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Species Differences in Regional Patterns of ^{3}H-8-OH-DPAT and ^{3}H-Zolpidem Binding in the Rat and Human Brain

GARY E. DUNCAN,*†‡ DARIN J. KNAPP,†‡ GEORGE R. BREESE*†‡§
FULTON T. CREWS†§ AND KARLEY Y. LITTLE¶

*UNC Neuroscience Center, †Center for Alcohol Studies, ‡Department of Psychiatry, and §Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC
¶Psychobiology Laboratory, Ann Arbor VA Medical Center and Department of Psychiatry, University of Michigan, Ann Arbor, MI

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DUNCAN, G. E., D. J. KNAPP, G. R. BREESE, F. T. CREWS AND K. Y. LITTLE. *Species differences in regional patterns of ^{3}H-8-OH-DPAT and ^{3}H-zolpidem binding in the rat and human brain.* PHARMACOL BIOCHEM BEHAV **60**(2) 439–448, 1998.—The rat has proven to be a valuable preclinical model for characterizing effects of psychotrophic drugs and for identifying new psychotherapeutic agents in pharmacological screens. However, substantial differences have been described between the rat and human brain in regard to the neuroanatomical distribution of some drug and neurotransmitter receptor binding sites. To assess the utility of the rat as a model for the neuroanatomical topography of 5-HT$_{1A}$ and type 1 benzodiazepine (BDZ) receptors in humans, the distribution of binding sites for ^{3}H-8-OH-DPAT (5-HT$_{1A}$ agonist) and ^{3}H-zolpidem (type 1 BDZ agonist) was compared with autoradiography in select regions of the rat and human brain. Concordance in the binding patterns for the two ligands was observed in several brain regions for the two species. However, substantial differences were also found in the topography of binding sites for the ligands in the rat and human brain. High ^{3}H-8-OH-DPAT binding was seen in the dorsal raphe nucleus and hippocampal formation in both the rat and human brain. However, species differences were observed in the relative distribution of ligand binding among hippocampal subregions. In the cerebral cortex, the laminar distribution of ^{3}H-8-OH-DPAT binding sites was notably different for rats and humans. In humans, outer cortical layers were most densely labeled with ^{3}H-8-OH-DPAT, whereas in the rat cortex, the highest binding was in the inner layers. A striking difference between rats and humans was observed for ^{3}H-8-OH-DPAT binding in the lateral septal nucleus, which was densely labeled in the rat but weakly labeled in humans. Substantial differences between rats and humans were also observed for ^{3}H-zolpidem binding. In the rat brain, high densities of binding sites were found in the medial septum, inferior colliculus, and substantia nigra reticulata. These regions showed very low ^{3}H-zolpidem binding in the human brain. Intermediate binding was seen in the rat cerebral cortex, and low binding was found in the hippocampus. By contrast, in humans, cerebral cortical regions were the most densely labeled of all regions studied, and certain hippocampal subregions exhibited relatively high binding. The striking neuroanatomical differences in ^{3}H-8-OH-DPAT and ^{3}H-zolpidem binding observed between rats and humans suggest that different functional consequences may be produced within specific brain regions after administration of drugs that influence 5-HT$_{1A}$ and type 1 BZD receptors. © 1998 Elsevier Science Inc.

Human Rat Serotonin$_{1A}$ (5-HT$_{1A}$) receptors Benzodiazepine type 1 receptors Binding Autoradiography

MOST chemical neuroanatomical characterization of binding sites for psychoactive drugs and neurotransmitter receptor ligands has been performed in the rat brain, with the tacit assumption that data obtained in the rat may be generalized to other species, including humans. Although the rat has proven to be a valuable model system for neuropharmacological research, substantial differences have been described between rats and humans regarding the neuroanatomical distribution of binding sites for some drugs and neurotransmitter receptors (18,19,26,29,36,47,49). Given the heavy reliance on the

Requests for reprints should be addressed to Gary E. Duncan, Ph.D., Neurosciences Center, University of North Carolina at Chapel Hill, CB# 7250, Chapel Hill, NC 27599.

rat for investigation of neurobiological actions of psychotherapeutic agents, it is important to better understand the strengths and weakness of the rat as a model for human psychopharmacology. Accordingly, in the present investigation, the topographic distribution of binding sites was compared in select regions of the rat and human brain for the serotonin$_{1A}$ agonist (5-HT$_{1A}$),^{3}H-8-OH-DPAT, and the type 1 benzodiazepine (BZD) receptor ligand, ^{3}H-zolpidem.

Neuroanatomical evaluation of 5-HT$_{1A}$ receptor binding was performed because of the emerging importance of this receptor in the treatment of psychiatric disorders. For example, several drugs selective for the 5-HT$_{1A}$ receptors have been shown to have anxiolytic properties in animal models (13,20) and in clinical trials (45). Agonists of the 5-HT$_{1A}$ receptor also show positive effects in animal screens for antidepressant activity (30) and clinical studies have demonstrated efficacy of 5-HT$_{1A}$ selective agonists in major depressive disorder (45). The ability of preclinical rat models of anxiety and depression to identify drugs with efficacy in the respective human conditions demonstrates the utility of the rat as an important model species for human psychopathology. In addition, preclinical studies indicate that 5-HT$_{1A}$ agonists have antiaggressive properties (6,34) and may be useful for the treatment of alcoholism (10,24,27,46).

Published data on the distribution of 5-HT$_{1A}$ receptors in separate studies of the rat brain (9,22,41) and human brain (23,38) have demonstrated homology between species but also suggested significant species differences in the topography of these receptors. However, no study of 5-HT$_{1A}$ receptor binding in rats has mentioned the potential topographic differences in ^{3}H-8-OH-DPAT binding between rats and humans, and no direct comparative study is available for binding of this ligand in the two species. Therefore, one goal of the present study was to compare the distribution of ^{3}H-8-OH-DPAT in the rat and human brain.

Benzodiazepine (BZD) recognition sites on GABA$_A$ receptors have been divided into major classes (type 1 and type 2) based on distinctive pharmacological profiles (6,7,14,32, 43,48). Sedative–hypnotic properties of clinically employed mixed type 1–type 2 BZD agonist (e.g., diazepam) may be related to interactions with type 1 BZD receptors (12). This postulated role of the type 1 BZD receptor is supported by clinical experience with the type 1 BZD selective drug zolpidem, which has excellent sedative–hypnotic properties. Although there are extensive neuroanatomical data published for ^{3}H-zolpidem binding in the rat brain (6,16,35), there is limited information available on the distribution of binding of this BZD type 1 selective ligand in the human brain. The only published work in this regard (12) examined a single human subject, and certain regions showing high binding of ^{3}H-zolpidem in the rat, such as the medial septum and inferior colliculus, were not studied. In the present study, ^{3}H-zolpidem binding was directly compared in regions of the rat and human brain, and regions of the human brain not previously examined with regard to ^{3}H-zolpidem binding were evaluated.

METHOD

Subjects

Male Sprague–Dawley (Charles River, Raleigh, NC) rats weighing 300–400 g were maintained under a 12 L:12 D cycle with continuous access to Purina rat chow and water. Rats were killed by decapitation and brains were frozen with powdered dry ice and stored at −70°C before sectioning.

Human brain regions were dissected at autopsy as authorized by the Chief Medical Examiner, State of North Carolina. Subjects died suddenly from gun shot wounds, stab wounds, vehicular trauma, or sudden cardiac arrest. The mean (±SEM) postmortem interval was 10.8 ± 1.3 h (range 4–24 h) and mean age was 35.5 ± 3.0 years with a range of 24–49 years. Blocks of select brain regions were cut from coronal slabs, quickly frozen with powdered dry ice, and stored at −70°C. By dissection of specific regions of interest, in comparison to processing whole brain, the tissue could be frozen more rapidly and more precise sectioning of specific regions was possible. At least three subjects were evaluated for each brain region, but all brain regions were not available for every subject. Brain regions dissected were hippocampus and amygdala with overlying cortex, midbrain at levels of the substantia nigra and the dorsal raphe, frontal cortex (Broadmans areas 9 and 10), cingulate cortex, and a block that included the subcallosal gyrus, septal nuclei, and hypothalamus. Histological subdivisions of the various regions of the human brain are designated after Paxinos (39).

Autoradiographic analysis of ^{3}H-8-OH-DPAT binding. Cryostat sections were thaw mounted onto microscope slides and stored at −20°C before measurement of 5-HT$_{1A}$ receptor binding with ^{3}H-8-OH-DPAT (New England Nuclear, 154 Ci/mmol). Sections were incubated with 50 mM Tris buffer (pH 7.5) for 30 min and then incubated in the same buffer containing 4 mM $CaCl_2$, 0.1% ascorbic acid, and 2 nM ^{3}H-8-OH-DPAT for 60 min at room temperature. Previous studies have shown the K_d for ^{3}H-8-OH-DPAT binding to be between 0.5–1.0 nM (23,38), and thus at a concentration of 2 nM, approximately 70–80% of receptors will be occupied. Slides were washed in three successive changes of ice-cold Tris buffer for 1-, 5-, and 5-min periods, followed by a quick rinse in distilled water to remove buffer salts. Nonspecific binding was assessed in the presence of 1 M serotonin. After drying, sections and radioactive standards (^{3}H-microscale, Amersham) were apposed to Amersham Hyperfilm in X-ray cassettes for 3 weeks and then developed in Kodak D19 for 2 min at room temperature. Radioactivity in discrete brain regions was quantified by reference to the standards by digital image analysis with a

TABLE 1

COMPARISON OF ^{3}H-OH-DPAT BINDING IN RAT AND HUMAN BRAIN

Brain Region	Rat	Human
Hippocampus, CA-1	8.6 ± 0.7	19.6 ± 2.2*
Hippocampus, CA-3	3.2 ± 0.3	2.7 ± 0.3
Dentate gyrus molecular layer	14.8 ± 0.9	5.3 ± 0.6*
Perirhinal cortex, layers I–III	2.3 ± 0.1	12.8 ± 0.5*
Perirhinal cortex, inner layers	4.2 ± 0.3	3.9 ± 0.3
Cingulate cortex, layers I–II.	4.6 ± 0.4	6.8 ± 0.9
Cingulate cortex, inner layers	4.5 ± 0.3	3.3 ± 0.3
Frontal cortex, layers I–II	5.9 ± 0.2	6.7 ± 0.7
Frontal cortex, inner layers	7.4 ± 0.3	3.2 ± 0.4
Entorhinal cortex, outer layers	14.2 ± 1.2	6.8 ± 0.4*
Subcollosal gyrus, outer layers	—	12.4 ± 0.7
Subcollosal gyrus, inner layers	—	4.9 ± 0.5
Lateral septum	11.5 ± 0.4	2.8 ± 0.3*
Dorsal raphe	12.6 ± 2.4	15.3 ± 1.7
Median raphe	4.3 ± 0.5	12.8 ± 0.8*

Data are expressed as mean ± SEM (nCi/mg) with n = 3–4 for humans and n = 4 for rats. There is no cortical structure analogous to the subcallosal gyrus in the rat. Repeated measures ANOVA revealed a significant species × brain region interaction ($F = 32.6$, $p < 0.05$).

*Significantly different from the rat, $p < 0.005$.

MCID system from Imaging Research (Ontario). Depending on the size of the region, two to four individual measurements were made within each region and values averaged to obtain a value for each section. For each region two to three sections were analyzed for each rat and human subject.

Autoradiographic Evaluation of ^{3}H-Zolpidem Binding

Cryostat sections of rat and human brain were thaw mounted onto microscope slides and stored at −20°C. Sections were incubated with 5 nM ^{3}H-zolpidem (New England Nuclear, 54 Ci/mmol) at 4° for 30 min in Tris buffer (pH 7.5) containing 120 mM NaCl, 100 μM GABA, and 1 μM PK 11195. The K_d for ^{3}H-zolpidem in the rat and human brain has been reported to be between 4 and 6 nM (12,35). Thus, approximately 50% occupancy of receptors is expected at the concentration if ^{3}H-zolpidem used. PK 11195 was included in the incubation solutions because Dennis et al. (12) reported that a small amount of ^{3}H-zolpidem binding in the human brain was to the peripheral type BZD receptor. Flumazenil (Ro 15-1788; 1 μM) was used to define nonspecific binding (35). After incubation with the ligand, sections were washed to remove excess radioactivity by immersion into two successive baths of ice-cold Tris for a total time of 3 min, followed by a 15 s immersion in distilled water to re-

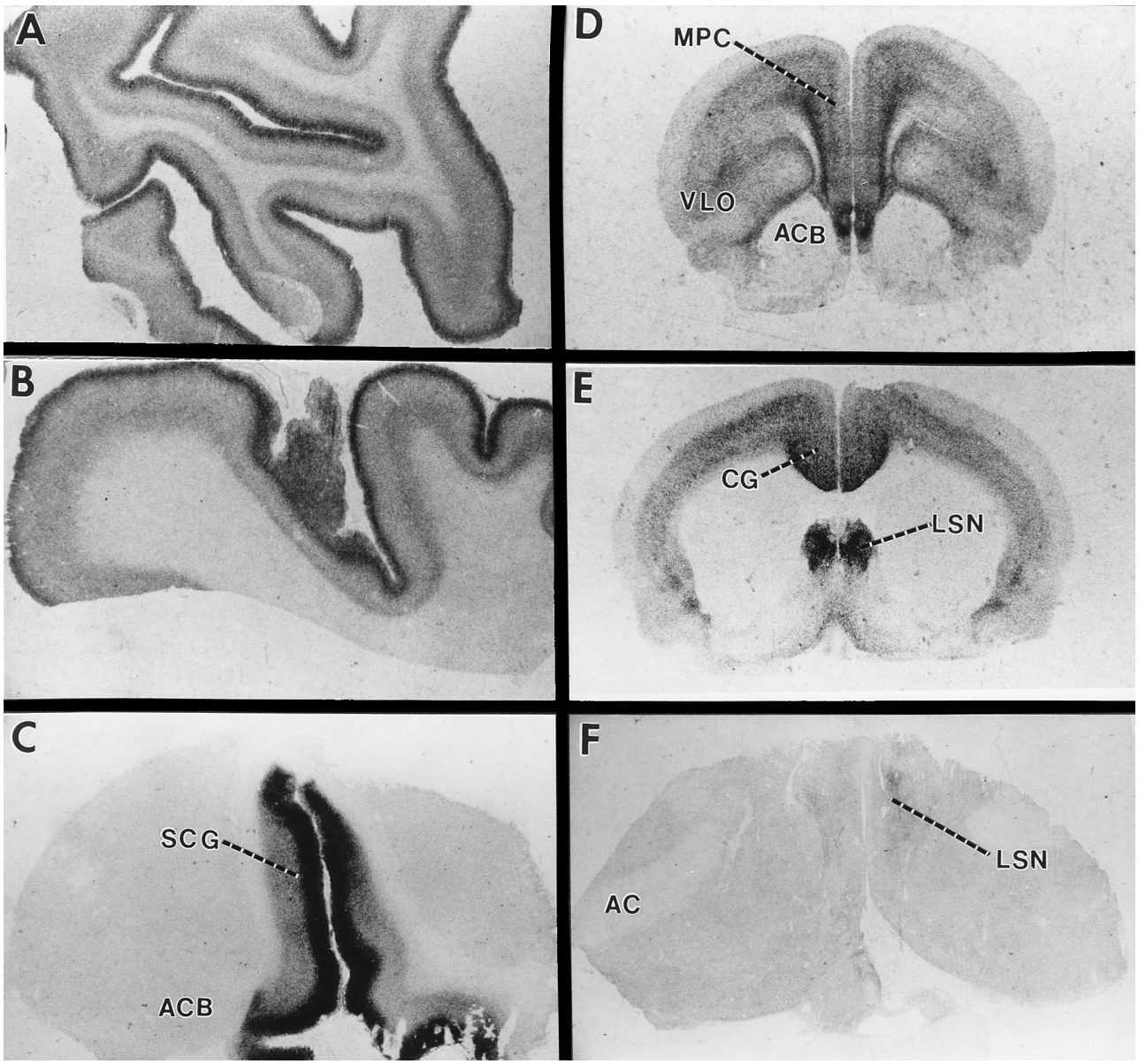

FIG. 1. Autoradiograms of ^{3}H-8-OH-DPAT binding in human and rat cererbral cortex and lateral septum. (A) Human frontal cortex (Broadman's areas 9 and 10). (B) Human cingulate cortex. (C) Human subcallosal gyrus. (D) Rat frontal cortex. (E) Rat cingulate cortex and lateral septum. (F) Human lateral septum. Auoradiographic exposure time was 3 weeks for rat and human sections. Abbreviations: SCG: subcallosal gyrus, ACB: nucleus accumbens, MPC: medial prefrontal cortex, VLO: ventrolateral orbital cortex, CG: cingulate cortex, LSN: lateral septal nucleus, AC: anterior commisure.

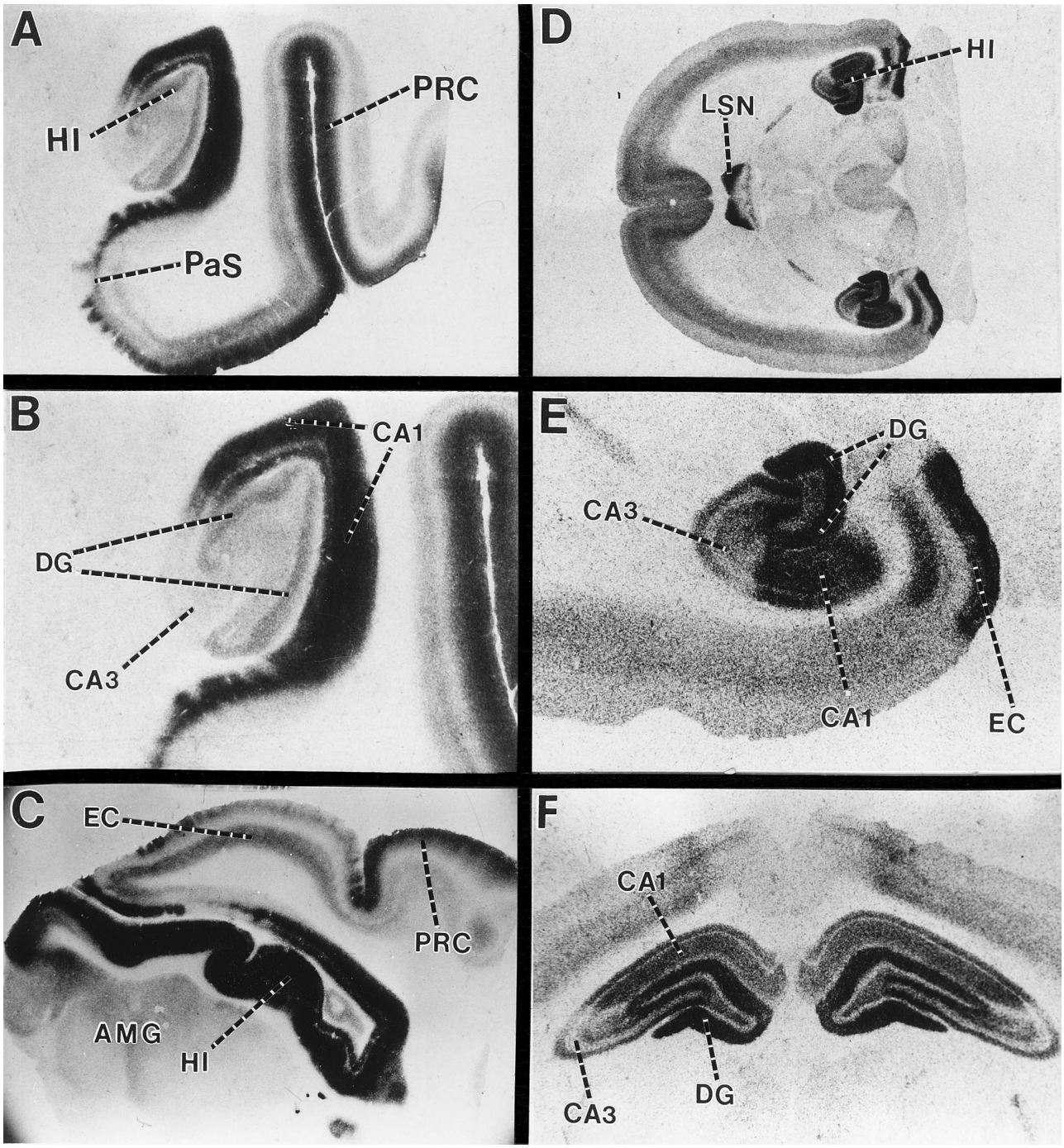

FIG. 2. Autoradiograms of ^{3}H-8-OH-DPAT binding in the human and rat hippocampus. (A–C) human, (D–F) rat. Sections B and E are higher magnification of autoradiograms in A and D, respectively. D and E are horizontal sections through the rat ventral hippocampus, and F is a coronal section through the rat dorsal hippocampus. Autoradiographic exposure time was 3 weeks for rat and human sections. Abbreviations: AMG: amygdala, HI: hippocampus, CA1: Cornu Ammonis region 1 of the hippocampus, PaS: parasubiculum; PRC: perirhinal cortex, DG: dentate gyrus, EC: entorhinal cortex. LSN: lateral septal nucleus.

move buffer salts. Autoradiograms were exposed for 5 weeks and developed and analyzed as described above.

Statistics

Data of ligand binding were analyzed by repeated measures analysis of variance and means for individual brain regions were compared with Tukey tests.

RESULTS

^{3}H-8-OH-DPAT Binding in Rat and Human Brain

Discrete and selective labeling of neuroanatomical regions was observed with ^{3}H-8-OH-DPAT in the rat and human brain. For both species, the topography of binding was identical across individual subjects. Although concordance in the neuroanatomical distribution ^{3}H-8-OH-DPAT binding was

found in some regions for rats and humans, substantial species differences were also observed.

^{3}H-8-OH-DPAT Binding in Rat and Human Cerebral Cortex

A distinctly different laminar pattern of ^{3}H-8-OH-DPAT binding was observed in human cerebral cortical regions in comparison to the rat (Table 1, Figs. 1 and 2). In general, outer cortical layers were more densely labeled in humans, whereas in the rat, the inner layers showed higher binding. In the rat, moderate binding was observed in the medial prefrontal and cingulate cortices and dense labeling was observed in the deepest part of the structures, adjacent to the corpus callosum (Fig. 1D and E). In humans, the prefrontal and anterior cingulate cortices showed a thin band of relatively high binding in the outer layers (I and II) and low binding in the deeper layers. In comparison to the cingulate and prefrontal cortices, higher ^{3}H-8-OH-DPAT binding was found in the human perirhinal cortex and subcallosal gyrus (Fig. 1C). Low binding was apparent in the perirhinal cortex of the rat. There is no rat cortical structure analogous to the human subcallosal gyrus for comparison. Binding in the human entorhinal cortex was less compared to the adjacent perirhinal cortex (Fig. 2C). The moderate binding in the human entorhinal cortex contrasts with the high binding in this cortical region of the rat (Fig. 2E).

^{3}H-8-OH-DPAT Binding in the Rat and Human Hippocampal Formation

In both the rat and humans, high densities of ^{3}H-8-OH-DPAT binding sites were found in the hippocampal formation (Table 1, Fig. 2). However, the topographic distribution of binding of the ligand within hippocampal subregions differed for the two species. For the rat, the highest binding was in the molecular layer of the dentate gyrus (Fig. 2D and E). In humans, relatively low binding was found in the dentate gyrus (Fig. 2B). High binding was observed in the CA-1 stratum radiatum of both the rat and human. However, unlike the rat, the CA-1 pyramidal cell layer of human hippocampus exhibited high binding of ^{3}H-8OH-DPAT.

^{3}H-8-OH-DPAT Binding in the Rat and Human Lateral Septum and Raphe Nuclei

The lateral septum exhibited very low binding in humans (Fig. 1F), whereas high binding was present in this region of

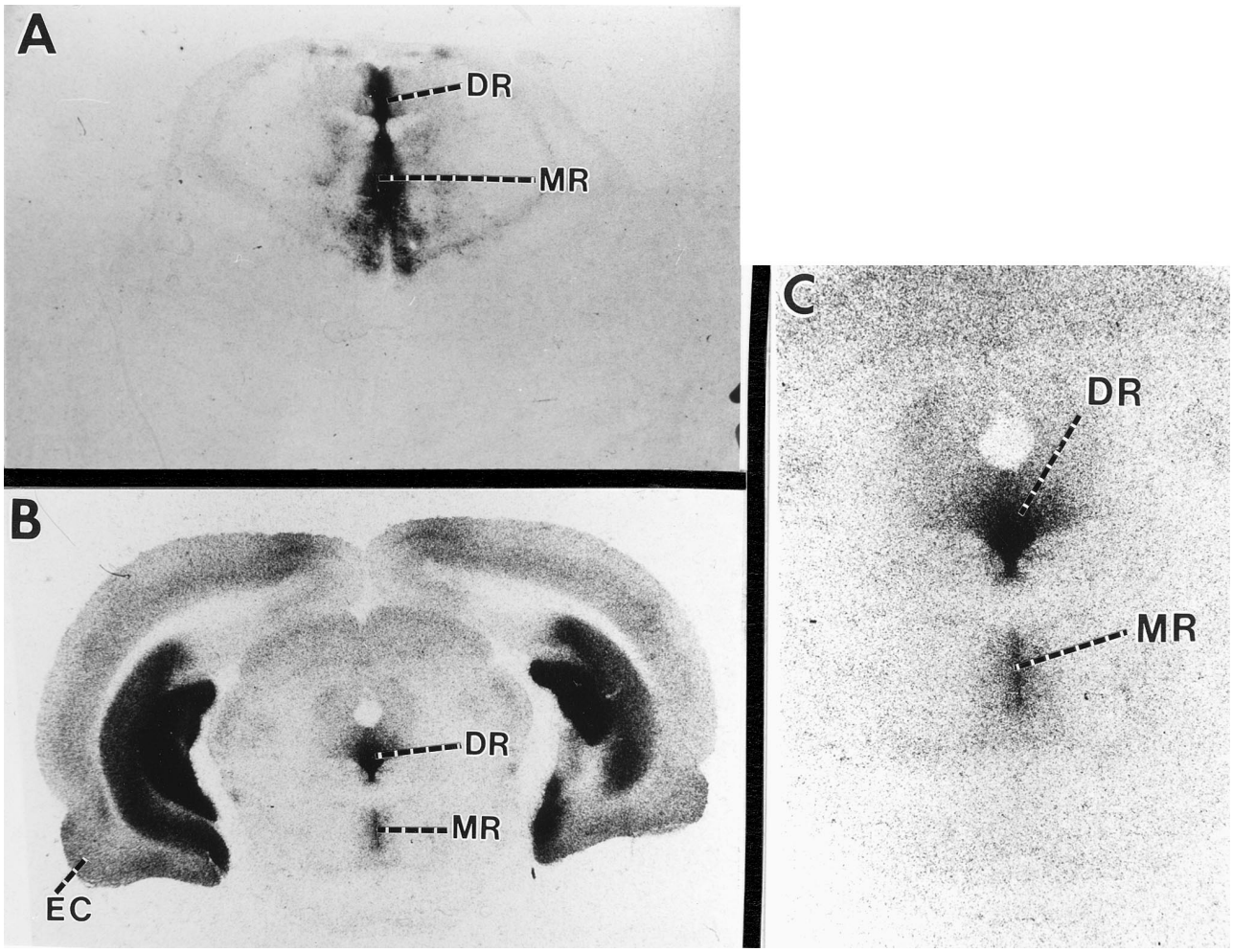

FIG. 3. Autoradiograms of ^{3}H-8-OH-DPAT binding in the human and rat dorsal and median raphe. Autoradiographic exposure time was 3 weeks for rat and human sections. In B, the heavily labeled region above the entorhinal cortex is the hippocampal formation, which was relatively overexposed to adequately document other more weakly labeled structures at this level. (A) human, (B) rat. C is a higher magnification of the raphe nuclei in B. Abbreviations: DR: dorsal raphe, MR: median raphe.

the rat (Fig. 1E). In humans, the dorsal and median raphe nuclei were both densely labeled with ^{3}H-8-OH-DPAT (Fig. 3A). In the rat, the dorsal raphe exhibited very high binding of ^{3}H-8-OH-DPAT but intermediate binding was present in the median raphe (Fig. 3B and C).

^{3}H-Zolpidem Binding in Rat and Human Brain

A discrete and consistent distribution of ^{3}H-zolpidem binding was found in all rat and human subjects examined. As observed with ^{3}H-8-OH-DPAT binding, both similarities and differences between rats and humans were observed in the neuroanatomical distribution of ^{3}H-zolpidem binding sites.

^{3}H-Zolpidem Binding in Rat and Human Cerebral Cortex and Hippocampal Formation

In the rat, cerebral cortical regions contained moderately high binding and a discrete laminar distribution was apparent, with layer 4 being the most densely labeled (Fig. 4B). In humans, cerebral cortical regions exhibited the highest ^{3}H-zolpidem binding of all regions examined, and the laminar pattern

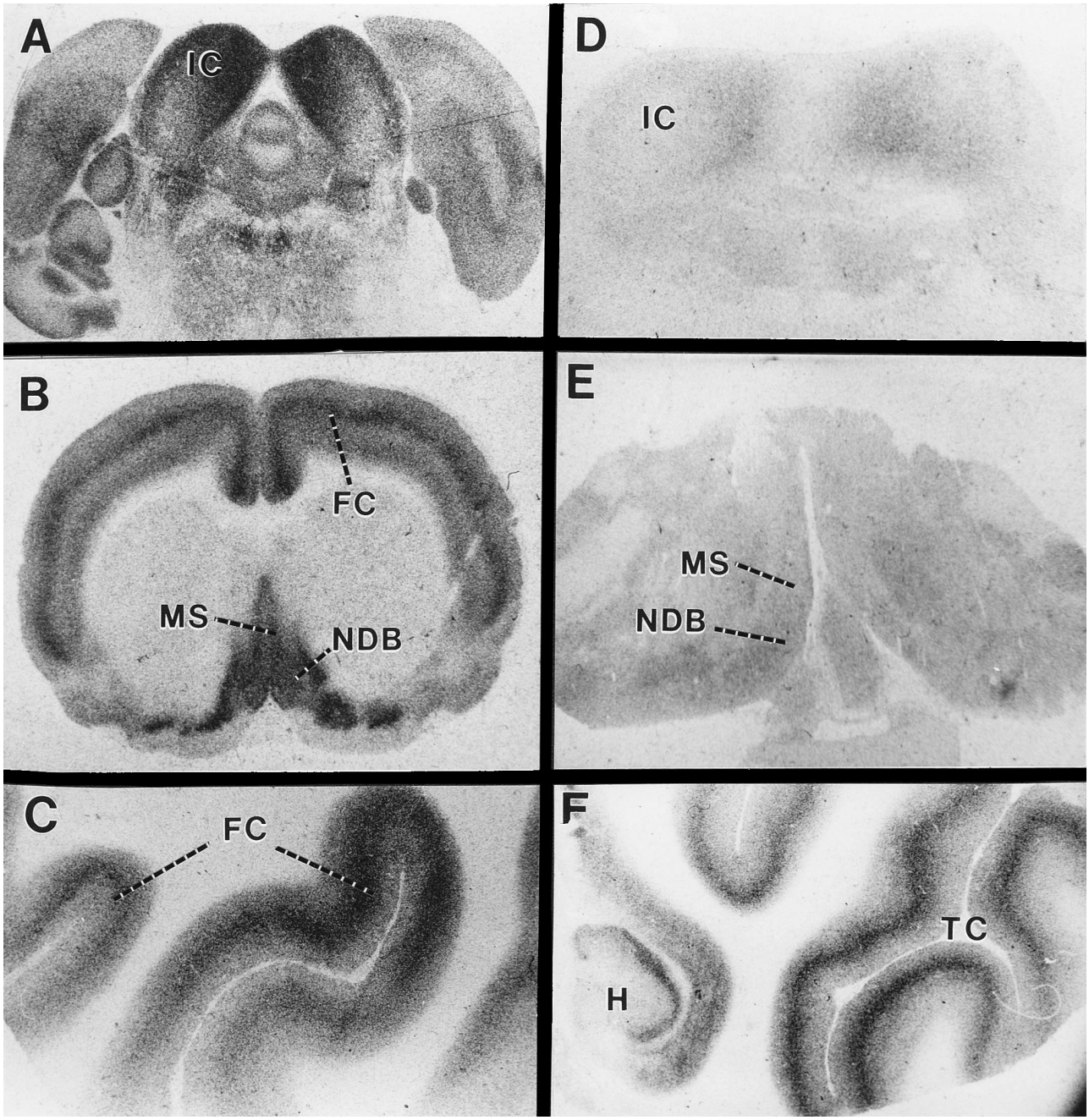

FIG. 4. Autoradiograms of ^{3}H-zolpidem binding in the rat and human brain. A and B are rat. C–F are human. Autoradiographic exposure time was 5 weeks for rat and human sections. Abbreviations: IC: inferior colliculus, MS: medial septum, NDB: nucleus of the diagonal band of Broca, FC: frontal cortex, TC: temporal cortex, H: hippocampus.

of labeling was similar to the rat (Fig. 4C and F). In the rat hippocampus, low binding was observed in all CA fields and the molecular layer of the dentate gyrus (Fig. 5A and B). In the human hippocampus, binding was low in the CA 3 stratum radiatum but the CA 1 stratum radiatum and the molecular layer of the dentate gyrus showed relatively high ^{3}H-zolpidem binding (Fig. 5D, Table 2).

^{3}H-Zolpidem Binding in Subcortical Regions of the Rat and Humans

In the rat brain, select subcortical regions exhibited high binding of ^{3}H-zolpidem, including the medial septum, inferior colliculus (Fig. 4A and B), and the substantia nigra reticulata (Fig. 5A). By contrast, low binding of was observed in these same regions of humans (Figs. 4 and 5). Binding of ^{3}H-zolpidem in the human substantia nigra reticulata, medial septum, and inferior colliculus was so weak that photographic documentation was difficult for autoradiographic exposure periods where cerebral cortical regions showed high optical density.

DISCUSSION

Similar binding affinities and pharmacological selectivity between rats and humans have been demonstrated with numerous ligands for neurotransmitter receptors, including ^{3}H-OH-DPAT and ^{3}H-zolpidem [see (12,18,23,36,37,38). Thus, at a molecular level, the rat appears to be an excellent model system for neuropharmacological research. At a behavioral level also, the rat has proven to be a valuable heuristic model to characterize actions of drugs and to identify compounds that modulate 5-HT$_{1A}$ and BZD receptors (5,10,12–14,24,30,32, 34,46). However, previous studies demonstrated substantial species differences between rats and humans with regard to the regional distribution of neurotransmitter receptors in brain (18,19,26,29,36,37). Therefore, it is important to define the strengths and limitations of the rat as model for human neurotransmitter receptor distribution, given the heavy reliance on rats for basic chemical neuroanatomical research. To clarify similarities and differences between rats and humans in the neuroanatomical distribution of 5-HT$_{1A}$ and BZD type 1 receptors, the topographic distribution of these receptors was assessed by autoradiographic analysis of ^{3}H-OH-DPAT binding and ^{3}H-zolpidem, respectively.

Although autoradiographic studies have described neuroanatomical patterns of ^{3}H-8-OH-DPAT binding in rats (9,22,23, 38,41) and humans (23,38), the present work represents the first direct and focused comparison of the topographic distribution of 5-HT$_{1A}$ binding sites for the two species. The topography of ^{3}H-8-OH-DPAT binding in the human brain observed in the present study was similar to that described by Pazos et al. (38) who used relatively aged subjects (mean age 66 years) that died after chronic illness. For our human subjects, there was no history of chronic illness, and the mean age was 34 years. The similar patterns of ^{3}H-8-OH-DPAT binding observed in our work and that of Pazos et al. (38) indicate that the fundamen-

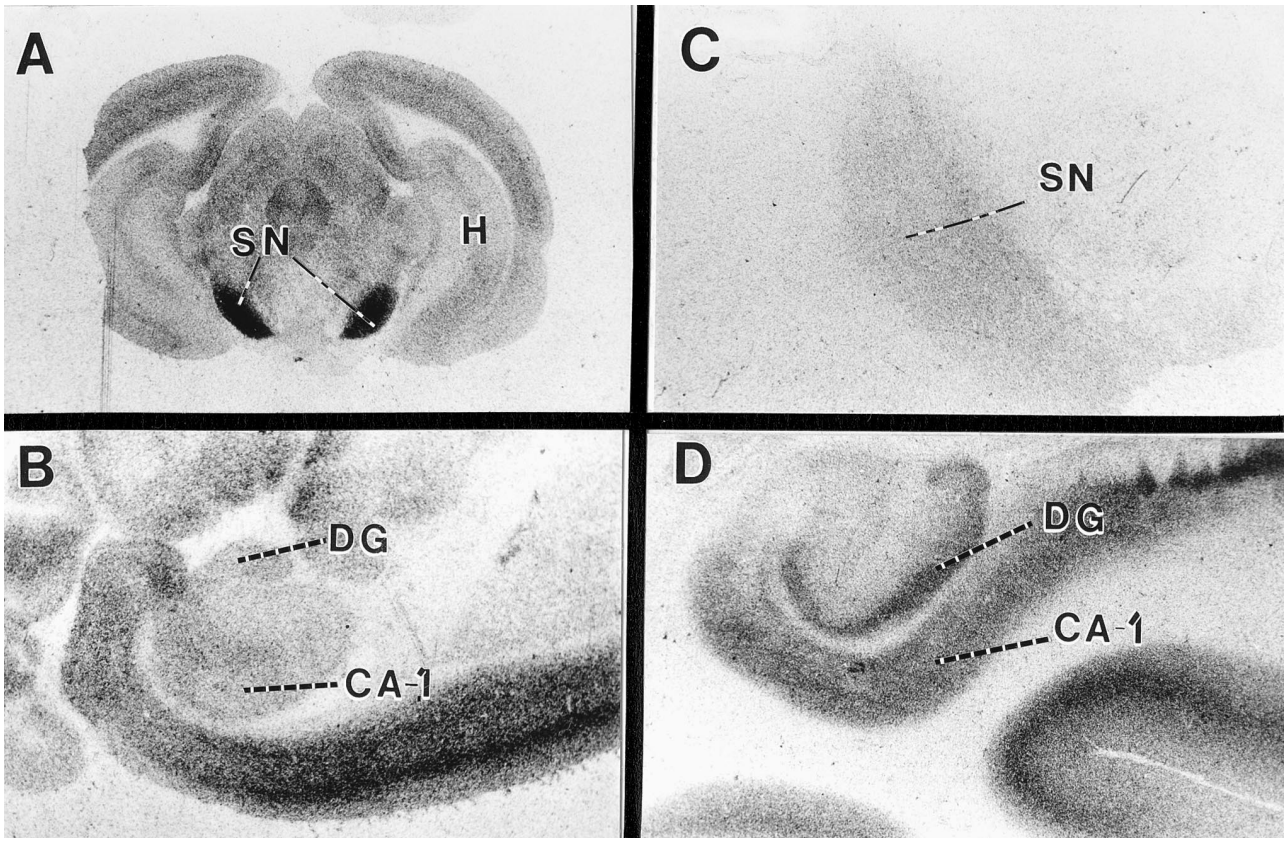

FIG. 5. Autoradiograms of ^{3}H-zolpidem binding in the rat and human substantia nigra and hippocampal formation. Autoradiographic exposure time was 5 weeks for rat and human sections. A and B are rat. C and D are human. Abbreviations: SN: substantia nigra, DG: dentate gyrus, CA1: cornu Ammonis region 1 of the hippocampus.

TABLE 2

COMPARISON OF ^{3}H-ZOLPIDEM BINDING IN RAT AND HUMAN BRAIN

Brain Region	Rat	Human
Hippocampus, CA-1	2.43 ± 0.20	3.93 ± 0.36
Hippocampus. CA-3	1.79 ± 0.32	1.49 ± 0.14
Hippocampus, dentate molecular layer	2.70 ± 0.17	4.29 ± 0.36*
Lateral septum	1.34 ± 0.10	1.26 ± 0.07
Medial septum	7.55 ± 0.55	2.67 ± 0.21*
Frontal cortex (all layers)	3.89 ± 0.28	5.53 ± 0.59
Substantia nigra reticulata	8.08 ± 0.52	1.93 ± 0.25*
Inferior colliculus	6.68 ± 0.54	2.29 ± 0.20*

Data are expressed as mean ± SEM (nCi/mg) with $n = 3$ for humans and $n = 4$ for rats. Repeated-measures ANOVA revealed a significant species × region interaction ($F = 42.63$, $p < .005$).

*Significantly different from the rat, $p < 0.05$.

tal distribution pattern of 5-HT$_{1A}$ receptors does not appear to be affected by age or chronic illness.

Some striking differences between rats and humans were observed for ^{3}H-8-OH-DPAT binding in specific brain regions. The most dramatic difference in ^{3}H-OH-DPAT binding in the rat and human brain was in the lateral septum, which contained high densities of binding sites in the rat but was weakly labeled in humans. The lateral septal nucleus of humans is well developed and has progressed in phylogeny to its greatest degree in humans (1–3). Work in humans involving direct electrical and chemical stimulation of the lateral septum has implicated this region in emotional behavior, specifically with pleasure responses (21). Functional mapping studies with Fos and ^{14}C-2-deoxglucose in rats have demonstrated that the lateral septum is activated in response to a variety of stress paradigms (4,11,15,17,40). In addition, 5-HT$_{1A}$ agonists are active in rat behavioral models of antidepressant drug action when microinjected into the lateral septum (31,42). The striking species differences observed for ^{3}H-OH-DPAT binding in lateral septum suggest that this brain region will be affected to a much greater extent by drugs acting on 5-HT$_{1A}$ receptors in rats compared to humans.

There were also notable differences between rats and humans in topographic patterns of ^{3}H-OH-DPAT binding in cerebral cortical regions and in the hippocampal formation. The laminar distribution of binding in the cortex was strikingly different for the two species, with the highest binding present in inner layers of the rat and outer layers of humans. These data suggest the possibility of distinct modulatory influences of 5-HT$_{1A}$ receptors in the rat and human cerebral cortex. High binding of ^{3}H-8-OH-DPAT was observed in both the rat and human hippocampus, but species differences were observed in the distribution of binding sites for the ligand within the various hippocampal subregions. The high densities of 5-HT$_{1A}$ binding sites in the both the rat and human hippocampus suggest an important role for these receptors in the regulation of hippocampal function for both species. However, the differences between rats and humans in the distribution of 5-HT$_{1A}$ receptors within hippocampal subregions suggest that hippocampal circuitry could be differentially affected by 5-HT$_{1A}$ agonists in these two species.

For both the rat and humans, very high densities of ^{3}H-OH-DPAT binding sites were apparent in the dorsal raphe nucleus. In the rat, 5-HT$_{1A}$ receptors in the dorsal raphe nuclei are localized to serotonergic cell bodies, and activation of these receptors reduces the firing rate of the serotonin neurons (8,25,44). It is likely that the binding of ^{3}H-8-OH-DPAT in the human raphe nuclei also represents binding to serotonin-containing cells and that the function of the 5-HT$_{1A}$ receptors in these regions is similar to that observed for the rat.

Although binding of ^{3}H-OH-DPAT in the raphe nuclei is primarily associated with serotonergic neurons, binding of the ligand in forebrain regions is apparently localized to cells postsynaptic to these neurons, because binding is not reduced after 5-7-dihydroxytryptamine treatment (22,41). In addition, RNA for the 5-HT$_{1A}$ receptor is highly expressed in brain regions showing high densities of ^{3}H-OH-DPAT binding sites in the rat (9) and human brain (33). The high densities of 5-HT$_{1A}$ receptors in limbic regions of the rat and human brain suggest an important role for this receptor subtype in regulation of limbic circuits for both species.

To further compare receptor localization in the rat and human brain, the distribution of binding sites for ^{3}H-zolpidem was examined. For the common regions examined, the distribution of ^{3}H-zolpidem binding in the human brain was similar to that observed for the single human subject examined by Dennis et al. (12). The highest binding of ^{3}H-zolpidem in the human brain was in cerebral cortical regions, with layer 4 containing the highest densities of binding sites. In the rat, intermediate levels of binding were seen in the cerebral cortex, and layer 4 was also distinctly labeled. The hippocampal formation was in general weakly labeled by ^{3}H-zolpidem in the rat. By contrast, in the human hippocampus, CA 1 stratum radiatum and the dentate gyrus were relatively densely labeled.

Although ^{3}H-zolpidem binding in the cerebral cortical regions appeared similar in rats and humans, marked species differences were apparent for binding in subcortical structures. Regions of the rat brain that contained the highest densities of ^{3}H-zolpidem binding sites had very low densities of binding sites for the drug in the human brain. For example, the inferior colliculus, substantia nigra reticullata, and medial septum were densely labeled by ^{3}H-zolpidem in the rat brain, but were very weakly labeled in autoradiograms of the human brain. Dennis et al. (12) also reported low ^{3}H-zolpidem binding in the human SNR. However, the present work is the first to examine ^{3}H-zolpidem binding in the human inferior colliculus and medial septum. These substantial species differences in ^{3}H-zolpidem binding suggests that certain neuroanatomical regions will be affected differently by zolpidem in rats compared to humans, with subcortical regions presumably being affected to a considerably greater extent by zolpidem in the rat than in humans.

The rat has served as a valuable model for neuropharmacological research, and the fundamental neuroanatomical structure of the rat and human brain are remarkably similar in subcortical regions. For the cerebral cortex, there is also remarkable homology between rats and humans in cytoarchitecture and basic subdivision, despite the relative expansion of the cortical mantle in human evolution. However, the present study reinforces the view that substantial species differences can exist between rats and humans in the neuroanatomical topography of neurotransmitter receptors. Previous work from our laboratory has documented substantial species differences between rats and humans in the topographic distribution of beta-adrenergic receptors in the hippocampal formation (19) and hypothalamus (29). In addition, the beta-1 adrenergic receptor subtype was predominant in the rat hippocampus and hypothalamus, while the beta-2 adrenergic subtype was more abundant in humans for those brain regions. Striking differences were

also found between rats and humans in neuroanatomical binding patterns of ^{3}H-imipramine and ^{3}H-citalopram [a selective serotonin uptake inhibitor; (18)]. Other investigators have described considerable differences between rats and humans in the neuroanatomical distribution of α-1 adrenergic (37), 2 adrenergic (49), GABA$_A$ (28), neuropeptide-Y (47), and cholecystokinin (26) receptors.

Although there are well-documented species difference between rats and humans in regard to the topographic distribution of neurotransmitter receptors, the rat has proven to be a critical model for investigating the effects of psychotherapeutic drugs. Indeed, most of the drugs used to treat mental disorders today were discovered in preclinical behavioral and pharmacological screens. Still, it is important to be aware of potential species differences in drug and neurotransmitter receptor distribution. There has been a tendency to overlook such differences and assume that drug and neurotransmitter receptor topography in the rat brain can be generalized to humans. The major differences observed between rats and humans in regard to the neuroanatomical distribution of neurotransmitter receptors emphasize the importance of comparative studies to assess the strengths and weaknesses of the rat as a model for chemical neuroanatomical research.

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REFERENCES

1. Andy, O. J.; Stephan, H.: Phylogeny of the primate septum telencephali. In: Stephan, H., ed. Evolution of the forebrain. Stuttgart: Thieme; 1966:389–399.
2. Andy, O. J.; Stephan, H.: Septal nuclei in primate phylogeny: A quantitative investigation. J. Comp. Neurol. 126:157–170; 1966.
3. Andy, O. J.; Stephan, H.: The septum in the human brain. J. Comp. Neurol. 133:383–410; 1968.
4. Arnold, F. J. L.; de Lucas Bueno, M.; Shiers, H.; Hancock, D. C.; Evan, G. I.; Herbert, J.: Expression of c-*fos* in regions of the basal limbic forebrain following intracerebroventricular corticotropin-releasing factor in unstressed or stressed male rats. Neuroscience 51:377–390; 1992.
5. Bell, R.; Hobson, H.: 5-HT$_{1A}$ receptor influences on rodent social and agonistic behavior: A review and empirical study. Neurosci. Biobehav. Rev. 18:325–338; 1994.
6. Benavides, J.; Peny, B.; Ruano, D.; Vitorica, J.; Scatton, B.: Comparative autoradiographic distribution of central (benzodiazepine) modulatory site subtypes with high, intermediate and low affinity for zolpidem and alpidem. Brain Res. 604:240–250; 1993.
7. Billard, W.; Crosby, G.; Iorio, L.; Chipkin, R.; Barnett, A.: Selective affinity of the benzodiazepines quazepam and 2-oxo-quazepam for BZ1 binding site and demonstration of ^{3}H-2-oxo-quazepam as a BZ1 selective radioligand. Life Sci. 42:179–187; 1987.
8. Blier, P.; de Montigny, C.: Modification of 5-HT neuron properties by sustained administration of the 5-HT$_{1A}$ agonist gepione: Electrophysiological studies in the rat brain. Synapse 1:470–480; 1987.
9. Chalmers, D. T.; Watson, S. J.: Comparative anatomical distribution of 5-HT$_{1A}$ receptor mRNA and 5-HT$_{1A}$ binding in rat brain—A combined in situ hybridization/in vitro receptor autoradiographic study. Brain Res. 561:51–60; 1991.
10. Chen, C. C.; Fujiwara, Y.; Akiyama, K.; Ujike, H.; Moriya, F.; Otsuki, S.: Voluntary intake of alcohol is attenuated by ipsapirone in mice and role of 5-HT$_{1A}$ receptor. Jpn. J. Psychiatr. Neurol. 46:197–203; 1992.
11. Cullinan, W. E.; Herman, J. P.; Battaglia, D. F.; Akil, H.; Watson, S. J.: Pattern and time course of immediate early gene expression in rat brain following acute stress. Neuroscience 64:477–505;1995.
12. Dennis, T.; Dubois, A.; Benavedes, J.; Scatton, B.: Distribution of central 1 (benzodiazepine1) and 2 (Benzodiazepine 2) receptor subtypes in the monkey and human brain. An autoradiographic study with ^{3}H-flunitrazepam and the 1 selective ligand ^{3}H-zolpidem. J. Pharmacol. Exp. Ther. 247:309–322; 1988.
13. De Vry, J.; Glaser, S. R.; Traber, J.: Behavioral pharmacology of 5-HT$_{1A}$ agonists: Animal models of anxiety and depression. In: Stahl, S. M.; Gastpar, M.; Keppel Hesselin, J. M.; Traber, J., eds. Serotonin 1A receptors in depression and anxiety. New York: Raven Press; 1992:55–81.
14. Doble, A.; Martin, I. L.: Multiple benzodiazepine receptors: No reason for anxiety. Trends Pharmacol. Sci. 13:76–81; 1992.
15. Duncan, G. E.; Johnson, K. B.; Breese, G. R.: Topographic patterns of brain activity in response to swim stress: Assessment by 2-deoxyglucose uptake and expression of Fos-like immunoreactivity, J. Neurosci. 13:3932–3943; 1993.
16. Duncan, G. E.; Breese, G. R.; Criswell, H. E.; McCown, T. J.; Herbert, J. S.; Devaud, L. L.; Morrow, A. L.: Distribution of ^{3}H-zolpidem binding in relation to mRNA encoding the 1, 2, and 2 subunits of GABA$_A$ receptors in rat brain. Neuroscience 64: 1113–1128; 1995.
17. Duncan, G. E.; Knapp, D. J.; Breese, G. R.: Neuroanatomical characterization of the induction of Fos in rat behavioral models of anxiety. Brain Res. 713:79–91; 1996.
18. Duncan, G. E.; Little, K. Y.; Kirkman, J. A.; Kaldas, R. S.; Stumpf, W. E.; Breese, G. R.: Autoradiographic characterization of ^{3}H-imipramine and ^{3}H-citalopram binding in rat and human brain: Species differences and relationships to serotonin innervation patterns. Brain Res. 591:181–197; 1992.
19. Duncan, G. E.; Little, K. Y.; Koplas, P. A.; Kirkman, J. A.; Breese, G. R.; Stumpf, W. E.: β-Adrenergic receptor distribution in human and rat hippocampal formation: marked species differences. Brain Res. 561:84–92; 1991.
20. Handley, S. L.; McBlane, J. W.: 5-HT drugs in animal models of anxiety. Psychopharmacology (Berlin) 112:13–20; 1993.
21. Heath, R. G.: Pleasure response of subjects to direct stimulation of the brain: Physiological and psychodynamic considerations. In: Heath, R. G., ed. The role of pleasure in behavior. New York: Harper and Row; 1964:219–244.
22. Hensler, J. G.; Kovachich, G. B.; Frazer, A. A.: Quantitative autoradiographic study of serotonin$_{1A}$ receptor regulation: Effect of 5,7-dihydroxytryptamine and antidepressant treatments. Neuropsychopharmacology 4:131–144; 1991.
23. Hoyer, D.; Pazos, A.; Probst, A.; Palacios, J. M.: Serotonin receptors in the human brain. I. Characterization and autoradiographic localization of 5-HT$_{1A}$ recognition sites. Apparent absence of 5-HT$_{1B}$ recognition sites. Brain Res. 376:85–96; 1986.
24. Jankowska, E.; Bidzinski, A.; Kostowski, W.: Alcohol drinking in rats treated with 5,-7-dihydroxytryptamine: Effect of 8-OH-DPAT and tropisetron (ICS 205-930). Alcohol 11:283–288; 1994.
25. Jolas, T.; Haj-Dahmane, S.; Lanfumey, L.; Fattaccini, C. M.; Kidd, E. J.; Adrien, J.; Gozlan, H.; Guardiola-Lemaitre, B.; Hamon, M.: (−)Tertatolol is a potent antagonist at pre- and postsynaptic serotonin 5-HT$_{1A}$ receptors in the rat brain. Naunyn Schmiedebergs Arch. Pharmacol. 347:453–463; 1993.
26. Kohler, C.; Chan-Palay, V.: Cholecystokinin-octapeptide (CCK-8) receptors in the hippocampal region: A comparative in vitro autoradiographic study in rat, monkey and the postmortem human brain. Neurosci. Lett. 90:5156; 1988.
27. Kostowski, W.; Dyr, W.: Effects of 5-HT$_{1A}$ receptor agonists on ethanol preference in the rat. Alcohol 9:283–286; 1992.
28. Kume, A.; Albin, R. L.: Quantitative autoradiography of 4′-ethynyl-4-n-[2,3-^{3}H] propylbicycloorthobenzoate binding to the GABA$_A$ receptor complex. Eur. J. Pharmacol. 263:163–173; 1994.
29. Little, K. Y.; Duncan, G. E.; Breese, G. R.: Autoradiographic

analysis of beta-adrenergic receptor distribution in the human and rat hypothalamus. Biol. Psychiatry 32:512–522; 1992.
30. Lucki, I.; Singh, A.; Kreiss, D. S.: Antidepressant-like behavioral effects of serotonin receptor agonists. Neurosci. Biobehav. Rev. 18:85–95; 1994.
31. Martin, P.; Tissier, M. H.; Adrien, J.; Peuch, A. J.: Antidepressant-like effects of buspirone mediated by the 5-HT_{1A} postsynaptic receptors in the learned helplessness paradigm. Life Sci. 48:2505–2511; 1991.
32. Massoti, M.; Schlichting, J. L.; Antonacci, M. D.; Giusti, P.; Memo, M.; Costa, E.; Guidotti, A.: Aminobutyric acidA receptor heterogeneity in rat central nervous system: Studies with clonazepam and other benzodiazepine ligands. J. Pharmacol. Exp. Ther. 256:154–1160; 1991.
33. Marazziti, D.; Marracci, S.; Palego, L.; Rotondo, A.; Mazzanti, C.; Nardi, I.; Ladinsky, H.; Giraldo, E.; Borsini, F.; Cassano, G. B.: Localization and gene expression of serotonin$_{1A}$ (5-HT_{1A}) receptors in human brain postmortem. Brain Res. 658:55–59; 1994.
34. Miczek, K. A.; Weerts, E. M.; Vivian, J. A.; Barros, H. M.: Aggression, anxiety and vocalizations in animals: $GABA_A$ and 5-HT anxiolytics. Psychopharmacology (Berlin) 121:38–56; 1995.
35. Niddam, R.; Dubois, A.; Scatton, B.; Arbilla, S.; Langer, S.: Autoradiographic localization of ^{3}H-zolpidem binding sites in the rat CNS: Comparison with the distribution of ^{3}H-flunitrazepam binding sites. J. Neurochem. 49:890–899; 1987.
36. Palacios, J. P.; Probst, A.; Cortes, R.: Mapping receptors in the human brain. Trends Neurosci. 9:284–289; 1986.
37. Palacios, J. M.; Hoyer, D.; Cortes, R.: α1-Adrenoceptors in the mammalian brain: Similar pharmacology but different distribution in rodents and primates. Brain Res. 419:65–75; 1987.
38. Pazos, A.; Probst, A.; Palacios, J. M.: Serotonin receptors in the human brain—III. Autoradiographic mapping of serotonin-1 receptors. Neuroscience 21:97–122; 1987.
39. Paxinos, G.: The human nervous system. New York: Academic Press; 1990.
40. Pezzone, M. A.; Lee, W. S.; Hoffman, G. E.; Rabin B. S.: Induction of c-*fos* immunoreactivity in the forebrain by conditioned and unconditioned aversive stimuli. Brain Res. 597:41–50; 1992.
41. Pranzatelli, M. R.; Durkin, M. M.; Barkai, A. I.: Quantitative autoradiography of 5-hydroxytryptamine$_{1A}$ binding sites in rats with chronic neonatal 5,7-dihydroxytryptamine lesions. Brain Res 80:1–6;1994.
42. Schreiber, R.; De Vry, J.: Neuroanatomical basis for the antidepressant like effects of the 5-HT_{1A} receptor agonists 8-OH-DPAT and ipsapirone in the rat forced swimming test. Behav. Pharmacol. 4:625–636; 1993.
43. Shannon, H. E.; Guzman, F.; Cook, J. M.: β-Carboline-3-carboxylate-t-butyl ester: A selective BZ1 benzodiazepine receptor antagonist. Life Sci. 35:2227–2236; 1984.
44. Sprouse, J. S.; Aghajanian, G. K.: Responses of hippocampal pyramidal cells to putative serotonin 5-HT_{1A} and 5-HT_{1B} agonists: A comparative study with dorsal raphe neurons. Neuropharmacology 27:707–715; 1988.
45. Stahl, S. M.; Gastpar, M.; Keppel-Hesselink, J. M.; Traber, J.: Serotonin$_{1A}$ receptors in depression and anxiety. New York: Raven Press; 1992.
46. Svensson, L.; Engel, J.; Hard, E.: Effects of the 5-HT receptor agonists, 8-OH-DPAT, on ethanol preference in the rat. Alcohol 6:17–21; 1989.
47. Widdowson, P. S.: Quantitative receptor autoradiography demonstrates a differential distribution of neuropeptide-Y Y1 and Y2 receptor subtypes in human and rat brain. Brain Res. 631:27–38; 1993.
48. Yakushiji, T.; Shirasaki, T.; Munakata, M.; Hirata, A.; Akaike, M.: Differential properties of type I and type II benzodiazepine receptors in mammalian CNS neurones. Br. J. Pharmacol. 109:819–825; 1993.
49. Zilles, K.; Qu, M.; Schleicher, A.: Regional distribution and heterogeneity of alpha-adrenoreceptors in the rat and human central nervous system. J. Hirnforsch. 34:123–132;1993.