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A RAT MODEL OF THE COGNITIVE IMPAIRMENTS IN PARKINSON’S DISEASE

UN MODELO EN RATA FRL DETERIORO COGNITIVO EN LA ENFERMEDAD DE PARKINSON

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ABSTRACT

Although Parkinson’s disease (PD) is classically considered to be a motor system disease, subtle cognitive impairments can be observed even during the early phases of PD. In this article we review behavioral and neurochemical studies on the cognitive alterations observed in rats treated with intranigral infusion of the neurotoxin MPTP. The critical role of dopamine release in the dorsal striatum and its modulation by adenosine receptors is also reviewed as a potential strategy to treat the cognitive disabilities of PD patients who do not improve with levodopa therapy. Most of the impairments presented by rats treated with intranigral infusion of MPTP are similar to those observed during the early phase of PD, when a moderate loss of nigral dopamine neurons (40-70%) results in sensory and memory deficits with no major motor impair-

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ments. These animals also model the working memory and habit learning deficits, with long-term spatial (episodic) memories being mostly spared as observed in non-demented PD patients. The intranigral infusion of MPTP in rats has led to the development of useful models, which do not present gross motor impairments that would otherwise compromise the interpretation of the performance of the animals in cognitive tasks.

Keywords: Parkinson’s disease, learning, memory, cognition, MPTP, rats, animal model.

RESUMEN

Aunque el mal de Parkinson (DP) es considerado clásicamente como un desorden del sistema motor, pueden observarse ligeros deterioros cognitivos aun en las fases iniciales del DP. En este artículo revisamos estudios conductuales y neuroquímicos sobre alteraciones cognitivas observadas en ratas tratadas con infusiones intranigrales de la neurotoxina MPTP. El papel crítico de la liberación de dopamina en el estriado dorsal y su modulación por los receptores de adenosina también es revisada como una estrategia potencial para tratar los deterioros cognitivos en pacientes con desorden de Parkinson (PD) que no mejoran con la terapia de levo dopa. Resultados: La mayoría de los daños presentados en ratas con infusiones intranigrales de MPTP son similares a los observados en las primeras fases de PD, una pérdida moderada de neuronas nigrales dopaminérgicas (40-70%) que causa déficits sensoriales y motores y poco deterioro motor. Estos animales también modelan los déficits de memoria de trabajo y aprendizaje de hábitos, con la memoria de largo plazo espacial (episódica) mayormente preservada como se observa en los pacientes sin DP. La infusión intranigral de MPTP en ratas a llevado al desarrollo de modelos útiles, ya que no presentan un deterioro motor excesivo que podrían de otra manera comprometer la interpretación de la ejecución de los animales en tareas cognitivas.

Palabras clave: mal de Parkinson, aprendizaje, memoria, cognición, MPTP, ratas, modelo animal.

Parkinson’s disease (PD) is the second most common neurodegenerative disorder, following Alzheimer’s disease, affecting approximately 1% of the population older than 50 years (Duvoisin 1991). Current estimates from the American Parkinson’s Disease Foundation put the number of American citizens suffering from this disease at more than 1.5 million individuals. Since the incidence of the disease increases with age (the most important risk factor), it is likely that the number of people suffering from PD will rise as improved health care lengthens the average life span.
Classically, PD is considered to be a motor system disease and its diagnosis is based on the presence of a set of cardinal motor signs (e.g. rigidity, bradykinesia, rest tremor and postural reflex disturbance). These symptoms of PD mainly result from the progressive degeneration of dopamine neurons of the substantia nigra pars compacta (SNc) that project predominantly to the striatum (Hirsch et al. 1988), a fact that contributes to the prevailing view that the basal ganglia are mainly concerned with motor control functions (Heikkila et al. 1989). More recently, an increasing amount of evidence has suggested that this system is also critically involved in learning and memory processes (Brown et al. 1997), as indicated by the fact that many cognitive impairments, including memory deficits, occur during the early stage of PD even before the development of its classical symptoms (Dubois and Pillon 1997; Owen et al. 1995). The non-motor symptoms that include cognitive deficits can be more important than the motor deficits to determine the patients' quality of life and represent an important factor to determine the need for nursing home care.

On the other hand, animal models are an invaluable tool for studying the pathogenesis and progression of human diseases, as well as for testing new therapeutic intervention strategies. PD is one of many human diseases which do not appear to have arisen spontaneously in animals. The characteristic features of the disease can, however, be more or less faithfully mimicked in animals through genetic approaches and the administration of various neurotoxic agents that interfere with dopaminergic neurotransmission. Despite the recent discovery of mutations in the alpha-synuclein gene (and some other genes) in a few PD patients that has led to the development of gene-based PD models (von Bohlen und Halbach et al. 2004), the administration of different neurotoxins such as 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP), which disrupt or destroy the dopaminergic system, remains the most widely used animal model for the study of PD. Although these models have undoubtedly contributed to a better understanding of many features of PD, most studies have focused on the ability of these models to induce nigrostriatal pathway damage and motor alterations associated with advanced phases of PD. However, until recently, no well-accepted model of the early phase of PD was available in the literature. The present review seeks to document these challenges using our earlier review (Da Cunha et al. 2002) as a basis for integrating the subsequent behavioral and neurochemical studies showing that the intranigral infusion of MPTP into rats causes a partial loss of dopamine neurons in the SNc and depletion of striatal dopamine, resulting in sensory and memory deficits with no major motor impairments, thus representing a model of the early phase of PD.

Finally, the fact that most of the drugs currently available for the treatment of PD (such as levodopa) are more efficient in alleviating motor than cognitive impairments has led many researchers to postulate non-dopaminergic
mechanisms for the cognitive symptoms of this disease. Here, we will briefly review clinical and non-clinical studies evaluating the potential of caffeine and other adenosine receptor antagonists to restore defective learning and memory processes in PD.

COGNITIVE IMPAIRMENTS IN PARKINSON’S DISEASE

In addition to the characteristic motor symptoms, subtle cognitive impairments can be observed even during the early phases of PD (Dubois and Pillon 1997; Bosboom et al. 2004). They comprise a dysexecutive syndrome that includes attentional and working memory impairments accompanied by secondary deficits in the internal representation of visuospatial stimuli and in the use of declarative memory storage (Bradley 1989; Owen et al. 1993; Dubois and Pillon 1997; Tamaru 1997; Bosboom et al. 2004). Skill and habit learning is also impaired in these patients (Knowlton et al. 1996). Almost one-third of patients may eventually progress to dementia (Aarsland et al. 1996).

Dysexecutive syndrome represents the core of the cognitive impairments and dementia observed in PD, and appears even during early stages of the disease (Dubois and Pillon 1997; Tamaru, 1997; Bosboom et al. 2004; Owen 2004; Zgaljardic et al. 2004). Executive function describes a wide range of cognitive functions required for goal-directed, adaptive behavior in response to new, challenging environmental situations, including planning, task management, attention, inhibition, monitoring, and coding. All of these functions are attributable to the prefrontal cortex and therefore, PD cognitive disabilities resemble cognitive deficits found in frontal cortex patients (Tamaru 1997; Marie et al. 1999; Owen 2004).

A recent positron emission tomography study by Aalto et al. (2005) has shown increased dopamine release in the frontal cortex of human subjects performing a working memory task. Working memory (Stebbins et al. 1999; Marie and Defer 2003), especially spatial working memory (Pillon et al. 1996, 1997; Owen et al. 1997), fails in non-demented PD patients. The articulatory (verbal-phonological) component of the working memory is usually preserved, but when a verbal working memory task demands more attention, a deficiency is also observed in these patients (Moreaud et al. 1997; Owen 2004). These impairments are possibly the consequence of failure of the central executive component that manages the short-term memory. Thus, these impairments appear when the working memory tasks present a higher demand on executive functions such as planning and attention shifting (Bosboom et al. 2004).

Many studies have tested whether PD patients, who are known to have a striatal depletion of dopamine, present non-declarative learning and memory deficits (Bondi and Kaszniak 1991). These patients fail to improve mirror
reading of words that appeared only once during the test, an impairment attributable to a skill learning deficit (Thomas et al. 1996; Koenig et al. 1999). PD patients have been shown to present impaired skill learning not only for visuoperceptual but also for motor skill tasks such as puzzle assembly, pressing specific keys on a computer keyboard in response to a stimulus presented on the computer screen, and drawing lines in hidden mazes (Bondi and Kaszniak 1991; Thomas et al. 1996; Moreaud et al. 1997). Many deficits of PD patients in performing non-declarative tasks relay on the initial learning phase (Dujardin and Laurent 2003). On the other hand, there is no consensus about whether PD spares declarative memory (Thomas et al. 1996; Bondi and Kaszniak 1991). PD patients are generally not impaired to encode and store consolidated new information, but they present difficulties in retrieving this information, particularly when they have to self-initiate remembering strategies (Dujardin and Laurent 2003). A failure in executive functions may explain this deficit. However, the non-intentional and automatic nature of a non-declarative task, such as learning a list of words or matching pairs of words, may also determine whether it can be learned normally or not by PD patients (Faglioni et al. 1995, 1997; Roncacci et al. 1996). Some authors explain the declarative deficits reported in some studies involving PD patients as resulting from the fact that they require a larger number of repetitions of the task to translate non-declarative (procedural) into declarative knowledge (Pascual-Leone et al. 1993).

Habits are by definition stimulus-response associations that are unconsciously learned through repetitively rewarded experiences. The main difficulty to model a habit task is to guarantee that the subjects will not respond consciously in order to receive the reward. One of the main well-designed studies planned to test whether non-demented PD patients are impaired in stimulus-response habit learning used a probabilistic classification task (Knowlton et al. 1996). The probabilistic structure of the task permitted the subjects to learn the task unconsciously by trial-and-error. PD patients scored worse than Alzheimer’s disease patients and healthy subjects, but when asked about it, they remembered to have participated in the previous training sessions. Alzheimer’s disease patients, on the other hand, learned this task like healthy subjects, but barely remembered the training episode. This study supports the double dissociation proposed for the medial temporal- and basal ganglia-mediated declarative (episodic) and non-declarative (implicit habit learning) memory systems, respectively (Packard and Knowlton 2002).

The risk of developing dementia is up to six times higher in PD patients than in healthy subjects of the same age (Aarsland et al. 1996). The core of the impairments lies in executive functions (e.g. set-shifting) (Girotti 1986). Mood (e.g. depression), and psychotic (e.g. visual hallucinations) symptoms are also common in demented PD patients. Other common im-
pairments include visuospatial and visuoconstructive skills. Speech and language difficulties, such as naming and sentence comprehension, are also common. Furthermore, poor verbal fluency would be predictive of dementia in PD. Declarative memory impairments are present, but are less severe, as compared to Alzheimer’s disease. There is a deficit in free recall, but it can be compensated for by semantic cueing. Furthermore, PD patients have more problems to recall than to encode declarative memories, i.e., their impairment relies on difficulties in activating processes involved in the functional use of memory storages, probably as a consequence of the dysexecutive syndrome. Recognition memory is relatively intact (Bosboom et al. 2004). Some of these cognitive impairments, especially attention impairment, are aggravated by a degeneration of cholinergic neurons in the nucleus basalis of Meynert and of noradrenaline neurons in the locus ceruleus that also occur in PD. On the other hand, impairments in declarative memory, aphasia and apraxia, when present, are related to cortical pathology indicative of Alzheimer’s disease or Lewy body dementia. Regarding the last co-morbidity, it is noteworthy how many characteristics of PD dementia resemble Lewy body dementia. Additionally, postmortem studies have revealed that many Lewy body disease patients had been wrongly diagnosed in life as having PD patients and many PD patients develop Lewy body disease later on (Zgaljardic et al. 2004).

THE BASAL GANGLIA SYSTEM OF LEARNING AND MEMORY

As important as knowing the cause of PD is to know the normal function of the brain components affected by this disease. Dopamine neurons of the SNC modulate the basal ganglia, which are composed of the caudate nucleus and putamen (altogether called striatum) and the globus pallidus. Due to their reciprocal connections with these core structures of the basal ganglia, the substantia nigra, ventral tegmental area and the subthalamic nucleus are considered to be associated basal ganglia structures (Alexander and Crutcher 1990). Neurons from all parts of the neocortex project to the striatum. Striatal neurons, in turn, project to the globus pallidus or to the substantia nigra pars reticulata which projects to the ventrolateral thalamus that, in turn, projects back to the frontal cortex (Alexander and Crutcher 1990). Therefore, the activity of sensory and motor parts of the cortex affects the activity of the basal ganglia that, in turn, modulate the activity of motor and cognitive parts of the frontal cortex. The positive modulation exerted by glutamate thalamic neurons on the frontal cortex is under inhibitory control of GABAergic neurons of the globus pallidus and the substantia nigra pars reticulata. This inhibition can be either blocked by a direct pathway or increased by an indirect pathway of neurons that arise in the striatum. Midbrain dopamine neurons play a dual
role in the modulation of the activity of these striatal neurons. Acting on D1-like or D2-like dopamine receptors, the dopamine released by these neurons activates the direct pathway and inhibits the indirect pathway, respectively. Both actions result in a positive modulation of the motor and cognitive functions of the frontal cortex (Alexander et al. 1986). According to this view, it is clear that the loss of midbrain dopamine neurons that occurs in PD results in the impairment of both motor and cognitive functions.

How does the decrease of dopamine concentration in the striatum, and its consequent decrease in the positive modulation exerted by the basal ganglia loop on the frontal cortex, causes the cognitive impairments observed in PD? Let us start with motor skills and habit learning. The primary motor cortex, supplementary motor area and somatosensory cortex neurons directly control the firing of spinal motor neurons, leading to consciously willed movements. Motor programs are the orchestrated sequences of commands to functional groups of muscles that govern movements at or around the joints (Alexander and Crutcher 1990). Where are these motor programs encoded and stored? The striatum is in a strategic position to participate in the encoding of such motor programs that will constitute the framework of the motor skills and habits (Packard and Knowlton 2002). The ability to perform a skill demands the coordinated activity of muscle groups from different parts of the body, the continuous integration of information about the contraction state of these muscles, and the visual follow-up of the movement in order to make fine adjustments for proper movements. Habit learning consists of increasing the probability that a sensory stimulus triggers a motor program designed for a particular behavioral response (White and McDonald 2002). As mentioned above, both sensory and motor regions of the entire cortex project to the striatum. The primary motor cortex also presents a somatotopic organization. Inputs from regions of the primary motor (MI) and sensory (SI) cortex that represent the same part of the body send projections to the same region within the striatum (Flaherty and Graybiel 1998). However, while the cortical regions form a single and continuous representation of the entire body, the representation of these areas of the body in the striatum is broken into a mosaic and is redundant, i.e., each part of the body is represented by multiple striatal units called matriosomes. After these somatosensormotor inputs are processed in the striatum, the multiple matriosomes representing the same body parts send overlapping projections to the globus pallidus, where a unique and continuous representation of the body is restored (Graybiel 1998). Primary cortex regions for other sensory modalities, i.e., vision, hearing and smell, also send projections to the striatum (Calabresi et al. 1996). Notice that the multiple mosaic representation of sensory and motor information in the striatum allows the association of different stimuli with the activation of movement sequences involving different parts of the body. The capacity of dopamine neurons to either
inhibit or stimulate the basal-cortical output and to induce firing-dependent plasticity in the corticostratial synapses enables this system to form experience-driven stimulus-response programs that are the basis of skills and habit learning (Graybiel et al. 1994; Graybiel 1998). Working memory and executive functions, also affected in PD, depend on the activity of the prefrontal cortex (Dirnberger et al. 2005). There are loops integrating the dorsolateral and the orbitofrontal areas of the prefrontal cortex with the basal ganglia (Alexander et al. 1986). A study by Postle and D’Esposito (1999) showed increased activity of these cortical regions and the dorsal striatum when subjects were performing spatial working memory tasks. Furthermore, Lewis et al. (2003) reported that cognitive impairments in early PD, including working memory, are accompanied by a reduced activity in the frontostriatal neural circuitry. The concept of working memory involves the integration and maintenance of information for its prospective use when selecting the appropriate behavior (Baddeley 2003). This process could involve the transformation of sensory cues into a code response. The prefrontal cortex is at the top hierarchy of the sensory and motor systems (Faw 2003). Like the striatum, it can receive information from all sensory modalities and control the motor output. While doing this, it works in consonance with basal ganglia loops. These corticobasal loops can run parallel subroutines that are unconsciously operated, while the prefrontal cortex is involved in solving conscious demands for the ongoing behavior. Like the striatum, the prefrontal cortex is also modulated by dopamine neurons arising in the midbrain (Costa et al. 2003). Therefore, it is easy to understand how the abnormal depletion of dopamine levels in these brain regions as observed in PD can affect working memory. In the same way, attention and other executive functions of the prefrontal cortex will be affected by dopamine depletion in the striatum and prefrontal cortex (Dubois and Pillon 1997; Owen 2004).

**MPTP-LESIONED RAT AS AN ANIMAL MODEL OF COGNITIVE IMPAIRMENTS OBSERVED DURING THE EARLY PHASE OF PARKINSON’S DISEASE**

In the early 1980s, the dopaminergic neurotoxin MPTP was accidentally discovered when a group of young drug addicts in California developed an idiopathic parkinsonian syndrome. Investigation revealed that the syndrome was caused by self-administration of a “synthetic heroin” analogue that had been contaminated with a byproduct (MPTP) during manufacturing (Davis et al. 1979; Langston et al. 1983). At present, MPTP represents the most important and most frequently used neurotoxin applied to animal models of PD, presenting advantages over all other toxic PD models since it causes a specific
loss of dopamine neurons and induces symptoms identical to PD in humans (Przedborski and Vila 2003).

MPTP is highly lipophilic and readily crosses the blood-brain barrier. It is then converted in the glia into its active metabolite, 1-methyl-4-phenylpyridinium cation (MPP⁺), by monoamine oxidase B, an enzyme involved in catecholamine degradation. MPP⁺ is taken up by the dopamine transporter and accumulates in dopamine neurons. Absorbed MPP⁺ concentrates in mitochondria where it inhibits complex I of the electron transport chain, thereby reducing ATP generation and causing the production of reactive oxygen species, inducing apoptotic death of dopamine neurons (see Beal 2001).

MPTP can be given in a variety of regimens (e.g. gavage or stereotactic injection), but the most common and reproducible form is systemic administration (e.g. subcutaneous, intravenous, intraperitoneal or intramuscular) (Przedborski et al. 2001). In primates such as humans, monkeys and baboons, MPTP causes irreversible and severe parkinsonian symptoms that are indistinguishable from those of sporadic PD (Bezard et al. 1997, 2001; Przedborski et al. 2001). In contrast to primates, rodents are less sensitive to MPTP toxicity (Schmidt and Ferger 2001). Nevertheless, the C57 black mouse strain was found to be sensitive to systemic injection of MPTP and was significantly more selective than other mouse strains in terms of affecting mesencephalic dopamine neurons (Sedelis et al. 2000, 2001; Schmidt and Ferger 2001). Therefore, because of the economical, logistic and ethical constraints related to experimental research in primates, the MPTP mouse model has become the most commonly used animal model of PD to study neuropathological and neurochemical changes (Schmidt and Ferger 2001; Schober 2004).

On the other hand, few studies have used MPTP-lesioned rats. The main reason for this is that shortly after the discovery that MPTP causes a parkinsonian syndrome when systemically administered to humans and non-human primates (Langston et al. 1983), no susceptibility of rats to MPTP has been reported when the drug was administered systemically (Chiueh et al. 1984; Kalaria et al. 1987). The conspicuous insensitivity of rats to MPTP toxicity may be related to a species-specific MPTP metabolism and/or sequestration of MPP⁺, which could be different in rats compared to mice and monkeys (Johannessen et al. 1985; Kalaria et al. 1987; Schmidt and Ferger 2001). For this reason some authors (see Schmidt and Ferger 2001; Schober 2004) did not recommend rats for MPTP research. Recently, this view has been re-evaluated following the findings that the infusion of MPTP directly into the rat SNc causes a partial loss of dopamine neurons and depletion of striatal dopamine that result in sensory and memory deficits (Harik et al. 1987; Da Cunha et al. 2001, 2002).

Rats with SNc lesion induced by intracerebral administration of 6-OHDA have been successfully used to study the physiology of nigrostriatal pathway
disruption, and have become a very popular model of motor alterations related to advanced phases of PD characterized by gross motor alterations (Ungerstedt 1968; Shwarting and Huston 1996). However, until recently, no well-accepted model of the early phase of PD was available in the literature. Such model is very important to study the mechanisms of the deficits characteristic of this phase and to screen putative drugs able to improve and maintain the quality of life of PD patients during a phase when they can better benefit from treatment and be more effectively cared for.

Since the early phase of PD is characterized by only partial lesion of the SNc (less than 70% cell loss), mild motor impairment and cognitive deficits, we have proposed that bilaterally MPTP-lesioned rats represent a good model of this early phase of the disease. This model of PD seems to be appropriate for this purpose because, in contrast to unilaterally SNc-lesioned rats, animals with bilateral lesions do not present gross motor alterations that would otherwise confound the interpretation of poor scores in memory tasks as indicative of cognitive impairment. Extensive tests have shown that 3 weeks after surgery these animals present no significant sensorimotor disturbances. At this time, the animals are not aphagic or adipsic and their exploratory behavior scored in an open field or in a shuttle-box, as well as their time of permanence in a rota-rod, is normal (Da Cunha et al. 2001; Miyoshi et al. 2002). The reason for this lack of motor impairment is probably due to a combination of the following factors: 1) the partial nature of the SNc lesion and striatal dopamine depletion induced by MPTP, 2) a compensatory neural plasticity in the basal ganglia circuit during the 3 weeks after surgery, and 3) the bilateral nature of the lesion.

Since bilateral lesion of the SNc by MPTP does not cause motor impairments in rats, the next step was to study what kinds of memory are affected in these animals. Nowadays, it is generally accepted that there are multiple memory systems. Two of the most studied examples are the hippocampal and the basal ganglia memory systems, which process and store information independently and in different styles. According to this view, the hippocampal system processes spatial-temporal memories involving relations among environmental cues (e.g. episodic memory in humans), while the basal ganglia system is involved in habit learning in which a single stimulus is repeatedly associated with a response (Packard and Knowlton 2002; McDonald et al. 2004; White 2004). As pointed out above, there is evidence to support the idea that PD patients present deficits to learn habit tasks (Knowlton et al. 1996; Dubois and Pillon 1997). Other studies consistently reported that PD patients are impaired in spatial working memory and other central executive functions (Owen et al. 1997; Owen 2004). Our studies using MPTP-induced SNc-lesioned rats as a model of PD are consistent with this view. Two different versions of the Morris water maze task proved to be particularly suitable
to test spatial memory or habit learning. In the spatial version, rats learn to escape to a submersed platform that is maintained in the same location in the water maze from the beginning to the end of the experiment. In this case, the animals need to make associations among the spatial environmental cues in order to form a cognitive map that helps them to find the platform (Morris et al. 1982). In the habit version, the animals learn to associate the position of a white ball attached to the platform and protruding above the water. The position of the platform is changed randomly among trials. In this case, a single stimulus (the ball) is repeatedly associated with a response of approaching the platform. Spatial memory critically depends on the integrity of the hippocampus but not of the dorsal striatum, whereas habit learning critically depends on the integrity of the dorsal striatum but not of the hippocampus (Packard and McGaugh 1992; White and McDonald 2002).

Studies from our laboratory have shown that SNc lesion does not affect learning or memory in the spatial version of the water maze, but hippocampal inactivation with lidocaine prevents animals from finding the submersed platform. An opposite response was observed with the cued version, since SNc lesion, but not hippocampal inactivation, impairs learning and memory. No significant interaction was observed between the SNc lesion and hippocampal inactivation conditions in terms of affecting scores in the spatial or in the cued version of the water maze (Miyoshi et al. 2002; Da Cunha et al. 2002). These results suggest that the nigrostriatal pathway is an essential part of the basal ganglia memory system which processes stimulus-response habit learning and works independently of the hippocampal memory system which processes spatial/relational memories.

MPTP rats also presented a deficit in the working memory version of the Morris water maze (Miyoshi et al. 2002). In this version, the position of the platform is maintained constant during four subsequent trials performed on the same day, but its position is changed on each subsequent training day. With this protocol, the animal cannot use the previous day reference memory to find the platform and, thus, has to use its working memory of the previous trial to find it. Another rat learning and memory task affected by bilateral lesion of the SNc is two-way active avoidance (Da Cunha et al. 2001). This task models multiple kinds of memory, but habit learning is an important component of this task, in which a single cue (a sound signal) is repeatedly associated with a foot shock that can be avoided by crossing to the opposite side of a shuttle box. The impairing effect of nonspecific (electrolytic) SNc lesion on this task has been previously reported by Mitcham and Thomas (1972). The dependency to learn this task on the integrity of the dorsal striatum has also been reported in other studies (Kirkby and Polgar 1974; El Massiou and Delatour 1997).

The deficit of MPTP rats has also been observed in another working
memory task named delayed alternation in a Y-maze (Braga et al. 2005). In this task, the rats have to alternate between two arms of a Y-maze in order to find a food pellet. During the 20-s intertrial intervals the animals have to maintain in their working memory which arm they had previously visited in order to alternate correctly. SNC lesion with MPTP increased the number of errors in both pretrained and naive rats. In another study, we have shown that the left SNC seemed to be more critical than the right SNC for the performance of the working memory of rats in a version of the Morris water maze (Bellissimo et al. 2004).

**EFFECTS OF DOPAMINERGIC DRUGS ON THE MPTP RAT MODEL OF MEMORY IMPAIRMENTS RELATED TO PD**

Controversy exists regarding the dopaminergic nature of the cognitive impairments in PD. Since neurons producing other neurotransmitters (e.g. acetylcholine, serotonin, noradrenaline) are also reported to degenerate in this disease (Braak et al. 2003), some authors consider that they may cause some cognitive and behavioral dysfunction, especially in demented patients (Dujardin and Laurent 2003; Zgaljardic et al. 2004). On the other hand, other investigators have reported a correlation between the loss of dopamine neurons of the nigrostriatal pathway and the degree of dementia (Rinne et al. 1999) and performance in neuropsychological tests in PD patients (Marie et al. 1999; Bruck et al. 2001). Animal models can contribute to establish the specific implications of each neurotransmitter system in the cognitive impairments of PD. The role of dopamine can be studied by using models that are specific for dopamine depletion, such as the MPTP models, and by investigating the effects of dopamine receptor agonists on cognition.

Ogren and Archer (1994) reported that haloperidol and other dopamine receptor antagonists impair acquisition and retention in the two-way active avoidance task, indicating that the performance of this task depends on normal dopaminergic neurotransmission. The sensitivity of this task to SNC lesions and striatal dopamine manipulations and the facility to perform this task – only two sessions are necessary in an automated apparatus – make it particularly suitable to test drugs with a potential to treat the cognitive symptoms of PD. Thus, we tested the effect of the most efficient drug used in the treatment of the motor symptoms of PD, levodopa, on SNC-lesioned rats. The administration of benserazide/levodopa to MPTP-lesioned rats, at a dose that restores the striatal level of dopamine, did not reverse the MPTP-induced learning and memory impairment (Gevaerd et al. 2001a).

In humans, the beneficial effect of levodopa on improving the cognitive function affected in PD is controversial. While some studies indicate an im-
provement of cognitive functions in PD patients treated with levodopa (Beardsley and Puletti 1971; Loranger et al. 1972; Girotti et al. 1986; Cooper et al. 1992; Cools et al. 2001), others have shown that this treatment may cause no or only mild improvement (Pillon et al. 1989; Growdon et al. 1998; Rektorova et al. 2005), or may even aggravate PD cognitive impairments (Huber et al. 1989; Poewe et al. 1991; Prasher and Findley 1991; Cools et al. 2001). Gotham et al. (1988) proposed that the detrimental effects of levodopa observed in some cognitive tasks may be due to excessively high concentrations of dopamine in areas such as the prefrontal cortex where dopamine depletion is less severe. We showed that this was the case for MPTP rats treated with levodopa (Gevaerd et al. 2001a). The levodopa dose necessary to restore a normal striatal level of dopamine caused a large increase of dopamine levels in extrastriatal brain regions. Therefore, that study proves that, at least for the MPTP rat model of PD, levodopa therapy is not effective in improving the observed memory impairment because it appears to tilt the balance between dopamine levels in the striatum and in extrastriatal regions such as the prefrontal cortex (and also limbic structures), resulting in a cognitive deficit. In accordance with this idea, a recent work by Bruck et al. (2005) showed that the finding of early phase PD patients scoring poorly in tests measuring frontal lobe functions was positively correlated with increased cortical Fdopa uptake.

Furthermore, the various cognitive impairments of PD may depend on different brain areas that are differently depleted of dopamine, such as the dorsal striatum and prefrontal cortex. A study by Swainson et al. (2000) has shown that non-medicated PD patients performed better than medicated patients in a reversal test that depends on the striatum and ventral frontal cortex. However, the same patients performed worse than medicated patients in a spatial recognition memory task that depends on the dorsolateral frontal cortex. The authors suggested that the levodopa treatment overdosed the dorsolateral frontal cortex, which was less affected by the disease, at the same time that it restored a normal level of dopamine in the striatum and ventral frontal cortex. Cools et al. (2001) reported similar results showing that levodopa-treated patients can perform better or worse in tasks depending on different components of the frontostriatal circuitry. In that study, levodopa withdrawal improved performance in probabilistic reversal learning, a task that depends on the orbitofrontal cortex, ventral frontal cortex, and ventral striatum. However, levodopa withdrawal impaired performance in a set-shifting task, which depends on the dorsolateral frontal cortex and dorsal caudate nucleus.

Therefore, although the above studies discourage the use of levodopa therapy to treat some PD cognitive symptoms, it does not imply that these cognitive symptoms are not related to the degeneration of the nigrostriatal pathway. In addition to the observed impairment in two-way active avoid-
ance learning caused by the depletion of striatal dopamine in MPTP-lesioned rats, other important findings suggest that mnemonic processes depend on a normal level of stimulation of the striatal dopamine receptors. Packard and White (1991) and Packard and McGaugh (1994) showed improved cognitive performance after intrastratial administration of a D2 receptor agonist to rats. Also, Schneider et al. (1994) observed a cognitive improving effect of the systemic administration of a D1-receptor agonist to MPTP-lesioned monkeys. D1-receptor agonists have also been reported to release acetylcholine in the frontal cortex and dorsal striatum and to improve cognitive performance in rats (Steele et al. 1997). More recently, other authors have suggested that D1 receptor agonists can be useful in the treatment of cognitive impairments of PD (Nichols and Lewis 2004; Salmi et al. 2004), and it would be interesting to test them in an animal model such as the MPTP rat model used here.

The failure of levodopa to reverse the memory impairment of MPTP rats in the two-way active avoidance task is likely to be related, at least in part, to the failure of this treatment to improve the cognitive impairments of PD patients, as mentioned above. Since this was equally observed in some clinical studies and in our rat model of memory impairments related to PD, these results encouraged the use of the rat MPTP model in studies on alternative drug therapies for the treatment of the cognitive impairments of PD.

**EFFECTS OF ADENOSINE RECEPTOR ANTAGONISTS ON THE MPTP RAT MODEL OF MEMORY IMPAIRMENTS RELATED TO PD**

It is well known that adenosine receptors are densely expressed in the striatum and exert a modulatory influence on dopamine neurotransmission (Moreau and Huber 1999; Svenningsson et al. 1999). The understanding of the role of adenosine in basal ganglia and its anatomical and functional relationship with the striatal dopamine D1 and D2 receptors has increased over the last years, providing evidence of an antagonistic interaction between A(2A)/D2 and A(1)/D1 receptors in the striatum (Fuxe et al. 1998; Franco et al. 2000). Moreover, neuroprotective properties of caffeine and A(2A) adenosine receptor antagonists have been reported for dopamine neurons in the SNc (Chen et al. 2001). Furthermore, adenosine receptor-related drugs seem to be promising candidates for the symptomatic treatment of PD, since there is evidence that caffeine directly increases dopamine release from striatal nerve terminals (Okada 1997). This dopamine-releasing effect of caffeine was also observed with the A(2A) adenosine receptor antagonist, ZM 241385, in striatal synaptosomes (Da Cunha et al. 2002). All these putative anti-Parkinson effects may explain the finding that the risk of PD is significantly reduced among coffee drinkers (Paganini-Hill 2001). Based on these promising effects, adenosine receptor
antagonists are being pursued as putative drugs to treat PD (Ferre et al. 2001; Wardas 2001).

Caffeine has also been reported to improve learning and memory in a variety of animal (Pare 1961; Molinengo et al. 1995; Cestari and Castellano 1996; Howell et al. 1997) and human studies (Riedel et al. 1995; Pollina and Calev 1997). In our laboratory we also demonstrated that pretraining and pretest systemic administration of caffeine can improve the memory of rats in various tasks (Angelucci et al. 1999, 2002; Prediger and Takahashi 2005; Prediger et al. 2005a,b,c). Due to the failure of levodopa to reverse the memory impairments caused by SNc lesion in rats, we decided to test whether caffeine is effective to do so. Caffeine (0.1 to 0.3 mg/kg, i.p.) reverses the impairing effect of the MPTP-induced SNc lesion of rats on the avoidance scores in the training and test sessions of a two-way active avoidance task (Gevaerd et al., 2001b). This result suggests that the effects of caffeine and other adenosine receptor antagonists acting on the striatal dopaminergic system can be useful to restore defective learning and memory processes in PD.

CONCLUDING REMARKS

The data reviewed here indicate the successful refinement of an experimental model of PD, and describe behavioral tests that can be used in rodents to study early PD-related cognitive deficits. Because an animal model cannot provide the full range of effects of such complex human neurodegenerative disease, a rodent model by injecting MPTP into the SNc was constructed by drawing from various sources, which included tests of spatial memory, working memory and habit learning. Measures of cognitive impairments in the absence of compromising sensory and/or motor disabilities have been obtained and tentatively related to current theoretical constructs of human cognition. The studies reviewed here stress the critical role of the dopaminergic nigrostriatal pathway as an essential element of the basal ganglia neural circuit, participating in specific learning and memory processes in the brain. The proposed MPTP rat model of PD-related memory impairments proved to be appropriate for studies of the neural circuits supporting this cognitive pathology. Moreover, our studies consistently suggest that adenosine receptor antagonists (e.g. caffeine), previously reported as putative drugs for treating the motor symptoms of PD, are also promising drugs to treat the cognitive impairments related to this disease. Considering the failure of levodopa to treat these cognitive disabilities, the development of a new class of drugs for incorporation into the pharmacological options for the treatment of PD is noteworthy. Certainly, additional studies are necessary to better understand the neurobiological substrates of early cognitive impairment in PD, as well as the development of novel therapeutic strategies for this neurodegenerative disorder.
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