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Efeitos do exercício físico sobre diferentes parâmetros locomotores e comportamentais em ratos Wistar tratados com Haloperidol

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RESUMO

Sintomas extrapiramidais (*extra-pyramidal symptoms*) (EPS), como acinesia, distonia, alterações de marcha e tremores, são observados quando receptores D2 de dopamina são bloqueados farmacologicamente por agentes comumente utilizados na clínica como o haloperidol. Os EPS combinados criam um estado que se assemelha a Doença de Parkinson (Parkinson´s Disease) (PD), conhecido como parkinsonismo. É amplamente conhecido que o exercício físico é capaz de melhorar a marcha e os sintomas locomotores da PD, contudo são escassos os estudos relativos a prática de exercício concomitantemente com o uso de bloqueadores de receptores D2. O objetivo deste trabalho foi compreender os possíveis efeitos benéficos do exercícios em ratos Wistar com parkinsonismo induzido pela administração crônica de haloperidol. Para isso, 48 ratos Wistar foram distribuidos em quatro grupos (n = 12 por grupo): 1- Controle + sedentário, 2- Controle + exercício, 3- Haloperidol + sedentário e 4- Haloperidol + exercício. Os animais receberam doses diárias de haloperidol ou salina (0,3 mg/kg/dia, i.p.), os animais dos grupos 2 e 4 foram submetidos a um protocolo de exercício que consistia de caminhada leve em esteira rolante a uma velocidade de 4 m/min nos primeiros 5 minutos, seguido de uma velocidade de 6 m/min por 25 minutos. Os animais foram pesados e submetidos a diferentes testes para avaliação da atividade locomotora e de parâmetros comportamentais: o teste da pata-impresssa para análise de marcha, o teste da barra horizontal para avaliar acinesia e o teste de campo aberto para mensurar bradicinesia. A análise da marcha mostrou uma diminuição do passo e um alargamento da base com o uso de haloperidol em relação ao grupo controle ($p<0.05$), estas alterações foram revertidos pelo exercício. O tempo dispendido na barra, utilizado como medida do grau de acinesia, aumentou significativamente durante o tratamento com haloperidol ($p<0.001$), contudo o exercício foi capaz de atenuar este aumento ($p<0.05$). O teste de campo aberto apresentou um aumento nos parametros de bradicinesia quando comparados pré- e pós-exercício, mas sem diferenciação entre os grupos. Estes resultados indicam que exercício físico pode se constituir como um coadjuvante terapêutico eficiente para o tratamento dos EPS causado pelo uso de haloperidol.

Pavavras-chaves: Dopamina, Parkinsonismo, Atividade Locomotora, Exercício Físico, Comportamento.

ABSTRACT

Extra-pyramidal symptoms (EPS), such as akinesia, dystonia, gait alterations, and tremors, are observed when dopamine D2 receptors are pharmacologically blocked by agents such as haloperidol, a widely used clinical drug. These EPS produce a Parkinson´s Disease (PD)-like state, known as a parkinsonism. It is widely accepted that physical exercise is capable to improve gait and locomotor deficits caused by PD, however studies are scarce when comparing physical exercise with dopamine D2 receptor blockers. The objective of this study was to understand the possible benefits of physical exercise in Wistar rats with chronic haloperidol-induced parkinsonism. To this end, 48 Wistar rats were allocated into four groups (n = 12 per group): 1- Control + sedentary, 2- Control + exercise, 3- Haloperidol + sedentary, 4- Haloperidol + exercise. The animals received daily doses of either haloperidol or saline (0.3 mg/kg/day, i.p.). Furthermore, groups 2 and 4 underwent a physical exercise protocol which consisted of a light-intensity walk at a speed of 4 m/min for the first five minutes, and then 6 m/min for 25 minutes. The animals were subjected to different tests to evaluate locomotor activity and behavioral parameters: the ink-paw test for a gait analysis, the bar test to measure akinesia, and the open-field test to measure bradykinesia. The gait analysis showed a shorter stride length and a wider stance width with the use of haloperidol when compared to the control group ($p<0.05$), these alterations were reverted with physical exercise. The time on bar, used as a measure of akinesia, increase significantly during the haloperidol treatment ($p<0.001$), however physical exercise was capable to attenuate this increment ($p<0.05$). The open-field test presented an increase of the parameters of bradykinesia when comparing pre- and post-exercise time frame, but with no differentiation between groups. These results indicate that physical exercise can be used as an efficient approach as an adjuvant treatment to haloperidol induced EPS.

Keywords: Dopamine, Parkinsonism, Locomotor Activity, Physical Exercise, Behavior.

SUMÁRIO

1. CAPÍTULO 1

1.1. INTRODUÇÃO

1.1.1 Haloperidol, bloqueador dos receptores de dopamina D2

A dopamina é um neurotrasmissor que está relacionado a diferentes funções encefálicas. A mais notável é a função associada às vias motoras (Lieberman, 2005). A ação dopaminérgica inerente ao controle de postura e movimento voluntário é relacionada principalmente ao conjunto de fibras que parte de corpos neuronais localizados na substância nigra do mesencéfalo e projeta-se até o estriado (via nigro-striatal), que é uma das principais vias responsáveis pela modulação dos impulsos motores provenientes do córtex cerebral (Lieberman, 2005).

Intervenções farmacológicas no sistema dopaminérgico são usuais para o tratamento de diferentes patologias que afetam o sistema nervoso central (SNC) desde 1952, destacando-se o uso de bloqueadores dos receptores D2 de dopamina, como o haloperidol (Frota, 2004).

O haloperidol é a droga antipsicótica de alta potência mais utilizada no mundo, sendo referência no tratamento de indivíduos com esquizofrenia. Este fármaco é um importante antagonista de receptores de dopamina D2, localizados predominantemente em neurônios estriatais. O perfil famacocinético do haloperidol mostra que esta droga apresenta dissociação lenta e grande afinidade por receptores D2 (Frota, 2004).

Estudos com tomografia por emissão de pósitrons demonstraram que com um bloqueio significativo dos receptores de dopamina, mais de 80% dos receptores, os

pacientes começam a exibir também sintomas extrapiramidais que incluem discinesia, distonia, rigidez muscular, alteração de marcha e tremor ao repouso (Kapur, 2000; Miyamoto, 2008).

Estes paraefeitos extrapiramidais são mais evidentes com o uso de antipsicóticos de primeira geração, típicos, como haloperidol, quando comparados aos paraefeitos inerentes ao tratamento com drogas antipsicóticas de segunda geração (drogas atípicas) que aparentemente funcionam sob a teoria de antagonismo serotonina-dopamina, na qual estes fármacos teriam uma maior afinidade pelos receptores de serotonina 5-HT2A em relação ao receptor D2, o qual explicaria a redução dos sintomas extrapiramidais com o uso destas (Miyamoto, 2008).

Deste modo é postulado que as drogas utilizadas no tratamento da esquizofrenia e outros distúrbios psiquiátricos, principalmente os antipsicóticos típicos, induzem o parkinsonismo em humanos, sendo inclusive utilizadas como indutores de parkinsonismo em modelos animais (Farde, 1992; Miwa, 2000; Inada, 2001; Miyamoto, 2008; Wu, 2009).

Ademais, pacientes esquizofrenicos, que portanto podem fazer uso de bloqueadores de receptores D2, apresentam falta de motivação e dificuldades na realização de diferentes atividades da vida cotidiana, sendo que grande parte destes indivíduos tornam-se tabagistas pesados, e relatam que a sua vontade de fumar está intimamente relacionada a um desejo de melhora em suas percepções sensoriais e motoras (Barr, 2008; Zhao, 2009; Choi, 2009).

Mais recentemente, tem se sugerido que o sistema dopaminérgico está intimamente relacionado à realização de atividade física voluntária. Esta relação está fundamentada em diferentes componentes do sistema dopaminérgico, que variam desde alterações nas enzimas responsáveis pela síntese catecolaminérgica, como

tirosina hidroxilase, afetando portanto os diferentes receptores de dopamina, noradrenalina e adrenalina, até alterações mais pontuais, como variações na expressão de receptores dopaminérgicos do tipo D1 (Knab, 2009).

Um estudo com camundongos da linhagem C57/L1, que apresentam uma grande atividade motora espontânea, demonstrou que nestes animais observa-se uma redução da expressão de genes relacionados ao receptor D1 de dopamina e reduzida produção de tirosina hidroxilase, enzima limitante na síntese dopaminérgica, indicando quedas na função dopaminérgica estriatal. Estes dados indicam que reduções da atividade dopaminérgica podem conduzir a um aumento da atividade física espontânea (Knab, 2009). Neste mesmo estudo foi demonstrado que um aumento na expressão de genes relacionados ao receptor D1 e tirosina hidroxilase, característica de camundongos da linhagem C3H/HeJ, conduzem a uma diminuição significativa da atividade motora (Knab, 2009). Contudo, estes dados são relativamente paradoxais quando comparados a seres humanos com Mal de Parkinson, onde a queda da atividade dopaminérgica conduz invariavelmente à acinesia. Estes achados sugerem que o controle da atividade física voluntária não depende apenas de aumento ou diminuição da quantidade de dopamina disponível, sendo um evento bem mais complexo que provavelmente está intimamente relacionado a um ajuste adequado da atividade dopaminérgica em seus diferentes receptores. (Knab, 2009).

1.1.2 Modelo animal para marcha

Os roedores são modelos animais freqüentemente utilizados para analisar disfunções de movimentos induzidos por diferentes agentes farmacológicos. Alguns agentes neurotóxicos são utilizados para mimetizar o Mal de Parkinson (Parkinson´s Disease) (PD) em roedores, como o MPTP (Amende, 2005; Pothakos, 2009; Kurz, 2007), e o 6-OHDA (Metz, 2005; Ferraz, 2008).

As alterações na marcha de ratos com PD induzido é bem descrita e assemelha-se com as alterações de pacientes que desenvolvem a doença (Kurz, 2007; Amende, 2005). O aumento da frequência de passos, a diminuição do comprimento da passada (marcha festinante) e a diminuição da uniformidade do padrão da marcha são exemplos dessas mudanças (Amende, 2005; Kurz, 2007; Pothakos, 2009). Existem poucos estudos que detalhem precisamente as alterações motoras de pacientes que utilizam bloqueadores dos receptores D2 de dopamina, sendo que alguns destes estudos simplesmente citam que há um déficit motor durante a marcha desses indivíduos, com alterações neuromusculares que prejudicariam a marcha normal (Lieberman, 2005; Inada, 2001; Hansen, 1997). Também é documentado, em ratos, que o uso de bloqueadores de receptores dopaminérgico D2 induz uma marcha parkinsoniana festinante nestes animais, indicando que este modelo animal pode ser de extrema valia no estudo do parkinsonismo induzido por antipsicóticos típicos, assim como no estudo de alternativas terapêuticas para minimizar os paraefeitos de fármacos desta natureza (Fernagut, 2002).

1.1.3 Exercício físico influenciando a marcha no parkinsonismo

Exercícios aplicados a pacientes com Mal de Parkinson, com intuito de melhorar a marcha e qualidade de vida, são bem conhecidos e prescritos (Herman, 2009). Os exercícios recomendados para esses pacientes são, geralmente, a fisioterapia aquática, o treino em esteira rolante e atividades que estimulem movimentos contínuos, considerando que o parkinsoniano tem um déficit no controle motor e não sofre de fraqueza muscular (Herman, 2009). Indivíduos parkinsonianos submetidos a treino em esteira rolante apresentam uma melhora na qualidade da marcha, com menor festinação e aumento da mobilidade (Herman, 2009). Em modelos animais para o estudo do Mal de Parkinson observa-se que ratos submetidos a exercícios físicos apresentam um aumento significativo do comprimento da passada, retornando a padrões típicos de animais normais (Pothakos, 2009). Ademais, a atividade física melhora importantes parâmetros fisiológicos em ratos parkinsonianos, uma vez que esta induz uma diminuição da frequência cardíaca de repouso e faz com que os animais apresentem melhores valores de índices de esforço quando submetidos a um teste de esforço máximo, sugerindo um aprimoramento de sua condição cardiorrespiratória (Al-Jarrah, 2007).

O exercício físico tem se apresentado como uma boa alternativa na redução da sintomatologia encontrada em patologias como o Mal de Parkinson. Deste modo, esta abordagem pode trazer efeitos benéficos aos pacientes, que devido ao seu quadro clínico, tem a necessidade de uso de haloperidol. Contudo, são escassos os estudos relativos ao uso de bloqueadores de receptores D2 associados à atividade física, e estes são fundamentais para o correto entendimento desta possível interação.

1.2. OBJETIVOS

1.2.1. OBJETIVO GERAL

Avaliar o efeito de um protocolo de exercício físico sobre diferentes parâmetros de marcha, comportamento motor e peso corporal, em ratos Wistar submetidos ao bloqueio dos receptores de dopamina D2.

1.2.2. OBJETIVOS ESPECÍFICOS

1- Avaliar as alterações na marcha de ratos causadas por um protocolo de exercício físico em esteira rolante através do método de pata-impressa.
2- Avaliar a influência de um protocolo de exercício físico em esteira rolante no comportamento motor e exploratório de ratos com o uso do teste da barra horizontal e campo aberto.
3- Avaliar as alterações do peso corpóreo de ratos induzido por um protocolo de exercício físico em esteira rolante.

2. CAPÍTULO 2

2.1. ARTIGO

Physical exercise reverts locomotor side effects induced by haloperidol treatment in Wistar rats.

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Physical exercise reverts locomotor side effects induced by haloperidol treatment in Wistar rats.

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ABSTRACT

Extra-pyramidal symptoms (EPS) such as akinesia, dystonia, gait alteration and tremors are observed when dopamine D2-receptors are pharmacologically blocked by agents such as haloperidol. These alterations produce a Parkinson´s Disease (PD)-like state in patients, known as parkinsonism. Physical exercise has been proven to improve gait and locomotor effects in PD, so we sought to elucidate the action of physical exercise on parkinsonism induced by chronic haloperidol administration in adult Wistar rats. 48 rats were distributed into four groups: control + sedentary, control + exercise, haloperidol + sedentary and haloperidol + exercise. They underwent daily injection of saline or haloperidol (0.3 mg/kg/day, i.p.) and a 30-minute daily exercise program for the corresponding groups. The animals were also weighed weekly and subjected to the ink-paw test for gait analysis, the bar test for akinesia and the open-field test for bradykinesia throughout the training period. Assessment of these tests showed shortened stride length and increased stance width with the use of haloperidol, which were reversed with exercise. Haloperidol- induced akinesia increased over treatment days, but exercise was shown to attenuate the increment. These results indicate that physical exercise could be an efficient approach for dealing with unwanted EPS caused by haloperidol.

Keywords: Exercise, Antipsychotics, Dopamine, Parkinsonism, Gait.

1. INTRODUCTION

Dopamine is a neurotransmissor that is associated to different encephalic functions. The most noteworthy are those related to the motor functions (Lieberman et al., 2005). Pharmacological interventions to the dopaminergic system are common in various diseases that affect the central nervous system, such as the use of D2 dopamine receptor blockers like haloperidol, which is the most commonly employed antipsychotic drug (APD), being widely used in schizophrenia treatment (Lieberman et al., 2005).

Like other neuroleptic drugs, haloperidol presents strong motor side effects. PET-scan studies have shown that with a substantial blockage of the dopamine receptors of over 80%, patients present extra-pyramidal symptoms (EPS) which include akinesia, dystonia, gait alterations and tremors (Lieberman et al., 2005; Covell et al., 2004; Miyamoto et al., 2005). Therefore, it is accepted that drugs, mainly typical APDs, used in the treatment of schizophrenia and other psychiatric alterations induce parkinsonism in humans. APDs have been also used as agents to produce parkinsonism in animal models (Miyamoto et al., 2005; Farde et al., 1992; Miwa et al., 2000; Inada et al., 2001).

Gait alterations in animal models of induced Parkinson´s Disease (PD) are well described and are in accordance with findings in patients diagnosed with PD (Kurz et al., 2007; Amende et al., 2005). Increased stride frequency, shortened stride length, and reduced gait pattern certainty are examples of these changes (Kurz et al., 2007; Amende et al., 2005; Pothakos et al., 2009). Little is known about gait pattern alterations in patients that make use of D2 receptor blockers, some studies simply mention that there is a motor deficit during gait, with neuromuscular alterations that impair normal gait (Lieberman et al., 2005; Inada et al., 2001; Hansen et al., 1997).

Physical exercise is widely prescribed to PD patients in an attempt to ameliorate gait and enhance life quality. PD patients submitted to physical training on treadmill presented

better gait quality (Herman et al., 2008). In animal models of PD it has been shown that physical exercise brought on an increase in stride length and reestablished a normal gait pattern (Pothakos et al., 2009).

In this study, we used adult Wistar rats to further examine how physical exercise can alleviate some EPS, such as gait alterations, akinesia and bradykinesia caused by dopamine D2 receptor blockage.

2. RESULTS

2.1. Weight

In this study no significant change to the animals' weight was observed during the evaluated period (Data not shown).

2.2. Ink-paw test

The ink-paw test demonstrated relevant haloperidol induced gait alterations that were prevented by exercise. After 4 weeks of treatment the Hal+Sed group showed a reduced stride length ($p<0.05$) (Figure 2A) and larger stance width ($p<0.001$) when compared to all groups)(Figure 2B). These effects were prevented in those animals that maintained a continuous exercise routine while under haloperidol treatment, as observed in the Hal+Exe group (Figure 2A and 2B). The paw placement measurements (paw length and paw width) were highly variable. There were differences between the groups in the pre-training period - paw length was longer in the Ctrl+Exe group when compared with the Ctrl+Sed and Hal+Exe groups ($p<0.01$) (Figure 2C) and paw width was shorter in Hal+Sed group when compared with all other groups ($p<0.01$) (Figure 2D). However, the statistical analysis showed there was no significant change during the course of the experiment in any of the groups (Figure 2C and 2D).

2.3. Bar test

The bar test showed a significant increase in akinesia associated with haloperidol treatment, as represented by the time spent on the bar at the different test intervals.

In the Hal+Sed group, PT was different from the 2W and 4W measurements ($p<0.05$ and $p<0.001$ respectively), while 2W and 4W also differed ($p<0.05$). The Hal+Exe group demonstrated a less intense progression of akinesia, PT being statistically different from 4W ($p<0.05$ (Figure 3).

When the groups were compared in the same period of treatment (2W or 4W) a significant increase in the time spent on the bar was observed in both haloperidol treated groups in comparison with the control groups (Figure 3). The most important finding of this test was that the increase in akinesia found in the Hal+Sed group was attenuated by 4 weeks of physical exercise (Figure 3).

2.4. Open-field test

The open-field test showed, in general, a significant decrease in exploratory parameters over the experiment duration, when PT and 4W measurements were compared within groups. In accordance with the observed decrease in the number of lines crossed, total travelled distance, average speed and mobile time, there was an increase in the time immobile over the course of 4 weeks. Intergroup analysis showed no differences in the same period (PT or 4W) (Figure 4).

3. DISCUSSION

Our findings on the body mass of the animals are in accordance with a previous study that showed that despite increased fat deposition, the weight in male rats does not change with APD (Minet-Ringuet et al., 2006). These results go against what is normally known about antipsychotic-induced weight gain in humans. It is widely accepted that the chronic use of

APD increases the body mass of both humans and rats (Lieberman et al., 2005; Covell et al., 2004; Bobes et al., 2003). It is possible that the treatment interval used in our study, of only 28 days, was insufficient to induce significant changes in the rats' body weight. The lack of weight changes observed could also be attributed to the use of male rats, since a previous study using rats showed that APD affected females more rapidly than males (Pouzet et al., 2003).

Our study shows that haloperidol is responsible for reducing stride length and increasing stance width, and these changes are reverted by associated exercise. (Figure 2A and 2B). Currently there is little literature available on haloperidol-induced gait alteration. Comparison is traditionally made with PD models because of the similarities between EPS and the symptoms of PD. In PD animal models, the gait presents a faster stride frequency, a shorter stride length, and smaller stance width (Kurz et al., 2007; Amende et al., 2005; Pothakos et al., 2009), which is consistent with findings in humans. The reduced stride length found in our study is very similar to the findings obtained using PD animal models (Kurz et al., 2007; Amende et al., 2005; Hansen et al., 1997).

Our experiments also showed that the reduction in stride length persisted 24 hours after the last haloperidol injection, which is typical characteristic of chronic, but not acute, use of haloperidol. In a previous study, stride length was shown to recover its regular size 24 hours after a single acute dose of haloperidol in mice (Fernagut et al. 2002).

Furthermore, our findings showed an increase in stance width, which is an indicator of loss of balance (Cheng et al., 1997) (Figure 2B). Our study is, to the best of our knowledge, the first to demonstrate changes in this parameter promoted by haloperidol use. Measurements of stance width are usually evaluated in animal models of PD using different neurotoxic agents, such as 6OHDA and MPTP (Amende et al., 2005; Pothakos et al., 2009; Metz et al., 2005). In these studies, reductions in stance width are normally observed (Amende et al., 2005; Pothakos et al., 2009). On the other hand, a previous study showed a tendency towards

increased stance width in unilateral PD rat models (Metz et al., 2005). These PD models are only based on lesioned dopaminergic neurons in the substantia nigra pars compacta (Amende et al., 2005; Pothakos et al., 2009; Metz et al., 2005), whereas the use of haloperidol is a more complex model, because it affects different neuronal populations. Moreover, haloperidol is not only responsible for dopamine binding reduction, it may also be responsible for depolarization blockage in other neuronal populations, including other motor pathways (Boye and Rompré, 2000), which could explain this discrepancy with the results obtained in PD models.

One of the most important findings of our study is that the stride length and stance width of the Hal+Exe group remained the same after 4 weeks, indicating that this approach could be a very efficient therapy to manage haloperidol-induced gait alterations.

We observed that use of haloperidol caused no paw placement (paw length and paw width) alterations (Figure 2C and 2D). Changes in these parameters are often found in studies with sciatic nerve lesion and mesencephalic lesions (Ilha et al., 2008; Metz et al., 2005). The lack of changes in paw length and paw width found in our study could be related to the fact that haloperidol only impairs neurotransmission and does not induce neuronal lesion. Studies into drug-induced changes to paw length and paw width are scarce, and it is probable that paw placement parameters are only, or mainly, affected by more aggressive insults, such as lesions to the nervous system and neuromuscular diseases.

Along the 4 weeks of haloperidol treatments an increase was observed in the amount of time taken by the animal to conclude the bar test, i.e. time on bar. In our study this parameter was considered a measurement of akinesia, but not catalepsy. Catalepsy is considered a lack of movement for more than 30 seconds (Wu et al., 2009). The haloperidol dose used in our study and the interval between the last dose and locomotor tests was unable to induce catalepsy. The dose used in our study was similar to that previously described (Ohno et al., 2010).

In the Hal+Sed group the time on bar value increases significantly between each time frame suggesting a worsening of akinesia throughout the treatment. However, physical exercise attenuates this increase in akinesia in the animals treated for four weeks (Figure 3); this suggests that exercise helps to soothe this chronic symptomatology.

The locomotor effects of haloperidol are usually measured immediately after administration (Vascolcelos et al., 2003; Wu et al., 2009; Karl et al., 2006). Our results for akinesia are similar to those reported using the same drug dose (Ohno et al., 2010). We only found one study where the chronic effects of haloperidol were measured 1 hour, 1, 3, 7 and 15 days after haloperidol withdrawal (Vascolcelos et al., 2003). Unfortunately our results are not in accordance with this study because it presents a significant degree of catalepsy 24 hours after the withdrawal of haloperidol treatment (Vascolcelos et al., 2003).

In our study none of the animals were classified as cataleptic because for such a classification the bar test has to take longer than 30 seconds (Wu et al., 2009), whereas akinesia, and it´s increment, is the response under this 30 seconds threshold (Wu et al., 2009 já está dito lá em cima). Typically, catalepsy is seen after acute administration of typical APD (Vascolcelos et al., 2003; Wu et al., 2009). In fact, the half-life of haloperidol in rats is 4-6 times faster than in humans, hence multiple doses are necessary throughout the day to maintain the same intensity of symptoms (Kapur et al., 2003).

Exploration and habituation to the open-field, analyzed using ANY-maze software did not differ between experimental groups during the analyzed time frame. The lack of differences between the groups treated for 4 weeks shows that haloperidol treatment was unable to induce a significant impairment in these locomotor parameters, indicating that this drug does not induce bradykinesia in these treatment conditions. These findings are in accordance with the results obtained in a previous study in which a similar dose of haloperidol was found to be insufficient to promote bradykinesia in the pole test (Ohno et al., 2010). When exploratory parameters such as lines crossed, total distance travelled, average speed

and time mobile from PT and 4W sessions were compared within each experimental group, a significant decrease in exploration was observed over time (Figure 4). The reduced exploratory behavior observed after 4 weeks could be related to the reduced stress due to constant manipulation during this period, but it is probably an indication of task apparatus recognition and habituation (Vianna et al., 2000). Long-term habituation to novel stimuli is one of the most elementary forms of nonassociative learning and has been shown to be long-lasting even in its most elementary forms (Carew et al., 1972). Our findings therefore suggest that, despite having effects on gait and akinesia, haloperidol treatment for 4 weeks does not impact hippocampal-dependent memory formation and retrieval.

The main findings in our study are that chronic exposure to haloperidol induces substantial gait alterations and increased akinesia. These changes in locomotor activity are reverted, or at least attenuated, by physical exercise, indicating that this approach could reduce some unwanted effects of haloperidol and help patients that need to use this drug. Clinically, when the motor side-effects of haloperidol become unbearable for a patient the drug dosage is reduced, and it may even be replaced. However, this study further elucidates the some aspects of the motor effects of haloperidol.

4. MATERIALS AND METHODS

4.1. Animals

48 male Wistar rats, aged approximately three months and weighing about 200-300g were obtained from the Institute of Basic Health Sciences (ICBS) – UFRGS. They were maintained in a controlled environment and housed in groups of five with food and water *ad libitum*, at a 12:12h dark:light schedule. The animals were allocated into four groups with twelve animals each: 1-Saline and sedentary (Ctrl+Sed), 2-Saline and exercise (Ctrl+Exe), 3-Haloperidol and sedentary- (Hal+Sed) and 4-Haloperidol and exercise (Hal+Exe). All experiments were performed in accordance with the Guide for the Care and Use of Laboratory

Animals adopted by the National Institute of Health (USA). All efforts were made to minimize animal suffering and reduce the number of animals needed.

4.2. Drug and exercise program

Saline solution (0.9% NaCl) or Haloperidol (Janssen-Cilag Farmaceutica Ltda., Brazil) at a dosage of 0.3mg/kg/day were administered intraperitonially (i.p.) during 28 days. A single dose was applied per day, 20-30 minutes before the exercise training session.

The animals in the exercise groups walked on an adapted motorized treadmill for thirty minutes, five days a week for four consecutive weeks, , totaling 28 days (the first two days of drug injections were used to find the proper speed used in this study). The exercise load consisted of a light-intensity treadmill exercise at a speed of 4 m/min for the first five minutes, and then 6 m/min for the remaining 25 minutes. The exercise protocol used in our study was adapted from a previous study using PD rat models (Yoon et al., 2007).

4.3. Tests and measurements

The animals were weighed weekly, starting with a pre-training (PT) evaluation and at the end of each week (1W, 2W, 3W, and 4W). The ink-paw test was carried out before (PT) and after (4W) of treatments and training. The bar test was used before (PT), after 2 weeks (2W) and after (4W) of treatments and training, and the open-maze test was also applied before (PT) and after (4W) of treatments and training. . All tests were performed before the daily dose of haloperidol or saline or exercise program, that is, all tests were performed at least 24 hours after the last injection of the drugs, thus, ensuring that only chronic (long-term) effects of the drug would be evaluated avoiding the acute (immediate) effects. Immediately after the tests, Haloperidol or saline solution were injected and the daily exercise session started.

For the ink-paw test, the hind paws of the animals were dyed with non-toxic ink before animals were individually placed on a catwalk with the floor covered by white paper. As the animals walked on the apparatus, paw prints were left on the sheet of paper. Six consecutive steps were selected and analyzed using these parameters: 1- Stride length (longitudinal distance between consecutive prints); 2- Stance width (latitudinal distance between consecutive left and right paw prints); 3- Paw length (distance between the print of the tip of the third toe and the ankle); and 4- Paw width (distance between the tip of the first toe and the tip of the fifth toe) (Ilha et al., 2008) (Figure 1).

In the bar test the degree of akinesia was evaluated by placing the animals' forelimbs on a 9 cm high horizontal bar and measuring the time taken by the animals to remove both paws from the bar (Vascolcelos et al., 2003; Wu et al., 2009; Ohno et al., 2010).

The open-field apparatus consisted of a 50 cm high, 60 cm X 40 cm box made of plywood, with one glass side. The floor is divided by drawn lines, composing 12 equal rectangles. Animals were individually placed in a corner of the apparatus and their behavior was recorded from above using a video camera for three minutes. Animal behavior was analyzed using ANY-Maze software (Version 4.70, Stoelting), which tracked the following parameters: Lines crossed, total distance travelled, average speed, time mobile, time immobile and immobile episodes.

4.4. Statistics

One-way ANOVA followed by Tukey´s *post hoc* analysis was performed to evaluate the differences among the groups in the same period. The differences in the same group in different periods (PT and 4W) were analyzed using a t-paired test. All procedures were performed in the SPSS 11.0 software ($p<0.05$).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTORS

All authors contributed to and have approved the final manuscript.

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LEGENDS

Figure 1 – Schematic view of paw prints and measures. 1 – Stride length, 2 – Stance width, 3 – Paw length, and 4 – Paw width.

Figure 2 – Effects of haloperidol and exercise on gait in the ink-paw test. Where (A) a = $p<0.01$ when compared to all other groups; (B) a = $p<0.001$ when compared to all other groups; (C) a = $p<0.01$ when compared to the Ctrl+Sed and Hal+Exe groups, b = $p<0.05$ when compared to the Ctrl+Sed and Hal+ Exe groups; (D) a = $p<0.01$ when compared to the Ctrl+Exe and Hal+Exe groups. (Mean distance $\pm$ SE).

Figure 3 – Effect of haloperidol and exercise on akinesia in the bar test. A = $p<0.001$ when compared to the Ctrl+Sed and Ctrl+Exe groups, b = $p<0.05$ when compared to the Ctrl+Sed and Ctrl+Exe groups, and c = $p<0.05$ when compared to all other groups. *$p<0.05$, and ***$p<0.001$ when compared to the groups indicated by the lines. (Mean time on bar $\pm$ SE).

Figure 4 – Effects of haloperidol and exercise on bradykinesia in the open-field test. In all graphs *$p<0.05$, **$p<0.01$, and ***$p<0.001$ when PT was compared with 4W in all groups. (Mean values $\pm$ SE).

Figure 1

1- Stride length
2- Stance width
3- Paw length
4- Paw width

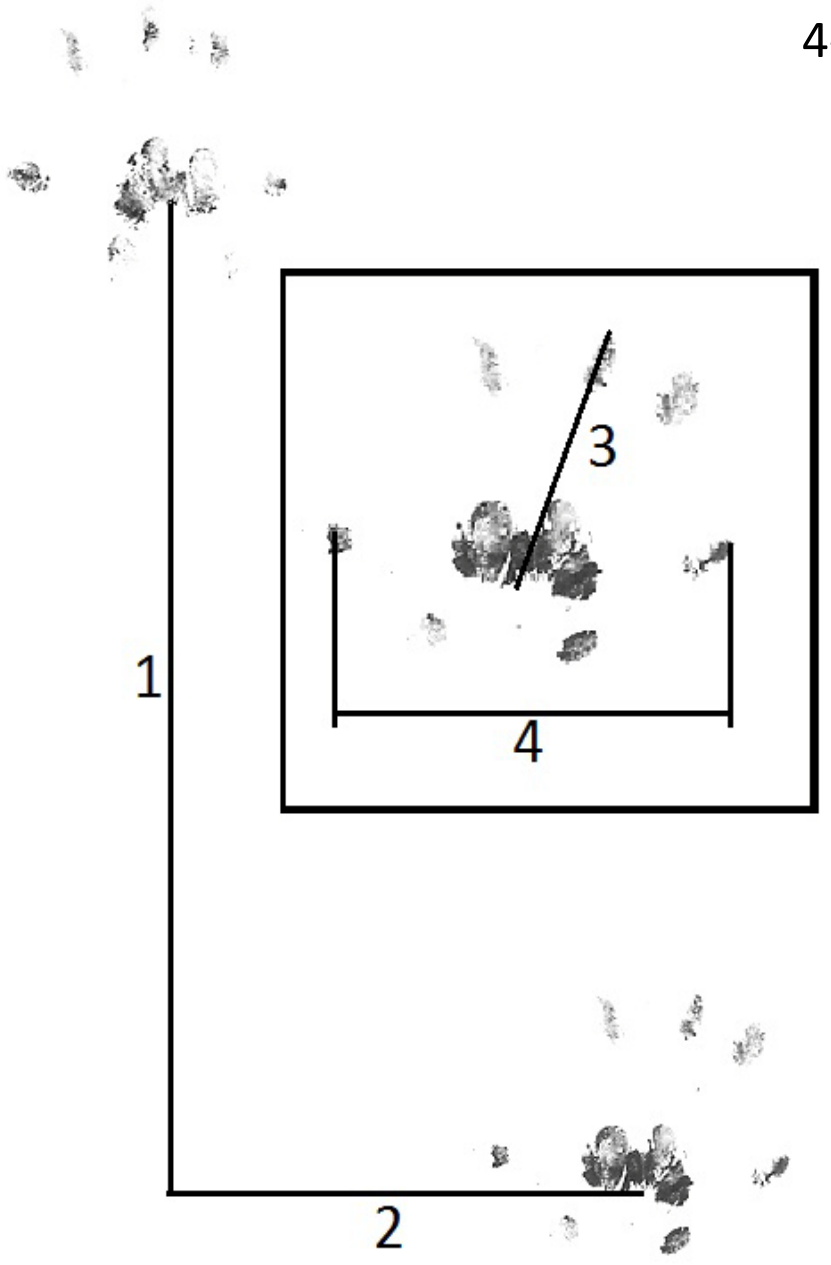


Figure 2

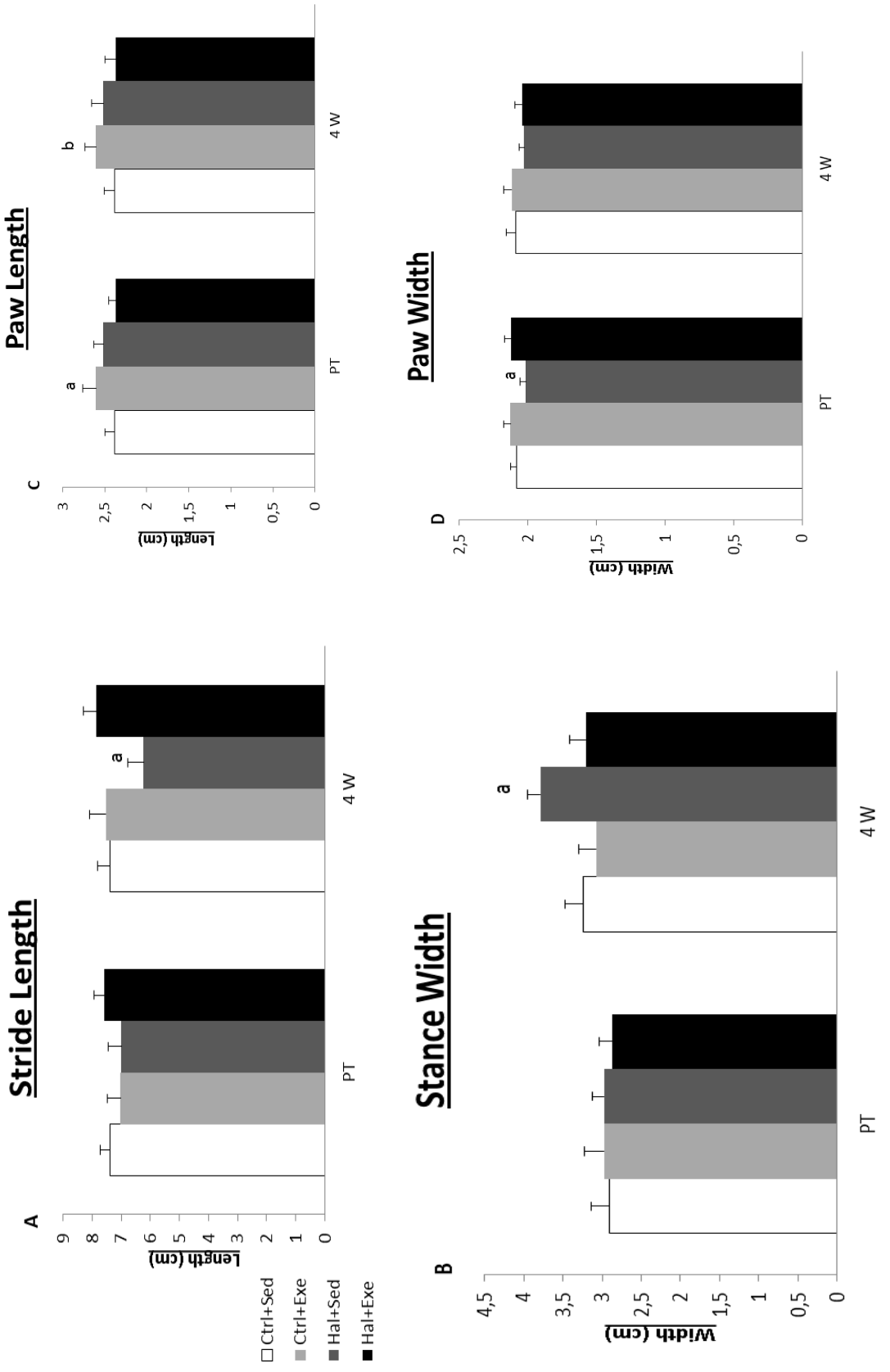

A
Stride Length
Length (cm)
9
8
7
6
5
4
3
2
1
0
a
PT
4 W
Ctrl+Sed
Ctrl+Exe
Hal+Sed
Hal+Exe
B
Stance Width
Width (cm)
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
a
PT
4 W
C
Paw Length
Length (cm)
3
2,5
2
1,5
1
0,5
0
a
b
PT
4 W
D
Paw Width
Width (cm)
2,5
2
1,5
1
0,5
0
a
PT
4 W

Figure 3

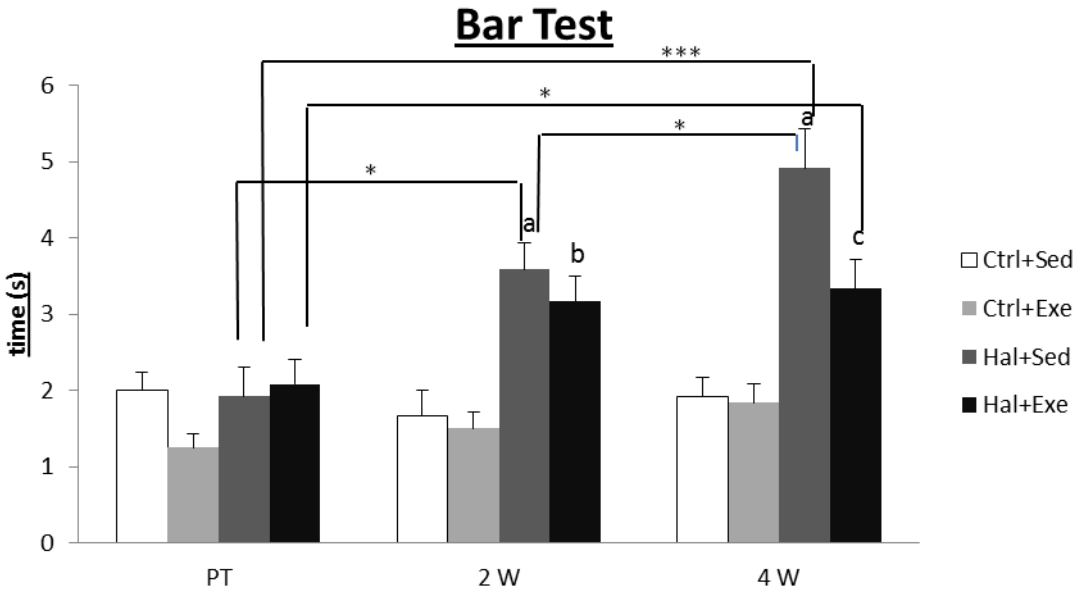
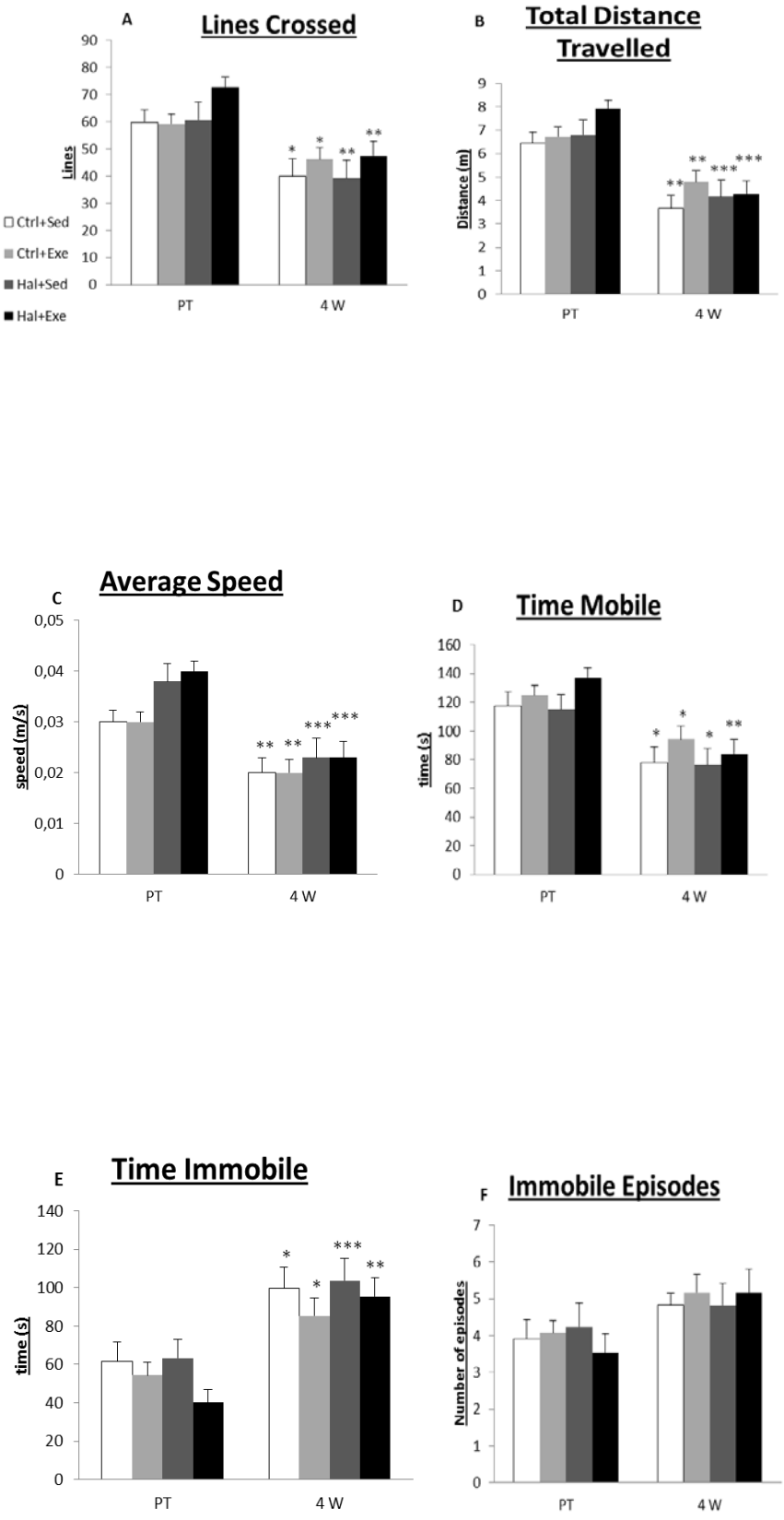

Bar Test
time (s)
0
1
2
3
4
5
6
PT
2 W
4 W
***
*
*
*
a
a
b
c
Ctrl+Sed
Ctrl+Exe
Hal+Sed
Hal+Exe

Figure 4


A
Lines Crossed
Lines
Ctrl+Sed
Ctrl+Exe
Hal+Sed
Hal+Exe
PT
4 W
B
Total Distance Travelled
Distance (m)
PT
4 W
C
Average Speed
speed (m/s)
PT
4 W
D
Time Mobile
time (s)
PT
4 W
E
Time Immobile
time (s)
PT
4 W
F
Immobile Episodes
Number of episodes
PT
4 W

3. CAPÍTULO 3

3.1. CONSIDERAÇÕES FINAIS

Em nosso estudo foi possível observar os seguintes achados:

1- As doses utilizadas e o tempo de tratamento com haloperidol empregados em nosso estudo não alteraram significativamente a massa corporal dos animais. Estes dados estão de acordo com os achados de um estudo prévio que mostrou que o peso de ratos machos não se altera com o uso de antipsicóticos em períodos e doses semelhantes as empregadas em nosso estudo (Minet-Ringuet, 2006). Estes dados são relativamente paradoxais em relação aos achados clínicos em humanos, onde o uso crônico de haloperidol é responsável pelo aumento de peso corpóreo (Hansen, 1997; Lieberman, 2005). É possível que estudos futuros, com doses mais elevadas haloperidol, associadas a tratamentos com maior duração possam levar a alterações significativas de peso corpóreo também em animais experimentais.

2- As doses e o tempo de tratamento com haloperidol utilizados em nosso estudo causaram uma diminuição significativa do comprimento do passo e um aumento na largura da base, que foram revertidos pelo exercício físico. A diminuição do comprimento do passo é um achado semelhante aos resultados usualmente encontrados nos modelos animais de parkinsonismo (Pothakos, 2009). Por outro lado, o aumento da largura da base, encontrada em nosso estudo, não está de acordo com os achados encontrados em modelos tradicionais de parkinsonismo com uso de agentes neurotóxicos (Kurz, 2007). Nossos achados indicam que possivelmente o bloqueio de receptores D2 pode afetar não apenas a via nigro estriatal, mas também outras vias motoras superioras relacionadas ao controle de

musculatura distal, como as vias córtico espinhal lateral e a rubro espinhal, além de alterar toda a dinâmica de neurotransmissores dos núcleos da base (Hansen, 1997). Deste modo, o parkinsonismo induzido por haloperidol parece ser um fenômeno neuroquímico bem mais complexo em termos motores do que a simples destruição nigral por agentes neurotóxicos.

3- O halopeperidol não causou alterações significativas nas medidas de comprimento da pegada e largura da pegada . Estes parâmetros são comumente utilizados para avaliação de insultos mais agressivos, como lesões ao sistema nervoso central ou periférico e doenças neuromusculares (Ilha, 2008). A ausência de alterações nestes parâmetros pode estar relacionada ao fato de que o comprometimento na neurotransmissão causado pelo haloperidol, nas doses utilizadas em nosso estudo, é capaz de compremeter a neurotransmissão dopaminérgica, contudo não gera distúrbios maiores, como uma lesão encefálica, medular ou nervosa capaz de alterar parâmetros mais “sólidos” como comprimento e largura da pegada.

4- O haloperidol causou um aumento gradual da acinesia, que foi significativamente atenuado pelo exercício físico. Em nosso estudo o tempo despendido até os animais finalizarem do teste da barra horizontal foi utilizado para mensurar o grau de acinesia. Os animais tratados com haloperidol em nosso estudo não foram considerados catalépticos, uma vez que o padrão de catalepsia é estabelecido quando o animal demora mais de 30 segundos para descer da barra horizontal (Wu, 2009) e nenhum dos animais tratados com haloperidol, em nosso estudo, permaneceu por mais de 30 segundos na barra horizontal.

5- O haloperidol não foi capaz induzir bradicinesia. A ausência de diferenças entre os grupos indica que o haloperidol não foi capaz de induzir nenhum comprometimento locomotor sob essas condições de tratamento. A dose utilizada em nosso estudo (0.3 mg/kg) foi previamente utilizada em outro estudo, neste foi observado que a mesma dose não induziu bradicinesia em modelos que foram submetidos ao *pole test* (Ohno, 2010). Nossos resultados são similares, e estão de acordo com este estudo. Ademais, durante o teste de bradicinesia, encontramos que após quatro semanas de tratamento todos os grupos apresentaram diminuição significativa em diferentes parâmetros exploratórios. Este achado provavelmente está relacionado ao fenômeno de habituação ao aparato do teste e consolidação da memória de longo prazo relativa ao teste (Vianna, 2000).

Estes resultados indicam que exercício físico foi capaz de atenuar e até mesmo reverter alguns efeitos colaterias locomotores causados pelo uso crônico de haloperidol. Possivelmente este efeito esteja relacionado aumento da atividade dopaminérgica na via nigro estriatal de ratos, induzido pelo treinamento físico em esteira (Do Nascimento, 2011).

Deste modo, nosso estudo caracteriza o exercício físico como uma alternativa terapêutica coadjuvante interessante em pacientes que necessitam o uso de antipsicóticos.

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ANEXO A – COMPROVANTE DE SUBMISSÃO DO ARTIGO

De: ees.bbr.0.e6ab2.7c2bd2c7@eesmail.elsevier.com em nome de Behavioural Brain Research
Enviada: seg 10/1/2011 04:43
Para: Leder Leal Xavier
Assunto: Editor handles BBR-D-11-00033

Ms. Ref. No.: BBR-D-11-00033
Title: Physical exercise reverts locomotor side effects induced by haloperidol treatment in Wistar rats.
Behavioural Brain Research

Dear Leder,

Your submission entitled "Physical exercise reverts locomotor side effects induced by haloperidol treatment in Wistar rats." will be handled by Editor-in-Chief Joe Huston.

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