completely, when CA terminals still had a weak to medium fluorescence in certain areas of the brain (e.g. in the mesencephalon, hypothalamus, preoptic and septal area). Therefore, this findings suggest that there is a higher concentration of monoamines in the terminals than in the cells.

There are significant differences in rate of recovery of fluorescence between CA and 5-HT containing cells following reserpine treatment. Recovery of the specific yellow fluorescence in 5-HT containing cells seems to begin at 8 hours later at the latest (Table 3). 5-HT containing cells show a distinctly yellow fluorescence at 12 hours later, when most of CA containing cells exhibit no certain fluorescence. Moreover, they show an approximately same fluorescence intensity as those in normal animals at 18 hours after administration of reserpine.
but CA containing cells still show a somewhat weaker fluorescence intensity. In addition to this fact, 5-HT containing cells in untreated birds have a stronger fluorescence than those in untreated mammals. These findings seem to suggest that 5-HT is of more importance in the avian central nervous system than in the mammalian.

The changes induced by the administration of nialamide or reserpine plus nialamide are roughly the same sequences as in the rat or pigeon (17, 22). The specific yellow fluorescence of 5-HT increased markedly following these treatment. Therefore, monoamine oxidase appears to be significantly important for 5-HT metabolism even in the chicken brain as in the pigeon and rat brain than for CA metabolism.

Either dopa or 5-HT after nialamide treatment caused a marked increase in the background fluorescence, especially in the superficial layers of the tectum opticum, in the regions of the posterior hypothalamus and the fasciculus solitarius, or in the preoptic region and the corpus striatum. These phenomena appear to be due to the presence of dopa or 5-HTP in the brain tissue. Thus, these five regions might be considered as a major site of uptake and accumulation of dopa or 5-HTP. In contrast to the findings in mammals (29, 30), any of fluorescence was not observed in the endothelium of brain capillaries of the chicken. This fact suggests strongly that the endothelium of the chicken brain capillaries contains neither dopa decarboxylase nor 5-HTP decarboxylase. The absence of this enzymic mechanism was also observed in fish, amphibian and reptile (unpublished data).

**SUMMARY**

The differential distributions of catecholamine (CA)-containing and 5-hydroxytryptamine (5-HT)-containing cells were established in the central nervous system of the chicken. Moreover, these cell groups were mapped topographically. The localization of CA containing cells was divided four groups in the medulla oblongata, five groups in the mesencephalon and one group in the diencephalon. The localization of 5-HT containing cells was divided four groups in the medulla oblongata, four groups in the mesencephalon and one group in the diencephalon. However, there were no monoamine-containing cells in the spinal cord, cerebellum and telencephalon, although it was observed that two regions except the cerebellum contained CA and 5-HT containing nerve terminals. Many of the monoamine-containing cells in the chicken brain were situated within the mesencephalon. The topographical distributions of these cells in the mesencephalon accord with those in the pigeon.

5-HT containing cells in untreated chickens have a stronger fluorescence than those in untreated rats. Moreover, the rate of recovery of fluorescence in the reserpinized animals was significantly quicker in 5-HT than in CA. These findings suggest that 5-HT is of more importance in the avian central nervous system as compared with that in the mammalian.

In contrast to the findings in the mammalian brain, any of fluorescence was not observed in the endothelium of the brain capillaries of chickens following nialamide plus dopa or 5-HTP treatments.

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Abbreviations used in textfigures

A: Nucleus vagus motorius ventralis (Nucleus ambiguus)
AN: Nucleus annularis
C: Cerebellum
CCD: Commissura cochlearis dorsalis
CL: Nucleus cochlearis laminaris
CM: Nucleus cochlearis magnocellularis
CMa: Corpus mamillare
DCS: Decussatio cerebellaris superior
DLP: Nucleus dorsolateralis posterior
DMP: Nucleus dorsomedialis posterior
DSD: Nucleus decussationis supraopticae dorsalis
EM: Nucleus ectomammillaris
FS: Fasciculus solitarius
GV: Nucleus geniculatus lateralis
GVS: Ganglion vestibulare scarpae
HL: Nucleus habenularis lateralis
HMd: Nucleus habenularis medialis pars dorsolateralis
HMv: Nucleus habenularis medialis pars ventromedialis
IM: Nucleus vagus motorius intermedius
Imc: Corpus mammillare
IO: Nucleus isthmi pars principalis magnocellularis
IO: Nucleus isthmo-opticus
IP: Nucleus interpeduncularis
Ipc: Nucleus isthmi pars principalis parvocellularis
LC: Nucleus linearis caudalis
LLd: Nucleus lemnisci lateralis dorsalis
LLv: Nucleus lemnisci lateralis ventralis
MLd: Nucleus mesencephalicus lateralis pars dorsalis
MLf: Medial longitudinal fasciculus
MPI: Nucleus mesencephalicus profundus pars lateralis
MPp: Nucleus mesencephalicus profundus pars posterior
MPv: Nucleus mesencephalicus profundus pars ventralis
NIII: Nervus oculomotorius
NIV: Nervus trochlearis
NVI: Nervus abducens
NVII: Nervus acusticus
NX: Nervus vagus
nDCS: Nucleus decussationis cerebellaris superior
nIV: Nucleus trochlearis
nVI: Nervus abducens major
nVII: Nervus facialis motorius ventralis
nX: Nervus vagus motorius dorsalis
nXII: Nucleus hypoglossus
O1: Nucleus olivaris inferior
OM: Nucleus oculomotorius
OMa: Nucleus oculomotorius accessorius
OSd: Nucleus olivaris superior pars dorsalis
OSv: Nucleus olivaris superior pars ventralis
OV: Nucleus ovoidalis
Qf: Tractus quinto-frontalis
R: Nucleus raphis
RHP: Regio hypothalamica posterior
RS: Nucleus reticularius superior
Rt: Nucleus rotundus

MONOAMINE CELLS IN CHICKEN BRAIN
Ru : Nucleus ruber
SL : Nucleus semilunaris
SP : Nucleus subpretectalis
SR : Nucleus subrotundus
TD : Nucleus tegmental is dorsalis
TeO : Tectum opticum
TL : Nucleus tegmental is laterodorsalis
TRD : Nucleus trigeminus radicis descend entis
TrO : Tractus opticus
TrOM : Tractus occipito-mesencephalicus et bulbaris
V : Ventricul
VDM : Nucleus vestibularis dorsomedialis
VM : Ventriculus mamillaris
VTa : Nucleus vestibularis tangentialis
VVL : Nucleus vestibularis ventrolateralis
TEXT-FIG. 1. Transverse section through the medulla oblongata at the level of the nucleus vagus motorius dorsalis. The topography of group A1 and A2 and of groups B1 and B2 is illustrated.

TEXT-FIG. 2. Transverse section through the medulla oblongata at the level of the nucleus abducens major. The topography of groups A3 and A4 and of groups B2-B4 is illustrated.

TEXT-FIG. 3. Transverse section through the medulla oblongata at the level just behind the nucleus trochlearis. The topography of group A5 and of groups B2, B5 and B6 is illustrated.
**Text-FIG. 4.** Transverse section through the mesencephalon at the level of the nucleus trochlearis. The topography of group A5 and of groups B5 and B6 is illustrated.

**Text-FIG. 5.** Transverse section through the mesencephalon at the level of the nucleus interpeduncularis. The topography of groups A6 and A7 and of groups B7 and B8 is illustrated.
TEXT-FIG. 6. Transverse section through the mesencephalon at the level of the nucleus oculomotorius. The topography of groups A6-A10 and of group B9 is illustrated.

TEXT-FIG. 7. Transverse section through the diencephalon at the level of the nucleus ovoidalis. The topography of group A10 and of group B9 is illustrated.
Fig. 1. Group A1. Transverse section. Small to medium-sized, round to oval cells of medium to strongly green fluorescence are situated immediately dorso-lateral to the nucleus vagus motorius ventralis (A). ×100.

Fig. 2. Group A1. A multipolar cell with three long green-fluorescent processes (A) from the same cell group as in Fig. 1. ×400.

Fig. 3. Group A2. Transverse section. Small-sized, round to oval cells (→) with a weakly green fluorescence are observed dorso-lateral to the nucleus vagus motorius dorsalis (A). The cells are surrounded with a number of fine, varicose green-fluorescent terminals. ×100.

Fig. 4. Group A2. Round to oval, weakly fluorescent cells (→) correspond to those in Fig. 3. ×400.
Fig. 5. Group A3. Transverse section. A small cell with a weakly fluorescence indicates CA containing cell which is present immediately dorso-lateral to the nucleus olivaris superior pars dorsalis (A). Fine, varicose green-fluorescent terminals are seen between the lamniscus spinalis (B) and the nucleus olivaris superior pars dorsalis (A). ×100.

Fig. 6. Group A5. Transverse section through the level just caudal to the nucleus trochlearis. A fairly large number of small-sized, round to oval nerve cells of strongly green fluorescence (A) are observed immediately under the forth ventricle (C). These cells are situated within the nucleus tegmentalis dorsalis. In the direction ventro-lateral to this group, very strongly green-fluorescent terminals and fibers (B) are present. ×40.

Fig. 7. Group B1. Nialamide treatment. Transverse section. The medium-sized, round to oval, closely packed cells of very weakly to weakly yellow fluorescence are situated between the medial longitudinal fasciculus (A). ×100.

Fig. 8. Group B2. Transverse section. Large-sized, round to oval cells with weakly to medium fluorescence indicate 5-HT containing cells within the nucleus raphe at the level of the nucleus abducens major. Non-fluorescent cells (--) are also seen in this area. ×100.
Fig. 9. Group A5. Transverse section. This figure shows strongly fluorescent cells which belong to group A5 at the level of the caudal part of the mesencephalon. × 100.

Fig. 10. Group A5 and group B5. Transverse section through the nucleus trochlearis. CA containing cells which belong to group A5 are present at A. At B, C and D can be seen a number of round to oval, small to medium-sized cells with medium to strongly yellow fluorescence, which form group B5. The medial longitudinal fasciculus is indicated at F. × 40.

Fig. 11. Group A5 and group B5. This figure corresponds to the lateral part of figure 10. CA (A) and 5-HT (B) containing cells are obviously distinguished one another. × 100.
Fig. 12. Groups A6, A7, A8 and A9. Transverse section through the rostral part of the mesencephalon. The left side of the figure indicates the midline of the brain. A large number of small to medium-sized, round to oval nerve cells of medium to strongly green fluorescence are seen from the midline up to the lateral part of the brain. Group A6 is observed in the lateral part of the figure (A) and is surrounded by a relatively few of fine, green-fluorescent terminals. Group A7 is present immediately lateral to the midline (B). Group A8 (C) is situated just lateral to the nervus oculomotorius (F). Group A9 (D) is seen from the dorso-medial to the dorso-lateral parts of the nucleus ectomammilaris (E) and is surrounded by numerous terminals and fibers with very strongly green fluorescence. ×40.

Fig. 13. Group A8. This figure shows the same cell group as in Fig. 12. The nervus oculomotorius is seen at A. ×100.

Fig. 14. Group A6. These medium-sized, round to oval nerve cells of medium to strong fluorescence belong to the same cell group as in Fig. 12. ×100.
Fig. 15. Group B7. Transverse section through the middle part of the mesencephalon. A large number of medium-sized, round to oval cells of medium yellow fluorescence are situated mainly within the nucleus decussationis cerebellaris superior. ×40.

Fig. 16. Group B8. Transverse section. This cell group is present lateral to the nucleus interpeduncularis. Cells are medium-sized, round to oval and often multipolar, and have medium yellow fluorescence. ×100.

Fig. 17. Group A10 and group B9. Transverse section through the posterior part of the hypothalamus. The modified ependyma consists of strongly to very strongly fluorescent cells which contain either CA or 5-HT. The third ventricle is present at A. ×100.

Fig. 18. Group A10 and B9. The processes arising from the cells within the modified ependyma run both toward the ventricle (→†) and the lateral part of the brain (→‡), ×100.