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The distribution of serotonin receptors in the human brain: high density of [^{3}H]LSD binding sites in the raphe nuclei of the brainstem

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Serotonin receptors were localized autoradiographically in the human brainstem after in vitro labeling using [^{3}H]lysergic acid diethylamide (LSD). Very high concentration of [^{3}H]LSD binding sites, apparently belonging to the 5-HT$_1$ class were localized in the raphe nuclei. Other areas of the brainstem presented only moderate or low receptor densities. Labeled areas were the nucleus interpeduncularis, periaqueductal gray matter, locus coeruleus and nucleus tractus solitarius. The choroid plexus was also labeled by [^{3}H]LSD. The use of [^{3}H]LSD binding as a marker for serotonin cells in the brainstem is suggested.

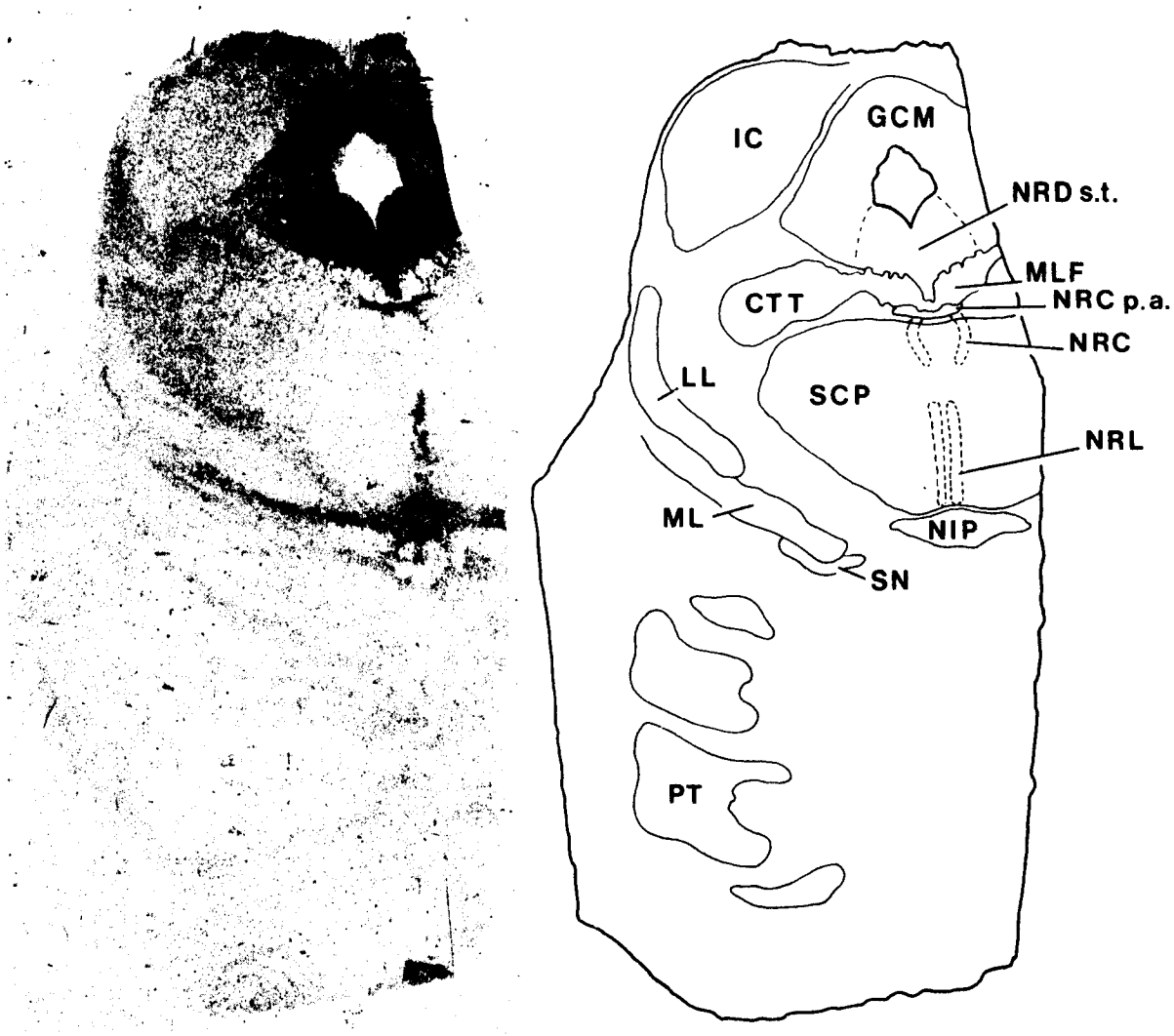
Receptor sites for serotonin (5-HT) in brain have been characterized using several tritiated high affinity ligands[3,7,15,17]. The existence of multiple 5-HT receptors has been postulated in order to account for the results of these ligand binding studies. In particular Peroutka and Snyder[15] have suggested two types of 5-HT sites in brain membranes: one that is preferentially labeled by [^{3}H]5-HT and has a high affinity for 5-HT itself, named 5-HT$_1$, and a second that is labeled with high affinity by the neuroleptic [^{3}H]spiperone in rat cortical membranes, denominated 5-HT$_2$ receptors. Tritiated LSD labels both receptors with the same affinity and is therefore useful to label both populations of receptors.

Serotonin receptors have been also studied in human brain by binding techniques, using [^{3}H]5-HT, [^{3}H]spiperone and [^{3}H]LSD as ligands. The properties of the site labeled by these ligands in human material appear to be similar to those described in tissue from experimental animals[2,6,9].

Alterations in pre- and postsynaptic serotonergic mechanism have been reported in some diseases of the human brain[2,6,9]. Because most of these studies have been performed using biochemical techniques that have a very limited anatomical resolution, we considered it of interest to apply a quantitative autoradiographic technique to the study of localization of 5-HT receptors[23]. This technique has already been succesfully applied to the localization of 5-HT receptors in the brains of experimental animals[4,8,11,14,24] and for receptor localization in human brain[10,13].

In this report we describe the localization of 5-HT receptors labeled with [^{3}H]LSD in the brainstem of the human brain, the region containing the cell bodies of the serotoninergic neurons[21]. Human brains were obtained at autopsy, dissected and frozen at — 80 °C until use. The results described in this report are based on observations obtained from 4 brains and from patients dying without a history of neurological diseases. The sexes and ages were male 26, female 49, male 60 and male 67. The autolysis times (time between death and autopsy) were 7 h 30 min, 6 h 30 min, 6 h 15 min and 3 h, respectively.

Sections (10 μm) were cut with a microtome cryostat and mounted onto gelatin coated slides. Serotonin receptors were labeled with [^{3}H]LSD (48.5 Ci/mmol, New England Nuclear, Boston, MA, U.S.A.) as previously described: consecutive sections were incubated with 5 nM [^{3}H]LSD alone or in combination with 0.1 μM ketanserin, to block [^{3}H]LSD binding to 5-HT$_2$ receptors[8,14], or with 30 nM 5-HT, to block binding to 5-HT$_1$ receptors. Blanks were obtained with 100 μM 5-HT. Autoradiograms were obtained by apposing the labeled tissues to [^{3}H]Ultrofilm (LKB, Sweden)[12]. Standards containing known amounts of tritium, prepared as described by Un-



Figs. 1-4. Autoradiographic localization of [^{3}H]LSD binding sites in the human brainstem. On the left side of the figures is a bright-field photograph of the autoradiographic image. Dark regions represent areas with high receptor densities. On the right side of the figures is a schematic drawing showing the main anatomical structures at the corresponding levels. Abbreviations used in the drawings are listed separately in the text. Fig. 1 is a slightly oblique section through the upper brainstem showing the upper pons basis ventrally and the lower midbrain dorsally. Note the dense labeling of the nucleus raphe dorsalis (NRD s.t.), of the nucleus raphe centralis pars annularis (NRC p.a.) and of the nucleus raphe linearis (NRL). The central gray (GCM) and the nucleus interpeduncularis (NIP) present an intermediate degree of labeling.

nerstall et al.[22] were exposed along with the tissues. Exposure time was 56 and 72 days. Films were developed and analyzed as previously described[12,22].

Binding of [^{3}H]LSD was observed in many areas and nuclei of the brainstem. A wide variation in the density of autoradiographic grains was evident, with the white matter tracts showing the lowest level of labeling. The presence of 100 μM 5-HT in the incubation medium blocked the binding to all the areas producing a uniform low density labeling. Katenserin produced non-significant changes, while 30 nM 5-HT blocked the binding to all the nuclei described in this work, indicating that these sites belong probably to the so called 5-HT$_1$ class of 5-HT receptors[15,16]. Examples of the distribution of [^{3}H]LSD binding sites in the human brainstem are illustrated in Figs. 1–4. The areas showing the highest levels of labeling were the different raphe nuclei. The dorsal raphe nuclei, both at the supratrochlear (Fig. 1) and more caudal levels (Fig. 2), presented a very high density of autoradio-

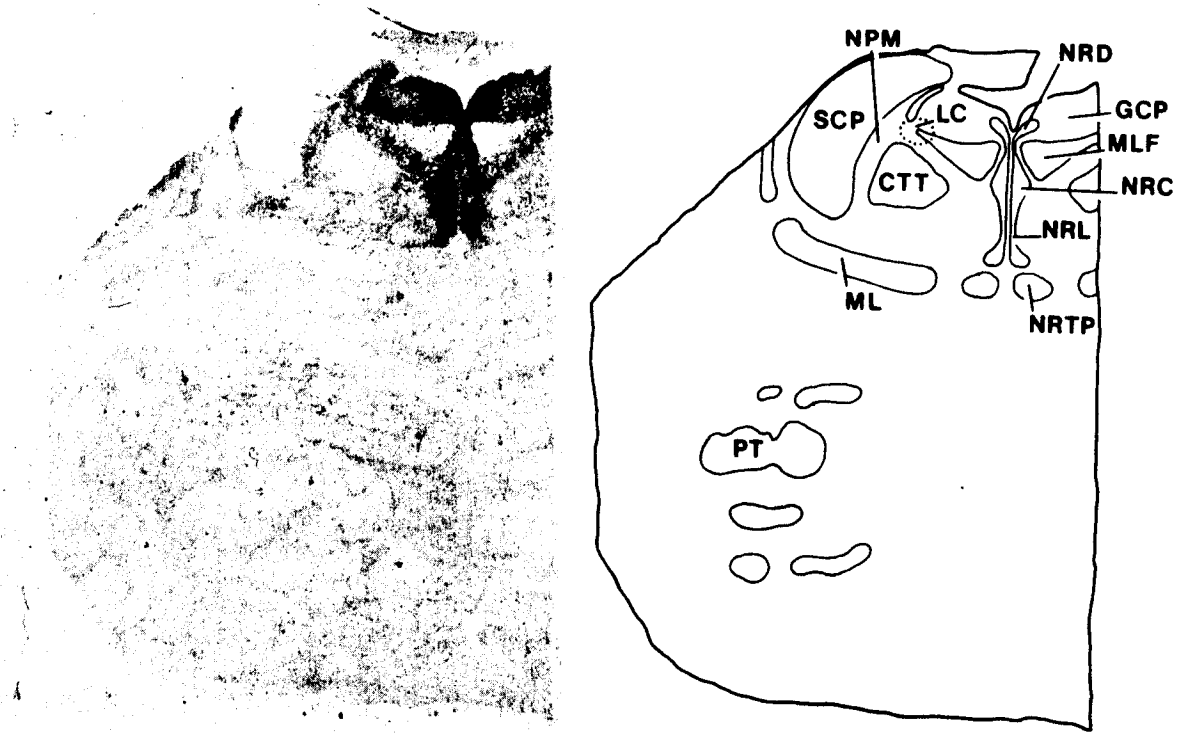


Fig. 2. Transverse section through the upper pons at a slightly lower level than Fig. 1. The highest density of autoradiographic grains is observed in the nucleus raphe dorsalis (NRD), nucleus raphe centralis (NRC) and linearis (NRL). The central gray (GCP) presents intermediate degrees of labeling. Low levels of receptor binding are observed in area containing the locus coeruleus (LC) and the medial parabrachial nucleus (NPM).

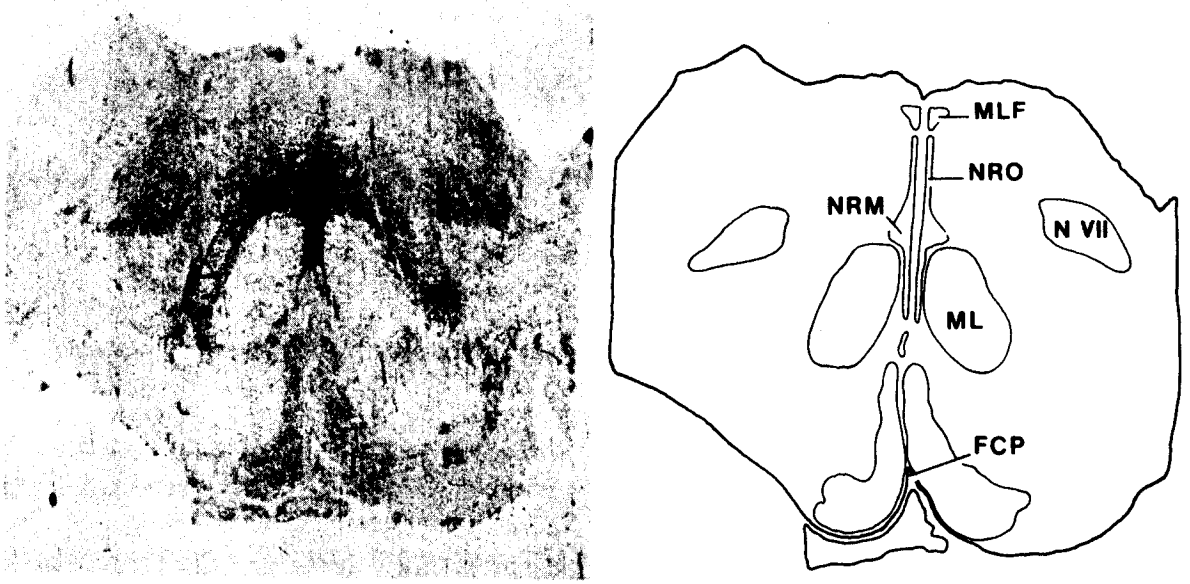


Fig. 3. Transverse section through the lower pons at the transition zone to the medulla oblongata. Strongest labeling is seen in the nucleus raphe magnus (NRM) and the nucleus raphe obscurus (NRO). Note also intermediate to low degree of labeling dorsal and lateral to the medial lemniscus (ML).

graphic grains. The density was similar to that observed in the human hippocampus and entorhinal cortex (unpublished observations), that contain one of the highest densities of [^{3}H]LSD binding sites in the brain. At both levels the highly labeled areas extended beyond the limits of the raphe nuclei, as described in the human brain by Braak[5], for example. Although we cannot deduce the precise cellular localization of the binding sites from our autoradiograms, the picture suggests that these sites are not restricted to the cell body areas, but extend beyond, probably over the dendritic field surrounding the cell bodies. The localization in the raphe nucleus also suggests that the binding is probably to 5-HT 'autoreceptors'.

A more restricted localization of the autoradiographic grains was observed in the raphe linearis (Fig. 1), raphe centralis (Fig. 2), raphe magnus (Fig. 3) and raphe obscurus and pallidus (Fig. 4). In these nuclei the [^{3}H]LSD labeling seemed to be more concentrated over the cell body areas. By visual examination the density of autoradiographic grains over these nuclei appeared lower than that seen in the dorsal raphe. Although the [^{3}H]LSD binding sites were predominantly concentrated over the raphe nuclei, other structures of the human brainstem were also labeled. The periaqueductal gray matter (GCM in Fig. 1) presented an intermediate degree of labeling without important regional variation. At the same level, the interpeduncular nucleus (NIP in Fig. 1) showed a low density of [^{3}H]LSD sites. Some cell bodies, probably belonging to the substantia nigra pars compacta, were also labeled at this level. The substantia nigra pars compacta and reticulata are intensely labeled (not shwon). In the upper pons (Fig. 2) the central gray (GCP in Fig. 2) had intermediate levels of binding, similarly to the periaqueductal gray. Low levels of labeling were observed in areas containing the locus coeruleus and the medial parabrachial nucleus. A band of labeling lying below the medial longitudinal fasciculus, including the 'regio stellata' of Braak[5], was observed at this level. In the lower pons (Fig. 3) some labeling was seen dorsal to the medial lemniscus in areas that could be associated with the raphe magnus. Laterally placed indoleamine-containing cells have been observed in primate and non-primate mammals[18,19], although this is not characteristic of the rat[21].

In the medulla oblongata (Fig. 4) the nucleus tractus solitarius presented intermediate to high levels of ligand binding. A similar density of [^{3}H]LSD binding sites was observed in the choroid plexus of the IVth ventricle, whereas a low level of receptor binding was found in the inferior olive.

The main finding of this study is the presence of high densities of [^{3}H]LSD binding to 5-HT$_1$ receptors

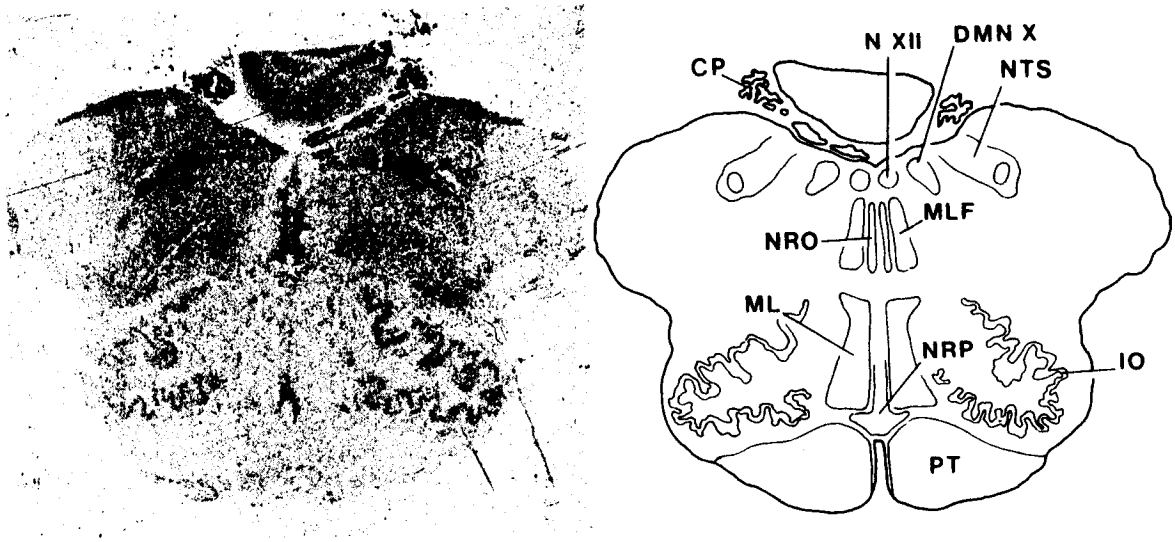


Fig. 4. Transverse section through the upper third of the medulla oblongata. Highest level of labeling is seen over the nucleus raphe obscurus (NRO). Intermediate to high levels of receptor binding are seen in the nucleus of tractus solitarius (NTS) and over the nucleus raphe pallidus (NRP). Note also intermediate to high level of labeling in the choroid plexus of the IVth ventricle.

in the human brainstem with a predominant localization over the raphe nuclei, i.e. the nuclei that contain the cell bodies of the serotoninergic neurons. Because of this relatively restricted anatomical localization, [^{3}H]LSD binding could be a good marker for serotoninergic neurons in this area and could be used to study the effects of aging or disease on these neurons. Changes of binding of [^{3}H]LSD or other markers of the serotoninergic neurons, for example [^{3}H]imipramine binding, have been reported in the brains of schizophrenic, Huntington's chorea or depressed (suicide) patients[6,9,20], mainly in forebrain areas. Our autoradiographic studies indicate that the extension of these analyses to brainstem areas would be of interest.

The localization of [^{3}H]LSD sites in the human brainstem is similar, although not identical, to that reported in the rat brain. The rat dorsal raphe, like the human, also contains very high densities of [^{3}H]LSD[4,8,24] and [^{3}H]5-HT binding sites, apparently also belonging to the 5-HT$_1$ class. In contrast, other areas such as the nucleus interpeduncularis, locus coeruleus and the dorsal tegmental nucleus have a lower density of [^{3}H]LSD binding sites in our samples of human brain than those observed in the rat brain[4,8,24].

Noteworthy is the localization of [^{3}H]LSD binding in the choroid plexus. The density of receptors was lower than that reported in the rat brain, where it is very high[4,8], but in the human brain it appears to be lower than that observed in, for example, the raphe nuclei. A possible role of 5-HT in regulating the blood–cerebrospinal fluid barrier has been suggested by this and other findings (see ref. 8 for a discussion) and these considerations could now be extended to the human brain.

Finally, the presence of high densities of [^{3}H]LSD binding sites in the human raphe nuclei is important in the frame of the well-known psychostimulant actions of LSD and related drugs in the human. In experimental animals[1] raphe neurons have been found to be extremely sensitive to locally applied LSD, and these cells have been proposed as the primary target of low doses of intravenously injected LSD.

ABBREVIATIONS
USED IN SCHEMATIC DRAWINGS

CP	choroid plexus
CTT	central tegmental tract
DMN X	dorsal motor nucleus of vagus
FCP	foramen coecum posterior
GCM	griseum centrale mesencephali
GCP	griseum centrale pontis
IC	inferior colliculus
IO	inferior olive
LC	locus coeruleus
LL	lemniscus lateralis
ML	medial lemniscus
MLF	medial longitudinal fasciculus
N VII	nucleus facialis
N XII	nucleus hypoglossus
NIP	nucleus interpeduncularis
NPM	nucleus parabrachialis medialis
NRC	nucleus raphe centralis
NRC pa	nucleus raphe centralis, pars annularis
NRD	nucleus raphe dorsalis
NRD st	nucleus raphe dorsalis, pars supratrochlearis
NRL	nucleus raphe linearis
NRM	nucleus raphe magnus
NRO	nucleus raphe obscurus
NRP	nucleus raphe pallidus
NRTP	nucleus reticulo tegmentalis pontis
NTS	nucleus of tractus solitarius
PT	pyramidal tract
SCP	superior cerebellar peduncle
SN	substantia nigra

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