Glutamate Transporters in the Rat Basal Ganglia:
Localization and Modulations in Normal and Parkinsonian Rats

CHUNG Ka Yin

A thesis submitted in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy

Principal Supervisor: Prof. YUNG Kin Lam

Hong Kong Baptist University

April 2006
Abstract

Parkinson’s disease (PD) is a serious motor disorder in human and it is caused by a degeneration of dopaminergic neurons in the basal ganglia. One damaging effect after dopamine denervation is an overactivity of glutamate pathways in the brain and this is related to the onset of symptoms of Parkinson’s disease. Glutamate transmission is regulated by vesicular glutamate transporters (VGLUT1-T3) and excitatory amino acid transporters (EAATs: GLT1, GLAST and EAAC1). In normal rats, double labeling experiments revealed that pattern of distribution of VGLUT1 and VGLUT2 immunoreactivities was found to be primarily segregated in the neuropilar elements, whereas VGLUT3 immunoreactivity was found in the perikarya of different subpopulations of neurons in the basal ganglia. The present results indicate that VGLUT1 and T2 proteins are likely to be expressed by sub-groups of glutamatergic terminals that differ in origin. VGLUT3 is only found in subpopulations of neurons in the basal ganglia suggests possible roles of glutamate in these neurons. In 6-hydroxydopamine (6-OHDA)-lesioned rats, an animal model of Parkinson’s disease, immunoreactivity for GLT1 and GLAST was found to be decreased in the striatum of the lesioned side. Prominent decreases in GLT1 and GLAST protein expressions were also found. These findings indicate that excessive levels of extracellular glutamate after the onset of Parkinson’s disease may at least be due to a depletion of glial glutamate transporters, i.e., a deficit in re-cycling of glutamate as a neurotransmitter. In addition, immunoreactivity for EAAC1 and VGLUT3 was differentially modulated in the basal ganglia after 6-OHDA lesion. These results indicate that the expression of EAAC1 and VGLUT3 are related to change of activities of neurons in the basal ganglia after dopamine denervation. These findings may have important implications in the treatment of Parkinson’s disease. Last but not the least, GLT1 expression was found to be increased after the administration of an antibiotic ceftriaxone in normal and 6-OHDA lesioned rats. Immunoreactivity for GLT1 was found to be increased in the lesioned striatum compared to the non-lesioned side of the ceftriaxone-treated rats. Although, no amelioration in motor behavior was found, these results provide evidence that ceftriaxone is potent positive regulator for GLT1 expression. These findings may have potential clinical application in the treatment of Parkinson’s disease.
Table of Contents

Declaration ........................................................................................................... i
Abstract ............................................................................................................. ii
Acknowledgements ........................................................................................... iv
Table of Contents ............................................................................................. v
List of Tables ..................................................................................................... xxi
List of Figures .................................................................................................. xxiii
List of Abbreviations ......................................................................................... xxix

Chapter 1 Background and Literature Review ................................. 1

1.1 Glutamate ............................................................................................... 1
  1.1.1 Metabolism of Glutamate ................................................................. 1
  1.1.2 Glutamate Receptors ....................................................................... 2
  1.1.3 Glutamate Recycling at Axonal Terminals ................................. 3
  1.1.4 Glutamate as a Neurotoxin ........................................................... 5

1.2 Basal Ganglia ......................................................................................... 7
  1.2.1 Functional Anatomy of the Basal Ganglia ................................. 7
  1.2.2 Neurotransmitters in the Basal Ganglia ................................. 8
  1.2.3 Nuclei of the Basal Ganglia ..................................................... 9
    1.2.3.1 Striatum ................................................................. 9
    1.2.3.2 Globus Pallidus ..................................................... 12
    1.2.3.3 Subthalamic Nucleus .............................................. 13
    1.2.3.4 Substantia Nigra .................................................. 14
1.3 Basal Ganglia Circuitry in Parkinsonian State .......... 15
1.4 Parkinson’s Disease .................................................. 18
  1.4.1 Clinical Features of Parkinson’s Disease ................. 18
  1.4.2 Pathology of Parkinson’s Disease ......................... 19
  1.4.3 The Role of Astrocytes in Parkinson’s Disease ............ 20
  1.4.4 A Rat Model of PD: 6-Hydroxydopamine-Lesioned Rat
  1.5 Glutamate Transporters in Plasma Membrane (EAATs)
    1.5.1 Functional Properties of EAATs ......................... 23
    1.5.2 Expression of EAATs in Rat Brain ....................... 25
      1.5.2.1 Localization of Glial Glutamate Transporters
               (GLT1, GLAST) 25
      1.5.2.2 Localization of Neuronal Glutamate Transporter
               (EAAC1) 26
    1.5.3 Mechanism of Glutamate Uptake by EAATs ............... 28
    1.5.4 Physiological Functions of EAATs ....................... 29
1.6 Glutamate Transporters in Vesicular Plasma Membrane (VGLUTs)
    1.6.1 Functional Properties of VGLUTs ....................... 31
    1.6.2 Expression of VGLUTs in Rat Brain ..................... 32
      1.6.2.1 Vesicular Glutamate Transporter 1 (VGLUT1) 32
      1.6.2.2 Vesicular Glutamate Transporter 2 (VGLUT2) 33
      1.6.2.3 Vesicular Glutamate Transporter 3 (VGLUT3) 34
    1.6.3 VGLUTs and Neurological Disease ....................... 35
1.7 Objectives of the Thesis ........................................... 36
### 2. Materials and Methods

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Animals</td>
<td>38</td>
</tr>
<tr>
<td>2.2 Six-hydroxydopamine (6-OHDA) Lesion</td>
<td>39</td>
</tr>
<tr>
<td>2.2.1 Unilateral 6-hydroxydopamine Lesion</td>
<td>39</td>
</tr>
<tr>
<td>2.2.2 Rat Rotation Tests after 6-OHDA Lesion</td>
<td>39</td>
</tr>
<tr>
<td>2.3 Tissue Preparation</td>
<td>40</td>
</tr>
<tr>
<td>2.4 Immunocytochemistry</td>
<td>41</td>
</tr>
<tr>
<td>2.4.1 Immunocytochemistry</td>
<td>41</td>
</tr>
<tr>
<td>2.4.2 Single Immunofluorescence</td>
<td>41</td>
</tr>
<tr>
<td>2.4.3 EAATs Double Immunocytochemistry</td>
<td>43</td>
</tr>
<tr>
<td>2.4.4 VGLUTs Double Immunocytochemistry</td>
<td>44</td>
</tr>
<tr>
<td>2.4.5 Control of Immunofluorescence</td>
<td>44</td>
</tr>
<tr>
<td>2.5 Western Blot Analysis</td>
<td>45</td>
</tr>
<tr>
<td>2.6 Data Analysis</td>
<td>47</td>
</tr>
<tr>
<td>2.6.1 Proportions of Axonal Terminals in Striatum Displaying Immunolabeling for Each of the Antibodies to Vesicular Glutamate Transporters</td>
<td>47</td>
</tr>
<tr>
<td>2.6.2 Percentage of Co-localization of VGLUT3 and Specific Neuronal Markers in Basal Ganglia</td>
<td>47</td>
</tr>
<tr>
<td>2.6.3 Digital Image Analyses of Immunofluorescence and Statistics for Chapter 4 and 5</td>
<td>48</td>
</tr>
<tr>
<td>2.6.4 Semi-quantitative Analysis of Western Blot Results and Statistics</td>
<td>50</td>
</tr>
</tbody>
</table>

**Chapter 3 Comparative Localization of Vesicular Glutamate Transporters in the Rat Basal Ganglia**
3.1 Introduction ................................................................. 51
3.2 Objectives ................................................................. 54
3.3 Materials and Methods .................................................. 55
3.4 Results ................................................................. 56
  3.4.1 Specificity of Primary Antibodies ......................... 56
  3.4.2 Light Microscopic Observations – Immunohistochemical
       Detection of VGLUT1 and VGLUT2 in the Basal Ganglia
  3.4.3 Localization of VGLUT1 and VGLUT2 in the Striatum
  3.4.4 Co-localization of VGLUT1 and VGLUT2 in the Striatum
  3.4.5 Expression of VGLUT3 in a Subset of Striatal Interneuron
       but not Synaptic Vesicles Expressing VGLUT1 and
       VGLUT2 in Striatum
     3.4.5.1 Double Labeling for VGLUT3 and Either VGLUT1
           or VGLUT2
     3.4.5.2 Striatal Cholinergic Interneurons Displayed
           VGLUT3 Immunoreactivity
     3.4.5.3 Other Striatal Interneurons Display Less VGLUT3
           Immunoreactivity
  3.4.6 Expression of VGLUT3 in the Globus Pallidus (GP) ...... 63
     3.4.6.1 Pallidal Cholinergic Neurons also Displayed
           VGLUT3 Immunoreactivity
     3.4.6.2 Expression of VGLUT3 in Pallidal GABAergic
           Neurons and Axonal Terminals
  3.4.7 VGLUT3 is Expressed in Glutamatergic Neurons in the
       Subthalamic Nucleus
     3.4.7.1 Double Labeling for VGLUT3 and PV .................
3.4.7.2 Double Labeling for VGLUT3 and GluR1 .......... 65
3.4.7.3 Double Labeling for VGLUT3 and GluR2 .......... 65
3.4.8 VGLUT3 is Expressed in Dopaminergic Neurons and GABAergic Neurons in the Substantia Nigra
   3.4.8.1 Double Labeling for VGLUT3 and TH .............. 66
   3.4.8.2 Double Labeling for VGLUT3 and PV .............. 67
3.5 Discussion ...................................................... 68
   3.5.1 VGLUT1 and T2 are Likely to be Localized in Distinct Subsets of Glutamatergic Terminals in the Striatum
   3.5.2 VGLUT1 and T2 are Differentially Expressed in the Rat Basal Ganglia
   3.5.3 Physiological Roles for VGLUT1 and T2 at Excitatory Synapse in Basal Ganglia
   3.5.4 Localization of VGLUT3 in the Basal Ganglia Suggests Novel Roles for Glutamate Signaling in Non-glutamatergic Neurons
      3.5.4.1 Expression of VGLUT3 in Cholinergic Neurons .... 75
      3.5.4.2 Expression of VGLUT3 in GABAergic Neurons ..... 77
      3.5.4.3 VGLUT3 Immunoreactive Interneurons are Immunopositive for GluR1/2 Subtypes
      3.5.4.4 Expression of VGLUT3 in Dopaminergic Neurons 79
   3.5.5 The Physiological Significance for the Expression of VGLUT3 in Different Types of Neurons in the Basal Ganglia

Chapter 4 Changes in Expression of Neuronal and Glial Glutamate Transporters in 6-hydroxydopamine Lesioned Rat, an Animal Model of Parkinson’s Disease

4.1 Introduction ...................................................... 117
4.2 Objectives .............................................................. 120

4.3 Materials and Methods ........................................... 121

4.4 Results ................................................................. 122

4.4.1 Control for Immunocytochemistry ......................... 122

4.4.2 Localization of Glutamate Transporters in Normal Striatum

4.4.2.1 Localization of GLT1 (EAAT2) ......................... 122

4.4.2.2 Localization of GLAST (EAAT1) ....................... 123

4.4.2.3 Localization of EAAC1 (EAAT3) ....................... 124

4.4.3 Dopaminergic Neuronal Loss in SN after 6-OHDA Lesion

4.4.4 Changes in Expression of Glial Fibrillary Acidic Protein Immunoreactivity in 6-OHDA-lesioned Rat

4.4.5 Changes in Expression of GLT1, GLAST and EAAC1 in the Striatum of 6-OHDA-lesioned Rat

4.4.6 Changes in Expression of GLT1, GLAST and EAAC1 in the Globus Pallidus of 6-OHDA-lesioned Rat

4.4.7 Changes in Expression of GLT1, GLAST and EAAC1 in the Subthalamic Nucleus of 6-OHDA-lesioned Rat

4.4.8 Changes in Expression of GLT1, GLAST and EAAC1 in the Substantia Nigra of 6-OHDA-lesioned Rat

4.5 Discussion ............................................................. 132

4.5.1 Specificity of the Primary Antibodies ...................... 132

4.5.2 GLT1 and GLAST Immunoreactivity are Primarily Found in Astrocytes, whereas EAAC1 are Found in Subpopulation of Neurons in the Basal Ganglia
4.5.3 Significant Up-regulation of GFAP Immunoreactivity in the Striatum and Substantia Nigra of 6-OHDA-lesioned Rat

4.5.4 Changes in Glutamate Transporter Expressions in 6-OHDA-lesioned Rat

4.5.4.1 Down Regulation of Glial Glutamate Transporter GLT1 and GLAST, but not the Neuronal Glutamate Transporter EAAC1 in the Striatum after Dopamine Denervation

4.5.4.2 Glial Glutamate Transporters are not Involved in Cell Death in the Substantia Nigra

4.5.4.3 Modulation of the Neuronal Glutamate Transporter EAAC1 in Basal Ganglia after Dopamine Denervation

4.5.5 Modulations of Glial Glutamate Transporter Expressions as a Potential Treatment of PD

Chapter 5 Changes in Expression of Vesicular Glutamate Transporters VGLUTs in 6-OHDA Lesioned Rat, an Animal Model of Parkinson’s Disease

5.1 Introduction ......................................................... 183

5.2 Objectives ............................................................ 187

5.3 Materials and Methods ............................................ 188

5.4 Results ................................................................. 189

5.4.1 Antibody Specificity ............................................. 189

5.4.2 Striatal Expression of VGLUT1, VGLUT2 and VGLUT3 Immunoreactivity after 6-OHDA Lesion

5.4.2.1 Expression of VGLUT1 Immunoreactivity in Striatum after 6-OHDA Lesion

5.4.2.2 Expression of VGLUT2 Immunoreactivity in 191
Striatum after 6-OHDA Lesion

5.4.2.3 Expression of VGLUT3 Immunoreactivity in Striatum after 6-OHDA Lesion

5.4.3 Expression of VGLUT1, VGLUT2 and VGLUT3 Protein in Striatum after 6-OHDA Lesion

5.4.4 VGLUT2 and VGLUT3 Expression in the Globus Pallidus after 6-OHDA Lesion

5.4.4.1 Expression of VGLUT2 in the Globus Pallidus after 6-OHDA Lesion

5.4.4.2 Down Regulation of VGLUT3 Expression in the Globus Pallidus after 6-OHDA Lesion

5.4.5 VGLUT2 and VGLUT3 Expression in the Subthalamic Nucleus after 6-OHDA Lesion

5.4.5.1 VGLUT2 Expression in the Subthalamic Nucleus after 6-OHDA Lesion

5.4.5.2 VGLUT3 Expression in the Subthalamic Nucleus after 6-OHDA Lesion

5.4.6 VGLUT2 and VGLUT3 Expression in the Substantia Nigra after 6-OHDA Lesion

5.4.6.1 Expression of VGLUT2 in SN after 6-OHDA Lesion

5.4.6.2 Up-regulation of VGLUT3 Expression in SNr after 6-OHDA Lesion

5.4.7 Expression of VGLUT2 and VGLUT3 Protein in Substantia Nigra after 6-OHDA Lesion

5.5 Discussion .........................................................

5.5.1 Dopamine Denervation does not Alter the Expression of VGLUTs in the Striatum

5.5.1.1 Expression of VGLUT1 and VGLUT2 in Striatum after Dopamine Denervation
5.5.1.2 VGLUT3 Expression is not Modulated in the Striatum after Dopamine Denervation 202

5.5.2 VGLUT2 Expression is not Modulated in Other Basal Ganglia Structures after 6-OHDA Lesion 203

5.5.3 VGLUT3 Expression is Differentially Modulated in Other Basal Ganglia Structures after 6-OHDA Lesion 205

5.5.4 Functional Implications of the Roles of VGLUTs in PD 208

Chapter 6 Up-regulation of GLT1 by Antibiotic Ceftriaxone in 6-OHDA Rats: A Potential Treatment Strategy for Parkinson’s Disease 232

6.1 Introduction ................................................................. 232

6.2 Objectives ................................................................. 235

6.3 Materials and Methods ................................................. 236

6.4 Results ................................................................. 237

6.4.1 Expression of GLT1 after Ceftriaxone Treatment .......... 237

6.4.2 Rat Rotation and Cell Loss of Dopaminergic Neurons after Ceftriaxone Treatment in 6-OHDA-lesioned Rats 238

6.4.3 Expression of GLT1 after Ceftriaxone Treatment in 6-OHDA Lesioned Rats 239

6.4.3.1 Immunoreactivity for GLT1 in the Striatum and Substantia Nigra 239

6.4.3.2 GLT1 Protein Levels in the Striatum and Substantia Nigra 240

6.5 Discussion ................................................................. 241

Chapter 7 VGLUTs and EAATs are Differentially Modulated in Rat Basal Ganglia after 6-OHDA Lesion: An Implication on Parkinson’s Disease Pathogenesis 262
List of References ................................................................. 267
Appendix I ................................................................. 289
Appendix II ................................................................. 291
Appendix III ................................................................. 292
Curriculum Vitae ........................................................... 295