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# Developmental Pattern of 3H-Spiperone Binding Sites in Rat Brain

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## *Abstract*

Development pattern of 3H-Spiperone binding to 5-HT2 receptors was studied in cerebral cortex of new born, 45 days, 3 month, 8 month and 18 month old rats. Regional distribution of 3H-Spiperone binding revealed maximal density of 5-HT2 sites in hippocampus (B max 543.33 ± 17.97 f moles/mg protein), followed by cerebral cortex (B max = 392.72 ± 41.10 f moles/mg protein), cerebellum (B max = 272.00 ± 22.75 f moles/mg protein) and brain stem (B max = 162.00 ± 10.44 f moles/mg of protein). In these regions the Hill coefficient value was 0.98 suggesting that 3H-Spiperone binds to one class of non-interacting sites. Developmental pattern of 5-HT2 sites in rat cerebral cortex revealed that there was a gradual increase in 5-HT2 density during postnatal development. The maximum density of the 5-HT2 receptors (B max-392.72 ± 17.97 f moles/mg protein) was observed in three month old rats, which was 3 fold more than new born rats (B max-125.17 ± 57.00 f moles/mg protein). During the course of aging there was, however, a gradual decline in the density of 5-HT2 sites. The affinity of 3H-Spiperone to 5-HT2 sites (Kd), which did not change with age, was high in brain stem and cerebellum than in cortex and hippocampus.

Key words -

**HT2 Receptors,**
**H Spiperone,**
**Rat brain,**
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**5-HT2 Receptors,**
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Serotonin (5-HT), a putative neurotransmitter, is implicated in regulation of mood, body temperature and pain in rat and humans [1], [2], [3], [4]. Several classes and subclasses of serotonin receptors have been identified (5-HT1 to 5-HT7) in central nervous system [5]. Among the classes, 5-HT2 receptors have been reported to be positively coupled to Phospholipase C (PLC), via G-proteins [6]. Many radioligands have been used to label 5-HT2 receptors in brain, viz;

3H-Spiperone, 3H-Ketanserin, 3H-LSD and 3H-DOB (4-bromo-2, 5-dimethoxy phenylisopropylamine [5]). 5-HT has been implicated in certain pathological and psychopathological conditions. Alteration in 5-HT2 receptors are very well documented in the pathophysiology and biochemistry of depression which might be corrected by antidepressant drugs treatment [7], [8]. Hypersensitivity of 5-HT2 receptors has been reported in postmortem brain tissues from drug free depressives and suicide victims [9], [10], [11] which by chronic antidepressant treatment, has been shown to be down regulated [12], 5-HT2 receptors, as recently summarized [13], may be regulated by many factors including antipsychotic agents, antidepressants, receptor agonists and antagonists and unknown developmentally regulated substances [14], apart from other exogenous agents. Depleting brain 5-HT levels with parachlorophenylalanine (PCPA), but not with 5,7, dihydroxytryptamine (5,7 DHT) [14], [15], increases cortical 5-HT2 receptors. Preliminary findings, however, suggest that cortical 5-HT2 receptors may be increased by neonatal, 5,7 DHT treatment [16]. 5-HT2 receptors, which have been implicated in the neuronal growth [17] have been reported to be increased during perinatal development of rat brain [14]. Decreased 5-HT2 receptors have been seen in Alzheimer's disease and Schizophrenia [18]. There is also evidence for altered 5-HT2 receptor sensitivity in Obsessive Compulsive disorder [19] and following clozapine treatment of Schizophrenic and certain depressed patients [20], [21]. All these findings suggest that alterations in 5-HT2 and possibly 5-HT1C receptors may be important for many pharmacological, developmental, psychological and pathophysiological events. The molecular and biochemical details responsible for these changes remain largely undefined. Insight into these mechanism of processes and differences occurring with development could shed light on a number of important processes.

In view of these findings, it is of great importance to study the developmental pattern and regional distribution of 5-HT2 receptors in rat brain. Radioligand binding assays were carried out in isolated synaptosomal membranes from cerebral cortex, hippocampus, cerebellum and brain stem of rats. Postnatal developmental pattern of 5-HT2 receptor density is studied in cerebral cortex of new born, 45 days, three month, eight month and eighteen months old rats.

## Material and Methods

Adult male Sprague Dawley rats, weighing 250-290 gms, were sacrified by decapitation. Brain was immediately removed and different regions like cerebral cortex, hippocampus, cerebellum and brain stem, were dissected out. For developmental pattern, cerebral cortex tissues were obtained from newborn, 45 days, three month, eight month and eighteen months old rats. Synaptosomal membranes were prepared from these regions according to the method of Creese and Snyder [22]. In brief tissue homogenates (1:20 w/v) in 50 mM Tris-HCI buffer (pH7.7) were centrifuged once at 1000g for 10 minutes at 4° C. The resulting supernatant was centrifuged twice at 45000g for 20 minutes. The final pellet was resuspended in 50 mM Tris-HCI buffer and protein content was determined by Lowry's method [23]. Protein concentration of the pellet was adjusted to 1mg/ml with buffer.

### Radioligand Binding Assay

3H-Spiperone was used to label 5-HT2 receptor sites in synaptosomal membranes according to the method described by Creese and Snyder [22]. Different concentrations of 3H-Spiperone (0.2-2.0 nM; Specific activity 24 Ci/mmole) were incubated at 37° C for 20 minutes in 50 mM Tris-HCI buffer (pH.7.7) with an aliquot of membrane protein (200µ gms) in presence and absence of 10µ M mianserin (to determine the non specific binding). The assay was terminated by the addition of 2 ml of ice cold 50 mM Tris-HCI buffer and rapidly filtered through Whatman GF/B filters. Filters were washed three times with 2 ml of same buffer and transferred to counting vials containing 10ml of scintillation fluid. Radioactivity was measured in a liquid scintillation counter (LKB, UK) at an efficiency of 54%. Maximum binding (Bmax) and ligand dissociation constant (Kd) were determined from Scatchard plot data obtained by using computer assisted software program 'LIGAND' (McPherson's).

## Materials

3H-Spiperone (24 Ci/mmol) was obtained from Amersham Life Sciences, UK., Mianserin from sigma chemicals (USA) and other chemicals were from local chemical suppliers and were of excellar grade.

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# Results

The 5-HT2 Sites in rat brain were labelled by 3H-Spiperone. It is observed that the specific binding of 3H-Spiperone to 5-HT2 sites was saturable over a range of 0.2-2.0 nM (fig 1). The Scatchard plot of the data revealed a linear regression with Hill values of 0.98 ± 0.13, thus suggesting that, at this concentration, 3H-Spiperone binds to a single class of non-interacting 5-HT2 sites in rat brain. The regional distribution of 5-HT2 sites showed maximal binding in hippocampus (B max 543.33 ± 17.97 f moles/mg protein) compared to other regions. When compared to hippocampus, cerebral cortex showed 78% (392.72 ± 41.10 f moles/mg protein), cerebellum showed 50% (272.00 ± 22.75 f moles/mg protein) and brain stem showed 30% (162.00 ± 10.44 f moles/mg protein) of 5-HT2 sites. The affinity of 3H-Spiperone (Kd) to 5-HT2 sites was significantly different in all the regions. It is observed that brain stem with Kd value of 0.70 ± 0.04 nM and cerebellum with 0.77 ± 0.19 nM and cerebellum with 0.77 ± 0.19 nM showed high affinity compared to cortex (Kd = 0.85 ± 0.13 nM) and hippocampus (Kd = 1.3 ± 0.25 nM) (Fig.2).

***Binding experiments were done with 3H-Spiperone (0.2-2.0 nM) in presence and absence of mianserin (10μM) using synaptosomal membranes from cerebral cortex of adult rat brain, as described in methods***

***5-HT2, receptor density in synaptosomal membranes obtained from different regions of adult rat brain was estimated by binding experiment with 3H-Spiperone (0.2-2.0 nM) in presence and absence of mianserin (10μM), as described in methods***

## Profile of Developmental Pattern of 5-HT2 Sites

5-HT2 sites were labelled using 3H-Spiperone in newborn, 45 days, 3 month, 8 month and 18 month old rats. It is observed that there is a significant increase in 3H-Spiperone binding from newborn to three months old rats, which decreases after 8 months and 18 months of age. New-born rat cortex showed lowest density (125.17 ± 57.00 f moles/mg protein) of 5-HT2 sites. 45 days old rats did not show significant increase in the density of 5-HT2 sites (131.12 ± 36.80 f moles/mg protein). However three month old rats showed three fold increase in 3H-Spiperone binding sites (392.72 ± 17.97 f moles/mg protein) when compared to newborn rats. A significant decrease in 3H-Spiperone binding was seen with increase in age. A significant decrease in 5-HT2 sites was seen in eight month (30%; B max = 284.00 ± 51.53 f moles/mg protein) and 18 month (B max = 182.00 ± 15.50 f moles/mg protein) old rats when compared to 3 months old rats. The affinity of 3H-Spiperone binding to 5-HT2 sites, however, did not change significantly with age (Fig. 3).

***5-HT2 reporter density was obtained by conducting binding experiments with 3H-Spiperone (0.2-2.0 nM) in synaptosomal membranes obtained from cortex of brain of rats at different stages of development.***

## Discussion

In the present study, we found higher binding capacity of 3H-Siperone to 5-HT2 sites in hippocampus and cortex than in cerebellum and brain stem. Similar results were reported by Pazos et al [24]. The binding affinity of 3H-Spiperone has revealed Kd values less than 1 nM in these regions which agreed with earlier data [25]. Cerebellum and brain stem, however, showed lower densities of 5-HT2 receptor with higher affinity for 3H-Spiperone. The developmental pattern of 3H-Spiperone binding to 5-HT2 sites in cerebral cortical membranes of rat brain showed a significant increase from new born to 3 months old rats. The loss of 5-HT receptors in frontal cortex with age was observed without significant change in affinity of 3H-Spiperone. These findings are partially in line with previous findings [26]. 5-HT2 receptors have been shown to increase by eight fold along with the concomitant increase in the 5-HT2 receptor mRNA in rat brain [26]. These findings suggest that regulation of 5-HT2 receptor gene expression may be important for developmentally induced changes in 5-HT2 receptors. Recently age related changes have been reported to be critically important in receptor regulation and drug effects.

Roth et al [13] demonstrated that, during the prenatal period of brain there is increased levels of 5-HT2 receptor mRNAs, Secondly, mianserin, a prototypical receptor antagonist, alters 5-HT2 and 5-HT1C levels without altering steady-state receptor mRNAs. These results imply that a number of biochemical and molecular events might be important for regulating 5-HT2 and 5-HT1C receptors in vivo. It is also observed that 5-HT2 receptors were perodominant in cerebral cortex and display striking developmental expression over a relatively restricted time period. There is a 8 fold increase in the density with 13 fold increase in receptor mRNA. These results suggests that a burst of receptor gene expression occurs during the immediate perinatal period in rat brain. This roughly corresponds to the period of synaptogenesis in the rat. Available data suggests that serotonin levels and serotonergic synapses are necessary for the expression of serotonergic receptors, although recent studies have revealed that blockage of 5-HT2 receptors by mainserin treatment during ontogeny does not alter the expression of 5-HT2 receptors. Thus it is suggested that expression of 5-HT2 receptors may be regulated by factors other than serotonergic innervation [27], [28], [29].

Since the 5-HT2 receptors are expressed around the time period corresponding to the brain growth spurt, it has been hypothesized that neonatal 5-HT2 receptors serve to promote cortical growth during developmental epoch. Studies of regional distribution in human brain 5-HT receptors have shown high density of 5-HT1 receptors in frontal cortex and hippocampus regions with decrease in number of receptors with age [26]. Decrease in hippocampal 5-HT content with decrease in tryptophan hydroxylase activity has also been reported with age [26]. MAO Activity has also been shown to be decreased in rat cortex and hippocampus without changes in human brain. Further studies are required to understand 5-HT2 receptor regulation occurring during the postnatal period. Various studies have suggested existence of developmentally regulated periods of over expression of neurotransmitter binding sites, which may be of functional significance for human brain maturation and various pathological conditions. Structural correlates of neurotransmission such as synaptic terminals and dentritic spines exhibit a developmental time course parallel to that of neurotransmitter bidding sites in several regions of brain. Even the monoaminergic neurotransmission undergoes similar developmental changes. Since there is a growing evidence suggesting neurotransmitters to be involved in various psychiatric disorders, ontogenic changes in regulation and expression of receptors may be critical of studying the pathophysiology of neurological and psychiatric disorders. Available data on the ontogeny of other receptors like glutamate and dopamine [29] suggests that human brain may be

vulnerable in postnatal period between the age 1 and 2 years. Decrease in number of serotonergic binding sites with aging, as seen in this study, might suggest the vulnerability of brain regions for various exogenotoxins and also efficacy of therapeutic agents which depend on the receptor density. The knowledge of basic ontogenic functions of neurotransmitters and their specific receptors may represent not only clue in understanding pre-and perinatal disturbances of brain maturation, but may also help to find new strategies against ensuing disorders [30]. However it must be remembered that receptor binding make no assumption on function of the sites and thus functional status of these receptors needs to be studied with respect to aging.


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