Parkinson’s disease was first described by James Parkinson in a monograph published in 1817 (Parkinson, 1817). It is a degenerative neurological condition and the major neurological manifestations of this disease are: tremor, rigidity, bradykinesia (slowness of movement) and postural instability. PD primarily affects people over the age of 50 years and prevalence and incidence rates increase with age. Therefore, aging of the general population is likely to result in a dramatic increase in the number of people diagnosed with PD. One study projected that by the year 2030, the number of people over the age of 50 and consequently the number of persons with PD will double, resulting in an estimated 9 million persons with PD worldwide (Dorsey et al., 2007; Pahwa & Lyons, 2010). Such an increase will place a significant burden on healthcare systems and caregivers given the progressive nature of PD, associated disability and significant caregiving required in the later stages of the disease. With the expected increase in PD prevalence, it can be anticipated that the disease will continue to exact a significant direct and indirect economic cost.

The primary pathology of PD is the degeneration of dopaminergic neurons in the SNpc with subsequent depletion of nigrostriatal DA and the development of Lewy bodies, proteinaceous intracytoplasmic inclusions (Forno, 1996; Choi et al., 2011). Recent studies have shown that PD is also associated with extensive non-dopaminergic pathology involving noradrenergic neurons in the locus coeruleus, cholinergic neurons in the nucleus basalis of Meynert, serotonergic neurons in the midline raphe and neurons of the autonomic nervous system. Braak et al (2003) has demonstrated that the pathological changes in PD occur in a relatively predictable, topographically distinct sequence of events beginning with the olfactory structures and medulla oblongata, spreading to the SN and eventually affecting neocortical structures. A great deal of the brain, especially the regions beneath the cortex, is heavily involved with movement regulation. Such areas include the connected set of
basal ganglia, portions of the thalamus and the cerebellum (Schiff, 2010). In PD, there is degeneration of neurons that use DA as a neurotransmitter, which have their cell bodies in the SN at the upper edge of the midbrain. The decrease in neural output from the SN causes a disturbance in the network balance of excitation and inhibition. The result is a net increase in inhibition from the globus pallidus internus (GPI) to thalamus (Obeso et al., 2008).

Pathology, aetiology and pathogenesis

The hallmark of PD is the cell loss within the SN particularly affecting the ventral component of the pars compacta. By the time of death, this region of the brain has lost 50–70% of its neurons compared with the same region in unaffected individuals. The earliest documented pathological changes in PD (Braak et al., 2006) have been observed in the medulla oblongata/pontine tegmentum and olfactory bulb. In these early stages Braak stages 1 and 2 patients are pre-symptomatic. As the disease advances Braak stages 3 and 4 the SN, areas of the midbrain and basal forebrain become involved. Finally, the pathological changes appear in the neocortex. This pathological staging is based on the distribution of lewy bodies. Lewy bodies are the pathological hallmark of PD. They are α-synuclein-immunoreactive inclusions made up of a number of neurofilament proteins together with proteins responsible for proteolysis. These include ubiquitin, a heat shock protein which plays an important role in targeting other proteins for breakdown. Mutations in the α-synuclein gene are responsible for some familial forms of PD in which lewy bodies are also seen. Mutations in the parkin protein produce a Parkinsonian syndrome without lewy bodies in juvenile cases suggesting that the parkin protein plays an important role in the development of the lewy body. It has been shown that parkin facilitates the binding of ubiquitin (ubiquination) to other proteins such as the α-synuclein interacting protein synphilin-1 leading to the formation of lewy bodies (Chung et al., 2001). Lewy bodies
are found in PD and Dementia with lewy bodies (DLB), but are not a pathological hallmark of any other neurodegenerative disease.

Identifying environmental factors that predispose to the development of PD has proved elusive. Living in a rural environment appears to confer an increased risk of PD, and perhaps causally linked to this some but not all epidemiological studies have shown a correlation between exposure to pesticide use and wood preservatives (Dick, 2006). Despite intensive research efforts during recent years, fundamental questions regarding the etiology and pathogenesis of the disease are still unresolved (Beal, 1995; Calne & Takahashi, 1991; Youdim & Riederer, 1997). With the progression of the disease, there are a number of non-motor complications in PD like sleep disorders (Frucht et al., 1999; Vendette, et al., 2007), cognitive impairment (Emre et al., 2004; Ravina et al., 2005), dementia (McKeith 2005; McKeith 2007), Mood disturbance (Richard et al., 2004), Psychosis and confusion (Naimark et al., 1996) that are often seen. In many cases, these are not directly related to involvement of dopaminergic pathways and therefore develop even in patients where motor symptoms are well controlled.

**Problems associated with current treatments of Parkinson’s disease**

The understanding of DA receptor function has expanded enormously since the recognition of their existence in brain and the realization of their importance in PD in the early 1970s. But for patients with PD, the pharmacological treatment options have not really changed since then. Recent years have witnessed a number of choices for the therapy of PD. At this time, no therapy has been firmly established to have a neuroprotective role. Preliminary data suggest that high doses (at least 1200 mg/day) of Coenzyme Q10 is associated with slower deterioration of motor disability (Shults et al., 2002), but this finding awaits further confirmation. The propargylamine MAO-B inhibitor, rasagiline was recently reported to have a modest symptomatic effect in patients with early Parkinson’s (Parkinson Study Group, 2002), but whether it has an
effect on rate of progression has not been established. A trial of the sodium-dependent glutamate release inhibitor riluzole was terminated early based on futility analysis and trials of antiapoptotic agents, including non-MAO-inhibitor propargylamines and jun kinase inhibitors, are still under way. Although very preliminary results have suggested symptomatic benefit from glial cell line-derived neurotrophic factor (GDNF) (Gill et al., 2003), there is to date no evidence that GDNF or other trophic factors that interfere with disease progression. Thus, for the time being, the pharmacological management of PD is based entirely on symptom control and is in general instituted only when justified by disability.

Since the introduction of L-3,4-dihydroxyphenyalanine (L-DOPA) to treat PD over 40 years ago, numerous studies have examined the status of DA receptors in brain in an attempt to understand the mechanisms that underlie the decline in the efficacy of L-DOPA and the increase in the adverse effects of L-DOPA treatment (Péchevis et al., 2005, Hollingworth et al., 2011). In the early stages of PD treatment with L-DOPA or/and DA receptor agonists provides effective relief from the motor symptoms. After 4–6 years of treatment, 40% of patients experience motor side effects. The motor side effects increase with time so that following 10 years of L-DOPA and/or DA agonist treatment most individuals (95% in some studies) will exhibit some treatment-induced motor complications (Ahlskog & Muentner, 2001). In addition to the systemic side effects (nausea, vomiting and postural hypotension) produced by acute treatment with L-DOPA and DA agonists, chronic administration can result in the development of more serious adverse effects, namely, fluctuations in motor control (end of dose deterioration, on–off phenomenon) and dyskinesias (chorea, dystonia, athetosis). The debilitating motor side effects are compounded by treatment induced psychiatric disturbances such as, psychosis, mania or delirium (Schrag, 2004). Motor side effects were caused by alterations in DA receptor expression due to progression of the disease process and/or adaptive responses to the
drug treatment (Crossman, 1990). The psychotic effects presumably stop from actions on DA receptors in limbic or cortical regions of the brain. The consequence of these motor complications is that the dose of L-DOPA has to be reduced to levels which do not provide the desired reversal of Parkinsonian symptoms.

**The Unilateral 6-Hydroxydopamine Lesion Model**

All commonly accepted models of PD, like the actual disease itself, are thought to involve oxidative processes at the heart of dopaminergic injury. Examples of such models include neuronal death induced by exposure to the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydroxypyridine (MPTP) (Cadet & Brannock, 1998), methamphetamine (O'Dell et al., 1991) and 6-OHDA. The DA analog 6-OHDA, because of its similarity in molecular structure, can be taken up into dopaminergic terminals through the DA transporter. Once inside the cell, it is metabolized, resulting in the production of hydrogen peroxide and free radicals. Ultimately these toxic molecules induce neuronal death through mitochondrial dysfunction.

Like DA itself, 6-OHDA is not able to cross the blood–brain barrier and therefore must be delivered directly to the brain of experimental animals through stereotaxic surgery. In 1968, Ungerstedt and colleagues demonstrated the utility of 6-OHDA lesions as animal models of PD. In their study, 6-OHDA was unilaterally injected into the medial forebrain bundle, extensively depleting the nigrostriatal pathway on one side. Ungerstedt noted that lesioned animals rotated toward the side of their lesions spontaneously as well as after administration of the dopaminergic drug d-amphetamine. Conversely, apomorphine, a drug that acts upon up regulated DA receptors on the side ipsilateral to the lesion, induces rotations contralateral to the lesion. The number of rotations performed by a lesioned animal can be quantified to serve as an index of the integrity of nigrostriatal function. Experimental therapeutic strategies, such as neural or stem cell transplantation and gene therapy, can use the number of rotations an animal performs as an index of the intervention’s efficacy.
Using this model, neuronal loss is detected as soon as 12 h postinjection and peaks at 48 h. In addition, striatal fibers are found to degenerate between 1 and 7 days after 6-OHDA delivery, ultimately resulting in more than 90% striatal DA depletion. This provides an ideal environment to evaluate cellular replacement strategies. Alternatively, 6-OHDA delivery to the striatum can result in levels of DA depletion more representative of early-stage PD. Kirik et al., (2001) demonstrated the location of striatal injections, either ‘terminal’ (within the caudate–putamen) or ‘preterminal’ (at the caudate–putamen boundary), greatly affected the resulting lesion, with preterminal injections creating greater levels of DA depletion. In addition, they found variable reductions in tyrosine hydroxylase-positive (TH+) fiber densities and TH+ SN neurons after either single or multiple 6-OHDA intrastriatal injections.

Role of neurotransmitters in PD

Dopamine

DA is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake and endocrine regulation. This catecholamine also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function and gastrointestinal motility (Missale et al., 1998). DA containing neurons arise mainly from DA cell bodies in the SN and ventral tegmental area in mid-brain region (Carlsson, 1993; Lookingland et al., 1995; Tepper et. al., 1997; Tarazi et al., 1997 a, b, 1998, 2001). Dopaminergic system is organized into four major subsystems (i) the nigrostriatal system involving neurons projecting from the SNpc to the caudate-putamen of the basal ganglia. This is the major DA system in the brain as it accounts for about 70% of the total DA in the brain and its degeneration makes a major contribution to the pathophysiology of PD; (ii) the mesolimbic system that originates in the midbrain tegmentum and projects to the nucleus accumbens septi.
and lateral septal nuclei of the basal forebrain as well as the amygdala, hippocampus and the entorhinal cortex. They are all considered components of the limbic system and hence of particular interest for the pathophysiology of idiopathic psychiatric disorders; (iii) the mesocortical system, which also arises from neuronal cell bodies in the tegmentum which project their axons to the cerebral cortex, particularly the medial prefrontal regions; (iv) the tuberinfundibular pathway, which is a neuroendocrinological pathway arising from the arcuate and other nuclei of the hypothalamus and ending in the median eminence of the inferior hypothalamus.

There are 5 types of DA receptor, which can be subdivided into DA D₁-like (D₁, D₅) and D₂-like (D₂, D₃, D₄), based on their sequence homologies, pharmacology and functional properties (Sokoloff & Schwartz, 1995). In the striatum, DA D₁ and D₂ receptors are mainly present on dendrites of GABAergic striatopallidal neurons which receive input from afferent DA neurons. DA D₁ receptors are also found on the terminals of glutamatergic projections from the cortex and thalamus. Expression of each receptor subtype is enriched on subpopulations of striatopallidal neurons. DA D₁ receptors are more highly expressed on GABAergic neurons which innervate the internal segment of the globus pallidus and SNpr (the direct pathway) and co-localize with substance P and dynorphin, while DA D₂ receptors have higher levels of expression on GABAergic neurons which innervate the external segment of the globus pallidus (the indirect pathway) and co-localize with enkephalin (Gerfen et al., 1995; Le Moine & Bloch, 1995; Aubert et al., 2000). However, there is a degree of overlap, with co-expression of each receptor subtype on most striatal GABAergic neurons, such that the division of striatal neurons should be based on the relative levels of DA D₁ or D₂ receptors, rather than the presence or absence of a particular receptor subtype (Surmeier et al., 1993; Aizman et al., 2000). DA D₂ receptors are also present on the terminals of DA neurons and therefore also function as autoreceptors. Cholinergic interneurons express DA D₂ receptor mRNA, indicating that a proportion of DA D₂
receptors found in the striatum is present on these neurons. DA D₃ receptors have a similar distribution to DA D₂ receptors, except that their density is very low in the CN and putamen, with higher levels only found in the islands of Calleja and ventral areas of the striatum. DA D₃ receptors co-localize with either DA D₁ or D₂ receptors in up to a quarter of ventral striatal neurons (Le Moine & Bloch, 1996). DA D₅ receptors, like the DA D₃ receptor, are found at highest densities in the ventral striatum, but unlike the DA D₂ and D₃ receptors, they are not located on DArgic neuron terminals, but are found on cholinergic interneurons (Bergson et al., 1995). DA D₄ receptors have a very low level of expression in the striatum. The significance of DA D₄ and D₅ receptors in the symptoms or treatment of PD is unknown.

The function of DA receptors in PD is altered not only by the disease but also as a consequence of drug treatment. Alterations in the abundance of receptor density contribute to the complications of treatment. But, for the DA D₂ receptor in particular, there is no temporal correlation between the alterations in expression levels and the occurrence of motor complications of treatment. It is increasingly recognised however that DA receptor signaling cascades are altered both as a consequence of the denervation occurring in PD and as a result of the dopaminergic drug treatment used to treat the disorder. The functional response of DA receptors can therefore change despite no alteration in their expression level by virtue of changes in their coupling to second messengers. Before the cloning and definitive demonstration of 5 DA receptor subtypes, DA D₁ receptors were defined as being positively linked to adenylate cyclase, while DA D₂ receptors had negative coupling to the enzyme (Kebabian & Calne, 1979). The number of signaling cascades that DA receptors are known to interact with has grown considerably since then and has been extensively reviewed by Neve et al. (2004). The majority of data was derived from studies using transfected cells or animal models since the experimental techniques can not be used in post-mortem human tissue or living human subjects. However, it is reasonable to assume
that DA receptors couple to a similar repertoire of second messengers in human brain. At the molecular level DA receptors can have opposing actions, even though the final cellular response is similar. For example, in cell lines, arachidonate release was increased by both the DA D\textsubscript{2} and D\textsubscript{4} receptor receptor subtypes, but required activation of protein kinase A for the DA D\textsubscript{2} receptor and protein kinase C for the DA D\textsubscript{4} receptor (Di Marzo \textit{et al.}, 1993; Chio \textit{et al.}, 1994; Lee \textit{et al.}, 2004). It has also recently been demonstrated that different DA receptor subtypes (i.e. D\textsubscript{1} and D\textsubscript{2}) can form hetero-oligomers in cells and can cross phosphorylate each other (Lee \textit{et al.}, 2004; So \textit{et al.}, 2005). This means a DA D\textsubscript{1} receptor agonist can elicit a DA D\textsubscript{2} receptor-mediated cellular response and vice versa. Evidently, caution must be observed when extrapolating data derived from such studies to how the native receptors function in brain. But such interactions at the molecular level explain the synergy found between, for example, DA D\textsubscript{1} and D\textsubscript{3} receptors and the dysfunction of such interactions observed in animal models of PD (Ridray \textit{et al.}, 1998; Guigoni \textit{et al.}, 2005).

Receptor supersensitivity, leading to imbalance between the direct and indirect striatal output pathways, is believed to underlie some of the motor complications that occur following chronic treatment with L-DOPA or DA agonists (Obeso \textit{et al.}, 2000). DA D\textsubscript{2} receptor mediated effects in PD and animal models of the disorder can be explained, at least in part, by the increase in receptor DA D\textsubscript{2} receptor density which occurs following dopaminergic denervation of the striatum. In the absence of consistent alterations in the levels of receptor expression, altered functional responses of DA D\textsubscript{1} receptors results from changes in signaling mechanisms. DA receptors present on DA neuron perikarya and dopaminergic projections to areas other than the striatum are also affected by the neurodegeneration which occurs in PD. Also, chronic stimulation of extrastriatal DA receptors by L-DOPA-derived DA or dopaminergic drugs alters extrastriatal DA receptor expression (Hurley & Jenner, 2006).
Acetylcholine

Acetylcholine (ACh) is one of the principal neurotransmitters of the parasympathetic system. Extensive evidence supports the view that cholinergic mechanisms modulate learning and memory formation. Evidence for cholinergic regulation of multiple memory systems, noting that manipulations of cholinergic functions in many neural systems enhance or impair memory for tasks generally associated with those neural systems. The magnitude of ACh release in different neural systems regulates the relative contributions of these systems to learning. ACh is the neurotransmitter that is released by stimulation of the vagus nerve, which alters heart muscle contractions. It is important for the movement of other muscles as well. ACh induces movement by the locomotion of an impulse across a nerve that causes it to release neurotransmitter molecules onto the surface of the neighbouring cell. ACh is critical for an adequately functioning memory. Studies of ACh release, obtained with in vivo microdialysis samples during training, together with direct injections of cholinergic drugs into different neural systems, provide evidence that release of ACh is important in engaging these systems during learning and the extent to which the systems are engaged is associated with individual differences in learning and memory (Gold, 2003). Acetylcholine influences striatal DA release predominantly through an action at nicotinic acetylcholine receptors (nAChRs) (Exley et al., 2008), and also muscarinic receptors to a lesser extent (Grilli et al., 2008). These interactions of acetylcholine at the cellular level most likely have important behavioural consequences. Extensive work shows that nicotine, which acts at nAChRs, protects against nigrostriatal damage (Picciotto et al., 2008). In addition, recent studies demonstrate that nicotine administration reduces a major side effect of L-dopa, the primary treatment for PD (Bordia et al., 2008).
Epinephrine and Norepinephrine

Nondopaminergic mechanisms are also responsible for some of the sensory symptoms in patients with PD. In PD, the level of NE is reduced in the locus ceruleus (Zweig et al., 1993) and this is associated with a loss of pigmented neurons and the formation of Lewy body inclusions. Moreover, NE concentrations in the neocortex, nucleus accumbens, amygdala and hippocampus are 40% to 70% lower than normal. In limbic regions, the level of the major metabolite of NE, 3-methoxy-4-hydroxyphenylglycol (MHPG), is also reduced (Riederer et al., 1977) and depressive features commonly observed in PD patients is related to a central NE deficiency (Mayeaux et al., 1984). These changes taken together suggest that the dorsal NE system degenerates in PD. Noradrenergic projections from the locus coeruleus to the dorsal horn of the spinal cord, along with direct and indirect noradrenergic fibers from A5/A7 groups in the pontine tegmentum, reportedly inhibit ascending nociceptive pathways (Buzas & Max, 2004). There appears to be changes in both central and peripheral adrenergic receptors in PD. Studies have shown that α-2 receptors are decreased in number in the cerebral cortex (Cash et al., 1984). Other studies have noted a decrease in α-2 adrenoceptors (A2A) and decreased yohimbine-binding sites in platelets of untreated PD patients (Villeneuve et al., 1985). Bernal and coworkers (Bernal et al., 1989) suggest that untreated PD is associated with a significant reduction in A2A sensitivity. It is possible that patients with PD are more vulnerable to panic attacks because they have an alteration of A2A receptors. A2A receptor is thought to result in a decrease in the stimulation of GABA-enkephalin output neurons by striatal cholinergic interneurons and an increase in the GABA-mediated recurrent inhibition of these neurons. Antagonist activity at adenosine A2A receptors in the striatum might effectively compensate for the lack of DA-mediated inhibition of these neurons in PD (Richardson et al., 1997).
Serotonin

The 5-HT systems are widespread throughout the brain, with most of the cell bodies of serotonergic neurons located in the raphe nuclei of the midline brain stem (Palacios et al., 1990). The largest collections of 5-HT neurons are in the dorsal and median raphe nuclei of the caudal midbrain (Jacobs & Azmitia, 1992). The neurons of these nuclei project widely over the thalamus, hypothalamus, basal ganglia, basal forebrain and the entire neocortex. Interestingly, these 5-HT neurons also provide a dense subependymal plexus throughout the lateral and third ventricles. Activation of this innervations result in 5-HT release into the cerebrospinal fluid (CSF) and measurement of 5-HT content in CSF in disease states will largely reflect this pool (Chan-Palay, 1976).

Over the past four decades there have been numerous reports describing the involvement of serotonergic and dopaminergic systems in the mechanism of action of antiparkinsonian agents. Recent advances in our understanding of 5-HT receptor subtypes and their putative role in the control of movement suggest possible novel intervention strategies for modulating dopaminergic and non-dopaminergic systems in PD patients (Thomas, 2004). Post-mortem analysis reveals reductions in the number of 5-HT$_1$ and/or 5-HT$_2$ brain binding sites in patients having suffered from various neurodegenerative disorders including PD (Cross, 1988). Based on the distribution, localization and function in the basal ganglia, 5-HT$_{1B/D}$ and 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors are clearly linked with modulation of the nigrostriatal pathway (Barnes et al., 1999). Serotonergic terminals have been reported to make synaptic contacts with both DA-containing and non-DA containing GABA interneurones in the SNpc, substantia nigra pars reticulata (SNpr), striatum and ventral tegmental area (VTA) (Herve et al. 1987; Moukhels et al. 1997; Di Matteo et al., 2001). These brain areas contain the highest concentration of 5-HT, with the SNr receiving the greatest input. Raphe’ projections also innervate terminal areas to which the SNc and VTA project.
to, the striatum and nucleus accumbens (Azmita & Segal, 1978). Therefore, receptor subtype-specific serotonergic drugs can act at several sites within the extrapyramidal system to modify DA activity.

Advances in the production of DA neurons from stem or precursor cells for transplantation in PD patients have clearly established an intimacy between 5-HT-DA cells, to the extent that elimination of 5-HT cells induces a marked increase in the generation of DA neurones from mesencephalic precursors cells (Rodriguez-Pallares et al. 2003). Thus, scientific rationale strongly suggests that therapeutic strategies that target 5-HT-dopaminergic systems, such as drugs acting on 5-HT transporters, 5-HT1A, 5-HT1B/D and 5-HT2C receptor subtypes, can fulfill the medical need for the symptomatic treatment of PD and the motor fluctuations associated with long-term L-Dopa therapy (Barnes et al., 1999; Jones & Blackburn, 2002). Realistically, only with the use of these selective serotonergic agents will be able to unravel the complex monoaminergic circuitry in the basal ganglia and relate this to the pathophysiology of PD and drug-induced dyskinesias.

**Serotonin as co-mitogen**

In rats, 5-HT neurons in the brain stem raphe are among the first neurons to differentiate in the brain and play a key role in regulating neurogenesis (Kligman & Marshak, 1985). Lauder and Krebs (1978) reported that parachlorophenylalanine (PCPA), a 5-HT synthesis inhibitor, retarded neuronal maturation, while mild stress, a releaser of hormones, accelerated neuronal differentiation. These workers defined differentiation as the cessation of cell division measured by incorporation of $^{3}$H-thymidine. Since then, many other workers have shown a role for 5-HT in neuronal differentiation (Marois & Croll, 1992; Hernandez, 1994). The effects of 5-HT on morphology have long been known. For more than 50 years, 5-HT has been known to constrict blood vessels (indeed, this is the origin of the name) (Page, 1968) and induce shape changes in skeletal muscle (at both the light and electron microscope level)
(O’Steen, 1967), platelets (Leven et al., 1983), endothelial cells (Welles et al., 1985), and fibroblast (Boswell et al., 1992). In the periphery, 5-HT originates largely from mast cells, which can produce, release and re-uptake 5-HT. The released 5-HT, then act as a chemotactic, increase vascular permeability, vasodilatation, and smooth muscle spasm (Metcalfe et al., 1981). In addition to its role in morphological changes, 5-HT also has been shown to play a role in cell proliferation. In cultured rat pulmonary artery smooth muscle cells (SMC), 5-HT induces DNA synthesis and potentiates the mitogenic effect of platelet-derived growth factor-BB (Eddahibi et al., 1999). 5-HT effects on cell proliferation are involved with phosphorylation of GTPase-activating protein (GAP), an intermediate signal in 5-HT-induced mitogenesis of SMC (Lee et al., 1997). Earlier studies of from our laboratory showed that 5HT acting through specific receptor subtypes 5HT2 (Sudha & Paulose, 1998) control cell proliferation and act as co-mitogens. Thus, there is evidence that 5-HT is involved in a variety of cellular processes involved in regulating metabolism, proliferation and morphology.

**GABA**

The inputs to the basal ganglia portion of the motor circuit are focused principally on the putamen, whereas the caudate nucleus (CN) and the nucleus accumbens are the principal input sites of the limbic circuit depicts a simplified scheme of the 'motor circuit' (Albin et al., 1989). This postulates that in the normal brain there exists a balance between direct inhibitory input (GABA, co-localised with substance P) and indirect excitatory input (aspartate/glutamate) to the medial globus pallidus (GPm), which in turn controls thalamocortical activation. The deprivation of DA-ergic nigrostriatal input, as in PD, reduces the positive feedback through the direct system, and increases the negative feedback through the indirect system (Gerlach et al., 1996). The critical consequences are an overactivity of the
subthalamic nucleus (STN), GPm and SNpr, with the resulting inhibition of thalamocortical drive.

Because specific neural pathways are involved, one might propose that degeneration of nigrostriatal neurons in patients who had manifested a Parkinsonian syndrome causes a characteristic pathologic pattern of neurotransmission in the motor circuit. In fact, electrophysiological, neurochemical and pharmacological studies using experimental models of Parkinsonism have shown a secondary increase of glutamatergic neurotransmission in the STN, the GPm and the SNr, due to a decreased GABAergic input from the lateral GPi (Ossowska, 1994). On the other hand, these assumptions have been confirmed by studies using post mortem tissue. For example, Griffiths et al. (Griffiths et al., 1990) found decreased binding of flunitrazepam (a ligand to the GABA/benzodiazepine receptor) in the GPi of PD brains. Assuming that there is a simple relationship between increased pre-synaptic neural activity and post-synaptic receptor down-regulation and vice verse as in peripheral tissues, these data suggest that the GABA-ergic striatal neurons projecting to the GPi would be overactive in PD (Gerlach et al., 1996). As GABA helps "quiet" excessive neuronal firing and has been deficient in patients in the advanced stages of PD. So directly targeting GABA production rather than DA replacement is more effective way of improving brain function in late-stage PD, this also avoids the known therapeutic limitations and complications associated with the over-production of DA. GABA supplementation can help to decrease the over stimulation of neurotransmitters such as acetylcholine and can possibly be used in Parkinson's help to inhibit acetylcholine.

**GABA as co-mitogen**

GABA, the main inhibitory neurotransmitter in the mature CNS, was recently implicated in playing a complex role during neurogenesis (Ben-Ari et al., 1989; Baher et al., 1996; Behar et al., 2000; Haydar et al., 2000). Through embryonic development, GABA was demonstrated as acting as a chemo-attractant and being
involved in the regulation of progenitor cell proliferation. For example, GABA induces migration and motility of acutely dissociated embryonic cortical neurons (Baher et al., 1996; Behar et al., 2000). In addition, the neurotransmitters GABA and glutamate reportedly reduce the number of proliferating cells in dissociated or organotypic cultures of neocortex (LoTurco et al., 1995). In contrast, GABA was shown to promote cell proliferation in cultures of cerebellar progenitors (Fiszman et al., 1999). GABA also dramatically increases proliferation in the ventricular zone of the embryonic cerebrum in organotypic cultures by shortening the cell cycle. However, a reverse effect was observed in the subventricular zone (Haydar et al., 2000). Thus, during embryonic neurogenesis, GABA emerges as an important signal for cell proliferation and migration, but its precise regulation is depend on the region and cell type affected. Cellular response to GABA is mediated through its known receptors and the intracellular signals associated with them. The contribution of GABA$_A$-R to both chemo-attraction (Behar et al., 2000) and cell proliferation (Haydar et al., 2000) was indicated. However, in some aspects of cell motility there is an apparent involvement of GABA dependent G protein indicating a role of GABA$_B$-R (Behar et al., 2000). GABA acts as a trophic factor not solely during prenatal neurogenesis but also in the postnatal period in injured tissue. The effect of GABA involves stimulation of cell proliferation and Nerve growth factor (NGF) secretion (Ben-Yaakov & Golan, 2003). We have previously shown that GABA acting through specific receptor subtypes GABA$_B$ (Biju et al., 2002) control cell proliferation and act as comitogens.

**Glutamate**

Glutamate is the most prominent neurotransmitter in the body, being present in over 50% of nervous tissue. A large proportion of the glutamate present in the brain is produced by astrocytes through synthesis de novo (Hertz et al., 1999), but levels of glutamate in glial cells are lower than in neurons, 2–3 mM and 5–6 mM, respectively.
During excitatory neurotransmission, glutamate-filled vesicles are docked at a specialized region of the presynaptic plasma membrane known as the active zone. Packaging and storage of glutamate into glutamate vesicles requires Mg$^{2+}$/ATP-dependent vesicular glutamate uptake systems, which utilize an electrochemical proton gradient as a driving force. Substances that disturb the electrochemical gradient inhibit this glutamate uptake into vesicles. The concentration of glutamate in vesicle reaches as high as 20–100 mM (Nicholls & Attwell, 1990). In brain tissue, low concentrations of glutamate and aspartate perform as neurotransmitters, but at high concentration these amino acids act as neurotoxins.

There are two broad categories of glutamate receptors, the ion channel-forming or “ionotropic” receptors and the “metabotropic” receptors, those coupled to GTP-binding proteins (G proteins) and linked to the activation of phospholipase C (PLC) or the inhibition or activation of adenylyl cyclases (AC). The ionotropic receptors are further subdivided into three populations, those activated by N-methyl-D-aspartate (NMDA), those that respond to kainic acid (KA) and those sensitive to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). Activation of these receptors is responsible for basal excitatory synaptic transmission and many forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), which are thought to underlie learning and memory. It appears however, that aspartate aminotransferase and glutaminase account for a majority of glutamate production in brain tissue (McGeer et al., 1987).

The ionotropic receptors themselves are ligand gated ion channels, i.e. on binding glutamate that has been released from a companion cell, charged ions such as Na$^+$ and Ca$^{2+}$ pass through a channel in the centre of the receptor complex. This flow of ions results in a depolarisation of the plasma membrane and the generation of an electrical current that is propagated down the processes (dendrites and axons) of the neuron to the next in line. Metabotropic glutamate (mGlu) receptors are G-protein
coupled receptors (GPCR) that have been subdivided into three groups, based on sequence similarity, pharmacology and intracellular signalling mechanisms. Group I mGlu receptors are coupled to PLC and intracellular Ca\(^{2+}\) signalling, while group II and group III receptors are negatively coupled to adenylyl cyclase (Michaelis, 1998).

Glutamate functions as a fast excitatory transmitter in the mammalian brain. Glutamate triggers neuronal death when released in excessive concentrations by over excitation of its receptors (Vizi, 2000). Glutamate receptor activation and excitotoxicity has long been recognized as an upstream event in this cascade (Wieloch, 1985). In brain, glutamate accumulation is reported to cause neuronal degeneration (Berman & Murray, 1996; Budd & Nicholas, 1996; Atlante et al., 1997). The excitatory amino acid glutamate is the most prevalent transmitter in the brain; its effect on postsynaptic receptors is limited by uptake process (Erecinska, 1997) and by diffusion of glutamate from the cleft. The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA–kainate (Choi, 1988). The presence of G protein-coupled glutamate receptors (metabotropic Glu receptors) has been described and since 1991 (Conn & Pin, 1997), eight receptors have been discovered and classified into three groups based on their linkage to second messenger systems and their pharmacology: group I acts through the phosphoinositol system and groups II and III inhibit adenylyl cyclase.

In addition, the stimulation of receptors of these three groups directly influences voltage-gated Ca\(^{2+}\) and K\(^{+}\) channels through their G proteins, but their physiological correlate has not yet defined. The most consistent age-related change in the glutamatergic system is the loss of glutamate receptors. Significant decreases in the mRNA level of glutamate receptors were found in the aged cerebral cortex (Carpenter et al., 1992). Among different glutamate receptors, NMDA receptors are preferentially altered in the aged brain. Decrease in NMDA binding was shown in both rodents and mammalian brain (Cohen & Muller, 1992; Wenk et al., 1991).
mRNA level of both NMDAR1 and NMDA2B subunits of the NMDA receptors have been shown to decrease preferentially in the aged cerebral cortex, whereas no age-related change was observed in the NMDA2A subunit (Magnusson, 2000).

Neurons are expected to be more vulnerable to oxidative stress because of their high rate of metabolism, the presence of high amounts of lipids that is oxidized to form peroxides and the relatively low levels of some anti-oxidants when compared with other tissues. It is suspected that because neurons that are most vulnerable in PD are those that also receive strong input from glutamate pathways, for example striatum, that glutamate must play some role in the events that lead to neuronal damage during aging or PD. If this is the case, then cell degeneration or death is the result of a cumulative process of neurotoxicity produced by glutamate (Coyle & Puttfarcken, 1993).

**Metabotropic glutamate receptor**

Recent data on the development of motor dysfunctions in PD and related L-Dopa therapy suggest a critical involvement of enhanced glutamatergic transmission in the basal ganglia nuclei (Calabresi et al., 2000; Calon et al., 2003). In fact, pharmacological treatments that reduce NMDA receptors activity limit the extent of nigro-striatal damage (Sonsalla et al., 1998), improve motor symptoms of PD (Chase & Oh, 2000) and prevent or reduce L-Dopa induced dyskinesias (LIDs) (Blanchet et al., 1999; Hadj Tahar et al., 2004; Papa & Chase, 1996) in animal models of PD. However, alleviation of motor deficits in PD through the blockade of ionotrophic glutamate receptors revealed limitations to its clinical use because of considerable side effects (hallucinations, cognitive perturbations, postural imbalance) (Andine et al., 1999; Lee et al., 1999).

On the basis of these considerations, combined with the rich distribution and diverse physiological roles of mGluRs within the basal ganglia structures (Marino et al., 2002), recent attention has been placed on these receptors as an alternative targets
to modulate glutamate hyperactivity in PD. Eight subtypes of mGluRs have been cloned and they are classified into three subgroups based on their sequence similarity, preferred signal transduction mechanisms and relative pharmacology. Group I receptors (mGluR1 and mGluR5) are coupled to the activation of phospholipase C and generally mediate postsynaptic excitatory effects. Group II (mGluR2 and mGluR3) and group III (mGluR4, 6, 7 and 8) receptors are negatively coupled to adenylyl cyclase and inhibit cAMP formation (Ferraguti & Shigemoto, 2006). Recent studies in animal models indicate that antagonists of group I mGluRs, especially of mGluR5, could be considered as a suitable therapeutic approach in PD (Battaglia et al., 2004; Breysse et al., 2003, 2002; Ossowska et al., 2005; Oueslati et al., 2005; Dekundy et al., 2006). Moreover, strong expression of mGluR5 in the striatum, by medium spiny GABAergic neurons as well as by all interneurons and other basal ganglia nuclei including STN, SN and globus pallius (Marino & Conn, 2002; Ferraguti & Shigemoto, 2006) indicates that mGluR5 has a specific modulatory control of glutamatergic transmission through the basal ganglia circuit and is involved in the development of LIDs.

Eight different types of mGluRs, labeled mGluR1 to mGluR8 are divided into groups I, II, and III (Chu & Hablitz, 2000; Hinoi et al., 2001; Endoh, 2004; Bonsi et al., 2005). Receptor types are grouped based on receptor structure and physiological activity (Ohashi et al., 2002). The mGluRs are further divided into subtypes, such as mGluR7a and mGluR7b. The mGluRs in group I, including mGluR1 and mGluR5, are stimulated strongly by the excitatory amino acid analog L-quisqualic acid (Chu & Hablitz, 2000; Bates et al., 2002) Stimulating the receptors causes the associated enzyme phospholipase C to hydrolyze phosphoinositide phospholipids in the cell's plasma membrane (Chu & Hablitz, 2000; Endoh, 2004; Bonsi et al., 2005). This leads to the formation of inositol 1, 4, 5-trisphosphate (IP3) and diacyl glycerol. Due to its hydrophilic character IP3 can travel to the endoplasmic reticulum where it induces,
through fixation on its receptor, the opening of $\text{Ca}^{2+}$ channels increasing in this way the cytosolic $\text{Ca}^{2+}$ concentrations. The lipophilic diacylglycerol remains in the membrane acting as a cofactor for the activation of protein kinase C. These receptors are also associated with $\text{Na}^+$ and $\text{K}^+$ channels. Their action can be excitatory, increasing conductance, causing more glutamate to be released from the presynaptic cell, but they also increase inhibitory postsynaptic potentials (Chu & Hablitz, 2000). They can also inhibit glutamate release and can modulate voltage-dependent $\text{Ca}^{2+}$ channels (Endoh, 2004). Group I mGluRs, but not other groups, are activated by 3,5-dihydroxyphenylglycine (DHPG) (Shigemoto et al., 1997) a fact which is useful to experimenters because it allows them to isolate and identify them. The receptors in group II, including mGluRs 2 and 3 and group III, including mGluRs 4, 6, 7, and 8 prevent the formation of cyclic adenosine monophosphate, or cAMP, by activating a G protein that inhibits the enzyme adenylyl cyclase, which forms cAMP from ATP (Chu & Hablitz, 2000; Hinoi et al., 2001; Bonsi et al., 2005). These receptors are involved in presynaptic inhibition (Endoh, 2004) and do not appear to affect postsynaptic membrane potential by themselves. Receptors in groups II and III reduce the activity of postsynaptic potentials, both excitatory and inhibitory, in the cortex (Chu & Hablitz, 2000). Different types of mGluRs are distributed differently in cells. One study found that Group I mGluRs are mostly located on postsynaptic parts of cells while groups II and III are mostly located on presynaptic elements (Shigemoto et al., 1997), though they have been found on both pre- and postsynaptic membranes (Endoh, 2004). Also, different mGluR subtypes are found predominantly in different parts of the body. mGluR4 is located only in the brain, in locations such as the thalamus, hypothalamus and CN (InterPro, 2008). All mGluRs except mGluR6 are thought to exist in the hippocampus and entorhinal cortex (Shigemoto et al., 1997). Like other glutamate receptors, mGluRs have been shown to be involved in synaptic plasticity (Endoh, 2004; Bonsi et al., 2005) and in neurotoxicity and neuroprotection.
They participate in long term potentiation and long term depression and they are removed from the synaptic membrane in response to agonist binding (Siliprandi et al., 1992; Baskys et al., 2005).

**Ionotropic Receptors - NMDA Receptors**

The discovery of potent and selective agonists and antagonists has resulted in extensive information on the NMDA receptor-channel complex (Wood et al., 1990). It consists of four domains: (1) the transmitter recognition site with which NMDA and L-glutamate interact; (2) a cation binding site located inside the channel where Mg$^{2+}$ can bind and block transmembrane ion fluxes; (3) a phencyclidine (PCP) binding site that requires agonist binding to the transmitter recognition site, interacts with the cation binding site and at which a number of dissociative anesthetics PCP and ketamine, opiate N-allylnormetazocine (SKF-10047) and MK-801 bind and function as open channel blockers; and (4) a glycine binding site that appears to allosterically modulate the interaction between the transmitter recognition site and the PCP binding site (Fagg & Baud, 1988). NMDA is allosterically modulated by glycine, a co-agonist whose presence is an absolute requirement for receptor activation. Molecular cloning has identified to date cDNAs encoding NMDAR$_1$ and NMDAR2A, B, C, D subunits of the NMDA receptor, the deduced amino acid sequences of which are 18% belonging to NMDAR1 and NMDAR2, 55% belonging to NMDA2A and NMDA2C or 70% belonging to NMDA2A and NMDA2B are identical. Site-directed mutagenesis has revealed that the NMDAR2 subunit carries the binding site for glutamate within the N-terminal domain and the extracellular loop between membrane segments M3 and M4; whereas the homologous domains of the NMDAR1 subunit carry the binding site for the co-agonist glycine.

Normal functioning of the NMDA receptor complex depends on a dynamic equilibrium among various domain components. Loss of equilibrium during membrane perturbation causes the entire system to malfunction and result in abnormal
levels of glutamate in the synaptic cleft (Olney, 1989). An important consequence of NMDA receptor activation is the influx of Ca$^{2+}$ into neurons (MacDermott et al., 1986; Murphy & Miller, 1988; Holopainen et al., 1989, 1990). Collective evidence suggests that when the membrane is depolarized, the Mg$^{2+}$ block is relieved and the receptor can be activated by glutamate. Activation of the NMDA receptor therefore requires the association of two synaptic events: membrane depolarization and glutamate release. This associative property provides the logic for the role of the NMDA receptor in sensory integration, memory function, coordination and programming of motor activity (Collingridge & Bliss, 1987) associated with synaptogenesis and synaptic plasticity. Ca$^{2+}$ flux through NMDARs is thought to play a critical role in synaptic plasticity, a cellular mechanism for learning and memory. The NMDA receptor is distinct in that it is both ligand-gated and voltage-dependent. NMDA sensitive ionotropic glutamate receptors probably consist of tetrameric and heteromeric subunit assemblies that have different physiological and pharmacological properties. They are differentially distributed throughout the CNS (Seeburg, 1993; Hollmann & Heinemann, 1994; McBain & Mayer, 1994; Danysz et al., 1995; Parsons et al., 1998a).

To date, two major subunit families, designated NMDAR1 and NMDAR2, have been cloned. Various heteromeric NMDA receptor channels formed by combinations of NMDAR1 and NMDAR2 subunits are known to differ in gating properties, Mg$^{2+}$ sensitivity and pharmacological profile (Sucher et al., 1996; Parsons et al., 1998b). The heteromeric assembly of NMDAR1 and NMDA2C subunits, for instance, has much lower sensitivity to Mg$^{2+}$ but increased sensitivity to glycine and very restricted distribution in the brain. In situ hybridization has revealed overlapping but different expression profiles for NMDAR2 mRNA. For example, NMDA2A mRNA is distributed ubiquitously like NMDAR1, with the highest densities occurring in hippocampal regions and NMDA2B is expressed predominantly in forebrain, in
cerebellum NMDA2C predominates; NMDA2D is localized mainly in the brain stem (Moriyoshi et al., 1991; Monyer et al., 1992; Nakanishi, 1992; McBain & Mayer, 1994). The NMDA receptor antagonists have potential therapeutic applications. NMDA receptors are involved in learning and other forms of plasticity, such as drug dependence and addiction, chronic pain and CNS development, as well as in normal or disturbed synaptic transmission in some areas of the CNS. Activation of NMDA receptors depends not only on the level of synaptic activity but also on other factors, such as agonist affinity, gating kinetics and Mg²⁺ sensitivity. The role of NMDA receptors in various processes depends on the subtype composition and area of the CNS involved. In animals, most NMDA receptor antagonists produce impairment of learning when given at sufficiently high doses before the association phase but not when administered after this phase or during retrieval (Rogawski, 1993; Leeson & Iversen, 1994; Danysz et al., 1995; Avenet et al., 1996).

Antagonists of NMDA receptors have been shown to inhibit neurodegeneration of the DA in SN system induced by MPP⁺ and methamphetamine (Sonsaila et al., 1989; Greenamyre & O’Brien, 1991; Turski et al., 1991). NMDA receptor antagonists in general and aminoadamantanes in particular, have been suggested as potential neuroprotective therapies in PD (Kornhuber et al., 1994, Mizuno et al., 1994). In fact amantadine, an NMDA receptor antagonist increases life expectancy in Parkinson's patients, an effect attributed to neuroprotective activity of this agent (Uitti et al., 1996). An early exposition of CNS to increased Glutamate concentrations appear to modify, at least, the gene expression of NMDA-R subunits, it could represent changes in the neuronal connectivity as well as an increase in the neurodegeneration susceptibility the cerebral regions. NMDA glutamate receptors play a particularly important role in the function of the striatum. NMDA binding sites are very abundant in this region (Albin et al., 1992). Striatal NMDA receptors are involved in the regulation of GABA, acetylcholine, neuropeptide and glutamate
release and NMDA activation causes striatal neurons to dephosphorylate the DA-receptor-associated protein DARRP-32 (Damsma et al., 1991, Bustos et al., 1992, Morari et al., 1993, Young et al., 1993). The pharmacological properties of the receptors mediating some of these actions are distinct, suggesting segregation of different kinds of NMDA receptors among the types of striatal neurons (Nicolas et al., 1994). In addition to direct effects on striatal targets, NMDA receptors modulate the effect of DA on striatal function, an interaction is relevant to the therapy of PD (Starr et al., 1993; Kaur et al., 1997).

**Glutamate mediated excitotoxic cell death**

Excitotoxicity is the pathological process by which nerve cells are damaged and killed by glutamate and similar substances (Ashpole & Hudmon, 2011). Evidence is gathering that excitatory amino acid (EAA) neurotransmission contribute to neuronal ischemic injury during conditions of metabolic stress (Olney et al., 1973; Choi, 1988). Excessive synaptic accumulation of glutamate can cause neuronal over activation, precipitating a cascade of cellular events that lead ultimately to cell death, a phenomenon termed glutamate excitotoxicity. This occurs when receptors for the excitatory neurotransmitter glutamate such as the NMDA receptor and AMPA receptor are over activated. Glutamate is a prime example of an excitotoxin in the brain and it is also the major excitatory neurotransmitter in the mammalian CNS (Temple et al., 2001). During normal conditions, glutamate concentration can be increased up to 1mM in the synaptic cleft, which is rapidly decreased in the lapse of milliseconds. When the glutamate concentration around the synaptic cleft cannot be decreased or reaches higher levels, the neuron kills itself by a process called apoptosis. Glutamate receptors, including the NMDA subtype and several non-NMDA subtypes, are transiently overexpressed in neonates and infants, in as much as EAAs play a critical role in the development of the central nervous system (McDonald et al., 1990). Hardingham et al., (2002) noted that extrasynaptic NMDA receptor activation,
triggered by both glutamate exposure or hypoxic/ischemic conditions, activate a CREB (cAMP response element binding protein) shut-off, which in turn, caused loss of mitochondrial membrane potential and apoptosis. Excitotoxins like NMDA and kainic acid which bind to these receptors, as well as pathologically high levels of glutamate, cause excitotoxicity by allowing high levels of Ca\(^{2+}\) (Manev et al., 1989) to enter the cell. Ca\(^{2+}\) influx into cells activates a number of enzymes, including phospholipases, endonucleases, and proteases such as calpain. These enzymes go on to damage cell structures such as components of the cytoskeleton, membrane and DNA. Reports suggest that Ca\(^{2+}\) influx through NMDA receptors is involved in ROS production and neuronal damage resulting from moderate energy depletion (Hernández-Fonseca et al., 2008). Excitotoxicity is involved in spinal cord injury, stroke, traumatic brain injury and neurodegenerative diseases of the central nervous system such as Multiple sclerosis, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), PD, Alcoholism and Huntington's disease (Kim et al., 2002) and neurological disorders such as ischemia, cerebral trauma and some chronic neurodegenerative diseases. An excess of glutamate release, or a deficiency in its clearance from the synaptic cleft, which depends mainly on its transport by high affinity carriers, are potential sources for the accumulation of extracellular glutamate. The SN is a target for extensive glutamatergic inputs from the cortex and the subthalamic nucleus. More importantly, secondary or ‘weak’ excitotoxicity is a consequence of neuronal depolarization as it might ensue from a defect of cellular energy metabolism after MPTP. The activation of NMDA receptors allows Ca\(^{2+}\) influx into the cell with possible detrimental consequences such as NOS activation or free radical cytotoxicity. Secondary excitotoxicity involving NMDA receptors was implied in MPP+-induced cell death. In vivo, NMDA antagonists were effective against MPTP toxicity. Decortication (i.e. cutting the cortical glutamatergic output, especially to the basal ganglia) alleviated MPP+ toxicity in rats (Srivastava et al., 1993).
Glutamate Transporter

Glutamate transport is the major mechanism controlling extracellular glutamate levels, preventing excitotoxicity and averting neural damage associated with PD (McBean & Roberts, 1985; Robinson et al., 1993; Tanaka et al., 1997, Miller et al., 2011). Glutamate transporters are localized to the membranes of synaptic terminals and astroglial processes that ensheath synaptic complex (Conti et al., 1998). GLAST for glutamate–aspartate transporter, (EAAT-1) for excitatory amino acid transporter-1 (Arriza et al., 1994) and GLT-1 for glutamate transporter-1, EAAT-2 (Pines et al., 1992) are astroglial glutamate transporters and EAAC1 for excitatory amino acid carrier-1, EAAT-3 (Eskandari et al., 2000), EAAT-4 (Fairman et al., 1995) and EAAT-5 (Arriza et al., 1997) are neuronal proteins.

The concentration of glutamate is regulated to ensure neurotransmission with a high temporal and local resolution. Neuronal damage is associated with excitotoxicity, a type of cell death triggered by the over activation of glutamate receptors and the loss of Ca^{2+} homeostasis. The removal of glutamate from the extracellular fluid occurs by uptake and by diffusion (Tong & Jahr, 1994). Failure of glutamate clearance leads to neuronal damage, named excitotoxic damage, due to the prolonged activation of glutamate receptors. Extracellular glutamate must be cleared quickly, perhaps within 1ms, to maintain glutamate below toxic levels (Trotti et al., 1998). Glutamate transporters represent the only significant mechanism for the uptake of extracellular glutamate and their importance for the long-term maintenance of low non-toxic glutamate concentrations is well documented (Danbolt, 2001). When glutamate is taken up into glial cells by the EAATs, it is not reused directly but converted to glutamine and stored vesicles. Subsequently these vesicle are released from Glia cells and glutamine transported back into the presynaptic neuron, converted back into glutamate and store into vesicles by action of the vesicular glutamate transporter (VGLUTs) (Shigeri et al., 2004). This process is named the glutamate-
glutamine cycle. Given that glutamate transporters provide the main route by which glutamate is cleared, it is logically predicted that an aberration in transporter expression and function lead to toxic glutamate levels and thus promote neuronal degeneration (Tanaka et al., 1997). Studies have suggested the involvement of the glutamate transporters in radiation induced neurotoxicity (Martha et al., 2009).

**Signal transduction through Second Messengers**

**Inositol 1,4,5-trisphosphate (IP3)**

Many biological stimuli, such as neurotransmitters, hormones and growth factors, activate the hydrolysis of phosphatidyl inositol 4,5-bisphosphate (PIP2) in the plasma membrane which is hydrolyzed by phospholipase C (PLC) to produce IP3 and diacylglycerol (DAG). The IP3 mediates Ca$^{2+}$ release from intracellular Ca$^{2+}$ stores by binding to IP3 receptors (IP3R). IP3R are the IP3 gated intracellular Ca$^{2+}$ channels that are mainly present in the endoplasmic reticulum (ER) membrane. The IP3 induced Ca$^{2+}$ signaling plays a crucial role in the control of diverse physiological processes such as contraction, secretion, gene expression and synaptic plasticity (Berridge, 1987; Berridge, 1993). Furthermore, Morita et al., (2004) demonstrated that the expressed GFP-IP3R3 acts as a functional IP3-induced Ca$^{2+}$ channel. Frequently, IP3Rs are not uniformly distributed over the membrane but rather form discrete clusters (Bootman et al., 1997). The clustered distribution of IP3Rs has been predicted to be important in controlling elementary Ca$^{2+}$ release events, such as Ca$^{2+}$ puffs and blips, which act as triggers to induce the spatiotemporal patterns of global Ca$^{2+}$ signals, such as waves and oscillations (Thomas et al., 1998; Swillens et al., 1999; Shuai & Jung, 2003). The binding of IP3 changes the conformation of IP3Rs such that an integral channel is opened, thus allowing the Ca$^{2+}$ stored at high concentrations in the ER/SR to enter the cytoplasm. A critical feature of IP3Rs is that their opening is regulated by the cytosolic Ca$^{2+}$ concentration.
Group I mGluRs (mGluR1/5 subtypes) are also demonstrated to mainly affect intracellular Ca\(^{2+}\) mobilization (Conn & Pin, 1997; Bordi & Ugolini, 2000). To sequentially facilitate intracellular Ca\(^{2+}\) release, group I receptors activate the membrane-bound PLC, which stimulates phosphoinositide turnover by hydrolyzing PIP2 to IP3 and diacylglycerol. IP3 then causes the release of Ca\(^{2+}\) from intracellular Ca\(^{2+}\) stores (such as endoplasmic reticulum) by binding to specific IP3 receptors on the membrane of Ca\(^{2+}\) stores (Berridge, 1993). Altered Ca\(^{2+}\) levels could then engage in the modulation of broad cellular activities. Mitochondrial cytochrome c release and IP3R-mediated Ca\(^{2+}\) release from the endoplasmic reticulum mediate apoptosis in response to specific stimuli (Boehning et al. 2003). This is serving as a key event in glutamate mediated neurodegeneration in PD.

**Cyclic Adenosine Monophosphate (cAMP)**

Cyclic Adenosine Monophosphate (cAMP) is produced from ATP adenylyl cyclase (AC) in response to a variety of extracellular signals such as hormones, growth factors and neurotransmitters. The second messenger concept of signaling was born with the discovery of cAMP and its ability to influence metabolism, cell shape and gene transcription (Sutherland, 1972) through reversible protein phosphorylations. Elevated levels of cAMP in the cell lead to activation of different cAMP targets. It was long thought that the only target of cAMP was the cAMP-dependent protein kinase (cAPK), which has become a model of protein kinase structure and regulation (Francis & Corbin, 1999; Canaves & Taylor, 2002). It has become clear that not all effects of cAMP are mediated by a general activation of cAPK (Dremier et al., 1997). Several cAMP binding proteins have been described: cAPK (Walsh et al., 1968), the cAMP receptor of Dictyostelium discoideum, which participates in the regulation of development (Klein et al., 1997), cyclic nucleotide gated channels involved in transduction of olfactory and visual signals (Kaupp et al., 1989; Goulding et al., 1992) and the cAMP-activated guanine
exchange factors Epac 1, 2 which specifically activate the monomeric G protein Rap (Kawasaki et al., 1998).

Cyclic Guanosine Monophosphate (cGMP)

The most extensively studied cGMP signal transduction pathway is that triggered by nitric oxide (NO) (Bredt & Snyder, 1990). cGMP effects are primarily mediated by the activation of cGMP-dependent protein kinases (PKGs). cGMP generation has been associated with neurotransmission (Hofmann et al., 2000), vascular smooth muscle relaxation (Fiscus et al., 1985) and inhibition of aldosterone release from adrenal glomerulosa cell suspension (Matsuoka et al., 1985).

Cyclic nucleotide pathways can cross talk to modulate each other’s synthesis, degradation and actions. Increased cGMP can increase the activity of cGMP stimulated phosphodiesterase 2 (PDE2) to enhance hydrolysis of cAMP, or it can inhibit the PDE3 family and decrease the hydrolysis of cAMP (Pelligrino & Wang, 1998). cAMP and cGMP are involved in NMDA receptor-mediated signaling in cerebral cortical and hippocampal neuronal cultures. The influx of Ca\(^{2+}\) through the NMDA receptor stimulates Ca\(^{2+}\)/calmodulin dependent adenylyl cyclase, leading to production of cAMP. This increase in cAMP seems to be tightly regulated by PDE4. The Ca\(^{2+}\) influx also stimulates the production of NO and subsequent activation of guanylyl cyclase, leading to cGMP production (Suvarna & O'Donnell, 2002).

**cAMP-response element binding protein**

CAMP-response element binding protein (CREB) belongs to a family of transcription factors that have been implicated in many important neuronal functions (Walton & Dragunow 2000, Finkbeiner 2000; Shimamura et al. 2000). For example, CREB-dependent gene expression has been reported to play a role in such diverse processes as cell survival, plasticity, growth and development and most recently, cell death. In neurons and other cells, CREB and its family members function as effectors molecules that bring about changes within a cell in response to a wide range of
signals. Diverse extracellular stimuli such as growth factors, hormones, membrane depolarization and Ca\(^{2+}\) influx can all cause activation of CREB and the multiple different signaling cascades all converge to phosphorylate a critical CREB residue–Serine 133 (Mayr & Montminy, 2001). cAMP accumulates in the cytoplasm in response to stimulation of membrane (G)-protein coupled receptors and stimulates the dissociation of the protein kinase A (PKA) heterotetramer, which consists of a pair of regulatory (R) and a pair of catalytic (C) subunits. Once liberated, the catalytic subunits are free to enter the nucleus by passive diffusion where they phosphorylate CREB on its Ser-133 residue and gene expression can then be induced. CREB controls neuronal survival, in part, by controlling transcription of neuroprotective genes. For example, the promoter regions for both Brain Derived Neurotrophic Factor (BDNF) and the anti-apoptotic protein, B-cell lymphoma 2 (Bcl2), each contain CRE sites (Mayr & Montminy, 2001) and both of these gene products have been shown to play an important role in neuronal survival. Additionally, transgenic mice that overexpress Bcl2 are protected from naturally occurring neuronal loss as well as experimentally-induced ischemia (Martinou et al., 1994). BDNF is also known to affect neuronal survival, as this neurotrophic factor protect nigrostriatal dopaminergic neurons from neurotoxins in rodent and monkey models of PD (Sun et al. 2005). Interestingly, BDNF has also been shown to be able to stimulate proliferation of neuronal precursors and the possible generation of new dopaminergic neurons in the striatum and SN in the unilateral 6-OHDA lesion rat model of PD (Mohapel et al., 2005). Taken together, these results confirm that CREB, together with its downstream gene products, play an important role in the regulation of neuronal survival throughout the life of a neuron. Activation during development, as well as during times of stress is critical for determining neuronal fate, opening up the possibility that disruption of this important signaling pathway would have detrimental consequences.
Mode of cell death in PD

Defects in several cellular systems have been implicated as early triggers that start cells down the road towards neuronal death. These include abnormal protein accumulation, particularly of α-synuclein; altered protein degradation through multiple pathways; mitochondrial dysfunction; oxidative stress, neuroinflammation and dysregulated kinase signaling (Izumi et al., 2011). As dysfunction in these systems mounts, pathways that are more explicitly involved in cell death become recruited. These include JNK signaling, p53 activation, cell cycle re-activation, and signaling through Bcl-2 family proteins (Levy et al., 2009). Using DNA nick-end labeling, Mochizuki, et al. (1996) found in some patients intense nuclear staining indicative of apoptosis. In addition, Anglade et al (1997) found the typical features of apoptosis in the SNpc of patients with PD, using ultrastructural analysis, and fragments of melanized neurons were found in glial cells. Using fluorescent probes specific for both DNA cleavage and chromatin clumping, Tatton et al (1998) were able to confirm, under a light microscope level, the positive staining of melanized neurons in the SNpc. It is likely that a pro-apoptotic transduction pathway dependent on tumor necrosis factor α (TNF-α)-receptor activation is induced in PD, supporting the notion of apoptotic death. Furthermore, caspase-3 is one of the key players in apoptosis and was found to be activated in experimental models of PD (Oo, et al. 2002). Caspase-8 is a proximal effector protein of the TNF-receptor-family death pathway and a significantly higher percentage of dopaminergic neurons displaying caspase-8 activation were observed in PD patients than in controls (Green, 1998). In addition, using in situ hybridization, the level of B-cell lymphoma-extra large (Bcl-xL) mRNA expression per dopaminergic neuron in PD patients was shown to be almost double that of controls, possibly reflecting the activation of a defense mechanism in the dopaminergic cells (Hartmann et al., 2002).
Bcl-2 family members include pro-apoptotic molecules (Bax, Bak, Bok, Bad, Bid, Bik, Blk, Hrk, BNIP3 and BimL) and anti-apoptotic molecules (Bcl-2, Bcl-xL, Bcl-w, Mcl-1 and A1). Bcl-2 family proteins participate in the modulation and execution of cell death (Deigner et al., 2000; Marino & Piantelli, 2011) and can preserve or disrupt mitochondrial integrity by regulating the release of cytochrome c/second mitochondrion-derived activator of caspase (Smac)/apoptosis inducing factor (AIF)/endonuclease G (Danial & Korsmeyer, 2004). Cytosolic Bax translocates to mitochondria upon death stimulus, promoting cytochrome c release (Gross et al., 1998). Besides the involvement of the Fas/Caspase-8/Bid cascade, Bid also mediates cytochrome c release while binding to both pro-apoptotic members (e.g. Bax) and anti-apoptotic members Bcl-2 and Bcl-xL. Moreover, cleavage of Bid by caspase-8 and caspase-1 mediates the mitochondrial damage (Guegan et al., 2002). Bax mediates cell death relates with mitochondrial permeability transition (Jin & El-Deiry, 2005).

**α-Synuclein modifications, aggregation and fibril formation**

α-Synuclein is natively unfolded under physiological conditions *in vitro*, but it is very sensitive to environmental and intrinsic factors that can modify conformation (i.e. configuration of β-sheet species) and facilitate dimer formation, aggregation of soluble oligomers (protofibrillar species) and assembly into insoluble amorphous and fibril aggregates (Uversky, 2003, 2007; Gorbatyuk et al., 2008; Mollenhauer et al., 2011; Winner et al., 2011). Oxidative dimer formation represents the initial step in fibrillogenesis (Krishnan et al., 2003). High levels of α-synuclein, α-synuclein mutations, metal cations and oxidative stress favor α-synuclein aggregation *in vitro* (Narhi et al., 1999; Hashimoto et al., 1999; Paik et al., 2000). Protein tau and tubulin also facilitate α-synuclein aggregation (Giasson et al., 2002; Alim et al., 2002). The solubility of α-synuclein is altered as well, and the protein is prone to the formation of aggregates in Lewy body diseases (LBDs) and related transgenic models (Baba et
In Parkinsonian SN, α-synuclein is also modified by acrolein and accumulates in dopaminergic neurons; excess acrolein reduces proteasomal activity in vitro, thereby suggesting that acrolein accumulation in Lewy bodies compromises the ubiquitin–proteasome system in dopaminergic neurons (Shamoto-Nagai et al., 2007). Finally, modifications in α-synuclein have effects in other PD related proteins. α-Synuclein aggregates interfere with parkin and tubulin solubility and result in parkin insolubility and cytoskeletal alterations (Kawahara et al., 2008).

**Tumor necrosis factor-α in PD**

TNF-α is a potent pro-inflammatory molecule, which upon engagement with its cognate receptors on target cells, triggers downstream signaling cascades that control a number of cellular processes related to cell viability, gene expression, ion homeostasis and synaptic integrity (Park & Bowers, 2010). The correlative presence of inflammatory cytokines in the cerebrospinal fluid (CSF) of PD patients was described by Mogi *et al.*, (1994) who found that TNF-α levels were enhanced in CSF of those afflicted with the disease. The same group also observed enhanced TNF-α levels in the striatum of patients that had succumbed to PD. They have also reported that TNF-RI levels are elevated in the SN of PD patients (Mogi *et al.*, 2000), further suggesting altered TNF-α signaling is participating in or a result of PD-related pathogenesis. Infusion of either the toxin MPTP or 6-OHDA induces degeneration of the nigrostriatal pathway and concomitantly enhances the levels of TNF-α within the striatum and SN (Sriram *et al.*, 2002). Experimental ablation of the TNF-α receptors protects against MPTP-induced dopaminergic neurotoxicity. Other studies have focused on a possible role for TNF-α signaling in the microglial activation observed in PD pathogenesis. The administration of MPTP or 6-OHDA activates these brain-resident immune cells and if TNF-α receptor expression is genetically suppressed,
Microglial activation is absent and MPTP-induced neurotoxicity is significantly blunted (Sriram et al., 2006). These studies not only suggest that modulation of TNF-α expression enhances one's risk for the development of PD, but further implicate dysfunctional TNF-α signaling in neurodegeneration.

**Cellular transplantation to the rescue**

Pioneering work by Elizabeth Dunn in 1904 showed that transplanted fetal tissue can survive in the brain of another animal. For many years, fetal tissue has been used for treatment of human disorders, including fetal pancreatic transplants to treat diabetes mellitus and fetal thymic transplants to treat lymphogenic immunological deficiency. The defining basic science research that opened investigations on fetal tissue and brain transplantation was undertaken by Olson and Seiger (1972). They showed that fetal tissue grafted in the immunoprivileged anterior chamber of the eye has the capacity to integrate with the host target neurons and that these graft-host connections were functional. The proof of principle providing evidence that fetal tissue transplantation exerts efficacious benefits against neurodegeneration came from research in PD.

A large variety of cell replacement strategies are under investigation in animal models of PD, which began with the success of transplanted fetal neurons in reconstructing the lesioned nigrostriatal pathway and ameliorating behavioural impairments (Bjorklund & Stenevi, 1979; Perlow et al., 1979). Various types of cells have been tested, such as cells from the embryonic ventral mesencephalon which contains the primordial SN, neuronal stem or progenitor cells, dopaminergic cell lines, non-neuronal cells (usually fibroblasts or astrocytes) engineered to secrete DA or neurotrophic factors, adrenal medullary cells which naturally synthesize DA, testis-derived Sertoli cells which are rich in trophic factors and more recently, carotid body epithelial glomus cells which synthesize DA and co-grafting cells with fetal kidney cells which are rich in neurotrophic factors (Koutouzis et al., 1994; Dunnett, 1995;
Implanted cells are encapsulated in permselective polymer matrices or seeded on microcarrier beads (Borlongan et al., 1996). Combining various cell types in co-grafts has often resulted in improvements (Meyer et al., 1995; Takeyama et al., 1995; Costantini & Snyder-Keller, 1997; Sautter et al., 1998). Pretreating cells to be transplanted with trophic factors, antioxidants, or anti-apoptotic factors also improve graft survival and supplement behavioural recovery of the animal. Recently, treatment of ventral mesencephalic cells prior to transplantation with an inhibitor of the pro-apoptotic enzymes, the caspases, dramatically improved not only the survival of grafted dopaminergic neurons, but also the volume of the graft in 6-OHDA lesioned rats (Schierle et al., 1999). Indeed, treated grafts became so robust that they caused an over abundance of dopaminergic activity on the grafted side of the brain which led to an imbalance and turning behaviour in the opposite direction. Many of these approaches have proven successful in ameliorating dopaminergic deficits and/or behavioural impairments in rodent or primate animal models of PD. Several of these techniques have progressed to clinical application.

Cells are commonly grafted ectopically to striatum (which is the target tissue for dopaminergic nigral neurons) because DA is required in the striatum and neuronal or non-neuronal cells implanted into the adult degenerating SN will physically re-establish the long nigrostriatal pathway to innervate the striatum and supply it with DA. Fetal ventral mesencephalic cells transplanted into the striatum 2 weeks after 6-OHDA lesioning in rats have been found to survive out to a full 2 years with many TH neurons remaining and forming functional synaptic connections with host striatum, improving DA content and successfully eliminating methamphetamine-induced rotations (Nishino et al., 1990). Grafting fetal ventral mesencephalic tissue into the SN rather than the striatum has surprisingly also proven successful (Nikkhah et al., 1994). Grafted 6-OHDA lesioned rats showed a reduction of apomorphine-induced
rotations that correlated with the number of TH cells. In addition, grafted dopaminergic neurons integrated into the host SN. However, amphetamine-induced rotations were not affected by the intranigral grafts, which is due to the differing roles of the striatum and SN in rodent drug-induced turning asymmetry. Bridging grafts created by injecting mesencephalic cells at multiple sites to lay down a tract from SN to striatum (Zhou et al., 1996) have shown success in physically re-establishing the nigrostriatal pathway including reciprocal functional synapses, increased DA release and near full reduction of amphetamine-induced turning asymmetry (Zhou et al., 1996). In addition, a bridging tract can be laid with fetal ventral mesencephalic cells along with neurotrophic factor-secreting substrate cells (providing both a physical growing substrate and a trophic factor) resulting in an improved survival of TH fibers along the nigrostriatal bridge and a reduction in amphetamine-induced rotations (Brecknell et al., 1996). A large variety of cell types and strategies have evolved for creating bridging grafts (Olson, 1997).

However, major obstacles remain. The fetal brain tissue used in clinical transplantation studies is difficult or ethically challenging to obtain (Lindvall, 2001). Also, while under normal conditions the CNS immune response is limited (Boulanger & Shatz, 2004), the CNS is continuously patrolled by the immune system and mount a well-organized innate immune reaction in response to allogeneic antigens and cerebral injury (Tambur, 2004). Finally, striatal transplants are difficult to “tune” for appropriate dopaminergic output, causing side effects such as dyskinesia (Carlsson et al., 2006), thereby emphasizing the necessity to restore the complexity of nigrostriatal neuronal circuitry.

**Bone marrow cells**

Stem cells have been detected in multiple organs in the adult, leading to the emerging concept of stem cell plasticity. These cells exhibit the classical traits of self-renewal and multipotentiality. In addition to well known stem cells of the adult
marrow lymphohematopoietic and stromal mesenchymal lineages (Krause et al., 2001), stem cells have been provisionally identified in liver, muscle, CNS and skin (Toma et al., 2001). Bone marrow cells (BMC), offer an alternative source of cells for treatment of neurodegenerative diseases and CNS injury. These cells normally differentiate into bone, cartilage and adipose tissue (Pittenger et al., 1999), but can be experimentally induced to differentiate into cells with surface markers characteristic of neurons (Sanchez-Ramos et al., 2000). The cells assumed characteristic neuronal forms and expressed a variety of neuron-specific genes and proteins, including neuronspecific enolase, tau, neurofilament M, NeuN (neuronal-specific nuclear protein), β-III-tubulin, and synaptophysin. When injected into the brain or administered systemically, BMC migrate to sites of injury, proliferate, and engraft (Chen et al., 2001). These cells offer several advantages over other sources of stem-like precursor cells as therapy for PD: they are easily harvested, isolated and purified, can be produced in large quantities, and their use does not pose ethical concerns (Munoz-Elias et al., 2004). Potential roles for BMC in treatment of PD include their use as vectors for delivery of gene products to sites of tissue injury (Ye et al., 2007), facilitation of recovery from neuronal damage by replacing injured and/or lost cells (Levy et al., 2008), and production of trophic factors promoting survival and regeneration of host tissue (Crigler et al., 2006). In support of these therapeutic concepts, modest improvements in neurological function have been reported following BMC administration in animal models of PD, stroke, and acute CNS injury (Li et al., 2001; Mahmood et al., 2004; Himes et al., 2006).

**Role of Astrocytes in PD**

Until relatively recently, astrocytes, along with other cells of the glial lineage such as oligodendrocytes and microglia, were believed to be structural cells, the main function of which was to hold neurons together. It is now known, however, that astrocytes serve many housekeeping functions, including maintenance of the extra
cellular environment and stabilization of cell–cell communications in the CNS. The function of astrocytes in regulating cerebral blood flow and maintaining synaptic function is becoming increasingly recognized as being of paramount importance in the maintenance of the neuronal environment. Astrocytes are also central to the maintenance of neuronal metabolism and neurotransmitter synthesis. The studies that have been carried out to date appear to support a neuroprotective role for astrocytes in PD. From pathological examinations, an increase in the number of astrocytes as well as in GFAP expression is observed in PD, as with other neurodegenerative disorders (Forno et al. 1992). The pathological evidence indirectly indicates that antioxidant pathways contribute to this neuroprotective effect, because in control brains the density of glutathione-peroxidase-positive cells was higher in the vicinity of the dopaminergic cell groups known to be resistant to the pathological process of PD. The increase in glutathione peroxidase containing cells was inversely correlated with the severity of dopaminergic cell loss in patients with PD. The quantity of glutathione peroxidase containing cells, therefore, might be critical for a protective effect against oxidative stress (Damier et al. 1993) Conversely, the presence of synuclein-positive astrocytes in pathological samples has been shown to correlate with nigral neuronal cell death (Wakabayashi et al. 2000).

In a PD model generated by lesioning the brain with the neurotoxin MPTP, it appears that astrocytosis occurs after the death of dopaminergic neurons, and that this response remains elevated even after most dopaminergic neurons have died (Teismann & Schulz, 2004). A more rapid response consisting of increased GFAP immuno reactivity as early as 1 hour following the injection of 6-OHDA into the nigrostriatal DA bundle has been observed, indicating a more direct effect of this compound on astrocytosis. Several pathways for this neuroprotection have been implicated, including the increased activation of astrocytes and neuroprotection in 6-OHDA models following infusion of interleukin-1β (a cytokine released by activated
microglia) into the SN (Saura et al. 2003). Astrocytes contribute to homeostasis in the brain by providing neurons with energy and substrates for neurotransmission. They remove excess neurotransmitter molecules from the extracellular space, allowing discrete and precise encoding of synaptic signals and neurotransmission (Nicola & Ben, 2009). The role of astrocytes in PD and related syndromes is sparsely investigated and poorly understood (McGeer & McGeer, 2008). Astrocytes respond to all forms of CNS insults through a process referred to as reactive astrogliosis, which has become a pathological hallmark of CNS structural lesions (Sofroniew & Vinters, 2010). The discovery of endogenous stem cells that can generate neural tissue has raised new possibilities for repairing the nervous system. Glial progenitors, provides a reservoir for astrocyte that migrates to sites of traumatic, infectious or degenerative brain damage. But, the failure of these migrated cells in brain repair is due to the remarkable resistance to accept such cells into a mature neuronal network (Pasko 2004).