Autoradiographic Localization of Tryptamine Binding Sites in the Rat and Dog Central Nervous System

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Tryptamine, an endogenous trace amine, is currently postulated to be a neuromodulator or neurotransmitter in the mammalian CNS. High-affinity binding sites have been described for tryptamine in rat brain homogenate preparations. The present study further characterizes tryptamine binding throughout the CNS and delineates its distribution using *in vitro* receptor binding in conjunction with autoradiographic techniques. Saturation studies on 20- μ m-thick brain sections suggest a single class of binding sites (Hill coefficient = 0.97 ± 0.04) with a high affinity ($K_D = 4.79 \pm 1.55$ nM). In competition studies, kynuramine and tetrahydrobetacarboline significantly inhibited H^3 -tryptamine binding while serotonin, dopamine, and phenylethylamine failed to significantly inhibit it. The most potent inhibitor of H^3 -tryptamine binding was tryptamine ($K_I = 4.19 \pm 2.13$ nM). In rat brain sections processed for *in vitro* autoradiography, highest binding occurred in the following limbic structures: the accumbens nucleus, the amygdalohippocampal area, the lateral septal nucleus, the entorhinal cortex, and the anterior olfactory nucleus. At diencephalic levels, the highest binding was observed in the reuniens thalamic nucleus, the paraventricular thalamic nucleus, the medial habenular nucleus, the central medial thalamic nucleus, and the arcuate hypothalamic nucleus. In the midbrain of the rat, binding was most notable in the interpeduncular nucleus, the superficial layer of the superior colliculus, the periaqueductal gray, and the paranigral nucleus. In the lower brain stem of the dog, binding was evident in the external cuneate nucleus, the spinal trigeminal nucleus, and in the region of the solitary nucleus. Binding was also present in both the ventral and dorsal horns of the canine spinal cord. Tryptamine binding sites appear widely distributed throughout the CNS, exhibiting the highest densities of binding in the more rostral portions of the brain.

Tryptamine is present in all major brain regions in all species examined thus far (Marsden and Curzon, 1974; Martin et al., 1972; Philips et al., 1974; Saavedra and Axelrod, 1972; Sloan et al., 1975; Snodgrass and Horn, 1973). Mass spectrometric quantification reveals that the concentration of tryptamine in the caudate (2.93 ng/gm) is almost six times that of whole brain, and concentrations in the hypothalamus are almost double that of whole brain (Philips et al., 1974). Tryptamine has been previously postulated to be a neuromodulator or neurotransmitter in the mammalian CNS (Frankhuijzen and Bonta, 1974; Quock and Weick, 1978; Vane et al., 1961). Both supporting and op-

posing evidence for such a role has been reviewed by Jones (1982).

More recently, additional supporting evidence that documents a high-affinity binding site for H^3 -tryptamine in rat brain homogenates has been presented (Cascio and Kellar, 1982; Kellar and Cascio, 1982). Since then, other investigators have also demonstrated that tryptamine binding sites are present in the CNS and are distinct from those of serotonin binding sites (Rommelspacher and Kaufmann, 1983; Wood et al., 1984). Tryptamine binding has been found to be strongly inhibited by beta-carbolines (Cascio and Kellar, 1983; Rommelspacher and Kaufmann, 1983; Wood et al., 1984), kynuramine (Charlton et al., 1984), and derivatives of phenylethylamine (PEA) (Cascio and Kellar, 1983; Martin et al., 1984). This suggests that several endogenously found compounds are possible ligands for the tryptamine binding site.

All the binding studies described above have been performed on brain homogenate preparations. Although these homogenate studies have provided data on tryptamine binding in large brain regions, they fail to delineate the localization of tryptamine-binding sites among brain nuclear groups. The *in vitro* autoradiographic technique developed by Young and Kuhar (1979) allows precise delineation of binding sites among brain nuclei. Two preliminary reports have described the use of this technique to elucidate the localization of tryptamine binding sites in the CNS (McCormack et al., 1983; Perry et al., 1982). The purpose of the present investigation is to employ *in vitro* autoradiographic technique to map tryptamine binding sites in the rat and dog CNS.

Materials and Methods

Male Sprague-Dawley rats, weighing between 250 and 300 gm, were anesthetized with chloral hydrate and perfused transcardially with ice cold isotonic saline. Brains were immediately removed, slowly frozen to -20°C , and used for binding studies within 24 hr. Dogs of either sex, weighing between 10 and 15 kg, were premedicated with atropine and acetylpromazine and anesthetized with pentobarbital. Dogs were cannulated in the carotid artery and perfused with ice cold isotonic saline over a 30 min period. The spinal cord and lower brain stem were removed and slowly frozen to -20°C after gross dissection into the following six regions: medulla, C1 to C5, C6 to T2, T3 to T13, L1 to L3, and L4 to S3.

To establish appropriate biochemical parameters for H^3 -tryptamine binding, rat brains were first sectioned at 20 μ m on an American Optical cryostat at -20°C . Serial sections from the rostral commissure through the caudal hippocampus were thaw-mounted onto clean glass slides and refrozen until used. Slide-mounted sections were then placed in preincubation baths for 5 and 2 min each, transferred to an incubation bath for 60 min, and subsequently washed twice in trizma citrate buffer, as described in Table 1. After the last bath, the sections were scraped off the slides using a 2.4-cm-diameter glass microfiber filter (Whatman) and placed in 10 ml of Scintiverse E scintillation fluid. After a minimum of 12 hr, the sections were counted in a liquid scintillation counter (Beckman Model LS7000). The amount of protein in 20 μ m tissue

Received Apr. 2, 1985; revised June 17, 1985; accepted July 8, 1985.

This work was supported by NIH Grants NS19208 and NS17407, NSF Grant BNS83-11214, as well as by a grant from the American Veterinary Medical Association.

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Table 1. H^3 -tryptamine binding procedure

Bath	Total binding
Preincubation I	0.1 M trizma citrate
5 min, 25°C	pH 7.4
	5 mM ascorbic acid
Preincubation II	0.1 M trizma citrate
2 min, 25°C	pH 7.4
	5 mM ascorbic acid
	20 μ M pargyline
Incubation bath	0.1 M trizma citrate
For binding	pH 7.4
60 min, 25°C	5 mM ascorbic acid
For autoradio	20 μ M pargyline
30 min, 25°C	2 nM H^3 -tryptamine
Wash I	0.1 M trizma citrate
6.5 min, 4°C	pH 7.4
	5 mM ascorbic acid
	20 μ M pargyline
Wash II	0.1 M trizma citrate
6.25 min, 4°C	pH 7.4
	5 mM ascorbic acid
	20 μ M pargyline

Nonspecific binding was measured in the presence of 2 μ M unlabeled tryptamine.

sections was determined using the Coomassie Brilliant Blue method, as described by Macart and Gerbaut (1982).

For autoradiography, both canine and rodent tissue sections were cut at 20 μ m and processed according to the protocol described in Table 1 with the exception that tissues were incubated for only 30 min. This shorter incubation time was used because it provided a higher specific/nonspecific binding ratio. Brains from rats were sectioned from the rostral pole of the forebrain to the caudal medulla. The canine caudal brain stem and spinal cord were also sectioned, incubated and processed for autoradiography. Autoradiography of tissue sections was carried out as described previously (Beitz et al., 1984). After the last bath, sections were briefly dipped in distilled water, dried, and apposed to LKB Ul-trofilm. After an appropriate exposure period, the film was developed in D-19 developer and analyzed using a computerized photometer system. Since tritium standards were not used in this analysis, the density values obtained represent relative values for the nuclear groups studied. In addition, no attempt was made to account for the regional white-matter quenching, which varies according to the different ratios of gray to white matter in the brain regions studied (Unnerstall and Kuhar, 1985). Brain structures were identified according to the atlas of Paxinos and Watson (1982) for the rat and of Singer (1962) for the dog.

Tritiated tryptamine hydrochloride, with a specific activity ranging between 23.9 and 41.5 Ci/mmol, was purchased from New England Nuclear. Fluoxetine hydrochloride was a gift from Eli Lilly and Co. (Indianapolis, IN). Lysergic acid diethylamide (LSD) was a gift from The National Institute on Drug Abuse. All other chemicals, unless otherwise stated, were obtained from Sigma Chemical Co. (St. Louis, MO).

Results

Binding kinetics

The rate of association of tritiated tryptamine with its binding site was determined by varying the length of the incubation period, while maintaining the bath temperature at 25°C. H^3 -tryptamine bound rapidly with a half-time of association at 3.8 min and saturation in approximately 15 min (Fig. 1). Analysis by linear regression of the same data provides the association rate constant (K_{+1}) of $0.059 \text{ nM}^{-1} \text{ min}^{-1}$. In contrast, the rate of dissociation of tritiated tryptamine was determined by varying the total wash time while maintaining the wash bath temperature at 4°C. The half-time of dissociation under these con-

Figure 1. Effect of varying incubation times on the total ($\square$), specific ($\bullet$), and nonspecific ($\circ$) binding of H^3 -tryptamine to mounted tissue sections. See text for details. Each point represents the average of three or more experiments, except at 75 and 90 min, which are based on two and one experiments, respectively. The insert depicts the association curve, based on linear regression of data in the main graph. The association rate constant was calculated to be $0.059 \text{ nM}^{-1} \text{ min}^{-1}$.

ditions was determined to be 15.5 min while the dissociation rate constant (K_{-1}) was calculated to be 0.045 min^{-1} (Fig. 2). If pargyline hydrochloride was omitted, specific H^3 -tryptamine binding was dramatically reduced because of the rapid oxidation by MAO.

Saturation studies

Slide-mounted tissue sections were incubated with different concentrations of H^3 -tryptamine to determine total binding, and also in the presence of excess unlabeled tryptamine (1000-fold excess) to determine nonspecific binding. All sections were incubated for 60 min to establish equilibrium. This longer incubation bath time was needed primarily at the lower concentrations of tritiated tryptamine. Scatchard analysis of this data (Scatchard plot, Fig. 3B) suggests an equilibrium dissociation constant (K_D) of $4.79 \pm 1.55 \text{ nM}$ ($n = 3$, ranging from 3.12 to 7.89 nM) and the maximum number of binding sites (B_{max}) was $195 \pm 34 \text{ fmol/mg protein}$. Linear regression of the data yields a Hill coefficient (n_H) of 0.97 ± 0.04 (Fig. 3A), suggesting binding to a single class of sites and lack of any apparent cooperativity. Previous studies by Cascio and Kellar (1983) have shown saturation of specific tritiated tryptamine binding at 8–10 nM. Owing to the large quantities of radiolabeled tryptamine required in the incubation baths, the concentration required for saturation of specific binding was not determined.

Competition studies

Various concentrations of the compounds listed in Table 2 were added to both incubation baths to determine their effects on specific H^3 -tryptamine binding. The changes in the specific binding due to the compounds' competition were analyzed by Logit

Figure 2. Effect of varying wash times on the total ($\square$), specific ($\bullet$), and nonspecific ($\circ$) binding of H^3 -tryptamine to mounted tissue sections. Results are the average of two or more experiments. The insert depicts the dissociation curve, based on linear regression of data in the main graph. The dissociation rate constant was calculated to be 0.045 min^{-1} . See text for further details.

Transformation (Rodbard and Frazier, 1975) to determine the concentration of compound required to inhibit 50% of the specific H^3 -tryptamine binding (IC_{50}). As the Hill coefficient was found to be close to 1 for each of the competing drugs, the IC_{50} of each was then converted to an inhibitory constant, K_i , where $K_i = IC_{50}/[1 + (H^3\text{-tryptamine})/K_D]$. The use of K_i allows comparison of the abilities of these compounds to inhibit tryptamine binding with previous studies.

Tryptamine hydrochloride had a K_i of $4.19 \pm 2.13 \text{ nM}$, in agreement with the K_D of 4.79 nM for H^3 -tryptamine, indicating a similar affinity of these ligands for the binding sites (Table 2). Tryptophan, the precursor to tryptamine, and indoleacetic acid, an endogenously formed metabolite of tryptamine, both lacked the ability to inhibit specific H^3 -tryptamine binding at all concentrations tested. On the other hand, other metabolic products

Table 2. Competition studies: inhibitory constants

Compound	K_i (nM)
Tryptamine·HCl	4.19
Tryptophan (D and L isomers)	$> 1.00 \times 10^6$
Indole-3-acetic acid	$> 1.00 \times 10^6$
Kynuramine·dihydrobromide	27.01
Tetrahydrobetacarboline·HCl (Tryptoline)	70.68
5-Hydroxytryptamine·oxalate (Serotonin)	1620
Lysergic acid diethylamide	$> 7.00 \times 10^4$
Fluoxetine·HCl	3490
beta-Phenylethylamine·HCl	1480
5-Hydroxytyramine·HCl (Dopamine)	4000
gamma-Aminobutyric acid	$> 1.00 \times 10^5$
Diazepam	$> 1.00 \times 10^5$

Figure 3. Representative saturation and Scatchard plots of H^3 -tryptamine binding to mounted brain tissue sections. Each point represents the average of two to six experiments. Insert A shows an analysis of the data using the Hill plot, which yields a K_D of 4.0437 nM and a B_{max} of $174.9931 \text{ fmol/mg protein}$. Insert B shows an analysis of the data using the Rosenthal (Scatchard) analysis, which yields a K_D of 4.0729 nM . Open squares indicate total binding, closed circles indicate specific binding, and open circles indicate nonspecific binding. See text for further details.

of tryptamine, such as kynuramine and tetrahydrobetacarboline, had K_i values of 27.01 and 70.68 nM , respectively, indicating high affinities for the tryptamine binding site. Compounds previously reported to affect serotonin binding showed little ability to inhibit tryptamine binding. Serotonin itself produced a K_i of 1620 nM , while fluoxetine, a serotonin uptake inhibitor, yielded a K_i of 3490 nM . At concentrations up to $100 \mu\text{M}$, LSD, a commonly used ligand for serotonin binding sites, did not inhibit H^3 -tryptamine binding, implying a K_i of greater than $70,000 \text{ nM}$. Other drugs inhibiting H^3 -tryptamine binding include dopamine and phenylalanine, both producing K_i values comparable to that of serotonin— 4000 and 1480 nM , respectively. Gamma-aminobutyric acid and diazepam were ineffective in displacing H^3 -tryptamine at concentrations up to $200 \mu\text{M}$, implying K_i values of greater than $100,000 \text{ nM}$.

Autoradiography studies

The relative density values obtained from autoradiographic images are shown in Table 3 for the rat and Table 4 for the dog. Each specific photometer reading indicates the decrease in percentage of transmittance and thereby represents the relative density of specific H^3 -tryptamine binding. Nonspecific binding was found to be relatively homogeneous throughout the brain and spinal cord. Each value represents the mean of three animals and was calculated by subtracting the nonspecific binding and background film density from the total binding.

Telencephalon (Figs. 4 and 5, Table 3)

Tryptamine binding sites were densest in the limbic structures. The highest values were found in the frontal cortex, entorhinal cortex, nucleus accumbens, lateral septal area, anterior olfactory

Table 3. Density (in arbitrary optical density units) of specific H³-tryptamine binding in various brain regions of the rat

Brain region	Density	±SE	n ^a
Telencephalon			
Accumbens nucleus	36.0	3.8	4
Amygdalohippocampal area	35.8	3.5	4
Cortex			
Frontal	35.7	3.2	3
Entorhinal	32.2	6.5	4
Medial frontal	29.2	7.7	3
Primary olfactory	28.6	3.1	4
Anterior cingulate	26.0	3.3	4
Retrosplenial	24.2	3.4	4
Frontoparietal—motor area	24.0	5.0	4
—somatosensory	23.6	5.7	3
Temporal, auditory area	22.4	7.8	4
Area 17 (striate)	22.0	4.5	3
Area 18 (striate)	19.4	4.6	4
Posterior cingulate	19.0	2.7	4
Islands of Calleja	35.1	5.1	4
Lateral septal nucleus	32.0	5.1	4
Bed nucleus of stria terminalis	31.6	5.4	4
Anterior olfactory nucleus	31.2	2.0	3
Septohippocampal nucleus	31.1	8.8	3
Olfactory tubercle	30.0	5.0	4
Amygdaloid nuclei	27.0	5.3	4
Medial septal nucleus	26.1	3.6	4
Hippocampus	25.1	3.0	4
Caudate putamen (striatum)	23.6	1.7	4
Ventral pallidum	23.1	4.0	4
Dentate gyrus	19.4	3.3	4
Globus pallidus	16.2	2.0	3
Diencephalon			
Thalamus			
Reuniens nucleus	30.0	7.7	4
Paraventricular nucleus	29.3	5.7	4
Central medial nucleus	25.6	4.6	4
Paratenial nucleus	24.8	11.4	2
Mediodorsal nucleus	21.6	2.2	4
Lateral posterior nucleus	21.2	3.3	4
Dorsal lateral geniculate n.	19.7	3.3	4
Stria medullaris of thalamus	19.0	5.1	4
Posterior nuclear group	16.4	3.8	4
Laterodorsal thalamic nucleus	15.4	4.5	4
Medial geniculate nucleus	15.3	4.3	4
Ventromedial thalamic nucleus	15.0	6.5	2
Ventrolateral thalamic nucleus	14.0	7.6	3
Reticular thalamic nucleus	10.1	4.6	4
Ventral lateral geniculate n.	9.7	3.6	3
Hypothalamus			
Arcuate nucleus	26.0	6.6	4
Periventricular nucleus	23.6	6.2	4
Dorsomedial nucleus	23.4	6.2	4
Posterior nucleus	22.8	6.9	3
Medial preoptic area	21.8	2.6	4
Ventromedial hypothalamic n.	20.6	6.9	4
Lateral preoptic area	19.9	—	1

Table 3. *Continued*

Brain region	Density	±SE	n ^a
Lateral hypothalamic nucleus	16.8	5.4	4
Supra mammillary nucleus	12.1	2.6	3
Mammillary nucleus	11.8	3.0	3
Subthalamus: Zona incerta	9.3	3.3	4
Epithalamus			
Medial habenular nucleus	25.8	4.2	4
Lateral habenular nucleus	25.0	4.1	4
Mesencephalon			
Zonal layer of sup. colliculus	25.6	2.7	4
Superior colliculus	22.8	3.3	4
Interpeduncular nucleus	20.6	2.2	4
Periaqueductal gray	19.0	2.9	4
Paranigral nucleus	18.4	7.5	3
Ventral tegmental area	15.7	4.8	4
Substantia nigra	15.6	4.3	4
Median raphe nucleus	15.5	5.0	3
Rostral linear n. of raphe	13.5	4.6	3
Deep mesencephalic nucleus	11.2	4.0	2
Red nucleus	7.5	2.8	4
Myelencephalon			
Pons			
Pontine nuclei	17.2	3.6	2
Locus coeruleus	14.3	5.2	3
Pontine reticular n. oral part	13.0	1.8	3
Dorsal tegmental nucleus	12.2	4.6	4
Reticulo tegmental n. of pons	10.7	1.2	2
Nucleus of trapezoid body	9.8	4.6	4
Central gray of the pons	8.8	1.1	3
Dorsal parabrachial nucleus	8.3	2.4	2
Principal sensory trigeminal n.	7.6	1.8	4
Motor trigeminal nucleus	7.5	2.1	2
Pontine ret. n. caudal part	5.5	2.9	2
Superior olive	5.5	2.3	4
Raphe pontis	^b	—	4
Medulla			
Vestibular nucleus	11.7	2.4	4
Prepositus hypoglossal nucleus	9.6	3.8	4
External cuneate nucleus	9.4	4.4	2
Dorsal motor n. of vagus	8.9	5.7	4
Spinal trigeminal n. oral part	8.8	2.5	4
Dorsal cochlear nucleus	8.2	2.9	3
Nucleus of solitary tract	7.4	2.1	4
Hypoglossal nucleus	7.2	2.6	4
Spinal trigeminal n. caudal part	4.8	8.0	2
Parvocellular reticular n.	4.4	0.8	4
Facial nucleus	4.1	6.4	3
Gracile nucleus	3.8	6.5	2
Spinal trig. n. interpolaris	3.8	1.5	4
Ventral cochlear nucleus	3.6	1.8	2
Pyramidal tract	1.2	2.3	4
Gigantocellular reticular n.	^b	—	4
Paramedian reticular nucleus	^b	—	3
Lateral reticular nucleus	^b	—	3
Inferior olive	^b	—	4
Raphe pallidus	^b	—	4

→

Table 3. *Continued*

Brain region	Density	±SE	n ^a
Raphe obscurus	^b	—	4
Ambiguus nucleus	^b	—	4
Lateral cervical nucleus	^b	—	4
Cerebellum			
Molecular layer	12.4	1.8	4
Granular layer	11.4	2.4	4
Interpositus nucleus	2.2	4.0	3
Choroid plexus	26.2	2.6	4

^a n refers to the number of sections from each of the three animals from which density values were obtained.

^b Indicates average densities are no greater than background.

nucleus, and the amygdalohippocampal area. Moderate binding was seen in the amygdala, choroid plexus, striatum, hippocampus, temporal cortex, anterior cingulate gyrus, and the motor and somatosensory areas of the frontoparietal cortex. Compared to the areas listed above, binding was low in areas such as the globus pallidus and corpus callosum.

Diencephalon (Figs. 4 and 5, Table 3)

Within the hypothalamus, the highest binding occurred in the arcuate nucleus, the dorsomedial nucleus, and the periventricular nucleus. Moderate binding was observed in the medial and lateral preoptic areas and in the ventromedial nucleus. Tryptamine binding in the thalamus was highest in the reuniens nucleus, the paraventricular nucleus, and the central medial nucleus. In contrast, the ventrolateral and ventromedial thalamic nuclei, as well as the reticular nucleus, showed low levels of binding. Within the epithalamus, both the medial and lateral habenular nuclei exhibited high binding densities.

Mesencephalon (Figs. 4 and 5, Table 3)

The highest binding occurred in the zonal layer of the superior colliculus, the interpeduncular nucleus and the periaqueductal gray. Binding was substantially lower in the red nucleus and reticular formation.

Myelencephalon and spinal cord (Fig. 6, Tables 3 and 4)

Within the pons and medulla of the rat brain stem, the highest binding occurred in the pontine nuclei, the pontine reticular nucleus (pars oralis), the locus coeruleus and the dorsal tegmental nucleus. In the medulla of the dog, binding was generally high in areas similar to those in the rat. The highest binding in the medulla of the dog was present in the external cuneate nucleus, spinal trigeminal nucleus, gracile nucleus, hypoglossal nucleus, solitary nucleus, and parvocellular reticular nucleus. Bind-

ing was low in the gigantocellular reticular nucleus and the inferior olivary nucleus. White-matter tracts exhibited binding similar to or slightly greater than that of the background.

Within the spinal cord, tryptamine binding sites were present throughout the gray matter, being highest in the areas of the dorsal and intermediate horns (Fig. 6 and Table 4). When analyzed by rostral-caudal segments, binding was highest in the lumbosacral segments of the cord and lowest in the lower cervical region.

Discussion

The present investigation, using *in vitro* autoradiographic techniques, confirms the studies using tissue homogenates (Cascio and Kellar, 1982, 1983), indicating that the brain contains specific high-affinity binding sites for H³-tryptamine. This method was developed by Young and Kuhar (1979) and has been successfully used to study gamma-aminobutyric acid, serotonin, adrenergic, muscarinic, and opioid binding sites. The results of our kinetic studies showing rapid association and dissociation, the high-specific affinity for labeled and unlabeled tryptamine, and the autoradiographic distribution strongly suggest that the binding observed is a recognition site for tryptamine. The *K_D* obtained in the present study is remarkably similar to that obtained by Cascio and Kellar (1983).

Our competition studies distinguish this binding site from other binding sites of known and putative neurotransmitters such as serotonin, dopamine, gamma-aminobutyric acid, and phenylethylamine. The precursors D- and L-tryptophan, and the metabolite, indoleacetic acid, failed to inhibit tryptamine binding. However kynuramine, also an endogenous tryptamine metabolite, was found to be a potent inhibitor of tryptamine binding, as previously shown by Charlton et al. (1984) using tissue homogenate preparations. Similarly, another endogenous metabolite of tryptamine, tetrahydrobetacarboline, was found to be a fairly potent displacer of tryptamine binding, as was reported previously by several other investigators using homogenate preparations (Cascio and Kellar, 1982; Rommelspacher and Kaufmann, 1983; Wood et al., 1984).

Serotonin, whose three-dimensional structure is similar to tryptamine, was found to be only a weak inhibitor of H³-tryptamine binding, suggesting a class of binding sites distinct from those of serotonin. LSD, a commonly used ligand in serotonin binding studies, failed to inhibit tryptamine binding. Fluoxetine, a serotonin reuptake inhibitor, was as ineffective as serotonin in inhibiting tryptamine binding; it would thus appear that the tryptamine binding site is distinct from both the LSD and serotonin binding site and the serotonin reuptake site.

Phenylethylamine (PEA) has been previously reported to displace H³-tryptamine in tissue homogenate studies (Cascio and Kellar, 1983). Our results confirm the ability of PEA to displace H³-tryptamine binding. A recent abstract presented by Martin et al. (1984) comparing various PEA derivatives indicates that

Table 4. Density (in arbitrary optical density units) of specific H³-tryptamine binding in various segments of the spinal cord of the dog

Cord region (lamina no.)	Density (±SE)				
	Upper cervical	Lower cervical	Mid-thoracic	Upper lumbar	Lumbo-sacral
Superficial (I, II)	9.1 ± 6.3	2.9 ± 3.5	8.9 ± 6.1	9.5 ± 8.5	14.5 ± 3.1
Dorsal (III, IV, V)	12.0 ± 5.2	6.7 ± 3.1	14.2 ± 5.4	14.2 ± 10.7	22.4 ± 6.5
Intermediate (VI)	—	12.9 ± 4.6	13.1 ± 4.7	10.2 ± 5.0	19.0 ± 4.9
Ventral (VII, VIII, IX)	10.3 ± 4.9	7.0 ± 3.8	9.6 ± 1.8	14.8 ± 11.0	16.5 ± 4.4
Central (X)	12.0 ± 6.1	6.6 ± 4.3	7.4 ± 2.0	14.4 ± 12.4	14.0 ± 6.0
Ventral funiculus	4.6 ± 3.0	-1.7 ± 3.1	0.8 ± 3.2	1.4 ± 3.4	7.2 ± 1.4

The above density values were obtained from three dogs.

Figure 4. Representative autoradiographic images of H^3 -tryptamine binding from the forebrain (*A*), diencephalon (*B*) and midbrain (*C*) in the rat. Images *A*, *B*, and *C* correspond to Figs. 12, 22, and 26 in the atlas of Paxinos and Watson (1982). Image *A* shows high binding densities in the anterior cingulate cortex (*ACg*), the accumbens nucleus (*Acb*), the septal area (*SA*), and the primary olfactory cortex (*PO*). The corpus callosum (*cc*) and anterior commissure (*aca*) are also shown. *B*, High tryptamine binding in the choroid plexus of the lateral ventricle (*CP*), the motor area of the frontoparietal cortex (*FrPa*), the hippocampus (*Hi*), the amygdalohippocampal area (*AHi*), the habenular nuclei (*Hb*), and the central medial nuclei (*CM*). Tryptamine binding in the midbrain, *C*, is highest in the superficial layer of the superior colliculus (*sc*), the interpeduncular nucleus (*IP*), and the periaqueductal gray (*PAG*).

Figure 5. Computer-enhanced images of the three autoradiograms shown in Figure 4. The highest binding is illustrated in white and areas of lower binding in darker shades. A computerized image analysis system was used, as described previously (Beitz and Buggy, 1981).

para-methoxyphenylpropylamine is the most potent displacer of H^3 -tryptamine. By comparing the abilities of these PEA derivatives, they concluded that the tryptamine binding site is composed of a large flat lipophilic site that lies distal to an anionic site.

The *in vitro* autoradiographic localization of H^3 -tryptamine binding sites in the rat brain was first reported by Perry et al. (1982); they reported binding to be highest in the areas of the striatum, hippocampus, and cortex. Our results also indicate that binding is highest in the limbic structures and cortex, cor-

Figure 6. Representative autoradiographic image of H^3 -tryptamine binding in the ventral (VH) and dorsal (DH) horns of the thoracic region of the canine spinal cord. This image illustrates the high binding in gray matter relative to the surrounding white matter. In general, binding to white-matter structures was no greater than to background.

relating well with this initial autoradiographic study as well as with studies in which the binding was examined in homogenates of various brain regions (Cascio and Kellar, 1983; Wood et al., 1984). In general, tryptamine binding appears to be highest in more rostral structures and decreases progressively toward the more caudal brain areas. While the H^3 -tryptamine binding sites are distinct from those of serotonin, there appears to be some overlap in the distribution of the binding sites of these two indoleamines—for example, in cortical and hippocampal regions (Bigeon et al., 1982; Nakada et al., 1984; Palacios et al., 1983). However, serotonin₁ binding sites are highest in the subiculum and dentate gyrus while serotonin₂ binding sites are found in highest concentrations in the cerebral cortex, caudate-putamen, nucleus accumbens, mammillary nucleus, and the inferior olive. In the present study, tryptamine binding sites are most dense in the nucleus accumbens, the amygdalohippocampal area, and the cerebral cortex, where they appear to overlap with the distribution of serotonin₂ binding sites. However, tryptamine binding sites are low in mammillary nuclei and nondetectable in the inferior olivary nucleus, indicating differences between serotonin₂ and tryptamine binding sites.

The use of the dog for the autoradiographic localization of H^3 -tryptamine binding allows even greater delineation of areas containing tryptamine binding sites because of the large size of the canine spinal cord and brain stem. Tryptamine binding was found to be relatively low in canine spinal structures. A comparison of binding densities between the rat and the dog indicates that tryptamine binding sites are distributed in similar areas of the medulla in these two species.

The anatomical distribution of tryptamine binding sites in the CNS and the high degree of pharmacologic specificity of agents in their ability to inhibit this binding suggest that the H^3 -tryptamine binding site may be a receptor for tryptamine. The physiologic significance of the ability of kynuramine, tetrahydrobetacarboline and PEA to inhibit H^3 -tryptamine binding remains obscure.

As tryptamine has a high affinity for H^3 -tryptamine binding sites, a differential distribution of endogenously formed tryptamine could potentially result in a local inhibition of labeled tryptamine binding, thus falsifying the distribution of regional binding sites. However, one would expect this effect to be min-

imal in the brain and spinal cord, as mass spectrum analysis indicates that tryptamine is found in very low concentrations in this tissue (Philips et al., 1974) relative to the concentration necessary to displace a significant amount of labeled tryptamine. In addition, much of the endogenously formed tryptamine would be metabolized or diffuse from the tissue during the 5 min preincubation period. Since tryptamine undergoes an exceptionally rapid metabolism compared either to serotonin or norepinephrine, it is unlikely that endogenous tryptamine is present in sufficient amounts to affect the binding of tritiated tryptamine in the present study.

The concentration of endogenous tryptamine has been generally found to be highest in the caudate, hippocampus, and hypothalamus and lowest in the brain stem and cerebellum (Jones, 1982; Philips et al., 1974). This distribution of indoleamine correlates well with the distribution of binding reported in the present study, showing high binding in the limbic system and striatum and the lowest binding in the cerebellum and lower brain stem. It has been previously proposed that tryptamine acts in the CNS to modulate the activity of serotonin (Jones and Boulton, 1980). Other studies have shown that tryptamine produces effects that are opposite to those produced by serotonin on the C-fiber reflex, mono- and polysynaptic reflexes (Bell and Martin, 1974), and nociceptive activity after its intrathecal injection in conscious rats (Larson, 1983). This is consistent with our finding of H^3 -tryptamine binding sites in the dorsal and ventral horns of the spinal cord. The present study shows the highest density of H^3 -tryptamine binding sites in the nucleus accumbens. A recent report indicates that injection of tryptamine into this region evokes intense locomotor activity (Altar et al., 1985). In addition to its effects on locomotion, tryptamine has also been implicated in various psychiatric disorders and physiological functions, as reviewed by Jones (1982).

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