2010

# Alterations in 5-HT2A receptor binding in various brain regions among 6-hydroxydopamine-induced Parkinsonian rats

Yun Li
*University of Wollongong*

Xu-Feng Huang
*University of Wollongong*, xhuang@uow.edu.au

Chao Deng
*University of Wollongong*, chao@uow.edu.au

Barbara J. Meyer
*University of Wollongong*, bmeyer@uow.edu.au

Aimin Wu
*Sun Yat-Sen University*

***See next page for additional authors***

## Publication Details

Li, Y., Huang, X., Deng, C., Meyer, B., Wu, A., Yu, Y., Ying, W., Yang, G., Yenari, M. A. & Wang, Q. (2010). Alterations in 5-HT2A receptor binding in various brain regions among 6-hydroxydopamine-induced Parkinsonian rats. Synapse (New York), 64 (3), 224-230.

# Alterations in 5-HT2A receptor binding in various brain regions among 6-hydroxydopamine-induced Parkinsonian rats

**Abstract**

The serotonergic system has close interactions with the dopaminergic system and is strongly implicated in the pathophysiological mechanisms and therapeutic paradigms of Parkinson's disease (PD). This study aims to investigate regional changes in 5-hydroxytryptamine (5-HT) 2A receptors in the rat brain 3 weeks after unilateral medial forebrain bundle lesion by 6-hydroxydopamine (6-OHDA). 5-HT 2A receptor distributions and alterations in the postmortem rat brain were detected by [3H]ketanserin-binding autoradiography. In the 6-OHDA-induced Parkinson's rat model, nigrostriatal dopaminergic neuron loss significantly mediated the decreased [3H]ketanserin binding, predominantly in the agranular insular cortex (17.3%, P 5 0.03), cingulate cortex (18.2%, P < 0.001), prefrontal cortex (8%, P 5 0.043), primary somatosensory cortex (17.7%, P 5 0.002), and caudate putamen (14.5%, P 5 0.02) compared to controls while a profound reduction of tyrosine hydroxylase (TH) immunostaining in the striatum was also observed. Alterations in [3H]ketanserin binding in the examined brain areas may represent the specific regions that mediate cognitive dysfunctions via the serotonin system. The downregulation of 5-HT2A receptor binding in this study also provides indirect evidence for plasticity in the serotonergic system in the rat brains. This study contributes to a better understanding of the critical roles of 5-HT2A receptors in treating neurodegenerative disorders and implicates 5-HT2A receptors as a novel therapeutic target in the treatment of PD.

**Disciplines**

Arts and Humanities | Life Sciences | Medicine and Health Sciences | Social and Behavioral Sciences

**Publication Details**

Li, Y., Huang, X., Deng, C., Meyer, B., Wu, A., Yu, Y., Ying, W., Yang, G., Yenari, M. A. & Wang, Q. (2010). Alterations in 5-HT2A receptor binding in various brain regions among 6-hydroxydopamine-induced Parkinsonian rats. Synapse (New York), 64 (3), 224-230.

**Authors**

Yun Li, Xu-Feng Huang, Chao Deng, Barbara J. Meyer, Aimin Wu, Yinghua Yu, Weihai Ying, Guo-Yuan Yang, Midori Yenari, and Qing Wang

# Alterations in 5-$HT_{2A}$ receptor binding in various brain regions among 6-hydroxydopamine-induced Parkinsonian rats

Yun Li MBBS [b c d], Xu-Feng Huang Ph.D MBBS[b], Chao Deng Ph.D[b], Barbara Meyer Ph.D[b], Aimin Wu MD [a], Yinghua Yu Ph.D[b], Weihai Ying PhD [f], Guo-Yuan Yang MD [f], Midori A Yenari MD [e], Qing Wang MD, Ph.D [a b]*

[a] Department of Neurology, The Third Affiliated Hospital of Sun Yat-Sen University, Tianhe Road 600, Guangzhou, Guangdong 510630, P.R. China

[b] Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, NSW 2522, Australia

[c] Hunan Engineering Center of Combinatorial Biosynthesis and Natural Product Drugs, Changsha, Hunan, P.R.China

[d] Department of Ophthalmology and Visual Sciences, the Second Xiangya Hospital of Central South University, Changsha, Hunan, P.R.China

[e.] Department of Neurology, University of California, San Francisco and the San Francisco Veterans Affairs Medical Center, 4150 Clement Street, San Francisco, CA 94121, USA

[f.] Med-X Research Institute, Shanghai Jiao Tong University, 1954 Huashan Road, Shanghai 200032, P.R.China

Correspondence and reprint request to:
Prof. Qing Wang MD, Ph.D
Department of Neurology,
The Third Affiliated Hospital of Sun Yat-Sen University,
600 Tianhe Road, Guangzhou,
Guangdong 510630,
P.R. China
Phone: +86-20-85252238
FAX: +86-20-85253117
Email: denniswq@yahoo.com or dwang@uow.edu.au

**Running title: 5-HT 2A receptor decreased in 6-OHDA induced PD rat brain**

**Total words: 5088** **Figures: 4**

**Abstract**

The serotonergic system has close interactions with the dopaminergic system and is strongly implicated in the pathophysiological mechanisms and therapeutic paradigms of Parkinson's disease. This study aims to investigate regional changes in 5-hydroxytryptamine (5-HT) 2A receptors in the rat brain three weeks after unilateral medial forebrain bundle lesion by 6-hydroxydopamine. 5-hydroxytryptamine (5-HT) 2A receptor distributions and alterations in the post-mortem rat brain were detected by [$^3$H]ketanserin binding autoradiography. In the 6-hydroxydopamine-induced Parkinson's rat model, nigrostriatal dopaminergic neuron loss significantly mediated the decreased [$^3$H]ketanserin binding, predominantly in the agranular insular cortex (17.3%, $p$ = 0.03), cingulate cortex (18.2%, $p$< 0.001), prefrontal cortex (8%, $p$= 0.043), primary somatosensory cortex (17.7%, $p$= 0.002) and caudate putamen (14.5%, $p$= 0.02) compared to controls while a profound reduction of tyrosine hydroxylase (TH) immunostaining in the striatum was also observed. Alterations in [$^3$H]ketanserin binding in the examined brain areas may represent the specific regions that mediate cognitive dysfunctions via the serotonin system. The down-regulation of 5-$HT_{2A}$ receptor binding in the present study also provides indirect evidence for plasticity in the serotonergic system in the rat brains. The present study contributes to a better understanding of the critical roles of 5-$HT_{2A}$ receptors in treating neurodegenerative disorders, and implicates 5-$HT_{2A}$ receptors as a novel therapeutic target in the treatment of Parkinson's disease.

Keywords: Parkinson's disease, Serotonin, 5-$HT_{2A}$ receptors, Tyrosine hydroxylase, Striatum, Down-regulation.

## Introduction

5-hydroxytryptamine (5-HT) receptors (with main types from 1 to 7 for a total of 14 different subtypes) have been widely identified in the central nervous system. As the G protein-coupled receptors (GPCR), 5-HT receptors are involved in various signal transduction pathways, including inhibition or activation of adenylyl cyclise, phosphatidylinositol-specific phospholipase C or Janus kinase/signal transducers and activators of the transcription pathway, and regulation of extracellular signal-regulated mitogen-activated protein kinase, proliferation related pathways, or the extracellular signal-regulated kinase (Raymond, 2001).

It is well documented that there is a close interaction between brain serotonergic and monoamine dopaminergic systems (Alex, 2007). The dopaminergic disturbance in the brain may lead to serotonergic changes. *In vivo* microdialysis studies showed that local infusion of the $D_2$ receptor antagonist raclopride significantly inhibited the tail pinch-induced serotonin increases in the prefrontal cortex and corpus striatum (Mendlin, 1999). Similar results also indicated that local administration of the nonselective dopamine receptor agonist apomorphine into the hippocampus increased 5-HT release in a concentration-dependent manner, and this increase was abolished by pre-treatment with the selective $D_2$ receptor antagonist, S(–)-sulphide (Matsumoto, 1996). Reciprocally, serotonin afferents are able to facilitate the release of dopamine. It has been shown that dopamine release is induced in different brain regions following local cerebral application with serotonin (Parsons and Justice, 1993; West and Galloway, 1991).

In addition to the correlation between serotonin and dopamine transmission, there is tight functional interaction between these receptors. It has been shown that 5-$HT_{2A}$ receptors are co-expressed with dopamine 1-5 receptors in different brain

regions, such as the substantia nigra pars compacta, striatum and cortex (Goldman-Rakic, 1999). Immunohistochemistry with confocal microscopy showed that 5-$HT_{2A}$ receptors were co-localised with tyrosine hydroxylase and were expressed on dopaminergic neurons within the A10 dopamine subnuclei (Nocjar, 2002). Alterations of 5-HT receptors in the brain are closely associated with the dopaminergic system. One study by Pehek showed that intracortical infusion of the 5-$HT_{2A}$ receptor antagonist M100907 profoundly attenuated dopamine release induced by systemic administration of the 5-HT agonist, suggesting that stimulation of cortical 5-$HT_{2A}$ receptors increased dopamine release from the mesocortical system (Pehek, 2006). Similar dopamine modulation was also observed by systemic or local administration of the 5-$HT_{2C}$ receptor agonist mCPP or SB 206553 (Alex, 2005).

Parkinson's disease (PD) is the second most common neurodegenerative disorder following Alzheimer's disease (AD) with the typical disturbance of the central dopaminergic system (Wang and Ying, 2009) and imbalances in some non-dopaminergic systems, including the serotonergic system (Scholtissen, 2006a; Scholtissen, 2006b; Zgaljardic, 2004; Zhang, 2008). Considering the interactions between the dopaminergic and serotonin receptors are well documented (Alex, 2007), here we explore whether 5-$HT_{2A}$ receptors in the different rat brain regions are also changed in 6-hydroxydopamine (6-OHDA) lesioned Parkinsonian rats. To the best of our knowledge, the 5-$HT_{2A}$ receptor binding site in adult rat brain has not been systematically investigated. To address this issue, we used [$^3$H]ketanserin binding autoradiography, a serotonin antagonist with high affinity for the 5-$HT_{2A}$ receptors to study serotonin responses following 6-OHDA lesion treatment across a wide range of brain structures.

## Materials & Methods

*Animals and drug treatments*

Sixteen male Sprague-Dawley rats (230-250g) were obtained from the Animal Resources Centre (Perth, Western Australia, Australia) and housed individually in environmentally controlled conditions (22°C, 12 h light-dark cycle with light cycle from 0600 to 1800 h and dark cycle from 1800 to 0600 h) with *ad libitum* access to standard laboratory chow and water. Rats were allowed 1 week to adapt to their new environment before experiments began. They were randomized with eight rats for controls receiving vehicle and eight rats for 6-hydroxydopamine (6-OHDA)-induced Parkinsonian treatment (One rat from the latter group died during surgery). Three weeks after 6-OHDA lesion, rats from each group were sacrificed to examine 5-$HT_{2A}$ receptor binding. The reasons for the timepoint of 3-week observation are that some studies have indicated that 3 weeks after 6-OHDA injection, 85% nigral dopaminergic neurons lost in SNc (Anastasia, 2007), whilst we also would like to explore the short-term effects with 6-OHDA lesion. This study has been approved by the University of Wollongong Animal Ethics Committee and all animal experiments were conducted in compliance with the *National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23)* revised 1996 guidelines and National Health and Medical Research Council (NHMRC) *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004)*.

*6-OHDA lesioned Parkinsonian rats*

6-OHDA lesioned Parkinsonian rats were prepared as described in our previous works (Wang, 2005). Briefly, male Sprague–Dawley rats (weight 230–250 g) were anesthetized with 75 mg/kg ketamine and 10 mg/kg xylazine (Troy

laboratories Pty, Ltd., Australia). Lesions were made by injecting 6-OHDA unilaterally (4 µl of 8 µg/µl in normal saline containing 0.2 mg/ml ascorbic acid, Sigma-Aldrich, St. Louis, MO; 0.8 µl/min) into the medial forebrain bundle (MFB) at the Bregma level AP −4.4 mm, ML −1.4 mm and DV −7.8 mm (Paxinos and Watson, 1997; Wang, 2005). The control group received the vehicle. All experiments were carried out in accordance with the guidelines set by the University of Wollongong, adapted from Howard-Jones (Howard-Jones, 1985).

*Histology and [$^3$H]ketanserin binding autoradiography*

Three weeks later, rats were sacrificed by rapid $CO_2$ asphyxiation. In order to minimise the impact of circadian variation on binding density, the rats were sacrificed between 0700 and 0900 hours, and the brains were immediately removed and frozen in liquid nitrogen. Coronal brain sections (14um) were cut at -17$^o$C with a cryotome (Clinicut cryostat; Bright Instruments), and thaw-mounted onto poly-L-lysine coated microscope slides (Polysine™, Menzel GmbH & Co KG) (Wang and Huang, 2008). Consecutive sections were used for the detection of the 5-$HT_{2A}$ receptor binding site. Neuroanatomical structures were identified according to a standard rat brain atlas (Paxinos and Watson, 1997). [$^3$H]ketanserin autoradiography was performed as described in our previous works (du Bois, 2006). In summary, binding of [$^3$H]ketanserin (67.0Ci/mmol; PerkinElmer Life Sciences, Boston, MA, USA) to 5-$HT_{2A}$ receptors was measured by preincubating sections in 170 mM Tris–HCl buffer (pH 7.4) for 15 min at room temperature (RT). Sections were incubated for 120 min at room temperature in buffer containing 2 nM [$^3$H] ketanserin. Non-specific binding was determined by the addition of 2 µM spiperone to a third of the sections. Sections were washed in 4 °C buffer (2×10 min), dipped in distilled water and dried.

*Tyrosine hydroxylase immunohistochemistry staining*

Tyrosine hydroxylase (TH) staining was performed as described in Yuan's study (Yuan, 2005). Briefly, endogenous peroxidase was quenched with 0.3% $H_2O_2$ (30 min). Then the sections were washed with distilled water (3 × 5 min) and placed in citrate buffer 0.1 M pH 5.8 (20 min at 60–80 °C). They were then washed in distilled water followed by PBS (5 min). Non-specific binding was blocked with 1.5% normal goat serum (Vectastain rabbit IgG ABC kit) (60 min). This was followed by application of TH primary antibody (rabbit polyclonal anti-tyrosine hydroxylase, Millipore Corporation, AB152) diluted to 1:500 in blocking solution and incubated overnight at 4 °C. After washing in PBS (3 × 5 min), the sections were incubated with a 1:200 dilution of the biotinylated anti-rabbit secondary antibody (Vectastain rabbit IgG ABC kit) for 60 min followed by washes with PBS (3 × 5 min). The horseradish peroxidase conjugate ABC (Vectastain rabbit IgG ABC kit) was applied for 60 min followed by PBS rinses (3 × 5 min). 3,3′-Diaminobenzidine (Vector Labs), mixed with distilled water, buffer pH 7.5, $H_2O_2$, and Nickel stock (DAB, Vector SK-4100), was applied until staining was optimal as determined by light microscopy. The sections were then washed with tap water, dehydrated, cleared with xylene and coverslipped using DPX mountant. After TH-staining, the image of the sections from the striatum were digitalized with a camera (Nikon D80, Nikon Japan), connected to an Olympus microscope and a computer. Density in the striatum was measured using the NIH ImageJ Analysis software.

*Quantification and Statistical Analysis*

Quantification of binding sites was performed on a high-resolution Beta Imager (BioSpace, Paris, France) according to our previous study (Wang, 2008). In brief, sections were placed inside the detection chamber of the Beta Imager and

scanned for 3.5 h at a high-resolution setting. The levels of bound radioactivity in the brain sections were directly determined by counting the number of β-particles emerging from the tissue sections, which was followed by analysis of activities in the regions of interest using the Beta Vision Plus program (BioSpace). The radioligand binding signal was expressed in counts per minute per square millimetre (cpm/$mm^2$) and a series of sections with known quantities of ligands were used as standards in all scans allowing the measurement of radioligand binding signals to be converted to nCi/mg tissue equivalents. The [$^3$H] ketanserin binding density in various brain regions was quantified by measuring the average density of each region in three to five adjacent brain sections. Different brain regions were identified with reference to a standard rat brain atlas (Paxinos and Watson, 1997). Data were expressed as mean ± SEM. [$^3$H] ketanserin binding densities for each brain region were analysed using a one-way ANOVA followed by independent *t*-test analysis using the SPSS 15.0 program (Chicago, IL). *p* values of less than 0.05 were regarded as statistically significant.

## Results

Specific [$^3$H] ketanserin binding was observed in most brain regions examined, although there were regional variations in density (Fig 1). Nonspecific binding was observed to be less than 5% (Fig 1). The highest density of [$^3$H] ketanserin binding was observed in the agranular insular cortex and prefrontal cortex. Moderate levels of [$^3$H] ketanserin binding were observed in the cingulate cortex, the primary motor cortex, and some limbic regions including the nucleus accumbens and primary somatosensory cortex. Low levels of [$^3$H] ketanserin binding were observed in the amygdala and caudate putamen. In addition, we found that the [$^3$H] ketanserin binding level is very low in the substantia nigral area and hardly to detect it. Our result is consistent with Du Bois study (du Bois, 2006).

### Effects of 6-OHDA lesion on [$^3$H] ketanserin binding in different brain regions

The analysis revealed significant differences in [$^3$H] ketanserin binding in all brain regions examined except Acb, Amy and M1 among 6-OHDA lesioned rat. Three weeks after 6-OHDA lesion, [$^3$H] ketanserin binding was significantly lower in five of eight brain regions examined compared to the controls (Figs 2 and 3). Specifically, 6-OHDA lesion significantly decreased [$^3$H] ketanserin binding density in the agranular insular cortex (17.3%, $p$= 0.03), the cingulate cortex (18.2%, $p$< 0.001), the prefrontal cortex (8%, $p$= 0.043), S1 (17.7%, $p$= 0.002) and the caudate putamen (14.5%, $p$= 0.02) compared to the controls. However, compared to the controls, 6-OHDA lesion induced no significant changes in [$^3$H] ketanserin binding in the nucleus accumbens ($p$= 0.138), the amygdala ($p$= 0.079) and the primary motor cortex ($p$= 0.061) (Fig 3).

**Effects of 6-OHDA lesion on TH immunohistochemistry staining in the caudate putamen**

In the Striatum: Coronal section (Fig. 4) throughout the striatum indicating the extent of nigrostriatal denervation induced by injecting the neurotoxin 6-OHDA into the right MFB, as seen by tyrosine hydroxylase immunoreactivity. The right side of the photo (Fig. 4A) shows the dopamine-depleted striatum side characterized by a large absence of TH immunoreactive fibres. The density of TH-positive fibres was expressed as a mean percentage ± S.E.M relative to the intact side, in which the dense TH-immunoreactive fibres were found throughout the striatum. Compared to the controls, around 80% reduction in the density of TH-positive fibres in the striatum was observed in 6-OHDA induced PD rats (Student *t*-test: $t$=15.696, *** $p$< 0.001, Fig. 4C). The density of TH immunoreactive fibres in the striatum among the control group (Fig 4B) is the same as that of intact side of 6-OHDA induced PD rats.

**Discussion**

The present study was designed to investigate [$^3$H] ketanserin binding density in 6-OHDA lesioned PD rats. [$^3$H] ketanserin has a specific affinity for 5-$HT_{2A}$ receptors and the [$^3$H] ketanserin binding densities directly reflect the 5-$HT_{2A}$ receptor levels and distributions in the rat brain following 6-OHDA lesion. Although there were regional variations in 5-$HT_{2A}$ receptor binding density, our finding suggests that the decrease in 5-$HT_{2A}$ receptors due to 6-OHDA lesion probably plays an important role in the progression of Parkinson's disease.

Our study shows that a significant down-regulation of [$^3$H] ketanserin binding densities was observed three weeks among 6-OHDA lesioned PD rats, predominantly in the agranular insular cortex, the cingulate cortex, the primary somatosensory cortex, the caudate putamen and the prefrontal cortex. This suggests that 6-OHDA lesion may interfere with 5-$HT_{2A}$ receptor expression and may prevent [$^3$H] ketanserin binding to the receptors, or enhance the receptor degradation and desensitization. In addition, the down-regulation of 5-$HT_{2A}$ receptor binding in the present study provides indirect evidence for the plasticity of the serotonergic systems in the rat brain. The down-regulation of 5-HT2A receptors in some mesocortical regions is linked to increased anxiety and impaired memory. It is expected that the 5-HT2A receptors' agonist like DOI may have important clinical implication in improving the cognitive deficits in PD.

Our study showed that three weeks after MFB lesion by 6-OHDA TH-immunostaining displayed a profound decreased in density of TH-positive fibres in the striatum (Fig 4), which indicated an obvious dopaminergic neuronal degeneration and complete nerve terminal denervation. This result is consistent with observations made by Yuan (Yuan, 2005). Destruction of the dopaminergic nigrostriatal pathway

due to MFB lesion in this study mediated an obvious down-regulation of 5-$HT_{2A}$ receptor binding, indicating that the loss of dopaminergic afferents induced decreased 5-$HT_{2A}$ receptor expression in the striatum. This hypofunction of the 5-$HT_{2A}$ receptor could be due to 5-HT hypo-innervations or the reduced number of 5-HT terminals after 3-week MFB lesion. Interestingly, our result is different from Zhang's study that indicated an increase in 5-$HT_{2A}$ receptor mRNA expression in the striatum (Zhang, 2007). This discrepancy may result from the following reasons: 1) in our study, we injected in total 32 μg 6-OHDA into the right medial forebrain bundle but Zhang only injected 12.5 μg (thus, 2.56 times more 6-OHDA neurotoxin was used in our study; 2) we measured 5-$HT_{2A}$ receptor binding three weeks after MFB lesion and this showed one week earlier in comparison to Zhang's study, and the turnover of 5-$HT_{2A}$ receptors did not occur during the short period of three weeks. In our study, the high dose of neurotoxin and shorter observed time following 6-OHDA MFB lesion leading to the down-regulation of 5-$HT_{2A}$ receptor binding in the striatum in stead of elevation suggests that during the early stage of Parkinson's disease, dopaminergic denervation usually has a parallel influence on the expression of striatal 5-$HT_{2A}$ receptors. However, whether 5-$HT_{2A}$ receptors rebound in the striatum according to the prolonged timing course remains to be determined.

In addition to the down-regulation of 5-$HT_{2A}$ receptor expression in the striatum three weeks after MFB lesion, 5-$HT_{2A}$ receptor binding was also decreased in some mesocortical regions, such as the primary motor cortex, the cingulate cortex, the agranular insular cortex and the prefrontal cortex. It has been shown that the dopamine neurons in the mesocortical pathway project from the ventral tegmental area (VTA) and the medial substantia nigra pars compacta to the frontal lobe. Dopaminergic disruption in Parkinson's disease not only affects the nigrostriatal tract

but also the mesocortical pathway (Jellinger, 2001). Since the brain serotonergic and monoamines dopaminergic systems have close interactions, Parkinson's disease reflects abnormalities in the mesocortical pathways with the serotonergic system (Doder, 2003). The down-regulation of 5-$HT_{2A}$ receptor in examined mesocortical regions observed in this study may reflect 5-HT hypo-innervations following 6-OHDA lesion since some studies have indicated that unilateral 6-OHDA lesion of the nigrostriatal dopaminergic system abolished increases in cortical-striatal serotonin output (Mendlin, 1999).

. Increasing evidence shows that interruptions to the frontostriatal circuitry will elicit disturbances in cognitive, emotional, and memory disturbances among people afflicted with Parkinson's disease. Tamaru indicated that the dysfunction of the nigrostriatal dopaminergic system and cortical-basal ganglionic circuits connecting the frontal lobe and the basal ganglia obviously induced a series impairment of cognitive functions like memory, attention, and executive function in Parkinson's patients (Tamaru, 1997). As one of the main 5-HT receptors, the dysfunction of 5-$HT_{2A}$ receptors are recognised for their important roles and implications in a number of cognitive and stress-related disorders (Huang, 2007). Weisstaub demonstrated that the global disruption of 5-$HT_{2A}$ receptor signalling in mice significantly reduced inhibition in conflict anxiety without affecting fear-conditioned and depression related behaviours, indicating a specific role of cortical 5-$HT_{2A}$ receptor function in the modulation of conflict anxiety (Weisstaub, 2006). A recent study by Anseloni showed that the reduced anxiety was associated with the increase in expression of 5-$HT_{2A}$ receptors in the rat brain (Anseloni, 2005). Since 5-$HT_{2A}$ receptors localised in the mesocortical regions such as the prefrontal cortex have been found to be important modulators of executive functions including memory

and cognitive flexibility, evidences shows that the blockage or activation of 5-$HT_{2A}$ receptors can obviously attenuate (Williams, 2002) or enhance (Buhot, 1997) the learning and working memory. In our study, the decreased 5-$HT_{2A}$ receptors were observed in some mesocortical regions like the prefrontal cortex and the cingulate cortex (Fig. 3). Although the rat brain resembles the human brain to a certain degree, it does not mean we may directly translate the results from animal data to the human situation. However, this animal data could provide pre-clinical evidence for clinical studies on human being situation. Considering Parkinson's disease obviously results in cognitive and memory impairment (Aarsland, 2008), the down-regulation of 5-$HT_{2A}$ receptors in the examined mesocortical regions has important clinical implications, and may at least partially explain the memory impairment and stress dysfunction.

In summary, the present study examines tyrosine hydroxylase immuno-staining in the striatum, 5-$HT_{2A}$ receptor binding sites and the distributions in the rat brains three weeks after 6-OHDA MFB lesion. 6-OHDA lesion significantly decreased tyrosine hydroxylase immunostaining in the striatum, and the levels of 5-$HT_{2A}$ receptor densities predominantly in the agranular insular cortex, the cingulate cortex, the prefrontal cortex, the primary somatosensory cortex, the caudate putamen compared to controls. The anatomical distributions of these alterations suggest that the changes of 5-$HT_{2A}$ receptors observed in this study among 6-OHDA PD rats have important clinical implications and this result may contribute, at least partially, to cognitive dysfunctions such as memory impairment among Parkinson's disease patients via serotonin receptors. The applications of agonists or antagonist of 5-HT2A receptors would be a good strategy in the improvement of cognitive impairment;

therefore the present study implicates 5-$HT_{2A}$ receptors as a novel therapeutic target in the treatment of Parkinson's disease.

**Acknowledgments**

This work was supported by initiating grant of the Third Affiliated Hospital of Sun Yat-Sen University, FRC small grants (University of Wollongong 2007/2008, Australia) and Australia National Health and Medical Research Council Grant (NHMRC, ID: 514640) from Q. W. The study utilized the Schizophrenia Research Institute funded beta imager. We would like to thank Dr. Mandy Reid for critical reading of this manuscript and Mr. Nikolce Mackovski for preparation on the 6-OHDA induced PD rat model.

**Reference:**


Aarsland D, Brønnick, K., Larsen, J.P., Tysnes, O.B., Alves, G.; For the Norwegian ParkWest Study Group. 2008. Cognitive impairment in incident, untreated Parkinson disease: The Norwegian ParkWest Study. Neurology.

Alex KD, Pehek, E.A. 2007. Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. Pharmacol Ther 113(2):296-320.

Alex KD, Yavanian, G.J., McFarlane, H.G., Pluto, C.P., Pehek, E.A. 2005. Modulation of dopamine release by striatal 5-HT2C receptors. Synapse 55(4):242-251.

Anastasia A, de Erausquin, G.A., Wojnacki, J., Mascó, D.H. 2007. Protection of dopaminergic neurons by electroconvulsive shock in an animal model of Parkinson's disease. J Neurochem 103(4):1542-1552.

Anseloni VC, He, F., Novikova, S.I., Turnbach Robbins, M., Lidow, I.A., Ennis, M., Lidow, M.S. 2005. Alterations in stress-associated behaviors and neurochemical markers in adult rats after neonatal short-lasting local inflammatory insult. Neuroscience 131(3):635-645.

Buhot MC. 1997. Serotonin receptors in cognitive behaviors. Curr Opin Neurobiol 7(2):243-254. Review.

Doder M, Rabiner, E.A., Turjanski, N., Lees, A.J., Brooks, D.J. 2003. Tremor in Parkinson's disease and serotonergic dysfunction: an [11]CWAY 100635 PET study. Neurology 60:601-605.

du Bois TM, Deng, C., Bell, W., Huang, X.F. 2006. Fatty acids differentially affect serotonin receptor and transporter binding in the rat brain. Neuroscience 139(4):1397-1403.

Goldman-Rakic PS. 1999. The "psychic" neuron of the cerebral cortex. Ann N Y Acad Sci 868:13-26.

Howard-Jones N. 1985. A CIOMS ethical code for animal experimentation. WHO Chron 39:51-56.

Huang XF, Tan, Y.Y., Huang, X., Wang, Q. 2007. Effect of chronic treatment with clozapine and haloperidol on 5-HT(2A and 2C) receptor mRNA expression in the rat brain. Neurosci Res 59(3):314-321.

Jellinger KA. 2001. The pathology of Parkinson's disease. Adv Neurol 86:55-72.

Matsumoto M, Yoshioka, M., Togashi, H., Ikeda, T., Saito, H. 1996. Functional regulation by dopamine receptors of serotonin release from the rat hippocampus: in vivo microdialysis study. Naunyn-Schmiedeberg's Arch Pharmac 353 621-629.

Mendlin A, Martín, F.J., Jacobs, B.L. 1999. Dopaminergic input is required for increases in serotonin output produced by behavioral activation: an in vivo microdialysis study in rat forebrain. Neuroscience 93(3):897-905.

Nocjar C, Roth, B.L., Pehek, E.A. 2002. Localization of 5-HT(2A) receptors on dopamine cells in subnuclei of the midbrain A10 cell group. Neuroscience 111(1):163-176.

Parsons L, H.,, Justice J, B. 1993. Perfusate serotonin increases extracellular dopamine in the nucleus accumbens of the rat as measured by in vivo microdialysis. Brain Res 606:195-199.

Paxinos G, Watson C. 1997. The rat brain in stereotaxic coordinates. Academic Press, San Diego.

Pehek EA, Nocjar, C., Roth, B.L., Byrd, T.A., Mabrouk, O.S. 2006. Evidence for the preferential involvement of 5-HT2A serotonin receptors in stress- and drug-

induced dopamine release in the rat medial prefrontal cortex. Neuropsychopharmacology 31(2):265-277.
Raymond JR, Mukhin, Y.V., Gelasco, A., Turner, J., Collinsworth, G., Gettys, T.W., Grewal, J.S., Garnovskaya, M.N. 2001. Multiplicity of mechanisms of serotonin receptor signal transduction. Pharmacol Ther 92(2-3):179-212.
Scholtissen B, Deumens, R., Leentjens, A.F., Schmitz, C., Blokland, A., Steinbusch, H.W., Prickaerts, J. 2006a. Functional investigations into the role of dopamine and serotonin in partial bilateral striatal 6-hydroxydopamine lesioned rats. Pharmacol Biochem Behav 83(2):175-185.
Scholtissen B, Verhey, F.R., Steinbusch, H.W., Leentjens, A.F. 2006b. Serotonergic mechanisms in Parkinson's disease: opposing results from preclinical and clinical data. J Neural Transm 113(1):59-73.
Tamaru F. 1997. Disturbances in higher function in Parkinson's disease. Eur Neurol 38(Suppl 2):33-36.
Wang Q, Huang X, F. 2008. Effects of chronic treatment of olanzapine and haloperidol on peptide YY binding desities in the rat brain. Exp Neurol 209(1):261-267.
Wang Q, Wang, P.H., McLachlan, C., Wong, P.T. 2005. Simvastatin reverses the downregulation of dopamine D1 and D2 receptor expression in the prefrontal cortex of 6-hydroxydopamine-induced Parkinsonian rats. Brain Res 1045(1-2):229-233.
Wang Q, Ying W. 2009. New Frontiers in Neurological Research. Research Signpost ISBN: 978-81-308-0284-8:19-52.
Wang Q, Zengin, A., Ying, W., Newell, K.A., Wang, P., Yeo, W., Wong, P.T., Yenari, M.A., Huang, X.F. 2008. Chronic treatment with simvastatin upregulates muscarinic M1/4 receptor binding in the rat brain. Neuroscience 154(3):1100-1106.
Weisstaub NV, Zhou, M., Lira, A., Lambe, E., González-Maeso, J., Hornung, J.P., Sibille, E., Underwood, M., Itohara, S., Dauer, W.T., Ansorge, M.S., Morelli, E., Mann, J.J., Toth, M., Aghajanian, G., Sealfon, S.C., Hen, R., Gingrich, J.A. 2006. Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. Science 313(5786):536-540.
West A, R., Galloway M, P. 1991. Regulation of serotonin facilitated dopamine release in in vivo: the role of protein kinase A activating transduction mechanism. Synapse 23:20-27.
Williams GV, Rao, S.G., Goldman-Rakic, P.S. 2002. The physiological role of 5-HT2A receptors in working memory. J Neurosci 22(7):2843-2854.
Yuan H, Sarre, S., Ebinger, G., Michotte, Y. 2005. Histological, behavioural and neurochemical evaluation of medial forebrain bundle and striatal 6-OHDA lesions as rat models of Parkinson's disease. J Neurosci Methods 144(1):35-45.
Zgaljardic DJ, Foldi, N.S., Borod, J.C. . 2004. Cognitive and behavioral dysfunction in Parkinson's disease: neurochemical and clinicopathological contributions. J Neural Transm 111(10-11):1287-1301.
Zhang X, Andren, P.E., Greengard, P., Svenningsson, P. 2008. Evidence for a role of the 5-HT1B receptor and its adaptor protein, p11, in L-DOPA treatment of an animal model of Parkinsonism. Proc Natl Acad Sci U S A 105(6):2163-2168.
Zhang X, Andren, P.E., Svenningsson, P. 2007. Changes on 5-HT2 receptor mRNAs in striatum and subthalamic nucleus in Parkinson's disease model. Physiol Behav 92(1-2):29-33.

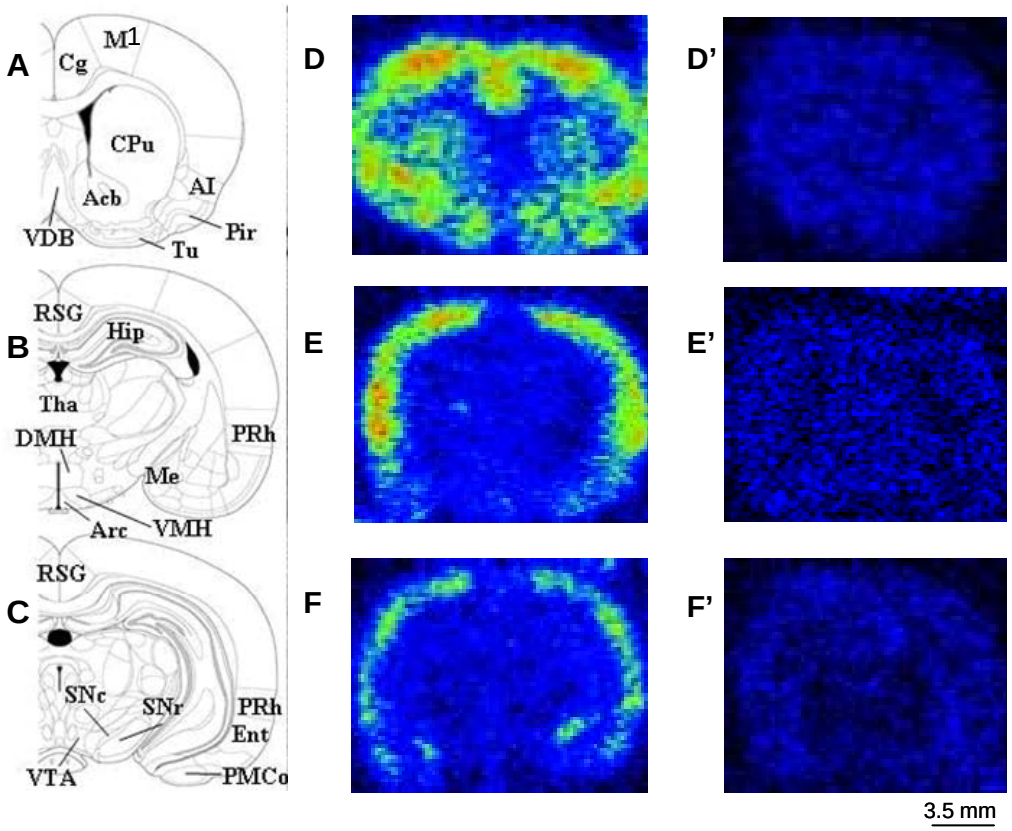



**Fig 1**. The maps of A, B and C are adopted from a rat brain atlas (Paxinos and Watson, 1997) indicating the levels where the [$^3$H]ketanserin binding density was measured. Autoradiographs (D, E, F) and (D', E', F') depict the expression of [$^3$H]ketanserin binding and non-specific [$^3$H]ketanserin binding at different rostro-caudal coronal levels of the rat brain. Abbreviations: Acb, accumbens nucleus; AI, agranular insular cortex; Arc, arcuate hypothalamic nucleus; Cg, cingulate cortex ; CPu, caudate putamen; DMH, dorsomedial hypothalamic nucleus; Ent, entorhinal cortex; Hip, hippocampus; M1, primary motor cortex; Me, medial amygdala; Pir, piriform cortex; PMCo, posteromedial cortical amygdala; PRh, perirhinal cortex; RSG, retrosplenial granular cortex; SNc, compact part of substantia nigra; SNr, reticular part of substantia nigra; Tha, thalamus; Tu, olfactory tubercle ; VDB, vertical limb of the diagonal band; VMH, ventromedial hypothalamus; VTA, ventral tegmental area.

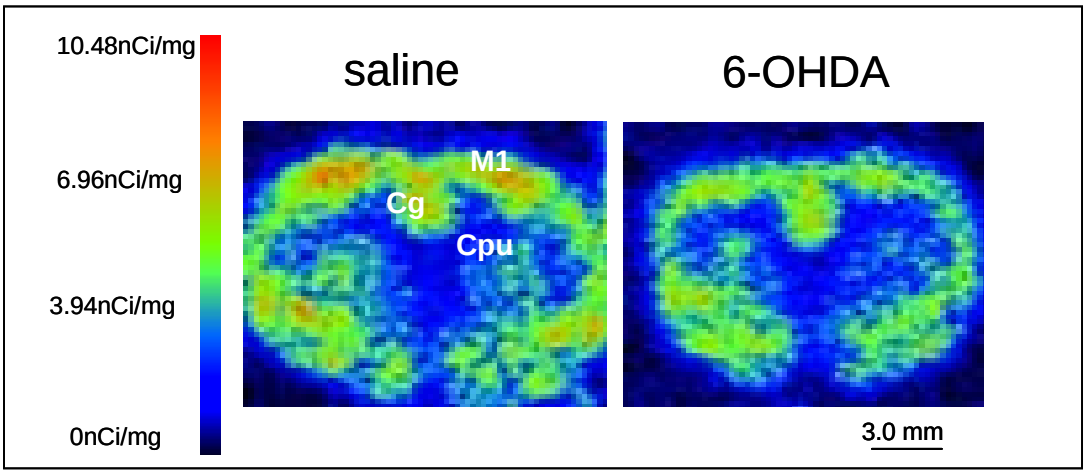



**Fig. 2.** Typical autoradiographs depict the expression of 5-$HT_{2A}$ receptors in the cingulate cortex (Cg), caudate putamen (Cpu) and primary motor cortex (M1) between 6-OHDA MFB lesioned rats and controls.

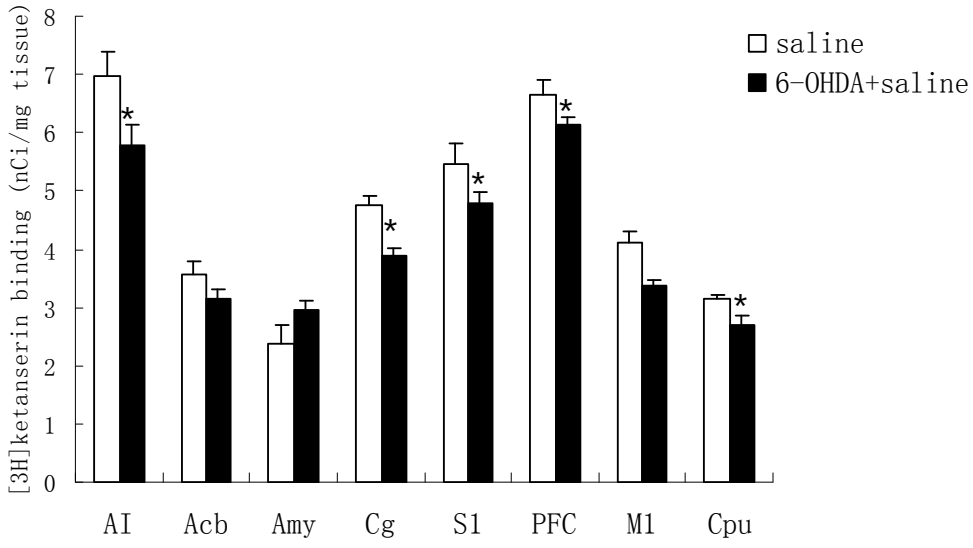
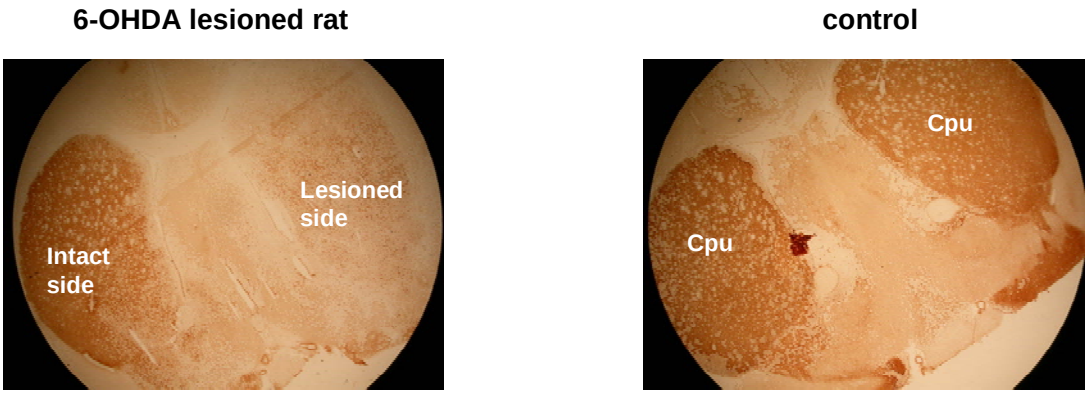
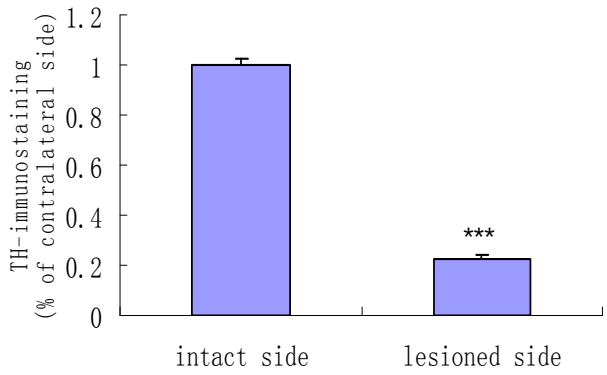



**Fig 3**. The effects of unilateral medial forebrain bundle lesion by 6-hydroxydopamine on [$^3$H] ketanserin binding in the rat brain regions. *Note*: Units of measurement are in nCi/mg tissue. Data shown are the mean ± SEM. Abbreviation: S1, primary somatosensory cortex; PFC, prefrontal cortex. For other abbreviation see Fig 1. Asterisks indicate significant differences between the 6-OHDA lesioned group and the control group (saline) (*$p$< 0.05; one-way ANOVA followed by independent *t*-test).




**(A)** **(B)**




**(C)**

**Fig 4.** Effects of 6-OHDA lesion on TH immunohistochemistry staining in the caudate putamen. The right side of the photo (A) shows the dopamine-depleted striatum side characterized by a significant lack of TH immunoreactive fibres, while the left side is the intact side. Photo (B) represents the striatum in the control group, in which the TH immunoreactive fibres are the same as in the intact side. (C) represents the density of TH-positive fibres relative to the contralateral (intact) side (Student *t*-test: $t = 15.696$, $^{***}p < 0.001$).