Discussion

Parkinson’s disease (PD) is a devastating neurodegenerative disorder that results in a wide range of motor and non-motor deficits that includes tremors, bradykinesia, rigidity, cardiovascular and gastrointestinal abnormalities, cognitive dysfunction and depression (Springer & Kahle, 2011). It is the second most prevalent neurodegenerative disease and affects an estimated 6 million people worldwide, with projections suggesting a two fold increase within 25 years (Richard & Serge, 2008). PD is largely characterized by the irreversible loss of DA neurons, although it is becoming increasingly clear that other neurotransmitter systems are likely involved in the pathogenesis. The great majority of cases of PD are sporadic, without any other family members being affected. One of the great advances in the last decade is that several gene mutations have been discovered to cause PD. But these monogenetic causes do not explain the great majority of sporadic cases (Dawson et al., 2010).

Weight loss in Parkinson's disease

Patients with PD frequently loose weight. The frequency of weight loss among such patients is 52 % as reported by Abbott et al., (1992) and 65% by Moroo et al., (1994). Weight loss is more prominent in women (average body weight loss 8.5 %) than in men (4.3 %) and becomes marked in patients with advanced disabilities. Energy expenditure decreases due to motor impairment but increase in parallel with worsening of muscle rigidity and the development of dopa-induced dyskinesias. Moreover, disturbed motility and absorption of the gastrointestinal tract impair energy intake. Dysphagia occurs in the advanced stage of PD, and anorexia caused by depression also could cause disturbed energy intake. Anti-Parkinsonian drugs accelerate anorexia and dysfunction of the gastrointestinal tract. Medical complications such as pneumonia, bone fracture and malignancy cause additional weight loss. Moreover, weight loss is also associated with insufficient nutrition, precipitating infection and decubitis and increasing the mortality rate (Kashihara
We observed a decreased body weight in the 6-OHDA rats compared to the control which is due to a prolonged disequilibrium between intake, digestion and absorption of energy from nutrients on the one hand, and energy expenditure on the other hand. It is a clinically relevant problem since weight loss can contribute substantially to both morbidity and mortality. Treatment of 6-OHDA rats with 5-HT, GABA and BMC in combination improved body weight significantly which indicate prevention of muscle tissue damage.

**Behavioural deficits in Parkinson's induced rats**

PD is often complicated by a variety of cognitive symptoms that range from isolated memory and thinking problems to severe dementia. While the motor symptoms of PD are well-known (tremor, rigidity, slowness of movement, imbalance), the commonly seen deficits in memory, attention and problem-solving are less understood. Studies have shown that over 50% of people with PD experience some form of cognitive impairment. About 20% have more substantial cognitive impairment (Harish *et al.*, 2010). 6-OHDA induces toxicity through intra- or extracellular auto-oxidation, hydrogen peroxide formation induced by monoamine oxidase-B (MAO-B) activity, or direct inhibition of the mitochondrial respiratory chain and consequent oxidative stress (Shim *et al.*, 2009). New Parkinsonian rat models have been developed with 6-OHDA injected directly into the SN to induce selective and moderate neurodegeneration of DA nerve terminals. Similarly, in PD the progressive degeneration of nigral dopaminergic neurons results in motor deficits only after 80% of the nigrostriatal system has degenerated. Therefore behavioural studies preferentially involve unilateral destruction of the nigrostriatal pathway with 6-OHDA to avoid the debilitating consequences of a bilateral lesion (Hritcu *et al.*, 2008). Depending on the dose and the site of infusion into the brain, unilateral 6-OHDA SNpc-lesioned rats present an almost complete loss of dopaminergic neurons in the SNpc, a proportional depletion of striatal DA and gross motor disturbances, like
turning behaviour (e.g. after a challenge with DA receptor agonists) and reduced locomotion. Ungerstedt (1968) reported preliminary findings that injection of the neurotoxin 6-OHDA into one nigrostriatal pathway of the rat produced an animal with loss of catecholamine histochemical fluorescence from the ipsilateral striatum and with marked motor asymmetry, turning spontaneously towards the side of the 6-OHDA injection. Unilateral injection of 6-OHDA into the SN caused degeneration of the ipsilateral nigrostriatal pathway and loss of DA from the ipsilateral striatum. Following injection of 6-OHDA into the nigrostriatal pathway, a rat exhibits rotational behaviour or a body asymmetry towards the operated side. This circling behaviour is exaggerated with systemic administration of amphetamine, which stimulates catecholamine release. Within a few weeks after 6-OHDA treatment, striatal denervation hypersensitivity to DA develops; it is demonstrated by circling behaviour in the opposite, away from the side of the injection site direction with administration of the DA agonist apomorphine. Our studies with apomorphine showed a reversal in the rotational behaviour in rats treated with 5-HT, GABA and BMC compared to 6-OHDA infused rats. This indicates the reduction of DA receptor hypersensitivity after the BMC transplantation with 5-HT and GABA.

We also investigated the ability of 5-HT, GABA and BMC to restore skilled forelimb performances in the PD rat model. Skilled forelimb use is dependent on an intact dopaminergic neurotransmission and is substantially impaired in animals with unilateral 6-OHDA lesions. The asymmetry score is calculated as the number of “ipsi” observations plus 1/2 the number of “both” observations, divided by the total number of observations (ipsi plus contra plus both). This provides an overall asymmetry percentage score, where 50% indicates an animal that explores symmetrically with both limbs, higher scores (> 50%) indicate a greater reliance on the ipsilesional limb, and lower scores (< 50%) indicate a greater reliance on the contralesional limb. In our study 6-OHDA infused rats showed higher scores in the forelimb use asymmetry test.
indicates its greater reliance on the ipsilesional limb. BMC treated alone didn’t reverse the condition. The higher scores seen also in the groups treated individually with 5-HT and GABA. Prominent reversal in the asymmetry score towards the control was observed in rats treated with 5-HT, GABA and BMC in combination. Previous studies by Vergara-Aragon et al., (2003) showed that severe unilateral DA depletion caused moderate impairments in skilled reaching of the “good” forelimb, which were improved by focused skilled reach training for 5 days/week for 45 days, beginning well after 6-OHDA infusions. Impairments in the “bad” forelimb could not be significantly rehabilitated.

PD has been reported to be accompanied by a number of behavioural and hormonal abnormalities, including reduced locomotor activity. Thus, for understanding the relationship between behavioural expression and underlying neuropathology in the 6-OHDA model, one should take into account that the behaviour is depend on the sensitivity of a test to measure striatal DA loss on the one hand, and the extent to which non-dopaminergic and extra-striatal neurotransmission are affected by the regimen on the other. To provide a behavioural assessment of the 6-OHDA-induced lesion, a rotarod test was done to determine the ability to perform well-coordinated locomotor activity (Cendelin et al., 2008). The rotarod test experiment demonstrated the impairment of the motor function and coordination in the 6-OHDA infused rats. 6-OHDA infused rats showed lower fall off time from the rotating rod when compared to control, suggesting impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture. Moreover, the 6-OHDA infused rats showed clear signs of deficiency in fine motor control as indicated by a reduced tendency to turn around and walk forward on the rotarod. At the same time, they were unable to adjust their limb movements on the metallic rod which is indicative of cerebellar dysfunction. The 5-HT, GABA and BMC treated rats showed an improved motor performance in rotarod, compared to
Discussion

6OHDA induced PD rats. Our findings indicate that 5-HT, GABA and BMC in combination reverses the motor abnormalities which assists in lowering their time for spatial recognition and thus helps to maintain their posture during movement on the rod.

Swim test, is an efficient technique to investigate the overall manifestation of motor dysfunction in PD is imminent for identifying relationship between the loss of dopaminergic neurons and behavioural changes observed in animal models of this disease. While tremor and akinesia are acute behavioural manifestations that are visible, swimming is latent and manifested only when tested in a new environment. Tremor and akinetic responses are short lasting, whereas swim deficit could be setting in slowly, along with depletion of striatal DA. The objective of the present study was to evaluate the suitability of swim endurance (as revealed by swim-score), for assessing motor deficit in rats with striatal DA depletion, following 6-OHDA administration. Two major criticisms for the applicability of swim-test are: (i) in highly akinetic state, the animals may drown and (ii) swimming in cold water may induce stress and interfere with the performance while they swim (Sedelis, 2001). We can always overcome the cold induced stress by maintaining the water temperature at the ambient level. Here, in our study, we have maintained the temperature at 27±2 °C. Swim-test is mostly used for animal models with bilateral lesions and with partial DA depletion. Here we tried to use this in the unilateral model along with the rotational analysis to observe the motor behaviour. In the present study 6-OHDA infused rat showed significant decrease in swim score compared to the control. This indicates a direct relationship between DA depletion and motor impairment in 6-OHDA infused Parkinsonian rat. Rats treated with 5-HT, GABA and BMC showed a considerable recovery in their swim-score as compared with the 6-OHDA infused rats.

Whether spatial deficits are one of the major characteristics of neuropsychological alterations in PD has been discussed for many years. A number of
studies however have demonstrated that spatial deficits are indeed associated with the motor component of the task (Lezak, 1995) or with the task’s speed component (Ogden et al., 1990). This pattern of results has been related to a gradually emerging dopaminergic deficit. Spatial deficits which are regularly seen in PD patients are often understood as deficits in the ability to sequence chains of motor responses, to plan these sequences in advance and to perform behavioural acts in multiple steps. The Y-maze test is a classic model behavioural test, with a strong aversive component, utilized for evaluating learning and memory in rats (Woo et al., 2008). Y-maze performance showed that intensity of derangement in 6-OHDA infused rats increased. Furthermore, spatial memory and exploratory activity have an influence on behavioural tests including Y-maze performance. In this regard, the number of novel arm entries was significantly lower in 6-OHDA infused PD rats. There was a prominent reversal towards the control in number of novel arm entry when 6-OHDA infused PD rats were treated with 5-HT, GABA and BMC in combination. These findings indicate that transplantation of BMC along with 5-HT and GABA were able to normalize the dopaminergic and glutaminergic receptor dysfunction which assists in lowering their time for spatial recognition and thus improving the cognitive functions.

DA, glutamate and ACh systems seem to have important interactions with regard to cognitive function. A variety of previous studies have found that DA ligands have significant interactions with ACh agonist and antagonist effects on memory performance (Levin et al., 1992). Alterations such as inhibition of DA neurotransmitter release, changes in pre- or postsynaptic receptor binding, and neurotransmitter transports contributes to the impairment in the brain blood flow on neurons or glial cells and consequently interfere with the information encoding processes. We evaluated the spatial memory and learning by radial arm maze to evaluate the memory deficit in the 6-OHDA infused rats. In radial arm maze, memory
errors like working memory error and reference memory error were scored along with the number of trials needed to attain the criterion. Working memory is a transient form of memory that maintains task relevant information during conditions of competing demands (Baddeley, 1986). Working memory can be operationalized as task accuracy in situations where information (or the appropriate response based on that information) changes frequently (i.e., from trial to trial), whereas reference memory reflects task accuracy when information (or the appropriate response based on that information) remains constant indefinitely (i.e. across trials and/or sessions) (Olton et al., 1979). The number of trials to attain five consecutive criterion performances increased significantly in the 6-OHDA infused rats. Increased numbers of trials to criterion performance indicates the learning and memory deficit in 6-OHDA infused rats. A significant increase in memory errors was also scored in 6-OHDA infused rats indicating the impairment in coordination of tasks. High reference memory error in 6-OHDA infused rats points to the impairment with respect to procedural or reference memory i.e., what to do with the information they have. A significant reversal towards the control was observed in the treatment groups: 5-HT, GABA, 5-HT + BMC, GABA+BMC. BMC treated alone did not show any significant reversal compared to 6-OHDA infused rats. Most prominent reversal in the number of trials to attain the criterion performance and memory errors was observed when rats treated with 5-HT, GABA and BMC in combination. The impairment in spatial learning, memory and task management was also reversed by BMC supplementation to 6-OHDA infused rats, in combination with 5-HT and GABA.

CENTRAL NERVOUS SYSTEM ALTERATIONS DURING PD

Corpus striatum

The corpus striatum is the largest component of the basal ganglia. Various anatomical, electrophysiological and pathological observations provide evidence that DA plays a major role in the control of striatal function and in the regulation of motor
control (Avale et al., 2008). Loss of dopaminergic neurons in the SN and nigrostriatal pathway disrupts the physiologic activity of the striatum (Penney & Young, 1983) and compromises the functioning of subcortical-cortical functional-anatomic loops. DA levels in the corpus striatum including the nucleus accumbens and Ventral tegmental area projection sites decreases significantly in 6-OHDA infused rats. The dopaminergic neurons in the SNpc extend its arm to the corpus striatum and hence are directly affected in PD. In the normal brain there exists a balance between direct inhibitory input through GABA and indirect excitatory input through glutamate to the lateral GPi, which in turn controls thalamocortical activation. The deprivation of dopaminergic 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). Thus, the low level of DA, in Parkinson’s, leads to enhanced glutamate function. Scatchard analysis of glutamate receptor in the corpus striatum of 6-OHDA infused rats showed a significant increase in $B_{\text{max}}$ compared to control rats. Increased glutamate content in the 6-OHDA infused rats leads to the up regulation of total glutamate receptors. There was no significant change in $K_d$ in all experimental groups. Significant reversal was seen in all treatment groups except the rats treated BMC alone. More prominent reversal was seen in rats supplemented with 5-HT and GABA treated with BMC. A key event in this process glutamate mediated neurotoxicity is the enhancement of the NMDA receptor-channel complex and a subsequent influx of $\text{Ca}^{2+}$. We observed an increase in NMDA receptors function in the corpus straitum of the 6-OHDA infused rats with no significant change in $K_d$. The increased receptor activity observed from the Scatchard plot was supported by the gene expression studies of NMDAR1, NMDA2B and mGluR5 glutamate receptor subtypes. Glutamate reuptake into neurons and glia cells is important for termination of glutamatergic transmission. Glutamate transporters are essential for the maintenance of low extracellular levels of glutamate. Our results showed a reduced
expression of GLAST glutamate transporter that indicates the reduced reuptake of the extracellular glutamate which is activated through glutamate receptor subtypes- NMDAR1, NMDA2B and mGluR5. The decreased glutamate transporter GLAST expression reduces the reuptake of the extracellular glutamate. Thus the results showed evidence for the dysfunction of the corpus striatum that is a reflection for manifestation of abnormal behavioural patterns. Combination treatment restored the impairment near to control.

NO-cGMP- Protein kinase G (PKG) signaling pathway plays an essential role in the neuroprotection through activating B-cell lymphoma 2 (Bcl-2), Mn Superoxide dismutase (MnSOD) and brain derived neurotrophic factor (BDNF) gene expression which reduces oxidative stress (Andoh et al., 2002). 6-OHDA induced oxidative damage and neurodegeneration leads to a decreased cGMP level in the corpus striatum. At the same time we obtained an increased production of IP3, cAMP in 6-OHDA infused rats which are mediated through the enhanced glutamate receptors. This will trigger the release of Ca\(^{2+}\) from the endoplasmic reticulum. IP3-mediated Ca\(^{2+}\) release in turn increase mitochondrial Ca\(^{2+}\) and consequently, increase respiration and ATP production (Hajnoczky et al., 2000). This causes metabolic stress on mitochondria that leads to excessive oxidative phosphorylation and increased production of reactive oxygen species. Excessive stimulation of glutamate receptor/ion channel complexes triggers Ca\(^{2+}\) flooding and a cascade of intracellular events that results in apoptosis (Johnston, 2005). Up regulation of pro-apoptotic Bax protein expression in the corpus striatum indicates the mitochondria mediated apoptosis in 6-OHDA infused rats. Enhanced glutamatergic, IP3 and cAMP activity leads to oxidative stress in the striatum. A plausible source of oxidative stress in striatum neurons is the redox reactions that specifically involve DA and produce various toxic molecules, i.e., free radicals and quinone species. We observed an increased expression of the \(\alpha\)-Synuclein in the striatum which advances the
neurodegeneration. Our findings showed a significant down regulation of CREB in the corpus straitum of 6-OHDA infused rats. Even though cAMP level was increased, the CREB expression was decreased. Enhanced activation of the glutamate receptors leads to the production of second messengers. But its acute and prolonged action triggers the cell death pathways by activating pro apoptotic genes like Bax, bad and destabilizing jun- fos complex. The activation of apoptotic pathways down regulates the CREB expression thereby blocking the cAMP signaling cascade in PD rats. These findings suggest that decreased CREB expression is the result of cell loss.

Astrocytes and microglia are thought to play a major role in brain inflammatory responses. Both of these cell types exhibit a reactive phenotype in association with neurodegenerative diseases as well as in response to neurotoxic insults. Cytokines as participants in the pathological processes underlying both neuronal and glial responses associated with PD. TNF-α is one of the most strongly implicated cytokines associated with PD. TNF-α is known to induce generation of reactive oxygen intermediates associated with necrotic cell death (Goossens et al., 1995), and it also induces changes in mitochondrial ultrastructure and function (Larrick & Wright, 1990). Up regulation of TNF-α in 6-OHDA infused rat confirms the mitochondrial impairment in the striatum area. We have demonstrated that enhanced expression of TNF-α is associated with the earliest stages of damage in the 6-OHDA model of dopaminergic neurotoxicity. Moreover, using 5-HT, GABA and BMC in combination we showed complete protection against 6-OHDA-induced neurotoxicity. The early onset of TNF-α expression after 6-OHDA and the neuroprotective effect afforded to dopaminergic neurons by 5-HT, GABA and BMC in combination implicate that this has a potential upstream effect in reducing the neurodegenerative processes underlying PD.
**Substantia nigra pars compacta**

One division of the basal ganglia, the SN, consists of two major components, the SNpr and SNpc. The SNpr contains one of the populations of basal ganglia output neurons and the SNpc contains the dopaminergic nigrostriatal neurons which are involved in the modulation of the flow of cortical information through the basal ganglia. SNpc is one of the main output nuclei of the basal ganglia structures and as such plays an important role in the motor activity. DA neuronal systems, originating in the SNpc constitute one of the main actors of such an important role (Campusano et al., 2002). Neuropathologic studies of PD suggested that patients with the earliest signs of disease have already lost as much as 50% of the pigmented dopaminergic neurons in the SN (Marsden, 1990). The principal pathological characteristic of PD is the progressive death of the pigmented neurons of the SNpc, the nigrostriatal DA neurons. Anatomical, behavioural, biochemical and electrophysiological studies have demonstrated an interaction between glutamatergic and dopaminergic systems at different levels of the SNpc. The SNpc represents the main output site of the basal ganglia and receives excitatory glutamatergic afferents mainly from the SN, cerebral cortex and pedunculopontine nucleus (Iribe et al., 1999). Glutamate receptor-mediated excitotoxicity has been suggested to be a contributory factor in the degeneration of dopaminergic neurons in PD (Blandini et al., 1996). The effects of glutamate in the SNpc are mediated by the two principal types of glutamate receptors, NMDA and metabotropic receptors (Hollmann & Heinemann, 1994). Fast synaptic transmission is proposed to be mediated by NMDA receptors and physiological evidence suggests that the stimulation of NMDA receptors is considered as a mechanism to modulate fast excitatory postsynaptic potentials (Standaert et al., 1994). Our observation in the mgluR5, NMDAR1 and NMDA2B gene expression showed an up regulation in the SNpc, this is due to an imbalance between excitation and inhibition which leads to excessive activation of excitatory amino acid receptors, leading to various types of
neuronal damage. Previous studies demonstrate that GABA counteracts such neuronal hyperactivity (Globus et al., 1991). We proved that GABA along with 5-HT and BMC reverse these abnormalities to near control level. This confirms that over activity of glutamatergic neurotransmission in the basal ganglia leads to the onset and pathogenesis of PD. Glutamate homeostasis around glutamatergic synapses is tightly regulated by two groups of glutamate transporters: glial glutamate transporters GLT1 (EAAT2) and GLAST (EAAT1) and neuronal glutamate transporter EAAC1. The present results indicate that reduction of GLAST which impair glutamate homeostasis around glutamatergic synapses in the SNpc and contribute to over-spills of glutamate in the system.

Glutamate receptor over activation in the SNpc results mitochondrial dysfunction and oxidative stress which “reset” the threshold for activation of apoptotic pathways in response to Bax and similar signals. Our analysis in the Bax gene expression gave an enhanced expression level which substantiates our observation. Previous studies on the role for Bax dependent pathway comes from the demonstration by Vila and colleagues that mice deficient in Bax are protected from the induction of apoptosis and loss of SN DA neurons in the MPTP model (Vila et al., 2001). From our study we can confirm that glutamate mediated neuronal damage occurring in the SNpc of 6-OHDA lesioned rats is Bax dependent. Inflammatory processes that involve a host of cytokines have been shown to be associated with ongoing neuronal degeneration seen in several neurodegenerative diseases, including PD. Proinflammatory cytokines are known to play a role in mitochondrial impairment and oxidative stress; therefore, an inflammatory response serve as an integral feature of the mechanistic underpinnings related to the pathogenesis of PD (Hunot et al., 2001). Previous studies showed an enhanced expression of the proinflammatory cytokine, TNF-α in association with glial cells in the SN of patients with PD (Krishnan et al., 2002). Our results also support the earlier studies. We got an
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increased TNF-α expression in the SNpc which confirms the role of inflammatory process in the PD progression.

CREB is a transcription factor which has various roles in development, learning, memory, plasticity, promotion and regulation of neuronal survival. CREB activated (phosphorylated) by a variety of signaling pathways. The most common and best elucidated is the cAMP-PKA pathway. Extracellular signals (ex: Hormones and neurotransmitters) activate heterotrimeric G-proteins, that directly stimulate adenyl cyclase, which can then catalyze the production of cAMP. cAMP then leads to the activation of PKA, which dissociates into active catalytic subunits which diffuse into the nucleus and phosphorylate CREB (Stewart & William, 2008). In our study increased ROS production and apoptotic pathways in the 6-OHDA infused rats caused a reduced CREB expression level in the SNpc which promote the neuronal damage. Abnormal folding and aggregation of neuronal proteins in the brain has been extensively investigated as one of the central mechanisms leading to neurodegeneration in PD. Accumulation of misfolded α-synuclein has been proposed to be centrally involved in the disease (Masliah & Hashimoto, 2002). 6-OHDA infusion to the SNpc increased the gene expression of the α-synuclein in the region which leads to the misfolding and aggregation.

There is increasing interest in the transplantation of stem cells as a means of recovering function in individuals with neurodegenerative disease. Although substantial improvements result from the systemic administration of L-dopa or DA agonists, such pharmacological interventions do not address the aetiology of the disease, provide a permanent remedy or prevent progression of the degenerative process (Snyder & Olanow, 2005). Implantation of stem cells will provide a more constitutive and relevant solution. This realisation has prompted a renewed interest in stem cells, which serves as a replenishable source of cells for the treatment of neurodegenerative disorders. The success of the cell transplantation will depend on
the ability of the cells to replace those neurons lost as a result of the disease process in the DA-deficient striatum and reverse, at least in part, the major symptoms of the disease. Previous studies on PD animal models have examined functional recovery after cell implantation, employing behaviour testing followed by post-mortem histology to establish cellular efficacy (Lu et al., 2005). A more recent study using MRI alone in PD rats, demonstrated the visualisation of implanted neural stem cells for several weeks with improved post-transplantation behavioural rotation (Yang et al., 2006). Other reports have shown that implanted mesenchymal stem cells tracked by MRI for 50 days in a rodent model of stroke (Jendelova et al., 2004) and oligodendrocyte progenitors were tracked for six weeks in a rodent model of demyelination (Bulte et al., 2002). A clinically relevant strategy is to implant BMC that are constitutively capable of neural differentiation and cytokine secretion. Allowing the cells to develop within the PD-affected brains, yields cells whose phenotypes, numbers, locations and regulation are determined by the interplay of donor elements and the local host milieu. A consequence of such donor-host interaction would result in a more pertinent homeostasis. We hypothesised that the BMC-based approach might better mitigate some of the limitations of previous strategies, where pre-programmed partially differentiated cells did not provide functional recovery (Brederlau et al., 2006). Furthermore, Autologous BMC to treat neurological disorders offers several unique advantages over other cell replacement therapies. Immunological reactions are avoided and it also bypasses ethical issues in the use of embryonic cells. They are also relatively easy to harvest in the clinic, with procedures used routinely in bone marrow donations (Jackson et al., 2009).

The success of this approach is hindered by the absence of methods that not only allow us to follow the fate of transplanted stem cells, but to monitor their efficacy non-invasively in the same subject. In vivo cell tracking techniques provide the most appropriate methodologies to achieve this goal in rats. Horan and Slezak
(1989) developed the Paul Karl Horan (PKH dyes) which are lipophilic, fluorescent membrane intercalating to provide improved cell tracking capabilities and to allow a better understanding of various disease processes. Three different PKH dyes are used for labeling cells PKH2, PKH26 and PKH67. Several authors have described the use of PKH2GL dyes to study proliferation (Traktuev et al., 2008). BMC were tagged with PKH2GL cell linker dye and it was infused individually and in combination stereotactically into the right SNpc. BMC differentiation analysis using primary antibody for nestin and GFAP proved that BMC differentiate to neuronal cells once the proper conditions are given. When autologous combination treatment was given to SNpc, they differentiated to both neuronal and glial cell types. PKH2GL tagged BMC when injected into the brain it started expressing both nestin and GFAP. The BMC observed in vitro changed its morphology once administered to SNpc within 12 days. The BMC differentiation was increased when it was administered along with 5-HT and GABA later differentiating to neurons in vivo. This was confirmed with the nestin gene expression analysis in the SNpc region in which it showed a maximum expression in the combinational groups compared to control rats. Our results confirmed the 5-HT and GABA comitogenic effect in proliferation and differentiation of the BMC to neurons in the brain by confocal studies using PKH2GL, Nestin and GFAP. 5-HT and GABA are involved in a variety of cellular processes involved in regulating metabolism, proliferation and morphology. The fine integration of these dynamic events appears to involve multiple receptor action.

Studies by Li et al., (Li et al., 2005) showed that neural transplants into the CNS lead to a marked activation of astrocytes. GFAP is an intermediate filament protein that is known to be localized to astrocytes (Maragakis & Rothstein, 2006). Up regulation of GFAP is the hallmark of reactive astrocytes (Eddleston & Mucke, 1993). We demonstrated here that the transplantation of BMC activate the migration of astrocytes to the SNpc. Our study using PKH2GL and GFAP confirmed the astrocyte...
migration in all the experimental groups. This was further proved by gene expression studies of GFAP which also showed an increased expression in 6-OHDA infused rats and the expression were further enhanced when the rats treated with BMC individually and in combination with 5-HT and GABA. In addition to providing structural and trophic supports for neurons, astrocytes are known to modulate the local environment around neural stem cells (Agulhon et al., 2010). Recent studies proved that these cells also release signalling molecules that helps in neuronal communication (Doetsch, 2003). Transplantation of adult neural progenitor cells into different brain regions has revealed neurogenic and non-neurogenic niches, underscoring the influence of environment on the fate of grafted cells (Dziewczapolski et al., 2003). Astrocytes from neurogenic regions of adult brain provide permissive or inductive environmental cues directing differentiation of neural progenitor cells (Song et al., 2002). Migrated astrocytes in our study made connections with the transplanted cells and helped its differentiation and proliferation. Our findings firmly support that the BMC infusion into the SNpc creates an environment which leads to the migration of astrocytes to the SNPC. These astrocytes are playing a crucial role in the differentiation and development of the transplanted BMC.

For confirming whether the transplanted cells can reverse back the normal DA production we checked the DA content analysis using HPLC, Tyrosine hydroxylase (TH) activity using immunohistochemistry and gene expression. A consistent neurochemical abnormality in PD is degeneration of dopaminergic neurons in SN, leading to a reduction of striatal DA levels. In our study 6-OHDA infusion into the SNpc region leads to a reduced DA content in the PD rats. TH catalyses the formation of L-DOPA, the rate-limiting step in the biosynthesis of DA, the disease can be considered as a TH-deficiency syndrome of the striatum. Like other cellular proteins, TH is also a possible target for damaging alterations induced by ROS (Haavik & Toska, 1998). 6-OHDA infusion severely reduced the number of TH-immunopositive
neurons in the SNpc compared to the control group. Confocal analysis confirmed with
the gene expression study which showed a reduction in the TH expression in 6-OHDA
infused rats. 5-HT, GABA along with BMC treatment reversed this alteration which
indicates that the differentiated BMC is able to synthesise DA.

Cerebral cortex

Changes in personality and moderate or mild cognitive debilitation are found
in PD. Cerebral glucose metabolism is reduced in the cerebral cortex in PD patients
suffering from cognitive impairment (Yong et al., 2007). Metabolic and neuroimaging
observations have recently documented decreased prefrontal and parietal 18F-
fluorodeoxyglycose uptake in PD cases with mild cognitive deficits (Huang et al.,
2007, 2009). Studies have demonstrated that complex I deficiency and abnormal ATP
synthase and inner protein membrane prohibiting expression levels in the frontal
cortex in PD (Parker et al., 2008). The cerebral cortex receives widespread inputs
from subcortical areas involved in sensorimotor and limbic functions. The integration
of these glutamatergic inputs is essential for the cerebral cortex role in executive
functions and goal-directed behaviour (Miller, 2000). It is also well known that
cerebral cortex activity is shaped by a number of neuromodulators, most notably
monoamines. Among these, DA stands out as having an important role in cerebral
cortex cognitive functions, including working memory, reward, and attention (Schultz,
2002). Several reports have highlighted the need of DA–glutamate coactivation for a
number of cortical functions (Gurden et al., 1999, Baldwin et al., 2002).

Glutamate neurotransmission plays an integral role in basal ganglia
functioning especially in the striatum, where the balance of glutamate and DA is
critical but also in the SN which receives glutamatergic input from the subthalamic
nucleus and cortex. Glutamatergic pathways also play a leading role in the structural
and functional organization of the cortico-basocortical loops involved in PD (Hirsch et
al., 2000). In addition, using morphological criteria, an 88% increase in glutamatergic
perforated synapses was reported in the putamen of PD patients (Anglade et al., 1996). Increased glutamate content in the cerebral cortex of 6-OHDA infused rats leads to the up regulation of total glutamate and NMDA receptors. This was confirmed by the gene expression studies of mGluR5, NMDAR1 and NMDA2B, where it showed an up regulation in 6-OHDA infused rats compared to control. The extracellular concentration of the glutamate in the CNS must be kept low to ensure a high signal to noise ratio during synaptic activation and to prevent excitotoxicity due to excessive activation of glutamate receptors (Katagiri et al., 2001). Glutamate uptake into neurons and glial cells is important for the termination of glutamatergic transmission. They are essential for the maintenance of low extracellular levels of glutamate. We observed a reduced expression of GLAST in 6-OHDA infused rats. The decreased glutamate transporter GLAST expression reduces the reuptake of the extracellular glutamate.

Nitric oxide synthesis can affect guanylyl cyclases and thus exert control on cGMP production. It has also been shown that it down regulates NMDA channels and thus reduces Ca\(^{2+}\) flow into the cytosol. We obtained decreased cGMP content in the cerebral cortex of 6-OHDA infused rats which is due to NMDA receptor activation and intracellular Ca\(^{2+}\) accumulation. All of glutamate receptors couple positively to phospholipase C through guanine nucleotide binding proteins whereby they stimulate phosphoinositide hydrolysis generating a second messenger cascade consisting of diacylglycerol and inositol 1,4,5 trisphosphate (Berridge, 1987). Jo et al., (2008) demonstrated that NMDA and mGluR receptors mediate Ca\(^{2+}\) release by stimulating IP3 and PKC. \(\beta_1\)-adrenoeceptors are highly expressed in PD which induced the up-regulation of cAMP/PKA signaling (Hara et al., 2010). In our studies we observed an elevated cAMP and IP3 level in the cerebral cortex of 6-OHDA induced rats. The elevated IP3 level causes extracellular release of Ca\(^{2+}\), which in turn enhanced metabolic stress on mitochondria that leads to excessive oxidative phosphorylation.
and increased production of reactive oxygen species. If the matrix Ca\(^{2+}\) level rises too high, then deleterious changes in mitochondrial structure occur. In particular, mitochondria can swell and rupture or undergo permeability transition, thereby releasing several pro-apoptotic factors into the cytoplasm, such as cytochrome C, second mitochondrial activator of caspases (SMAC/Diablo) or apoptosis-inducing factor (AIF) (Orrenius et al., 2003). Our study showed an increased activity of Bax gene expression in the cerebral cortex of the 6-OHDA infused rats which indicated the ROS mediated neurodegeneration in the cerebral cortex. Bax, one of the major pro-apoptotic family members, exerts its effects by compromising the membrane integrity leading to leakage of apoptogenic factors such as cytochrome c into the cytosol, resulting in caspase-3 activation and demise of the cell (Shacka & Roth, 2005). At the same time the CREB gene expression was down regulated in the 6-OHDA rats. This indicates the interruption of the cAMP signalling cascade in the PD rats due to enhanced pro-apoptotic factors which further enhances the neuronal death.

Abnormal brain mitochondrial function; oxidative damage on DNA, RNA and proteins; abnormal stress responses; modifications of crucial PD related proteins; and subcellular redistribution of proteins flow into one another and potentiate metabolic damage. Thus abnormal mitochondria function is a source of reactive oxygen species and oxidative stress, at the same time that they promote oxidative damage of glycolysis and energy metabolism-related proteins, mitochondrial proteins such as parkin and DJ1 and proteins involved in stress responses such as SOD1 and SOD2. Enhanced of \(\alpha\)-synuclein in the cerebral cortex are the seed of abnormal folding, oligomerization and aggregation. In the central nervous system, TNF-\(\alpha\) is produced by brain-resident astrocytes, microglia, and neurons in response to numerous intrinsic and extrinsic stimuli. TNF-\(\alpha\) induces neuronal apoptosis though an excitotoxic mechanism mediated through glutamate receptors especially AMPA receptors (Gelbard et al., 1993) and activates microglia, which leads to the propagation of
various neurological diseases (Hanisch et al., 2002). Gene expression studies of TNF-α in 6-OHDA infused rats showed an increased production which resulted in significant enhancement of Ca²⁺ signals downstream of glutamatergic stimulation. An increase in IP3 content positively correlated with this alteration in Ca²⁺ homeostasis. Modulation of Ca²⁺ responses arising from this receptor subtypes and its downstream effectors have exact significant consequences on neuronal function and underlie the compromise in neuronal activity observed in the setting of chronic neuroinflammation associated with PD. Based on these observations we confirm that irregular mood and behaviour and cognitive deficits observed even at early stages PD is the result of these molecular abnormalities (Nandhu et al., 2011).

**Hippocampus**

DA containing neurons participate in the regulation of certain cognitive processes (Prediger et al., 2011). Cognitive impairments are observed in Parkinson’s disease patients, especially on measures of memory, verbal fluency and other executive functions (McPherson & Cummings, 1996). Research suggests that the behavioural, motor and cognitive impairments found in Parkinson’s disease patients reflect dysfunction of hippocampal neural circuitry (Saint-Cyr, 2003). Additionally, cognitive and motor problems contribute to the existence of depression in Parkinson’s disease and conversely, symptoms of depression impact cognitive and motor deficits (Robinson et al., 2000). The hippocampal formation contains a rich glutamatergic and GABA-ergic input, GABA-ergic interneurones containing peptide co-transmitters and the glutamatergic perforant pathway interconnects with entorhinal cortex, subiculum, CA1, CA3 fields and dentate gyrus (Ottersen & Storm-Mathisen, 1984). Potentiation, defined as an increase in synaptic efficacy, is readily induced by high frequency stimulation (HFS) of the synapses between the Schaffer collaterals and the pyramidal cells in the hippocampus CA1 area (Malenka & Nicoll, 1999). The excitatory synapse
in the stratum radiatum of the CA1 area of the hippocampus has a number of features that have been attributed to various aspects of memory encoding (Martin et al., 2000).

Heightened responsiveness to the excitatory neurotransmitter glutamate and associated excitotoxicity has been implicated in the pathogenesis of PD (Dawson & Dawson, 2003). In the CNS, glutamate is an important factor for maintaining Ca\(^{2+}\) homeostasis; it is the most abundant excitatory neurotransmitter and it is widely distributed. Glutamate is associated with various brain functions, such as synaptic plasticity, learning and long-term potentiation (Collingridge & Singer, 1990; Groth et al., 2011). In this study, we focused on the glutamate receptor, which is abundantly expressed throughout the hippocampal formation which showed an increased glutamate content in the hippocampus of 6-OHDA rats compared to control. Our findings also report an increase in total glutamate and NMDA receptors function in the hippocampus with no significant change in K_d. This increased B_max observed shows the increased number of receptors with no change in the affinity of the receptors which was shown from the K_d. The increased receptor activity observed from the Scatchard plot was supported by the gene expression studies of NMDAR1, NMDA2B and mGluR5 glutamate receptor subtypes. Based on extensive supportive experimental data, the release of high levels of glutamate by neurons is thought to be the underlying mechanism for the initiation of neurodegeneration. The immunohistochemistry experiments in the present work supported the gene expression studies of NMDAR1, NMDA2B and mGluR5 receptors. This up regulation will increase the glutamate receptor activity and molecular cascades inside the cells. Other studies showed that mGluR5 blockade ameliorates motor abnormalities induced by lesions of the nigrostriatal dopaminergic system, or by dopaminergic receptor antagonists in animal models of PD (Dekundy et al., 2006). The major issue in the treatment of PD has been the occurrence of abnormal involuntary movements (AIMs) resulting from chronic L-dopa treatment of Parkinsonian humans. The AIMs,
occurring in up to 80% of chronically l-DOPA-treated Parkinson’s disease patients, are commonly referred to as l-DOPA-induced dyskinesia. Evidences suggest that mGluR5-mediated neurotransmission is involved in the pathogenesis of this disorder (Lundblad et al., 2002). Our experiments also support the same. 5-HT and GABA are playing a direct role in decreasing the activated glutamate receptors. We also obtained a decreased expression of GLAST glutamate transporter in the hippocampus of experimental rats compared to control. This decreased expression of glutamate transporter will lead to the decreased clearance of glutamate from the extracellular space and we report in our present study that glutamate content is high in the hippocampus of experimental group compared to control. Up regulation of NMDA receptor and down regulation of glutamate transporter expression suggests a response to altered synaptic glutamate levels (Lyon et al., 2008). It was found that GLAST glutamate transporter down regulation is involved in cell swelling in hippocampus (Ouyang et al., 2007).

The cyclic nucleotides cAMP and cGMP are involved in a number of intracellular processes such as signal transduction, gene transcription, activation of kinases, and regulation of channel function (Burns et al., 1996). Investigations of the cAMP content in the hippocampus of 6-OHDA rats revealed a significant increase when compared to control. At the same time the cGMP content was down regulated in the 6-OHDA rats. This rise in cAMP could be mainly due to the influence of increased mGLU5 receptors as is seen during PD rats. Treatment with 5-HT, GABA and BMC in combination reversed these alterations to near control. NMDA receptors mediate their function through the IP3 release. In our study the IP3 content increased in the brain stem of 6-OHDA infused rats. IP3 receptor activation leads to excessive Ca^{2+} overload in cells leading to apoptosis. Bax is a pro-apoptotic protein allowing apoptosis to occur through the intrinsic, damage-induced pathway and amplifying that one occurring through the extrinsic, receptor mediated pathway. Bax is present in
viable cells and activated by pro-apoptotic stimuli. Bax has multiple functions: it releases different mitochondrial factors such as cytochrome c, SMAC/diablo; it regulates mitochondrial fission, the mitochondrial permeability transition pore; it promotes Ca\(^{2+}\) leakage through ER membrane (Ghibelli & Diederich, 2010). The expression of pro-apoptotic protein Bax can be taken as an index of cell death. Increased Bax gene expression in the 6-OHDA infused rats confirmed the hippocampal neuronal damage. \(\alpha\)-synuclein has been identified as the main protein constituent of Lewy bodies. Our study in 6-OHDA infused rats showed increased expression of \(\alpha\) -synuclein which makes it more prone to aggregation. 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 (Hartmann et al., 2001). Our studies also support the earlier reports. We obtained an increased TNF-\(\alpha\) expression which further enhanced the neuronal damage.

CREB is activated in response to cAMP and this mechanism of activation is well-characterized. 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 and a pair of catalytic 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 is then induced. But in our study increased cAMP content didn’t influence on the CREB which showed a decreased expression. It is interesting to note that down regulation of CREB gene expression observed in 6-OHDA infused rats supporting a potential role for impaired nuclear import of phosphorylated signaling proteins in neuronal injury processes. Our evaluation in the hippocampus proves that glutamate over activity as a consequence of striatal DA depletion has a prominent role in the cognitive deficits in PD rats; in addition to motor dysfunction. 5-HT and GABA along
with BMC acting through its receptors ameliorating hippocampal dysfunction occurs in 6-OHDA rats.

**Cerebellum**

The basal ganglia and cerebellum are two groups of subcortical nuclei that have classically been regarded as motor structures. Damage to these brain regions produces well-described alterations in motor function. Cerebellar output abnormalities affects not only in the primary motor cortex but also subdivisions of premotor, oculomotor, prefrontal and infero temporal areas of cortex. In PD patients, increased activity was seen in the cerebellar vermis. Two distinct motivational systems in the brain have been described: the DA-dependent cortico-striatal-thalamocortical circuits and the DA independent brain stem pedunculopontine segmental nucleus, which has strong interconnections to the cerebellum. Parkinson’s patients utilize the latter circuit when the former has been damaged (Weintraub & Potenza, 2006).

Glutamate is the major excitatory neurotransmitter in vertebrate CNS, but inadequate regulation of extracellular glutamate and glutamate receptor agonists cause toxicity in the nervous system (Greene & Greenamyre, 1996). Glutamate neurotransmission is a paradox in that it is vital for essentially all excitatory synaptic transmission in the CNS, but excessive stimulation of glutamate receptors is toxic to neurons. This phenomenon, known as excitotoxicity, has been intensely studied and implicated as a potential pathogenic factor in a number of neurologic diseases, including PD. Studies have shown the involvement of NMDA receptor subunits-NMDAR1, NMDA2B in the cerebellum in motor learning of the mouse (Jiao et al., 2008). Experimental evidence indicate the involvement of the cerebellum in variety of human mental activities including language (Fiez et al., 1996), attention (Allen et al., 1997), cognitive affective syndromes (Schmahmann & Sherman, 1998), fear and anxiety caused by threats of pain, thirst sensation and fear for air hunger and motor relearning (Imazumi et al., 2004, Marvel et al., 2004).
Increased glutamate content in the 6-OHDA infused rats leads to the up-regulation of total glutamate receptors. There was no significant change in \( K_d \) in all experimental groups. Glutamate regulates neuronal activity by acting on ionotropic and mGluRs. The mGluR5 receptors, in particular, have received considerable attention in PD research, being major players, for example, in the excitatory drive to the SN from glutamatergic afferents. We observed an increase in total glutamate and NMDA receptors function in the cerebellum of 6-OHDA infused rats. This was supported by the real time PCR analysis which showed an increased receptor gene expression of NMDAR1, NMDA2B and mGluR5 in the PD rats. The extracellular concentration of the glutamate in the CNS must be kept low to ensure a high signal to noise ratio during synaptic activation and to prevent excitotoxicity due to excessive activation of glutamate receptors. Excess activation of NMDA receptors by glutamate increases cytoplasmic concentrations of \( \text{Na}^{2+} \) and \( \text{Ca}^{2+} \) to levels that exceed the capacity of neuronal homeostatic mechanisms, thereby altering transmembrane ion gradients. As with most classic neurotransmitters, glutamate is specifically concentrated into synaptic vesicles in nerve terminals, released during depolarization in a \( \text{Ca}^{2+} \)-dependent manner and inactivated by reuptake. However, unlike classic transmitters, glutamate uptake by glial cells is significant. Within glia, glutamate is transaminated by glutamine synthetase to form glutamine, which diffuses into nerve terminals and is converted. As seen in the other brain regions we observed a decreased gene expression of GLAST glutamate transporter in the cerebellum of 6-OHDA infused rats compared to control. This decreased expression of glutamate transporter will lead to the decreased clearance of glutamate from the extracellular space.

The activation of mGluR5 receptors leads to the potentiation of NMDA currents, possibly through the activation of protein kinase C and the subsequent increase in intracellular \( \text{Ca}^{2+} \), thereby acting as an indirect agonist of NMDA receptors. NMDA receptor activation in the cerebellum leads to an increase in the
Ca$^{2+}$ also through IP3 receptors. Glutamate stimulates adenylyl cyclase through a G protein to increase cAMP formation and the activity of cAMP-dependent protein kinase (protein kinase A, PKA) leads to phosphorylation of DARPP-32 on a single threonin residue. Our results also showed an increase in the IP3 and cAMP content and decreased cGMP content in the cerebellum of 6-OHDA infused rats. Group I mGluRs couple positively to phospholipase C, the activation of which leads to stimulation of protein kinase C and release of intracellular Ca$^{2+}$, or to adenylyl cyclase, activation of which stimulates cAMP formation. Over activation of NMDA receptors causes excessive influx of Ca$^{2+}$, and consequent production of damaging free radicals together with activation of proteolytic processes that contribute to neuronal injury and cell death. Up regulation in the glutamate receptor, IP3 and cAMP activity increased the intracellular Ca$^{2+}$ which caused enhanced metabolic stress on mitochondria that leads to excessive oxidative phosphorylation and increased production of ROS. Low levels of ROS are important for many life-sustaining processes of cells and tissues, but they induce cell damage and death at higher levels. Our study showed an increased activity of Bax gene expression in the cerebellum of the 6-OHDA infused rats which indicated the ROS mediated neurodegeneration in the cerebellum. 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. CREB is controlling neuronal survival, in part, by controlling transcription of neuroprotective genes. For example, the promoter regions for both BDNF and the anti-apoptotic protein, Bcl2, each contain CRE sites and both of these gene products have been shown to play an important role in neuronal survival. In the present study the gene expression of CREB was down regulated in cerebellum of 6-OHDA compared to control. Even though cAMP level was increased, the CREB expression was decreased.
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TNF-α plays an important role in the neurodegeneration of PD this is supported by the finding previous finding that that there is an increase in the number of melanized SN neurons expressing the activated form of caspase-8, demonstrated with an antibody specific for the cleaved form, in PD brains as compared to age-matched controls (Hartmann et al., 2001). Our experiment showed an increased gene expression of TNF-α which supports the earlier reports. Neurons that degenerate in PD accumulate cytoplasmic inclusion bodies composed of α-synuclein referred to as Lewy bodies. Increased α-synuclein gene expression proves that 6-OHDA infusion will lead to massive cell loss, particularly neuronal loss, along with other pathologic processes accompanying neurodegeneration that will influence its expression. Enhanced activation of the glutamate receptors leads to the production of second messengers. The activation of apoptotic pathways down regulates the CREB expression thereby blocking the cAMP signaling cascade in PD rats. 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. Thus the up regulation of glutamate receptor activity in the cerebellum caused the increase in second messengers which mediates the Ca^{2+} overload in the cells, leading to neuronal damage. The receptor analysis and gene expression studies along with the behavioural data implicate a role for glutamate, NMDA and mGluR5 receptors in the modulation of neuronal network excitability through changes in IP3 and cAMP. These neurofunctional deficits are one of the key contributors to motor abnormalities associated with PD. 5-HT and GABA along with BMC reversed these effects by functional recovery (Nandhu et al., 2011b).

Brain stem

There have been changes in our understanding of the pathology of the disease, where it begins in the nervous system and how it progresses. Indeed, there is now evidence to suggest that the PD does not affect the dopaminergic SNpc at the earliest
stages but instead begins in the lower brain stem, olfactory bulb and anterior olfactory nucleus. The brain stem is a part of the brain located beneath the cerebrum and in front of the cerebellum. It connects the spinal cord to the rest of the brain. The brain stem controls involuntary muscles such as the stomach and the heart. The brain stem also acts as a relay station between the brain and the rest of the body. Previous studies on deep brain stimulation at pedunculopontine nucleus (PPN) in the upper brain stem to the patients having PD with gait disturbance showed a significant improvement in gait, over 50%, which opens a whole new approach of the involvement of brain stem in PD (Plaha & Gill, 2005). Sections of the brain stem usually reveal loss of the normally dark black pigment in the locus ceruleus (LC), pigmentation that correlates with neuronal cytoplasmic neuromelanin pigment that accumulates in an age-related manner. Loss of pigment correlates with neuronal loss and with the duration of Parkinsonism. In our study total glutamate and NMDA receptors of the brain stem are found to be increased in 6-OHDA infused rats. 5-HT and GABA along with BMC treated PD rats, binding parameters were reversed back to near control values. The up regulation in the receptor expression is due to the increased Glutamate content which we observed in the PD rats. Depletion in the DA content in the SNpc will increase the glutamate firing rate into the brain stem. Neurophysiological studies have implicated overactivity at brain stem, due to altered glutamatergic neurotransmission, as one underlying mechanism for the development of motor impairment and L-dopa related motor complications in PD (Soares et al., 2004; Wichmann & Soares, 2006). Receptor binding analysis was confirmed with immunohistochemistry and real time PCR analysis which showed an up regulation in m-GluR5, NMDAR1 and NMDA2B expression. Reduced gene expression of the transporter GLAST indicates the accumulation of glutamate in the region.

The signaling from the neurotransmitters is carried to the cell nucleus by second messengers like cAMP, cGMP and IP3. Their expression and changes play a
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major role in the signaling cascade. The glutamatergic receptor stimulation leads to activation of PLC, which in turn hydrolyses phosphatidylinositol 4, 5-bisphosphate (PIP2) to produce IP3 and diacylglycerol (DAG). Increased IP3 and cAMP content in the brain stem is due to the over activation of these receptors. The elevated IP3 and cAMP level causes intra cellular release of Ca\(^{2+}\), which in turn results in the activation of apoptotic pathways. cGMP mediates physiological effects in the cardiovascular, endocrinological, and immunological systems as well as in CNS. In the CNS, activation of the NMDA receptor induces Ca\(^{2+}\)-dependent Nitric-oxide synthase (NOS) and Nitric-oxide release, which then activates soluble guanylate cyclase for the synthesis of cGMP. Both compounds appear to be important mediators in long-term potentiation and long-term depression and thus play an important role in the mechanisms of learning and memory. Although we obtained an increased NMDA expression, the cGMP level in the brain stem was significant reduced in 6-OHDA infused rats. Impaired energy metabolism resulting from mitochondrial dysfunction has been proposed to render cells vulnerable to excitotoxicity. Mitochondrial dysfunction could therefore potentially result in a lowering in the threshold for excitotoxic injury. This excitotoxic injury increases free radical generation and add to cellular injury. Mitochondria also play an integral role in the apoptotic cell death pathway. When the outer mitochondrial membrane is permeabilized by action of “death agonists” such as Bax, cytochrome c is released into the cytosol, leading to caspase activation and apoptosis. Up regulated Bax expression in the brain stem confirms the neuronal damage in the brain stem after the 6-OHDA infusion.

6-OHDA treatment results in impaired nuclear import of CREB which accumulate in the cytoplasm. Given the increasing reports of signaling proteins and transcription factors accumulating in the cytoplasm of degenerating neurons, strategies to bypass potential translocation deficits will aid in the development of new strategies for PD treatment. CREB gene expression studies in the brain stem showed a
decreased activity in the 6-OHDA infused rats. Treatment with 5-HT, GABA and BMC in combination reversed this alteration which indicates its ability to improve the translocation deficits occurs in the PD rats. Microglia-mediated neuroinflammation has been hypothesized to play an important role in the pathogenesis of PD, primarily based on findings from postmortem studies and animal experiments (McGeer et al., 2004). Consistently, concentrations of proinflammatory cytokines such as interleukin (IL)-1b, IL-2, IL-6 and TNF-α were elevated in the brain and cerebral spinal fluid of PD patients (Nagatsu et al., 2005). Increased TNF-α expression as we got in 6-OHDA rats will directly influence the further enhancement of neuronal damage. At the ultrastructural level Lewy bodies are composed of dense granular material and straight filaments approximately 10 to 15 nm in diameter (Galloway et al., 1992). Similar filaments can be created in the test tube with recombinant α-synuclein, which is normally an unfolded and structure less protein (Crowther et al., 2000). This fact, as well as the immunolocalization of α-synuclein to the filaments in tissue sections subjected to electron microscopy, indicates that the filaments in Lewy bodies are almost certainly derived from aggregates of α-synuclein that have an abnormal conformation. The presence of α-synuclein in cytoplasmic inclusions represents aberrant cytologic localization, since it is normally a protein enriched in presynaptic terminals. We obtained α-synuclein over expression in the 6-OHDA rats supporting the earlier studies. Overall observation in the brain stem comes to a conclusion that 5-HT and GABA along with BMC is directly helping in reversing back the glutamate receptor mediated abnormalities in PD and its antiglutamatergic action is useful in reducing the severity of the disease.

In conclusion, the present study demonstrated that unilateral lesion using 6-OHDA in to SNpc induces the glutamate receptor activity. Our molecular and behavioural results showed that 5-HT and GABA along with BMC potentiates a restorative effect by reversing the alterations in glutamate receptor binding and gene
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expression that occur during PD. 5-HT and GABA co-mitogenically induced BMC proliferation and differentiation to neurons. Thus, it is evident that 5-HT and GABA along with BMC to 6-OHDA infused rats renders protection against oxidative, related motor and cognitive deficits which makes them clinically significant for functional reestablishment and recovering from PD symptoms.