UC Irvine

UC Irvine Previously Published Works

Title

Development of neurotransmitter uptake in regions of the chick brain.

Permalink

<https://escholarship.org/uc/item/6883d6r9>

Journal

Brain research, 119(2)

ISSN

0006-8993

Authors

Bondy, SC
Purdy, JL

Publication Date

1977

DOI

10.1016/0006-8993(77)90319-5

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

DEVELOPMENT OF NEUROTRANSMITTER UPTAKE IN REGIONS OF THE CHICK BRAIN

STEPHEN C. BONDY and JANET L. PURDY

University of Colorado Medical Center, Departments of Neurology and Pharmacology, Denver, Colo. 80220 (U.S.A.)

(Accepted May 10th, 1976)

SUMMARY

The uptake of postulated neurotransmitters or their precursors into regions of the developing chick brain and retina has been examined. The transport of low concentrations (around 10^{-8} M) of GABA, glutamic acid, choline, dopamine and serotonin into homogenates was sodium and energy dependent and inhibited by a variety of pharmacological agents that are thought to act presynaptically. After morphological fractionation, the high affinity transport mechanism was concentrated in the nerve ending fraction.

Compounds were poorly accumulated by the cerebral regions of the 6 day incubated chick embryo. After this time, the uptake capacity of each brain region studied exhibited a characteristic developmental profile.

Mechanisms for GABA transport appeared early in development, while catecholamine and choline systems matured later. Homogenates of the cerebral hemispheres and optic lobes took up all compounds studied, while the retina and cerebellum of the young chick were able to take up only GABA to a significant extent.

INTRODUCTION

The presence of synapses specific for a given neurotransmitter may be detected by assaying the extent to which that neurotransmitter can be taken up by homogenates or synaptosomes prepared from cerebral tissue²¹. Synaptosomal elements are responsible for much of such uptake by brain homogenates, but neuronal and glial cell body fragments could also play a role in this process. Such non-synaptic events are minimized by the use of purified synaptosomal preparations in uptake studies.

The advantage of using homogenates to study the energy dependent uptake of postulated neurotransmitter is the lack of preparative loss of tissue. Thus, the uptake capacities of different samples of brain can be precisely compared. For these reasons,

we have examined this process in both homogenates and in synaptosomal fractions. These extracts are then incubated for 5 min with very low concentrations of radioactive compounds. The short incubation time minimizes any metabolic conversion of these compounds^{17,23,37}, and their low concentration ensures that any uptake is largely caused by high affinity mechanisms. There is evidence that a neurotransmitter is taken up by those synapses specific for it in a rather selective manner²¹. The degree of uptake of a given compound in brain regions allows an estimate to be made of the relative preponderance of synapses specific for that neurotransmitter in those regions. We have examined the developmental profile of neurotransmitter uptake in various regions of the maturing chick brain. The cerebellum, optic lobes and cerebral hemispheres are readily distinguishable even in the early embryo. In addition, the uptake capacity of the retina was examined, since this is a neural tissue derived from the central nervous system and can easily be dissected out. The eye develops very early in embryogenesis.

The onset of uptake may reflect synaptic differentiation, but glial maturation may also possibly play a role. Very little high affinity uptake was found in the early embryo but, during growth, major increases occurred which were characteristic for each region. We have also examined the uptake capacity of various morphological fractions. Even in the young embryo, the nerve ending fraction had the greatest ability to transport possible neurotransmitters.

MATERIAL AND METHODS

(a) *Synaptosome preparation*

Chick and chick embryos of a White Leghorn strain were used. After decapitation, tissues were rapidly dissected out and accurately weighed. Retinae were carefully removed largely free of the pigment layer. Tissues were then homogenized in 19 vols 0.32 *M* sucrose. In order to prepare synaptosomes, this homogenate was centrifuged at $1000 \times g$ for 10 min; the supernatant was then recentrifuged at $14,000 \times g$ for 20 min. The pellet from this latter centrifugation was rehomogenized in 0.32 *M* sucrose and recentrifuged ($14,000 \times g$ for 20 min). The precipitate was taken up in 2 ml 0.32 *M* sucrose and layered on a discontinuous sucrose gradient consisting of 5 ml 0.8 *M* sucrose and 5 ml 1.2 *M* sucrose¹². After centrifugation in an SW 36 rotor ($140,000 \times g$, 30 min), the material at the interface between the 0.8 *M* and 1.2 *M* sucrose was drawn off with a disposable pipet and diluted with 5 vols 0.32 *M* sucrose. This suspension was then centrifuged in SW 36 rotor ($88,000 \times g$, 30 min), and the pelleted material taken up in 2 ml 0.32 *M* sucrose. While this method was originally developed for the rat, a fraction considerably enriched in synaptosomes can also be made from avian brain³⁰.

(b) *Incubation*

The standard incubation medium consisted of Krebs–Ringer buffer containing 121 mM NaCl, 4.0 mM KCl, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 1 mM ascorbic acid, 0.015 mM EDTA, 20 mM glucose and 40 mM Tris · HCl, pH 7.6. An inhibitor of

monoamine oxidase (nialamide) was also present ($7.5 \times 10^{-5} M$) as was aminoacetic acid ($1 \times 10^{-5} M$), an inhibitor of γ -aminobutyric acid transaminase. To this medium was then added a single radioactive compound (New England Nuclear, Boston, Mass.). Final μM concentrations of each compound were: DL-[3-³H]glutamic acid 0.01 (8.2 Ci/mmol); [2,3-³H]GABA, 0.01 (10 Ci/mmol); [1,2-³H]5-hydroxytryptamine (serotonin) 0.024 (4.25 Ci/mmol); DL-[7-³H]norepinephrine, 0.013 (7.5 Ci/mmol); [methyl-³H]choline chloride, 0.017 (2.34 Ci/mmol); [ethyl-1-³H]-3,4-dihydroxyphenylethylamine (dopamine) 0.0125 (8.0 Ci/mmol); and [1,2-¹⁴C]-taurine 1.08 (4.61 mCi/mmol). Radioactive choline was used (rather than acetylcholine) since it is relatively stable and specifically transported by cholinergic neurons²².

This medium was gassed with 95% O₂-5% CO₂, and 0.9 ml of this was mixed with 0.1 ml of either a 5% (v/v) tissue homogenate sucrose or of a synaptosomal suspension in 0.32 M sucrose. The GABA uptake mechanism was intensely active. Thus, in order to maintain linear kinetics, only a 0.5% homogenate was used in these studies. In this case, 0.1 ml of 5% liver homogenate was added as a carrier at the end of the incubation. We found no mechanism for GABA uptake by liver homogenates. Incubation was at 37 °C for 5 min with continuous shaking. To allow for non-energy dependent neurotransmitter-binding, identical mixtures were held at 0 °C, and these served as controls. Inhibitors of the uptake process, when used, were added to the medium prior to the addition of tissue preparations. In the case of dopamine and serotonin, incubations were performed in dim light in order to retard photodecomposition.

All samples were then centrifuged at 0 °C and $28,000 \times g$ for 10 min. Supernatants were drawn off for determination of radioactivity remaining unbound to particulate matter. Pellets were resuspended in 4 ml isotonic (0.14 M) NaCl and recentrifuged ($28,000 \times g$, 10 min). The washed pellets were then dissolved in 0.5 ml tissue solubilizer (NCS, Amersham Searle, Arlington Heights, Ill.) at 45 °C. Radioactivity in these samples was determined. It was thus possible to calculate what percentage of the total radioactive compound in each incubation tube was actively

TABLE I

Sodium dependence of high affinity uptake by optic lobe homogenates from 3-day-old chicks

The concentration of labeled compounds was between $1.0 \times 10^{-8} M$ and $2.3 \times 10^{-8} M$. Tubes contained 0.1 ml of a 5% tissue homogenate except for the GABA uptake study where a 0.5% homogenate was used. Incubation was for 5 min at 37 °C in oxygenated Krebs-Ringer buffer. For detailed procedure see text. Standard errors of the mean are given.

³ H-compound	pmoles labeled compound accumulated/min/g tissue	
	Sodium-containing buffer	Sodium-free buffer
DL-Glutamate	14.8 ± 0.2	1.2 ± 0.02
GABA	840 ± 80	0
Dopamine	12.4 ± 1.2	2.0 ± 0.3
Choline	12.9 ± 1.9	4.5 ± 0.5
Serotonin	80.9 ± 4.6	12.4 ± 3.7

taken up by the particulate fraction. When the results were calculated on a protein basis, protein was assayed by the method of Lowry et al.²⁷.

(c) *Binding of GABA*

Non-energy dependent binding of GABA to synaptic membranes was determined in synaptosomal preparations⁴³. These were incubated with 10^{-8} M [³H]GABA (10 Ci/mmol) at 0 °C for 5 min in 0.1 M Tris · HCl, pH 7.5. In order to minimize any active uptake, the synaptosomal preparations were frozen and thawed prior to use and the incubation medium contained no sodium ion or glucose. At the end of the incubation, radioactivity in the precipitate and supernatant was determined as described previously, the only difference being that the pellet was washed in 5 ml distilled water, not isotonic saline.

RESULTS

(a) *Characteristics of the uptake system*

The ability of optic lobe homogenates from 3-day-old chicks to take up the compounds studied was severely depressed when incubation was in sodium-free Krebs–Ringer buffer, where sucrose was substituted for Na⁺ (Table I). All high affinity transport systems were sodium-dependent.

The uptake of suspected neurotransmitters and of choline into homogenates of chick optic lobes was examined in the presence of a variety of pharmacological agents which have been reported to block their synaptic uptake^{2,3,16,36,42}. The resulting inhibitions observed were consistent with a synaptic location of most of the uptake activity (Table II).

Transport of labeled neurotransmitter candidates and choline was examined in the presence of a large excess of the non-radioactive compounds (Table III). The

TABLE II

Inhibition of high affinity uptake by various agents

The concentration of labeled compounds was between 1.0 and 2.3×10^{-8} M. Tubes contained 0.1 ml of a 5% homogenate of optic lobes from newly hatched chicks. In the case of GABA uptake, a 0.5% homogenate was used. Incubation was for 5 min at 37 °C in oxygenated Krebs–Ringer buffer. Inhibitors and their final concentrations were: 10^{-3} M chlorpromazine (used with glutamate and GABA), 10^{-4} M L-amphetamine sulfate (used with dopamine), 10^{-3} M hemicholinium (used with choline), 10^{-4} M desipramine (used with serotonin). For detailed procedure see text. Standard errors of the mean are given.

Isotope	Uptake (pmoles/min/g tissue)		
	— Inhibitor	+ Inhibitor	% Inhibition
DL-Glutamate	8.9 ± 0.2	0.09 ± 0.01	99
GABA	1350 ± 71	67 ± 18	95
Serotonin	62 ± 3	1.3 ± 1.5	98
Dopamine	12.5 ± 0.7	0	100
Choline	11.2 ± 0.3	0.3 ± 0.1	98

TABLE III

Inhibition of uptake by non-radioactive homologous compounds

The concentration of labeled compounds was between 1.0 and 2.3×10^{-8} M. Tubes contained 0.1 ml of a 5% homogenate of optic lobes from 2-day-old chicks. In the case of GABA, a 0.5% homogenate was used. Incubation was for 5 min at 37°C in oxygenated Krebs-Ringer buffer. Inhibition caused by unlabeled compounds present at 10^{-4} M was determined. For detailed procedure, see text.

Non-radioactive compound (10^{-4} M)	% Inhibition				
	Glutamate	GABA	Serotonin	Dopamine	Choline
Glutamate	63	29	6	9	0
GABA	0	95	8	0	0
Serotonin	7	10	93	65	4
Dopamine	7	15	49	89	10
Choline	7	14	7	9	77

uptake of each radioactive chemical was severely inhibited by the unlabeled homologues while other compounds inhibited uptake to a much lesser extent. This suggested that these high affinity transport mechanisms were relatively specific for each substance studied. However there was considerable overlap and thus less selectivity between the dopamine and serotonin uptake systems.

(b) Localization of the transport mechanism

The ability of several morphological fractions to take up labeled compounds is shown in Table IV. The synaptosomal fraction was generally the most active toward compounds examined. This fraction contained the bulk of the total activity of the tissue. In the case of embryonic uptake, only GABA had an uptake that was active enough to allow cell fractions to be accurately tested (Table V). Even in the 11-day-old embryo, the nerve ending fraction was the most active. The nuclear fraction often had considerable activity; since this fraction was not further purified, it contained many unbroken cells and tissue fragments. The nerve ending fraction may thus represent the predominant site of uptake by brain homogenates.

TABLE IV

High affinity uptake capacity of morphological fractions from chick optic lobes 8 days after hatch

Figures in parentheses give the specific activity of uptake relative to the original tissue homogenate. Standard errors of the mean are given.

	pmoles ^3H -compound taken up/min/100 mg protein							
	Glutamate		GABA		Serotonin		Dopamine	
Homogenate	59	± 1	(1.0)	957	± 7	(1.0)	149	± 1 (1.0)
Nuclear pellet	166	± 2	(2.8)	337	± 4	(0.35)	93	± 4 (0.62)
Post-mito. supernat.	4.3	± 0.1	(0.07)	15.7	± 0.3	(0.02)	24	± 1 (0.16)
Mito. pellet	142	± 20	(2.4)	251	± 32	(0.26)	91	± 8 (0.61)
Synaptosomes	125	± 4	(2.1)	704	± 114	(0.74)	302	± 37 (2.03)
							23.0	± 1.9 (1.74)

TABLE V

High affinity GABA uptake capacity of morphological fractions from chick optic lobes at various stages of maturity

Figures in parentheses give the specific activity of uptake relative to the original tissue homogenate. Standard errors of the mean are given.

	<i>pmoles [³H]GABA taken up/min/100 mg protein</i>		
	<i>11 day embryo</i>	<i>18 day embryo</i>	<i>8-day-old chick</i>
Homogenate	87 ± 10 (1.0)	295 ± 15 (1.0)	957 ± 7 (1.0)
Nuclear pellet	26 ± 2 (0.30)	203 ± 14 (0.69)	337 ± 4 (0.35)
Post-mito. supernatant	28 ± 1 (0.32)	167 ± 1 (0.57)	46 ± 1 (0.05)
Mito. pellet	10 ± 5 (0.11)	80 ± 8 (0.27)	251 ± 32 (0.26)
Synaptosomes	146 ± 35 (1.68)	595 ± 35 (2.01)	704 ± 114 (0.74)

a

b

Fig. 1. Double reciprocal plot of the velocity of uptake of GABA by optic lobe homogenates from the 3-day-old chick. The standard incubation mixture and incubation conditions described in Methods were used in the presence of varying concentrations of sodium glutamate. Standard errors of the mean are shown. a: glutamate concentrations from $5 \times 10^{-6} M$ to $1 \times 10^{-3} M$. b: glutamate concentrations from $5 \times 10^{-3} M$ to $5 \times 10^{-2} M$.

Fig. 2. Development of high affinity uptake of $[^3\text{H}]\text{GABA}$ by homogenates of various chick brain regions. Incubation conditions are described in Materials and Methods. $\blacktriangle$ — $\blacktriangle$, optic lobes; $\triangle$ — $\triangle$, cerebral hemispheres; $\circ$ $\circ$, cerebellum; $\bullet$ ----- $\bullet$, retina. Standard errors are plotted.

If incubation was carried out at 0°C , there was an uptake of around 20% of the radioactivity normally accumulated during a 37°C incubation. When synaptosomes were frozen and thawed before use and then incubated with $[^3\text{H}]\text{GABA}$ or $[^3\text{H}]\text{serotonin}$ at 0°C in 10 mM Tris pH 7.5 for 5 min, almost no radioactivity was bound to the synaptosomal pellet. Since postsynaptic binding does not require glucose, sodium or intact synaptic structure, these results indicate that very little such binding was present in our preparations. The radioactivity binding to synaptosomes, incubat-

Fig. 3. Development of high affinity uptake of $[^3\text{H}]\text{serotonin}$ by homogenates of various chick brain regions. Incubation conditions are described in Material and Methods. $\blacktriangle$ — $\blacktriangle$, optic lobes; $\triangle$ — $\triangle$, cerebral hemispheres; $\circ$ $\circ$, cerebellum; $\bullet$ ----- $\bullet$, retina. Standard errors are plotted.

Fig. 4. Development of high affinity uptake of [^3H]dopamine by homogenates of various chick brain regions. Incubation conditions are described in Materials and Methods. $\blacktriangle$ — $\blacktriangle$, optic lobes; $\triangle$ — $\triangle$, cerebral hemispheres; $\circ$ $\circ$, cerebellum; $\bullet$ ---- $\bullet$, retina. Standard errors are plotted.

ed at 0°C in the presence of glucose and Na^+ under our usual incubation conditions, was largely due to residual uptake activity rather than postsynaptic binding.

The uptake of GABA was much greater than that of other compounds, and the kinetics of this process was examined in greater detail. Uptake was linear for at least 5 min at 37°C and was proportional to tissue concentration up to the concentrations of optic lobe homogenates that we routinely used (see Methods). A series of incubations were carried out over a wide range of GABA concentrations, and Lineweaver–Burk

Fig. 5. Development of high affinity uptake of [^3H]choline by homogenates of various chick brain regions. Incubation conditions are described in Materials and Methods. $\blacktriangle$ — $\blacktriangle$, optic lobes; $\triangle$ — $\triangle$, cerebral hemispheres; $\circ$ $\circ$, cerebellum; $\bullet$ ---- $\bullet$, retina. Standard errors are plotted.

Fig. 6. Development of high affinity uptake of [^3H]glutamate by homogenates of various chick brain regions. Incubation conditions are described in Materials and Methods. $\blacktriangle$ — $\blacktriangle$, optic lobes; $\triangle$ — $\triangle$, cerebral hemispheres; $\circ$ $\circ$, cerebellum; $\bullet$ — $\bullet$, retina. Standard errors are plotted.

curves²⁶ were plotted (Fig. 1). A high affinity uptake of GABA was found, with an apparent K_m of $6.25 \times 10^{-6} M$ and a $V_{\max}$ of 6.45×10^{-7} moles/min/g tissue. In addition, using higher GABA concentrations, a low affinity uptake mechanism was found. This had an apparent K_m of $3.33 \times 10^{-2} M$ and $V_{\max}$ of 4.17×10^{-6} moles/min/g tissue.

In view of the high K_m of the lower affinity transport system, the major uptake pathway for physiological concentrations of GABA is the high affinity mechanism. Taking the different $V_{\max}$ values into consideration, we have calculated that for 0.1 mM GABA, over 95% of any uptake would be due to the high affinity system.

Levi²⁵ reported embryonic chick brain to possess two mechanisms of differing affinities for GABA uptake. Only the high affinity system was present in 15-day-old chicks, and this had an apparent K_m of $0.72 \times 10^{-3} M$. Since the lowest GABA concentration used by Levi was $2 \times 10^{-5} M$, the very high affinity mechanism reported here may have not been observable under this conditions.

(c) Development of transport capacity

The uptake of a series of compounds during ontogenesis by various tissue homogenates was determined (Figs. 2–6). The 6 day incubated embryos had a very small ability to accumulate isotopes. Subsequently, each region had a distinctive developmental profile. The uptake of GABA reached maximal values at hatch, while serotonin, dopamine and choline uptake mechanisms continued to increase significantly after hatch. Choline uptake was not detectable before the 18th day of incubation while GABA, glutamate and serotonin uptake were pronounced at 11 days.

Uptake mechanisms in the optic lobes and cerebral hemispheres generally

developed in parallel except that the uptake of dopamine was much greater in the hemispheres than in any other brain region.

After 11 days of incubation, the cerebellum showed no major increase in its capacity to transport any chemical examined. There was no clear uptake of choline, and the uptake of serotonin gradually diminished to background values in the 8-day-old chick. Cerebellar glutamate uptake also declined after the 11th day of incubation. No major accumulation of serotonin, dopamine or choline was seen in the retina of any stage. Retinal capacity to accumulate GABA increased steadily, ultimately reaching a value of about 25% of the corresponding figure for the cerebral hemispheres.

DISCUSSION

A problem concerning neurotransmitter uptake studies is in the location of the active process since a Na^+ dependent high affinity uptake mechanism may exist in glia as well as neurons¹⁵. The uptake of labeled isotopes that we have observed is at least in part neuronal since it takes place in cerebral areas of the 8-day-old embryo where glial cells are almost completely absent. In addition, the concentration of the uptake process within the synaptosomes fraction suggests its preponderantly synaptic location. One cannot completely eliminate the possibility of glial elements being present in the synaptosomal fraction which may contain membrane components that are not readily identifiable. The neuronal site of uptake is further supported by the inhibition of the reaction by neuropharmacological agents thought to act at nerve endings. The possibility of uptake by whole neuronal or glial cell bodies is minimal since homogenization breaks up intact cells. Iversen and Schon¹⁷ reported 71% of GABA uptake into substantia nigra to be into nerve terminals and only 2% of uptake was glial. This uptake is depressed by the interruption of the nigro-striatal pathways and is thus thought to be synaptic⁴⁰.

A further question concerns the specificity of uptake. Such specificity has been reported (see Introduction) and is supported by our data on the differential developmental course of uptake of various radioactive compounds. Furthermore, there is a close parallel between the uptake of the compounds reported here and their concentration in avian brain^{1,33}. Thus, serotonin, which is taken up to the greatest extent by the forebrain, is found in maximal concentrations in that region. The optic lobes, which take up choline to the largest degree, have the greatest endogenous acetylcholine levels. Choline uptake has been reported to be an early index of cholinergic innervation³⁸ and is largely neuronal in chick embryos²⁹. This mechanism rather than endogenously synthesized choline may be the major source of substrate for synaptic acetylcholine synthesis¹³.

Dopamine uptake and also its concentration are both over 4-fold greater in the forebrain than in any other area examined. This may be related to the fact that the avian cerebral hemispheres are largely homologous with the mammalian basal ganglia, which contain many dopamine neurons³⁹. The relation between the uptake of a putative neurotransmitter and its concentration suggests that neurons synthesizing a specific neurotransmitter also possess a high affinity uptake mechanism for that

transmitter. There is, however, data suggesting that norepinephrine and dopamine are taken up by both norepinephrine and dopamine synapses. The much greater concentration of norepinephrine than dopamine in optic lobes⁴ suggests a preponderance of norepinephrine synapses in this region. Catecholaminergic uptake mechanisms matured relatively late, and so considerable posthatch elaboration of catecholamine neurons may occur. Vernadakis⁴¹ has showed that norepinephrine levels in several chick brain regions rise steeply after hatch. These neurons may then be more subject to environmental influences than systems which are largely developed at hatch. In the cat, the main increase in synaptic number of areas primarily or secondarily related to vision occurs while the visual system is in use⁸.

Considerable postnatal development of cerebral norepinephrine uptake has been reported in the rat⁷. In this case there was also a close relation between endogenous norepinephrine concentration and uptake capacity. However, the uptake of norepinephrine into chick cerebral hemisphere slices does not increase after the 15th day of incubation¹⁸.

The mechanism for the uptake of GABA is well developed in the new-hatched chick and declines somewhat in older birds. This is a similar result to that of Kelly et al.¹⁹ who found GABA uptake into mouse brain slices to decline during maturation. The uptake of GABA was detectable early in embryogenesis. Since GABA is thought to be an inhibitory neurotransmitter and since brain removal causes increased motility of the chick embryo¹⁴, it may be that the development of inhibitory pathway precedes that of excitatory ones. The development of GABA uptake in the optic lobes roughly parallels the rise of the levels of GABA and glutamic acid decarboxylase³⁵.

The retina of the new-hatched chick only takes up GABA to a marked extent. The location of this process has previously been shown to be largely neuronal (retinal ganglion and amacrine cells) rather than glial²⁸. There are conflicting data as to whether this uptake is neuronal or glial in mammals^{9,31}. Our homogenization conditions would tend to favor nerve endings rather than cell body uptake, whereas the other studies were carried out with intact retinæ. Although we found no differences in the GABA accumulation of light or dark adapted retinæ, Lam and Steinman²⁴ have reported that GABA uptake is increased by darkness in the goldfish retina. In addition, the GABA concentration of dark adapted frog retina is depressed¹¹. The release of GABA by chick retina is stimulated by light³⁹. However, several other retinal neurotransmitter candidates and their related enzymes may be also influenced by light. These include dopamine²⁰, taurine³² and acetylcholine¹⁰.

The quantitatively predominant cerebellar uptake mechanism was that for GABA. All other compounds were very poorly taken up. This is consistent with the largely inhibitory functions of inputs to this region. However, GABA uptake capacity was well below that of the cerebral hemispheres or optic lobes. Therefore, the major neurotransmitter of the cerebellum may be an unknown compound whose uptake we have not examined. The transient ability of the immature cerebellum to accumulate serotonin may reflect the appearance of a cell species that is only temporarily present. It may be significant that cerebellar serotonin concentrations are maximal in the 15-day-old embryo and decline sharply thereafter⁴¹. Programmed neuronal death takes place to a considerable degree during the maturation of the chick brain⁶.

Profiles of neurotransmitter uptake development have been expressed per unit wet weight. In view of the gradual decrease in water content during brain maturation³⁴ rates of increase would be steeper if expressed on a dry weight basis. The major onset of transport mechanisms is at the time of cell differentiation rather than proliferation. The ability to transport postulated neurotransmitters may be a valid index of the selective maturation of distinct neuronal species.

ACKNOWLEDGEMENTS

We are grateful to Mrs. Jane Mitchell for expert secretarial assistance.

This work was supported by NIH Grant NS09603 and Foundations' Fund for Research in Psychiatry Grant 70-487, S. C. B. is recipient of an NIMH Research Scientist Award K05-MH-00102.

REFERENCES

- 1 Aprison, M. H. and Takahashi, R., 5-Hydroxytryptamine, acetylcholine, 3,4 dihydroxyphenylethylamine and nor-epinephrine in several discrete areas of the pigeon brain, *J. Neurochem.*, 12 (1965) 221-230.
- 2 Azzaro, A. J., Ziance, R. J. and Rutledge, C. O., The importance of neuronal uptake of amines for amphetamine induced release of ³H-norepinephrine from isolated brain tissue, *J. Pharmacol. exp. Ther.*, 189 (1974) 110-118.
- 3 Blackburn, K. J., French, P. C. and Merrills, R. J., 5-Hydroxytryptamine uptake by rat brain in vitro, *Life Sci.*, 6 (1967) 1653-1663.
- 4 Bondy, S. C. and Purdy, J. L., Putative neurotransmitter of the avian visual pathway, *Brain Research*, 119 (1977) 417-426.
- 5 Cotman, C. W., Herschmann, H. and Taylor, D., Subcellular fractionation of cultured glial cells, *J. Neurobiol.*, 2 (1971) 168-180.
- 6 Cowan, W. M., Studies on the development of the avian visual system. In D. C. Pease (Ed.), *Cellular Aspects of Neural Growth and Differentiation*, University of California, Los Angeles, Calif., 1971, pp. 177-222.
- 7 Coyle, J. T. and Axelrod, J., Development of the uptake and storage of L-³H norepinephrine in the rat brain, *J. Neurochem.*, 18 (1971) 2061-2075.
- 8 Cragg, B. G., The development of synapses in the visual system of the cat, *J. comp. Neurol.*, 160 (1975) 147-166.
- 9 Ehinger, B., Autoradiographic identification of rabbit retinal neurons that take up GABA, *Experientia (Basel)*, 26 (1970) 1063-1064.
- 10 Glow, P. H. and Rose, S., Effects of light and dark on acetylcholinesterase activity of the retina, *Nature (Lond.)*, 202 (1964) 422-423.
- 11 Graham, L. T., Baxter, C. F. and Lolley, R. N., In vivo influence of light or darkness on the GABA system in the retina of the frog (*Rana pipiens*), *Brain Research*, 20 (1970) 379-388.
- 12 Gray, E. G. and Whittaker, V. P., The isolation of nerve endings from brain: an electron microscopic study of cell fragments derived by homogenization and centrifugation, *J. Anat. (Lond.)*, 96 (1962) 79-88.
- 13 Guyenet, P., Lefresne, P., Rossier, J., Beaujouan, J. C. and Glowinski, J., Effect of sodium, hemicholinium-3 and antiparkinson drugs on [¹⁴C]acetylcholine synthesis and [³H]choline uptake in rat brain synaptosomes, *Brain Research*, 62 (1973) 523-529.
- 14 Hamberger, V., Balabon, M., Oppenheim, R. and Wenger, E., Periodic motility of normal and spinal chick embryos between 8 and 17 days of incubation, *J. exp. Zool.*, 159 (1965) 1-14.
- 15 Henn, F. A. and Hamberger, A., Glial cell function: uptake of transmitter substances, *Proc. nat. Acad. Sci. (Wash.)*, 68 (1971) 2686-2690.

- 16 Iversen, L. L. and Johnston, G. A. R., GABA uptake in rat central nervous system comparison of uptake in slices and homogenates and the effects of some inhibitors, *J. Neurochem.*, 18 (1971) 1939–1950.
- 17 Iversen, L. L. and Schon, F. E., The use of autoradiographic techniques for the identification and mapping of neurotransmitter-specific neurons in the central nervous system. In A. J. Mandell (Ed.), *New Concepts in Transmitter Regulation*, Plenum Press, New York, 1965, pp. 153–193.
- 18 Kellogg, V., Vernadakis, A. and Rutledge, C. O., Uptake and metabolism of [^3H]norepinephrine in the central hemispheres of chick embryos, *J. Neurochem.*, 18 (1971) 1931–1938.
- 19 Kelly, P., Luttges, M., Johnson, T. and Grove, W., Maturation-dependent alterations in [^3H]GABA compartmentalization in neural tissue in vitro, *Brain Research*, 68 (1974) 267–280.
- 20 Kramer, S. G., Dopamine: a retinal transmitter. I. Retinal uptake, storage and light stimulation release of [^3H]dopamine in vivo, *Invest. Ophthalmol.*, 10 (1971) 438–452.
- 21 Kuhar, M. J., Neurotransmitter uptake: a tool in identifying neurotransmitter specific pathway, *Life Sci.*, 13 (1973) 1623–1634.
- 22 Kuhar, M. J., Sathy, V. H., Roth, R. H. and Aghajanian, G. K., Choline selective accumulation by central cholinergic neurons, *J. Neurochem.*, 20 (1973) 581–594.
- 23 Kuhar, M. J., Shaskan, E. G. and Snyder, S. H., The subcellular distribution of endogenous and exogenous serotonin in brain tissue. Comparison of synaptosomes storing serotonin, norepinephrine and γ -aminobutyric acid, *J. Neurochem.*, 18 (1971) 333–343.
- 24 Lam, D. M. K. and Steinman, L., The uptake of [^3H] γ -aminobutyric acid in the goldfish retina, *Proc. nat. Acad. Sci. (Wash.)*, 68 (1971) 2777–2781.
- 25 Levi, G., Cerebral amino acid transport in vitro during development: a kinetic analysis, *Arch. Biochem.*, 138 (1970) 347–349.
- 26 Lineweaver, H. and Burk, D., The determination of enzyme dissociation constants, *J. Amer. chem. Soc.*, 56 (1934) 658–666.
- 27 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the Folin phenol reagent, *J. biol. Chem.*, 193 (1951) 265–275.
- 28 Marshall, J. and Voaden, M., An autoradiographic study of the cell accumulating [^3H] γ -aminobutyric acid in the isolated retinas of pigeons and chickens, *Invest. Ophthalmol.*, 13 (1974) 602–607.
- 29 Massarelli, R., Sensenbrenner, M., Ebel, A. and Mandel, P., Kinetics of choline uptake in mixed neuronal glial and exclusively glial cultures, *Neurobiology*, 4 (1974) 414–418.
- 30 McBride, W. J., Aprison, M. H. and Hingtgen, J. N., Effects of 5-hydroxytryptophan on serotonin in nerve endings, *J. Neurochem.*, 23 (1974) 385–391.
- 31 Neal, M. J. and Iversen, L. L., Autoradiographic localization of [^3H]GABA in rat retina, *Nature New Biol.*, 235 (1972) 217–218.
- 32 Passantes-Morales, H., Kleithi, J., Urban, P. F. and Mandel, P., The effect of electrical stimulation, light and amino acids on the efflux of ^{35}S -taurine from the domestic fowl, *Exp. Brain Res.*, 19 (1974) 131–134.
- 33 Pschiedt, G. R. and Himwich, H. E., Chicken brain amines with special reference to cerebellar norepinephrine, *Life Sci.*, 2 (1963) 524–526.
- 34 Romanoff, A. L., *Biochemistry of the Avian Embryo*, Wiley, New York, 1967, pp. 78–79.
- 35 Siskin, B., Sano, K. and Roberts, E., γ -aminobutyric acid content and glutamic decarboxylase and γ -aminobutyrate transaminase activities in the optic lobe of the developing chick, *J. biol. Chem.*, 236 (1961) 503–507.
- 36 Snyder, S. H. and Coyle, J. T., Regional differences in [^3H]norepinephrine and [^3H]dopamine uptake into rat brain homogenates, *J. Pharmacol. exp. Ther.*, 165 (1969) 78–86.
- 37 Snyder, S. H., Green, A. I. and Hendley, E. D., Kinetics of ^3H -norepinephrine accumulation into slices from different regions of the rat brain, *J. Pharmacol. exp. Ther.*, 164 (1968) 90–102.
- 38 Sorimachi, M. and Kataoka, K., High affinity choline uptake: an early index of cholinergic innervation by rat brain, *Brain Research*, 94 (1975) 325–336.
- 39 Sourkes, T. L., Parkinson's disease and other disorders of the basal ganglia. In B. W. Albers, G. J. Siegel, R. Katzman and B. W. Agranoff (Eds.), *Basic Neurochemistry*, Little Brown, Boston, Mass., 1972, pp. 565–578.
- 40 Storm-Mathisen, J., High affinity uptake of GABA in presumed GABA-ergic nerve endings in rat brain, *Brain Research*, 84 (1975) 409–427.
- 41 Vernadakis, A., Comparative studies of neurotransmitter substances in the maturing and aging central nervous system of the chicken. In D. H. Ford (Ed.), *Neurobiological Aspects of Maturation and Aging*, *Prog. Brain Res.*, Vol. 40, Elsevier, Amsterdam, 1973, pp. 231–243.

- 42 Yamamura, H. I. and Snyder, S. H., High affinity transport of choline into synaptosomes of rat brain, *J. Neurochem.*, 21 (1973) 1355–1374.
- 43 Zukin, S. R., Young, A. B. and Snyder, S. H., Gamma-aminobutyric acid binding to receptor sites in the rat central nervous system, *Proc. nat. Acad. Sci. (Wash.)*, 71 (1974) 4802–4807.