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EFFECT OF ANTAGONIZATION OF M4 RECEPTORS WITH TROPICAMIDE, CATALYSIS AND MOTOR CHANGES INDUCED BY NITRERGIC INHIBITION, IN MICE

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ABSTRACT

The Parkinson's disease is characterized by the depletion of dopamine, however, there are strong indicators of interactions among other neurotransmitters in the base nuclei, such as in cholinergic neurotransmition. The aim of this study was to evaluate the effect of tropicamide, an M4 receptor antagonist, on the mice's motor behavior induced by inhibition of nitric oxide synthase with L-NOARG. 48 male Swiss mice were subdivided into 6 experimental groups. Each animal was subjected to stereotaxic surgery for implantation of cannulae, in the striatum. After 7 days, each animal received an intraperitoneal injection of saline or L-NOARG and then received intracerebral injection of saline or tropicamide at doses of 25 or50 nM. The animals were subjected to the bar catalepsy test and the Open Field test. $p<0.05$Tropicamide, both doses (was able to completely reverse the catalepsy of these animals at moments 5, 35 and 65 minutes after the application of the drugs ($p<0.05$). L-NOARG (L NOARG + saline) led to significant changes in the horizontal and vertical exploitation behavior in the animals (Tukey post-test, $p< 0.05$). These motor alterations were reversed by tropicamide (50 nM). In conclusion, L-NOARG significantly altered the animals' motor behavior, and these changes were reversed with tropicamide.

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INTRODUCTION

In the context of neurodegenerative diseases, Parkinson's disease (PD) affects about 1% of the world population over 65 years (Poewe *et al.,* 2017). It is a chronic and progressive condition of the nervous system involving a group of motor and non-motor disorders such as stiffness, akinesia, bradykinesia, tremor and postural instability, hyposmia, sleep disorder and gastrointestinal symptoms (Nasrolahi *et al.,* 2019). PD is primarily a disease of extrapyramidal motor function, caused by severe degeneration of dopaminergic neurons of substance nigra (Less *et al.,* 2009).

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The current treatment of PD is based on dopaminergic therapy, aiming to revert the effects of striatal dopamine depletion induced by the destruction of the nigrostriatal pathway (Jankovic, 2002; Obeso *et. al*., 2000; Olanow, 2008). Thus, the precursor of dopamine, L-dopa, has gold standard in the treatment of motor symptoms because it presents a significant improvement of the motor function in the early stages of the disease. However, its chronic use may produce adverse effects such as hallucination, insomnia, nausea and dyskinesia (Munhoz, *et al.*, 2015). The other therapies treat the neuromotor symptoms and do not significantly modify the progression of the disease. As a consequence, there is a need for the development of new pharmacological manipulations for the treatment of PD with novel manipulations capable of producing antiparkinsonian agents at all stages of the disease,

without loss of drug efficacy, and preventing the onset of dyskinesias (Dexter and Jenner, 2013; Jenner, 2014). In addition, the study of new therapies for PD is aimed at non-dopaminergic systems within the basal nuclei that go beyond the injured nigrostriatal pathways (Jenner, 2014). Various agents exhibiting a therapeutic potential have been described, including agents that act on glutamatergic, cannabinoid, opioid, α_2-adrenergic and nicotinic and muscarinic cholinergic receptors (Jenner, 2014). In the striatum (caudate nucleus and putamen), acetylcholine and dopamine interact strongly and play an important role in normal motor control (Calabresi *et al*., 2006; Perez-Lloret *et al.,* 2016). The imbalance of the transmission of acetylcholine and dopamine in the nigro-striatal system is the pathogenic basis of extrapyramidal disorders, as in the case of PD (Threlfell *et al*., 2010). According to the classic clinical hypothesis, the decrease in dopaminergic system activity is parallel to the hyperfunction of the cholinergic system (Tanimura *et al.,* 2019). In the SNC, M4 subtype muscarinic receptors are found with striatal abundance, and because their important role in motor function has been demonstrated, it is assumed that M4 muscarinic receptor antagonists could also be useful in restoring the balance between dopamine and acetylcholine and, therefore, be used as treatment in PD (Betz *et al*., 2007; Langmead *et al*., 2008). However, other neurotransmitters, in addition to acetylcholine and dopamine, are present in the base nuclei and participate in the behavior modulation. Among them, it is possible to mention the neuromodulator nitric oxide (NO), which has been increasingly recognized as an inter- and extracellular messenger in the SNC (Prast and Philippu, 2001). Nitric oxide synthase (NOS) inhibitors have been shown to produce catalepsy in mice (Del Bel *et al.*, 1998) and in rats (Del Bel *et al.,* 2004).

Moreover, interference with NOS formation can also produce deficits in other motor tests. Inhibitors of NOS decrease the locomotion and vertical exploration of mice in the open field test (Del Bel *et al*., 2002) and the rats' spontaneous motor activity (Halcák *et al*., 2000). Inhibitors of NOS, L-NOARG and 7-NI suppress the locomotor activity induced by dopaminergic D_1 and D_2 receptor agonists (Starr and Starr, 1995). These authors hypothesized that the continuous or tonic activity of constitutive NOS would be required to occur in normal body movements. Systemic administration of dopamine D1-receptor antagonists decreases NADPH-d activity in striatal neurons whereas D2-receptor antagonists produce the opposite effect (Morris *et al*., 1997). NO donors stimulate the release of acetylcholine into striatal slices whereas nitric oxide synthase (NOS) inhibitors reduce the levels of striatal acetylcholine (Hanania and Johnson, 1998). However, the interaction between NO and acetylcholine is not well understood at the behavioral level. The objective of this work was to evaluate the effect of an antagonist of M4 acetylcholine muscarinic receptor, applied intracerebrally, on the mice's motor behavior evaluated in the Open Field test and in Nitric-induced catalepsy, L-NOARG, an enzyme inhibitor Synthesis of Oxide.

MATERIAL AND METHODS

Animals: In this experiment Swiss male mice were used, from the laboratory of the Federal University of Mato Grosso do Sul, weighing between 30-50g. The animals were kept in the vivarium with water and food at libitum until the beginning of the tests. The light cycle (12/12 h, lights connected at 6:00 am) and theroom temperature (23 ± 1 ° C) were controlled. This study was approved by the Committee on Ethics in the Use of Animals / CEUA / UFMS, protocol nº 580/2013.

Drugs: Nitric oxide synthase inhibitor: NG-nitro-L-arginine (L-NOARG - Sigma); antagonist of muscarinic acetylcholine M4 receptors: tropicamide 10mg / ml (Mydriacyl - Alcon). All drugs were dissolved in 0.9% saline solution.

Drug route of administration: The drugs were administered intraperitoneally (i.p.) or intracerebrally (i.c.) route, according to the technique described below.

Intracerebral drug application: For intracerebral injections of tropicamide in the striatum, animals were anesthetized intraperitoneally with ketamine (100mg / kg) and xylasin (10mg / kg), by volume, seven days before the application of drugs and the evaluation of motor behavior and catalepsy of 10 ml / kg for each animal and subjected to stereotactic surgery (Figure 1A) for bilateral implantation of stainless steel cannulas, directed at the striatum (Figure 1B).According to the coordinates referenced by the atlas of Paxinos and Watson (1986): (anterior 0.5 mm, lateral 2,5 mm and vertical 2.7 mm of bregma). At the end of the implantation of the cannulas, they were fixed to the skull with an acrylic resin helmet and sealed with an internal guide of solid stainless steel. Soon after the surgery and until the third day afterwards, the animals received intraperitoneal analgesic therapy with buprenorphine injections at a dose of 0.05 mg / kg every 8 hours. On the seventh day after surgery, with the animal awake, the injection of the drugs was administered and for that, the obturators were removed and an injection cannula, attached to a microinfusion pump, was lowered by the guide cannula to the striatum. The drug or vehicle was infused bilaterally into the striatum (1µl per hemisphere), with an infusion rate of 0.5µl / minute. After the drug was infused, a 1-minute pause was taken to remove the injection cannula. Subsequently, the animal followed to perform the behavioral tests.

Experimental groups: For the present study, 48 mice were divided into 6 groups, as described below, each group consisting of 8 animals and that each animal was used only in one of the experimental groups. The animals received an i.p. of saline or L-NOARG (40mg / kg) followed by i.c. saline or tropicamide (25 and 50nM) after 60 minutes. In group 1 (G1) the animals received an injection i.p. of saline and after 60 minutes, another i.c. of saline; in group 2 (G2) the animals received i.p. of saline and after 60 minutes, i.c. tropicamide (25nM); in group 3 (G3) the animals received i.p. of saline and after 60 minutes, i.c. tropicamide (50nM); in group 4 (G4) the animals received i.p. of L-NOARG (40mg / kg) and after 60 minutes, i.c. of saline; in group 5 (G5) the animals received i.p. of L-NOARG (40mg / kg) and after 60 minutes, i.c. tropicamide (25nM); in group 6 (G6) the animals received i.p. of L-NOARG (40mg / kg) and after 60 minutes, i.c. of tropicamide (50nM).

Functional evaluation of animals: Catalepsy was evaluated according to the bar method (Figure 1C), where the mice was placed with both forepaws on a horizontal glass bar (diameter 0.5 cm), raised 4.5 cm from the soil (Sanberg *et al.,* 1988). The time in seconds, during which the animal remained in this position was recorded, up to a maximum time of 300 seconds (Zarrindast *et al.,* 1993), allowing three attempts to place the animal in a cataleptic position. The catalepsy time was

considered complete when the forepaws touched the ground or when the mouse climbed the bar. Measurements were performed after 5, 35 and 65 minutes after drug administration. The open field test (Figures 1D and 1E) was used to evaluate the animal's motor and emotional behavior (Whimbey and Denenberg, 1967; Walsh and Cummins, 1976; Ortiz and Souza, 2019). In our laboratory, the parameters evaluated during the open field test were: the frequency of the horizontal exploration (Squares crossed) and vertical (Rearings) behavior in a cylindrical arena, 40 cm in diameter, with translucent acrylic walls, 30 cm high, placed on a wood base covered with white Formica, which is subdivided into 12 quadrants of 104.7 cm^2 each. This test was performed only once, 30 minutes after drug application, measuring for 5 minutes the number of squares crossedand the number of rearing expressed by the animal.

Disposal of animals: After the behavioral tests, a small quantity of dye was infused by the cannula implanted in the stereotactic surgery. Then the animal was euthanized with cervical dislocation and thereafter had the brain removed to be sectioned on a microtome to check if the cannula was implanted at the desired location (striatum). If the cannula was not implanted in the striatum, that animal was discarded from the group. The animal was discarded for incineration through the biological waste collection system of UFMS.

Statistical analysis: For the analysis of the catalepsy time data, the original data were added from the constant 1 and were transformed into Base log 10. This data transformation was done with the intention of normalizing the data samples. The comparison between the moments of analysis in each experimental group was performed by means of the one-way ANOVA test, followed by the Tukey post-test. The comparison between the experimental groups, at each moment of analysis, was performed using the one-way ANOVA test, followed by the Tukey post-test. The comparison between the experimental groups in relation to the number of quadrants traveled (horizontal exploration) andto the number oflifting (vertical exploration) was performed by means of the one-way ANOVA test, followed by the Tukey post-test. The other results of the variables evaluated in this study were presented in the form of descriptive statistics or in the form of tables and graphs. Statistical analysis was performed using SigmaPlot Software, version 12.5, considering a level of significance of 5% (Norman and Streiner, 1994).

RESULTS

The data on catalepsy are presented in Table 1 and show that L-NOARG (L-NOARG + saline) caused catalepsy in the animals, being the dwell time in the bar greater than that observed in the animals of the other experimental groups (post-Tukey test $p<0.05$).

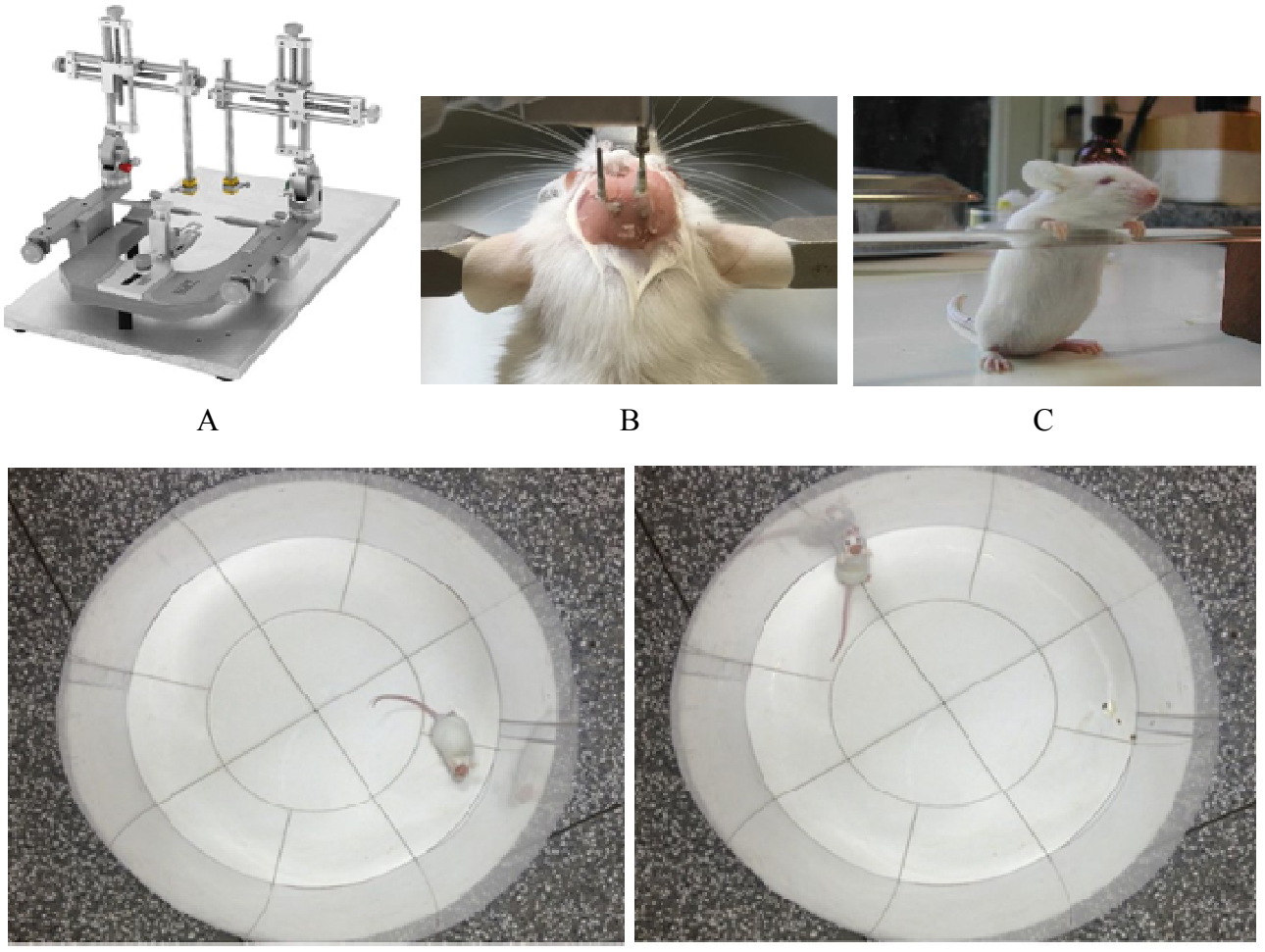

Figure 1. Motor behavior and catalepsy in bar. Stereotaxic apparatus(A), animal fixed in the stereotaxic apparatus withstainless steel cannulas positioned in the striatum region (bilateral) and fixed with acrylic resin helmet(B), evaluation of the catalepsy in the bar test(C), Open Field test, horizontal scanning(D), Open Field test, vertical exploration(E)

Table 1. Results regarding the time of catalepsy at each moment after drug application and in each experimental group

Group	Time after drug application			Value ofp*
	5	35	65	
Sal.+sal.	2,75±2,20c	2,38±2,24b	2,13±1,72c	0,960
Sal.+tropicam. 25nM	5,13±2,9bc	2,25±0,7b	3,5±1,69c	0,939
Sal.+ tropicam. 50nM	2,63±1,19bc	4,75±3,23b	5,5±1,73c	0,289
L-Noarg+sal.	168,86±29,06a	96,14±27,82[a]	209,29±25,94[a]	0,006
L-Noarg+ tropicam. 25nM	40,13±21,48b	88,88±37,14[a]	75,0±31,39b	0,187
L-Noarg+ tropicam. 50nM	4,88±1,71bc	2,5±0,85b	1,5±0,38c	0,405
Value of p**	<0,001	<0,001	<0,001	

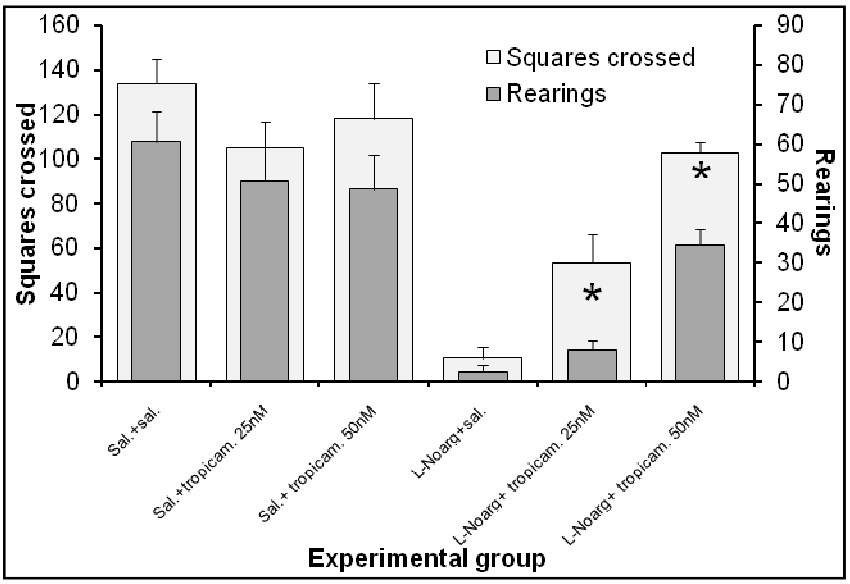

Figure 2. Graph showing the number of squares crossed and rearings, in each experimental group. Each column represents the mean and bar the standard error of the mean

At moments 5 and 65 minutes, the animals receiving LNOARG + saline had a longer catalepsy time than those in the other groups ($p<0.05$). At 35 minutes catalepsy in the animals of the L-NOARG + saline group was higher than that observed in the animals of the groups Saline + saline, Saline + tropicamide (25 and 50nM) and L-NOARG + tropicamide at the dose of 50 nM ($p < 0.05$). The application of tropicamide at the dose of 25 nM partially reversed catalepsy at moments 5 and 65 minutes ($p<0.05$) and at the dose of 50 nM totally reversed the catalepsy of the animals. The results for the horizontal and vertical exploration of the animals in the open field test, 30 minutes after the application of intracerebral drugs, for each of the experimental groups are shown in Figure 2. In the comparison among the experimental groups, there was a significant difference amongin relation to the behavior of horizontal exploration (squares crossed) and vertical exploration (rearings), and expressed by the animals (ANOVA one-way test, horizontal: $p <0.001$, vertical: $p <0.001$), and the animals of the L-NOARG + saline and NOARG + tropicamide (25nM) showed a significantly lower number of squares crossed (horizontal exploration) than Salina + Salina, Sal + tropicamide (25nM), Sal + tropicamide (50nM) and L-NOARG + tropicamide (50nM) (Tukey's post-test, $p<0.05$). In the L-NOARG + saline group, the animals presented a significantly lower number of animals compared to Salina + Salina, Sal + tropicamide (25nM), Sal + tropicamide (50nM) and NOARG + tropicamide (50nM) ($p<0.05$). In addition, animals of the NOARG + tropicamide group (25 nM) had a significantly lower number of rearings than those of the groups Salina + Salina, Sal. + Tropicamide (25 nM) and Sal + tropicamide (50 nM) ($p<0.05$).

DISCUSSION

PD is characterized as a disease of extrapyramidal motor function, caused by a marked degeneration and death of dopaminergic neurons of substance nigra. It presents a set of motor disorders, whose symptoms include bradykinesia, stiffness and tremor (Salamone, 2010; Nasrolahi *et al.,* 2019). The conventional pharmacological treatment for the therapeutic replacement of dopamine levels with L-3,4-dihydroxyphenylalanine (L DOPA) remains the most effective treatment for Parkinson's disease (Fahn, 2008; Lin and Laureno, 2019). However, new advances in the treatment of motor disorders based on the manipulation of non-dopaminergic systems such as glutamate, 5-hydroxytryptamine (5-HT), cannabinoids, adenosine, adrenergic, histaminergic, opioid and nicotinic and muscarinic cholinergic receptors have been proposed Cacciatore *et al.,* 2018; Silverdale *et al*., 2003). As well as in studies by Del Bel *et al.* (1998, 2004, 2010), in our study, L-NOARG also caused catalepsy in the animals, being the dwell time in the bar superior to that observed in the animals of the other experimental groups. In addition, the catalytic effect caused by L-NOARG (L-NOARG + Saline), corroborates with the literature (Del Bel *et al,* 1998; Del Bel *et al*., 2010); this drug has potential to be used in animal models of Parkinson's disease. It is possible that this effect is a consequence of the reduction of striatal dopamine caused by the inhibition of NOS (Mori *et al*., 2017; Bowyer *et al*., 1995; Sandor *et al*., 1995). In addition, NO acts as an NMDA modulator, which interacts with dopamine and has an effect on motor behavior (Koppula *et al.,* 2012; Lazzarini *et al*., 2004).

NO is a short-lived liposoluble molecule, generated from the amino acid L-Arginine, which belongs to the family of enzymes called nitric oxide synthase (NOS). Although the production of interneurons composes only 1% -2% of the population of striatal neurons, strong evidence has shown that NO has a function of control over the motor behavior modulating the integration and information processed by the base nuclei. Nitrogenic mechanisms may contribute to the severe degradation of AD neurons seen in Parkinson's disease (Gomes *et al*., 2008). The modulatory role played by NOreleases several neurotransmitters including DA, 5-hydroxytryptamine (5-HT), acetylcholine and GABA (Trabace and Kendrick, 2000; Cacciatore *et al*., 2018). Furthermore, nitric oxide synthase inhibitors (NOS) decrease the levels of striatal acetylcholine (Hanania and Johnson, 1998). However, the interaction between NO and acetylcholine is not well understood in terms of motor behavior.

Studies have shown that NO plays a central role in homeostatic control in the process that promotes stability to the DA-nigral system. In addition, the release of NO leads to the release of NO from striatal GABAergic interneurons (Sammut *et al*., 2006), which in turn influences the long-term induction of striatal depression (Calabresi *et al*., 1999). L-DOPA therapy has been shown to cause a marked increase in NO production (Itokawa *et al*., 2006). There are hypotheses that NO plays a modulatory role in the striatum, changing its input-output relationship and producing a significant functional impact on the target neurons (West and Grace, 2000). The release of NO from striatal interneurons facilitates concomitant dopamine release (West and Galloway, 1998; Trabace and Kendrick, 2000; Grace, 2008) and NO production by striatal interneurons that are regulated by phasic DA transmission (West and Grace, 2000). Such evidence demonstrates the strong interaction between the dopaminergic and nitrergic striatal systems. In our experiment, the catalytic effect produced by L-NOARG was partially reversed after application of tropicamide at a dose of 25 nM at moments 5 and 65 minutes ($p<0.05$) and at the dose of 50 nM completely reverted to catalepsy in animals. In view of this, our results support previous evidence that drugs acting on specific subtypes of muscarinic acetylcholine receptor increased interest in the modulation of striatal cholinergic signaling in order to reduce disturbances of the basal ganglia (Wess *et al*., 2007; Eskow Jaunarajs *et al*., 2015; Shen *et al*., 2015). Pathophysiological studies have suggested that in DP there is an imbalance between cholinergic and dopaminergic neurotransmission in the striatum and this plays a central role in the development of motor symptoms (Di Chiara *et al.,* 1994; Pisani *et al.,* 2007), although other authors have demonstrated complex and complementary interaction between these two systems after thalamic stimulation (Threlfell *et al*., 2012; Parker *et al.,* 2016).

In this study, the motor behavior evaluation, performed with the Open Field test, carried out a comparison among the experimental groups and showed that there was a significant difference among them, in relation to horizontal exploration (squares crossed) and vertical exploration (rearings), and the animals in the L-NOARG groups had a significantly lower number of squares crossed and rearings compared to the animals in the groups that did not receive L-NOARG intraperitoneal injection. In addition, tropicamide at the dose of 50 nM proved to be effective in reversing PD symptoms in animal model in the variables tested in this experiment (squares crossed and rearings). Previous studies have confirmed our findings on the role of NO control over several other neurotransmissions and have demonstrated that NOS inhibitors decrease the locomotion and vertical exploration of mice in the Open Field test (Del Bel *et al.,* 2002) and the spontaneous motor activity of rats (Halcák *et al*., 2000). In the present study, these hypokinetic findings are consistent with previous experiments, which found that nitric oxide synthase (NOS) inhibitors produce catalepsy and hypokinesia in mice, and in rats, similar to those observed in PD patients (Del Bel *et al.,* 2004; Marras *et al.,* 1995), and promote the understanding of the physiology of the Base Nuclei and the physiology of PD, and may contribute to the investigation of new pharmacological manipulations for the treatment of the disease capable of presenting a therapeutic potential in all phases of the disease, without the decline of efficiency and may also prevent complications. In the striatum, most muscarinic receptors are of the M1 and M4 subtypes (Oki *et al.,* 2005; Santiago and Potter, 2001). The M4 subtype receptors are predominant in the neostriate, especially in estriatonigral midline spinous neurons (Oki *et al*., 2005 Santiago and Potter 2001).

Concerning anti-Parkinsonian drugs, non-selective antagonists of muscarinic acetylcholine receptors have been used for several years. Evidence from the study of Betz *et al.* (2007) demonstrated strong affinity of tropicamide for M4 muscarinic receptors. Tropicamide is a muscarinic receptor antagonist that penetrates easily into the blood-brain barrier and is commonly used for ophthalmic purposes, with little known anti-parkinsonian effects. Thus, Betz *et al*., (2007) performed a comparison of the effects of tropicamide and atropine on the suppression of tremors induced by pilorcapine and pimozide in mice, where thetropicamide and atropine have similarly suppressed pilocarpine-induced tremor, but the tropicamide suppresed more powerfully the pimozide-induced tremors. These results indicate that the muscarinic antagonist tropicamide has shown tremolytic effects in this animal model of PD and that it can exert anti-parkinsonian actions, being this a possible alternative in the clinical treatment of Parkinsonism (Betz *et al.,* 2007), since anticholinergics which are usually used clinically, do not have particular selectivity. In order to demonstrate the effectiveness of M4 subtype muscarinic receptor antagonists the present study tested the effect of tropicamide on animal model of PD with L-NOARG. Our results demonstrated that the drug was able to completely reverse catalepsy and changes in the exploratory behavior of the animals at a dose of 50 nM. This indicates that acetylcholine M4 receptor antagonists have therapeutic potential for the treatment of the motor symptoms of Parkinson's disease.

Pharmacological blockade of muscarinic receptors with tropicamide (specific for M4 receptors) and telenzepine (specific for M1 receptors) in PD models with unilateral 6-OHDA injury, with selective inhibition of M4, has shown o decrease sensorimotor deficits in an animal model of PD and that this effect is lost in knockout mice for M4 receptors (Ztaou *et al*., 2016). These authors hypothesized that these effects are related to an interaction between M4 and D1 receptors in the middle spindle neurons of the striatum. These data provide important evidence related to the cholinergic interference of M4 in D1 receptors in medium spiny neurons that constitute about 95% of the striatal neuron population, thus strengthening the direct pathway of the nuclei of the base (Ztaou *et al.,* 2016). In our study the antagonism of M4

receptors had important effects on the reversal of motor alterations caused by inhibition of nitric oxide synthase, confirming the hypothesis of a strong cholinergic influence, through M4 receptors, on the striatum, showing the therapeutic potential of this neurotransmission in the pharmacological treatment in PD. However, further studies are needed to determine and confirm how M4 muscarinic receptors are involved in the basal ganglia physiology and their potential use for the treatment of Parkinson's disease. The motor effects observed in our study may possibly have occurred due to: (1) interaction between the dopaminergic and cholinergic neurotransmissions in the striatum between M4 and D1 receptors of the middle spinal neurons of the direct pathway; (2) direct interference of tropicamide in nitrergic neurotransmission; and (3) association of the two possibilities mentioned above.

Conclusion

The L-NOARG, as an inhibitor of nitric oxide synthase, had a cataleptic effect and significantly altered the horizontal and vertical exploration of the animals reproducing the symptoms of Parkinson's disease in the animals, confirming this pharmacological model of PD. These motor changes were reversed by both tropicamide concentrations, however, the reversal was partial at the dose of 25 nM and complete at the dose of 50 nM. More studies are needed to demonstrate the effectiveness of tropicamide in reversing motor changes in animal models of Parkinson's disease and in better elucidating the mechanisms of action of this drug in the base nuclei of the brain.

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REFERENCES

Betz, JA. McLaughlin PJ, Burgos M, Weber SM, Salamone JD 2007. The muscarinic receptor antagonist tropicamide suppresses tremulous jaw movements in a rodent model of parkinsonian tremor: possible role of M4 receptors. Psychopharmacology. 194: 347-359.

Bowyer JF, Clausing P, Gough B, Slikker WJR, Holson, RR 1995. Nitric oxide regulation of methamphetamine-induced dopamine release in caudate/putamen. Brain. Res. 699:62–70.

Cacciatore I, Ciulla M, Marinelli L, Eusepi, P, Di Stefano, A 2018. Advances in prodrug design for Parkinson's disease. Expert Opinion on Drug Discovery; 13(4): 295–305.

Calabresi P, Gubellini P, Centoze D. 1999. A critical role of the nitric oxide/cGMP pathway in corticostraiatal long-term depression. *J Neurosci.* 19(7): 2489-99.

Calabresi P, Picconi B, Pametti L, DiFilippo M. 2006. A convergent model for cognitive dysfunctions in parkinson's disease: the critical dopamine-acetylcholine synaptic balance. *Lancet Neurol.* 5: 974-983.

Del Bel EA, da Silva CA, Guimaraes FS, Bermudéz-Echeverey M. 2004. Catalepsy induced by intra-striatal administration of nitric oxide synthase inhibitors in rats. *Eur.J.Pharmacol.* 485 (1-3): 175-181.

Del Bel EA, Guimarães F, Joca, S, Echeverry M, Ferreira F. 2010. Tolerance to the cataleptic effect that follows repeated nitric oxide synthase inhibition may be related to functional enzymatic recovery. *Journal of Psychopharmacology,* 24(3):397–405.

Del Bel EA, Silva CA, Guimarães FS. 1998. Catalepsy induced by nitric oxide synthase inhibitors. Gen. Pharmacol. 30(2): 245-248.

Del Bel EA, Souza AS, Guimaraes FS, da Silva CA, Nucci-da-Silva LP. 2002. Motor effects of acute and chronic inhibition of nitric oxide synthesis in mice. Psychopharmacology (Berl). 161(1): 32-37.

Dexter DT, Jenner P. 2013. Parkinson disease: from pathology to molecular disease mechanisms. *Free Radical Biol Med;* 62:132-144.

Di Chiara G, Morelli M, Consolo S. 1994. Modulatory functions of neurotransmitters in the striatum: ACh/dopamine/NMDA interactions. Trends Neurosci. 17:228 –233.

Eskow Jaunarajs KL, Bonsi P, Chesselet MF, Standaert DG, Pisani A. 2015. Striatal cholinergic dysfunction as a unifying theme in the pathophysiology of dystonia. Prog Neurobiol. 127–128:91–107.

Fahn S. 2008. The history of dopamine and levodopa in the treatment of Parkinson´s disease. Mov Disord. 23:497-508.

Gomes MZ, Raisman-Vozari R, Del Bel EA. 2008. A nitric oxide synthase inhibitor decreases 6-OHDA effects on tyrosine hydroxylase and neuronal nitric oxide synthase in the rat nigrostriatal pathway. Brain Res. 1203: 160-69.

Grace AA. 2008. Physiology of the normal and dopamine-depleted basal ganglia: Insigths into levodopa pharmacotherapy. Mov Disord. 23: 560-69.

Halcák L, Pechángová O, Zigová Z, Klemová L, Novacký M 2000. Inhibition of NO synthase activity in nervous tissue leads to decreased motor activity in the rat. Physiol Res. 49(1): 143-149.

Hanania T, Johnson MK. 1998. Regulation of neurotransmitter release by endogenous nitric oxide in striatal slices. *Eur.J.Pharmacol.* 359: 111-117.

Itokawa K, Ohkuma A, Araki N, Tamura N, Shimazu K 2006. Effect of L-DOPA on nitric oxide production in striatum of freely mobile mice. *Neurosci Lett.* 402(1-2): 142-44.

Jankovic J. 2002. Levodopa strengths and weaknesses. Neurology. 58(4); 1 Suppl: S19-S32.

Jenner, P 2014. An Overview of Adenosine A2A Receptor Antagonists in Parkinson's Disease. Adenosine Receptors. Neurology and Psychiatry, 71–86.

Koppula S, Kumar H, Kim IS, Choi DK. 2012. Reactive oxygen species and inhibitors of inflammatory enzymes, NADPH oxidase, and iNOS in experimental models of Parkinson's disease. Mediators of Inflammation; 2012:823-902.

Langmead JC, Watson J, Reavill C. 2008. Muscarinic acetylcholine receptors as CNS drug targets. Pharmacolol. & Therap. 117: 232-243.

Lazzarini M, Salum C, Del Bel, EA. 2004. Combined treatment of ascorbic acid or alpha-tocopherol with dopamine receptor antagonist or nitric oxide synthase inhibitor potentiates cataleptic effect in mice. Psychopharmacology. 181: 71-79.

Lees AJ., Hardy J., Revesz T. 2009. Parkinson's disease. Lancet. 373(9680):2055-66.

Lin MM, Laureno R. 2019. Less Pulsatile Levodopa Therapy (6 Doses Daily) Is Associated with a Reduced Incidence of Dyskinesia. *J Mov Disord*; 12(1): 37-42.

Marras RA, Martins Ap, Del Bel EA, Guimaraes PS. 1995. L-NOARG, an inhibitor of nitric oxide synthase, induces catalepsy in mice. Neuroreport. 7(1): 158-160.

Mori, MA, Delattre, AM, Carabelli, B, Pudell, C, Bortolanza, M, Staziaki, PV, Ferraz, AC. 2017. Neuroprotective effect of omega-3 polyunsaturated fatty acids in the 6-OHDA model of Parkinson's disease is mediated by a reduction of inducible nitric oxide synthase. *Nutritional Neuroscience*, 21(5): 341–351.

Morris BJ, Simpson CS, Mundell S, Maceachern K, Johnston HM, Nolan AM. 1997. Dynamic changes in NADPH-diaphorase staining reflect activity of nitric oxide synthase: evidence for a dopaminergic regulation of striatal nitric oxide release. *Neuropharmacology*.36 (11-12): 1589-1599.

Munhoz RP, Moro A, Silveira-Moriyama L, Teive H. 2015. Non-motor signs in Parkinson's disease: a review. Arq. Neuropsiquiatr.73(5): 454-62.

Nasrolahi A, Safari F, Farhoudi M, Khosravi A, Farajdokht F, Bastaminejad S, Shotorbani SS, Mahmoudi J. 2018. Immune system and new avenues in Parkinson's disease research and treatment. Reviews in the Neurosciences.

Norman, GR, Streiner DL. 1994. Biostatistics the bare essentials. London: Mosby.

Obeso JA, Olanow, CW, Nutt JG. 2000. Levodopa motor complications in Parkinson's disease. Trends Neurosci. 23(10): S2-S7.

Oki T, Takagi Y, Inagaki S, Taketo MM, Manabe T, Matsui M, Yamada S. 2005. Quantitative analysis of binding parameters of [3H]N-methylscopolamine in central nervous system of muscarinic acetylcholine receptor knockout mice. Brain Res Mol Brain Res 133:6–11

Olanow CW. Levodopa/dopamine replacement strategies in Parkinson's disease: future directions. Mov Disord. 2008; 23: 613-22.

Ortiz HC, Souza AS. 2019. "Caffeine effect on non-motor symptoms in an animal model of Parkinson disease", International Journal of Development Research, 09, (01), 25408-25412.

Parker PR, Lalive AL, Kreitzer AC. 2016. Pathway-specific remodeling of thalamostriatal synapses in parkinsonian mice. Neuron 89:734 –740.

Perez-Lloret S, Peralta MC, Barrantes, FJ 2016. Pharmacotherapies for Parkinson's disease symptoms related to cholinergic degeneration. Expert Opinion on Pharmacotherapy, 17(18), 2405–2415.

Pisani A, Bernardi G, Ding J, Surmeier DJ 2007. Re-emergence of striatal cholinergic interneurons in movement disorders. Trends Neurosci 30: 545–553.

Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, Schrag AE, Lang AE 2017. Parkinson disease. *Nat Rev Dis Primers*. 3:17013.

Prast H, Philippu A 2001. Nitric oxide as modulator of neuronal function. Prog.Neurobiol. 64(1): 51-68.

Salamone JD 2010. Facing dyskinesia in Parkinson's Disease: Nondopaminergic approaches Drugs of the Future. 35(7): 567-573.

Sammut S, Dec A, Mitchell D, Linadarkis J, Ortiguela M, West AR 2006. Phasic dopaminergic transmission increases NO efflux in the rat dorsal striatum via a neuronal NOS and a dopamine D-1/5 receptor-dependent mechanism. Neuropsychopharmacology. 31(3): 493-505.

Sanberg, PR, Bunsey MD, Giordano M, Norman AB 1988. The catalepsy test: its ups and downs. Behav. Neurosci. 102(5):748-759.

Sandor NT, Brassai A, Puskas A, Lendvai B 1995. Role of nitric oxide in modulating neurotransmitter release from rat striatum. Brain Res. 36: 483-486.

Santiago MP, Potter LT 2001. Biotinylated m4-toxin demonstrates more M4 muscarinic receptor protein on direct than indirect striatal projection neurons. Brain Res 894:12–20.

Shen W, Plotkin JL, Francardo V, Ko WK, Xie Z, Li Q, Fieblinger T, Wess J, Neubig RR, Lindsley CW, Conn PJ, Greengard P, Bezard E, Cenci MA, Surmeier DJ 2015. M4 muscarinic receptor signaling ameliorates striatal plasticity deficits in models of L-DOPA-induced dyskinesia. Neuron. 88:762–773.

Silverdale MA, Fox SH, Crossman AR, Brotchie JM 2003. Potential nondopaminergic drugs for Parkinson´s disease. Adv Neurol. 91: 273-291.

Starr MS, Starr BS 1995. Do NMDA receptor-mediated changes in motor behaviour involve nitric oxide? Eur.J.Pharmacol. 272 (2-3): 211-217.

Tanimura, A, Du Y, Kondapalli, J, Wokosin DL, Surmeier, D 2019. Cholinergic Interneurons Amplify Thalamostriatal Excitation of Striatal Indirect Pathway Neurons in Parkinson's Disease Models. Neuron. 101(3):444-458.

Threlfell S, Clements AM, Khodai T, Pienaar S, Exley R, Wess J 2010. Striatal Muscarinic Receptors Promote Activity Dependece of Dopamine transmission via Destinct Receptor Subtypes versus Dorsal Striatum. The Journal of Neuroscience. 30(9):3398-3408.

Threlfell S, Lalic T, Platt NJ, Jennings KA, Deisseroth K, Cragg SJ 2012. Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. Neuron 75:58–64.

Trabace L, Kendrick KM. 2000. Nitric oxide can differentially modulate striatal neurotransmitter concentrations via soluble guanylate cyclase and peroxynitrite formation. J Neurochem. 75: 1664-74.

Walsh R, Cummins RA. 1976. The open-field test: A critical review. Psychological Bulletin, 83: 482-504.

Wess J, Eglen RM, Gautam D. 2007. Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. Nat Rev Drug Discov 6:721–733.

West AR, Galloway MP 1998. Nitric Oxide and potassium chloride facilitated striatal dopamine efflux in vivo: role of calcium-dependent release mechanisms. *Neurochem Int.* 33: 493-501.

West AR, Grace AA 2000. Striatal nitric oxide signaling regulates the neuronal activity of midbrain dopamine neurons in vivo. *J Neurophysiol.* 83: 1796-1808.

Whimbey AE, Denenberg VH 1967. Two independent behavioral dimensions in open-field performance. J.Comp Physiol Psychol. 63(3): 500-504.

Zarrindast MR, Modabber M, Sabetkasai M 1993. Influences of different adenosine receptor subtypes on catalepsy in mice. Psychopharmacology (Berl), 113(2):257-261.

Ztaou S, Maurice N, Camon J, Guiraudie-Capraz G, Kerkerian-Le Goff L, Beurrier C, Liberge M, Amalric M 2016. Involvement of striatal cholinergic interneurons and M1 and M4 muscarinic receptors in motor symptoms of Parkinson's disease. *J Neurosci* 36:9161–9172

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