UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

TRABALHO DE CONCLUSÃO DE CURSO

CURSO DE BIOMEDICINA

EFEITO DE ANTIPSICÓTICOS TÍPICOS E ATÍPICOS SOBRE DÉFICIT DE MEMORIA DE TRABALHO INDUZIDO POR MK801 EM TAREFA DE ESQUIVA INIBITÓRIA

MARÍLIA MOTA BESSA

PORTO ALEGRE, 2010

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PORTO ALEGRE, 2010

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ÍNDICE GERAL

RESUMO

Comprometimento da memória de trabalho é uma das alterações cognitivas mais relevantes no cotidiano do paciente esquizofrênico. A melhora cognitiva ainda é questionável, mesmo com uso de antipsicóticos, uma vez que resultados de ensaios clínicos são controversos e estudos pré-clínicos escassos. O objetivo desse trabalho foi avaliar o efeito de antipsicóticos típicos e atípicos na memória de trabalho e no déficit de memória de trabalho induzido por MK801 em camundongos. Para avaliar a memória de trabalho, bem como o seu déficit, usou-se o paradigma de esquiva inibitória, com intervalo de 10 segundos entre treino e teste e choque de 3 mA por 5 segundos. Somente doses de antipsicóticos que não alteraram a atividade locomotora foram utilizadas nos ensaios de memória. Todos os animais tratados com antipsicóticos apresentaram latência de teste significativamente maior que do treino ($p<0,05$) com exceção de clozapina ($p>0,05$). Apenas sulpirida e risperidona foram eficazes ($p<0,05$) quanto à prevenção de déficit induzido por MK801. Os dados sugerem que apenas sulpirida e risperidona apresentam efeito benéfico sobre o déficit de memória de trabalho induzido por antagonista de NMDA em camundongos. Clozapina, além de não prevenir esse déficit, também causou prejuízo na memória de trabalho na ausência de antagonista glutamatérgico.

Palavras-chave: Antipsicóticos, sintomas negativos, déficits cognitivos, farmacoeconomia

1. INTRODUÇÃO

A esquizofrenia é, entre os transtornos mentais, uma das doenças mais angustiantes e incapacitantes. Os primeiros sinais da esquizofrenia surgem, em geral, na adolescência ou no início da vida adulta. Os sintomas incluem distorções do pensamento e da percepção, afetos inapropriados ou embotados, bem como déficits cognitivos (World Health Organization, ICD 2007). Os sintomas da esquizofrenia são classicamente divididos em dois tipos: positivos, que parecem refletir um excesso ou distorção das funções normais, incluem alucinações (mais freqüentemente auditivas), delírios e transtorno do pensamento; e negativos, que parecem refletir uma diminuição ou perda de funções normais, que incluem retraimento social e perda de motivação (American Psychiatry Association, DSM-VI-TR 2000).

Um dos primeiros a classificar os transtornos mentais em diferentes categorias foi o médico alemão, Emile Kraepelin. O Dr. Kraepelin usou o termo “dementia praecox", devido à deterioração das habilidades mentais em pacientes jovens que apresentavam os sintomas que hoje associamos com esquizofrenia (Zec, 1995). A disfunção cognitiva é reconhecida como um déficit primário, que pode estar presente antes mesmo da manifestação clínica dos outros sintomas (Cornblatt et al., 1999), está quase totalmente desenvolvida já no primeiro episódio de psicose (Riley et al., 2000) e tende a persistir em pacientes crônicos independentemente da melhora de outros sintomas (Rund, 1998; Hughes et al., 2003; Laws e McKenna, 1997). Estas alterações cognitivas são um componente central da doença, presentes em até 80% dos pacientes (Keefe e Fenton, 2007) e podem limitar profundamente a capacidade dos doentes em adquirir, manter ou reaprender as habilidades necessárias para a vida (Green, 1996). Grande parte dos pacientes não se casa nem tem filhos, é desempregada ou trabalha em subemprego, bem como apresenta baixo rendimento acadêmico (Thornicroft et al., 2004; Carpenter e Koening, 2008). Estes fatos estão intimamente relacionados ao isolamento social e prejuízo cognitivo característicos da doença (Piskulic et al., 2009). Assim, a melhoria no desempenho cognitivo em pacientes com esquizofrenia tem sido vista como um alvo crucial para o tratamento farmacológico.

Entre as habilidades cognitivas mais prejudicadas em pacientes com esquizofrenia estão: memória verbal, atenção sustentada, função executiva e memória de trabalho (Bora et al., 2010). A memória de trabalho, que envolve o córtex pré-frontal (Goldman-Rakic, 1990), é um processo cognitivo que mantém grande parte do pensamento de ordem

superior, linguagem e comportamento. É a memória que nos permite manter temporariamente, atualizar e trabalhar com informações relevantes. O comprometimento da memória de trabalho é dos déficits cognitivos exibidos pelos pacientes com esquizofrenia o mais consistentemente observado (Park et al., 1999). O prejuízo na memória de trabalho pode ser a base do prejuízo de outras habilidades cognitivas que também estão alteradas na esquizofrenia (Silver et al., 2003).

Antes do advento dos antipsicóticos na década de 50, as opções de manejo da esquizofrenia eram muito limitadas. Lobotomia, tratamento com choque insulínico e eletroconvulsoterapia foram comuns, porém os pacientes permaneciam internados por longos períodos (ou mesmo ao longo da vida) (Berger, 1978).

Quando a clorpromazina foi sintetizada em 1950, procurava-se não um tratamento para psicose, mas sim um anti-histamínico mais eficaz para ser usado em medicação pré-operatória. Graças ao perfil particular de sedação da clorpromazina observado em pacientes no pré-operatório que se mantinham indiferentes ao meio, essa começou a ser usada como tranqüilizante em casos de mania e agitação. Clorpromazina e reserpina começaram a ser usadas em clínica para manejo de pacientes esquizofrênicos (Kapur e Mamo, 2003). Tais drogas foram denominadas primeiramente “tranqüilizantes maiores” (em contraposição aos ansiolíticos), e hoje são chamadas de neurolépticos (graças a indução da síndrome neuroléptica caracterizada por indiferença ao meio e afeto embotado) ou antipsicóticos típicos (em contraposição aos mais novos ou atípicos). Somente décadas mais tarde, com os trabalhos de Seeman e colaboradores (1975, 1976), se descobriu o mecanismo de ação da clorpormazina, ou seja, o bloqueio de receptores dopaminérgicos. Por serem potentes bloqueadores de receptores dopaminérgicos D_2, tais compostos apresentam alta incidência de efeitos adversos motores, especialmente sintomas extrapiramidais e discinesia tardia (Miyamoto et al., 2005). Apesar de suas limitações de eficácia e segurança, a disponibilidade dos antipsicóticos típicos tornou possível a alta hospitalar de pessoas com esquizofrenia e contribuiu para o desenvolvimento de vários tratamentos psicossociais, que mantém os pacientes em contato com a comunidade (Torrey et al., 2001).

Os antipsicóticos típicos mantiveram-se como base da farmacoterapia da esquizofrenia por aproximadamente 40 anos, até a introdução da clozapina, com alegada superioridade sobre os típicos no tratamento de esquizofrênicos. Uma vez que a clozapina tem pouca tendência em produzir efeitos extrapiramidais, discinesia tardia ou elevar os níveis de prolactina, foi descrita como um antipsicótico atípico (Garmendia et al., 1992);

porém pode causar outros efeitos adversos importantes tais como agranulocitose em 1% dos pacientes (Alvir et al., 1993).

No início da década de 90, a indústria farmacêutica produziu uma série de novos antipsicóticos atípicos (risperidona, olanzapina, quetiapina, ziprasidona e aripiprazole) que se assemelham à clozapina por terem uma menor tendência em produzir efeitos extrapiramidais, mas que não causam agranulocitose. Estes medicamentos foram lançados no mercado com supostas vantagens, incluindo melhor eficácia para sintomas positivos do que os típicos, eficácia em sintomas negativos, melhor tolerabilidade e eventual melhora nas funções cognitivas. Sobretudo esses fármacos foram apontados como mais eficientes no manejo dos sintomas negativos, com melhora na qualidade de vida e na redução dos sintomas extrapiramidais, quando comparados com os antipsicóticos típicos (Möller, 2000).

Embora os atípicos produzam menos efeitos extrapiramidais que os típicos, eles apresentam sérios efeitos colaterais metabólicos: aumento de índice de massa corpórea, hiperlipidemia, redução da sensibilidade à insulina, além do aumento da incidência de diabetes. Tais alterações metabólicas tendem a se agravar com o aumento da exposição aos fármacos, reduzindo significativamente o tempo de vida (Hennekens et al., 2005; Auquier et al., 2006; Seeman, 2007). Além disso, a melhoria acentuada dos sintomas negativos e déficits cognitivos por antipsicóticos atípicos ainda não está clara (Lieberman et al., 2005; Keefe et al., 2007).

Ademais, estudos de meta-análise e comparativos de longo prazo entre antipsicóticos típicos e atípicos têm mostrado que os benefícios presumidos, tais como aumento de eficácia sobre os sintomas negativos ou melhor adesão ao tratamento são modestos (Davis et al., 2003) ou inexistentes (Leucht et al., 2003). Somente a clozapina foi mais bem documentada como sendo mais eficaz no tratamento de casos refratários e resistentes à medicação tradicional (Kane et al, 1988; Lewis et al., 2006). Estudos também compararam a eficácia de típicos e atípicos para sintomas cognitivos, com pouca evidência de que estes agentes apresentem efeitos benéficos em funções cognitivas (Buchanan et al., 2010; Daban et al., 2005). Além disso, atualmente não há evidências suficientes que justifiquem o uso de drogas adjuvantes visando a melhora cognitiva de esquizofrênicos (Sumiyoshi et al., 2007).

A maior parte dos custos econômicos associados à esquizofrenia está associada com hospitalização. O custo do tratamento de esquizofrênicos aumentou em até 10 vezes desde a introdução dos antipsicóticos atípicos no mercado; a clozapina, por exemplo, além

de ser mais cara que os típicos requer acompanhamento hematológico periódico, o que aumenta ainda mais os custos do tratamento. Argumentou-se porém que como a medicação tem vantagens claras para pacientes refratários a outras medicações com as quais os pacientes necessitam de hospitalização freqüente e por longos períodos, sugeriu-se que os gastos adicionais da medicação podem ser recuperados através da redução de internações. Assim, foi alegado que os compostos atípicos, apesar de muito mais caros, apresentavam bom custo-benefício, seja por diminuir a freqüência de internações (Hanrahan et al., 2006) ou por melhorar o bem estar do paciente (Burton, 2006).

Um extenso estudo (Aguiar, 2008) realizado no Brasil buscou comparar os gastos do SUS com tratamento para esquizofrenia, entre 1999 e 2005. De acordo com os dados, um ano de vida com qualidade, associando antipsicóticos típicos e acompanhamento psicossocial, variava de U$1.743 a U$ 4.847 por paciente. Quando eram introduzidos antipsicóticos atípicos no lugar dos típicos, esse valor aumentava para U$10.232 a U$ 14.481. O estudo concluiu que, além do tratamento com antipsicóticos atípicos ser mais caro, não apresentou diferença significativa de efetividade.

Apesar de intensa investigação, a etiologia da esquizofrenia está longe de ser compreendida. A maioria das hipóteses evoluiu a partir das bases farmacodinâmicas (modo de ação) das drogas de uso clínico (antipsicóticos típicos e atípicos) ou pelas semelhanças entre os sintomas da esquizofrenia e os efeitos de certas drogas de abuso, como anfetamina (agonista de receptores dopaminérgicos), fenciclidina (PCP, antagonista de receptor NMDA de glutamato) e dietilamida do ácido lisérgico (LSD, agonista dos receptores 5-HT_2) (Tandon et al., 2008). Acredita-se hoje que uma hiperfunção dopaminérgica no sistema mesolímbico (responsável pelos sintomas positivos) acompanhada de uma hipofunção dopaminérgica no sistema mesocortical (responsável por sintomas negativos e cognitivos) - ambas possivelmente resultantes de uma hipofunção glutamatérgica cortical, desempenham papel central na fisiopatologia da doença (Abi-Dargham, 2004; Howes e Kapur, 2009).

Van Den Buuse e colaboradores (2005) publicaram uma revisão sobre a importância de modelos animais em pesquisas sobre esquizofrenia. Eles ressaltam que essa é uma doença mental complexa e não pode ser exatamente reproduzida em roedores. Porém, salientam que tais modelos são interessantes para as pesquisas difíceis de serem conduzidas em humanos - tanto por aspectos técnicos quanto éticos - além de ajudarem no desenvolvimento de novas drogas, na investigação da relação entre áreas do encéfalo com

o comportamento e de mecanismos tanto genéticos quanto neurodesenvolvimentais que estão envolvidos na patologia.

Apesar da complexidade da doença, os modelos com antagonistas de receptores glutamatérgicos NMDA apresentam alguma validade de predição, uma vez que oferecem a oportunidade de estudar drogas com potencial terapêutico para esquizofrenia, e forneceram uma importante base para o surgimento de novas hipóteses que vão além da transmissão dopaminérgica; apesar desse modelo estar intimamente relacionado com a hipótese de hipofunção glutamatérgica da doença. A validade de face desses modelos é razoável, uma vez que os sintomas apresentados por roedores se assemelham à clínica (pacientes sob efeito de antagonistas de NMDA apresentam comportamento comparável aos sintomas positivos, negativos e cognitivos da esquizofrenia); já a validade de construto é limitada já que a patofisiologia da doença ainda não foi completamente esclarecida (Large, 2007).

Assim, pode-se dizer que o custo do tratamento da esquizofrenia aumentou muito desde a introdução dos antipsicóticos atípicos, e que, no entanto, uma melhora igualmente significativa na qualidade de vida do paciente não está claramente comprovada. Os dados da literatura científica, clínicos e pré-clínicos, são controversos quanto às alegadas vantagens que esses novos antipsicóticos apresentam sobre os típicos. Especificamente quanto ao comprometimento da memória de trabalho ainda não há um consenso sobre os efeitos dos antipsicóticos sobre este processo mental.

2. OBJETIVO

Esse trabalho teve como objetivo comparar o efeito de antipsicóticos típicos e atípicos (e estes entre si) sobre a memória de trabalho, bem como sobre o déficit de memória de trabalho induzido por MK801 em camundongos.

3. ARTIGO CIENTÍFICO

Bessa, MM; Linck, VM; Menezes, CB; Herrmann, AP; Elisabetsky, E. **Effect of typical and atypical antipsychotics on working memory deficit in mice.**

3.1 Effect of typical and atypical antipsychotics on working memory deficit in mice.

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Abstract

Cognitive deficits are common in schizophrenics and are an important symptom of this disease. Working memory deficits are one of the most compromised cognitive aspects in schizophrenia, with important implications for the patient's daily life. Despite its clinical importance, the effect of antipsychotics in such deficit is not well documented, since clinical trial results are controversial and preclinical studies scarce. NMDA antagonists are widely used as pharmacological tools in animal models because it induces behaviors correlated to schizophrenia positive, negative and cognitive symptoms. The aim of this study was to evaluate the effect of typical and atypical antipsychotics on working memory and MK801-induced working memory deficits in mice. We evaluated the effects of haloperidol 0.125 mg/kg, chlorpromazine 1 mg/kg, clozapine 2 mg/kg sulpiride 10 mg/kg, risperidone 0.05 mg/kg or olanzapine 0.2 mg/kg in step down inhibitory avoidance with 10 sec training-test interval. The effects of this same antipsychotic were also analyzed in MK801-induced working memory deficit in the same task. The difference of latency between training and test sessions was taken as a measure of working memory. All groups showed significantly higher latency in test than in training session ($p<0.05$) except for clozapine ($p>0.05$). Only sulpiride and risperidone were effective ($p<0.05$) preventing MK801-induced working memory deficits. These data suggest that only sulpiride and risperidone have a beneficial effect on the working memory deficit induced by NMDA antagonists in mice. Clozapine did not prevent this deficit, and *per se* caused damage in working memory. These results collaborate to the discussion about the superiority of atypical on treating cognitive symptoms, studies with different doses and task may help in such discussion.

Keywords: classical and atypical antipsychotics, cognitive deficits, schizophrenia, step down, working memory.

1. Introduction

One of the first scientists to classify mental disorders into different categories was the German physician, Emile Kraepelin, who used the term "dementia praecox" to what we now associate with schizophrenia, due to the patients' mental abilities deterioration in an early age (Zec, 1995). Currently, the many symptoms of schizophrenia are subdivided into positive, negative and cognitive (American Psychiatry Association, DSM-VI-TR 2000).

Cognitive deficits are considered a primary cause of the long-term disability and a key feature of schizophrenia, with up to 80% of patients being cognitively impaired (Keefe and Fenton, 2007). The cognitive domains primarily affected in schizophrenia include attention, executive function, working memory, visual and verbal learning and memory (Bora et al., 2010). Deficit in working memory is considered as a core deficit underlying multiple neuropsychological deficits in schizophrenia patients (Silver et al., 2003). Working memory is defined as a temporary storing of necessary fundamental information required for certain tasks as well as for the planning of other more complex cognitive abilities (Baddeley, 2003). In schizophrenia there is a cortical dopaminergic dysfunction, leading to diminished dopamine stimulation, vital for the working memory impairments (Abi-Dargham, 2004).

First generation antipsychotic agents - discovered in the 1950s, also referred as typical antipsychotics - are effective in the treatment of psychotic symptoms, but often cause serious motor side-effects diminishing the adhesion to treatment (Miyamoto et al., 2005). In the early 90s, new antipsychotic agents that less frequently induce such adverse effects were introduced in the clinic. Initially, there was a considerable expectation that these new medication would improve not only schizophrenia positive symptoms, but also the negative and cognitive aspects of the syndrome (Möller, 2000). Unfortunately, however, large clinical trials have shown that second generation or atypical antipsychotics did not attend such initials expectations (Lieberman et al., 2005; Lewis et al., 2006; Leucht et al., 2003). Moreover, and relevant for a life time lasting disease, treatment with atypical antipsychotics is circa to 10 times more expensive than that with older antipsychotics, rightly generating a debate on the actual cost-benefit of the generalized use of atypical antipsychotics (Hanrahan et al., 2006). The lack of an effective treatment for the cognitive impairment in schizophrenics is particularly worrying, considering that deficits in attention, learning, memory and sensory modulation affect patients' ability to adequately perform daily activities, socialize and have a productive life with an acceptable quality.

Considering the allegations and marketing strategies of newer antipsychotics as being more effective in the management of cognitive symptoms in schizophrenics; that the data are scarce and controversial; and the implications of higher cost of such medications, comparative studies on the effect of typical and atypical antipsychotics in both negative and cognitive symptoms are sorely needed. Such comparisons in relevant animal models can contribute not only to the design of proper clinical trials, but also to a better understanding on the molecular basis of cognitive deficits and the effects of specific antipsychotic, ultimately supporting physicians prescribing choices.

The purpose of this study is to investigate the effects of typical (haloperidol and chlorpromazine) and atypical (clozapine, sulpiride, risperidone and olanzapine) antipsychotics in mice working memory (step down inhibitory avoidance task) as well as in MK801-induced working memory deficits.

2. Methods

2.1 Animals

Experiments were performed using male adult albino mice (CF1), from Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS) with 2 months of age (40–50g). Animals were maintained in our own animal facility under controlled environmental conditions (22 ± 1°C, 12h-light/dark cycle, free access to food [Nuvilab CR1] and water), for at least two weeks before the experiments. All animal procedures were approved by the University Ethical Committee (approval number 2007834). Strategies that minimized the number of animals used in each experiment and their suffering were applied.

2.2 Drugs

Haloperidol was used as commercial Haldol® (Janssen Farmacêutica Ltda, SP, Brazil); chlorpromazine, clozapine and sulpiride were purchased from Sigma Chemical Co. (St. Louis, MO); risperidone was purchased from Jassen-Cilag (Wien, Austria); olanzapine was used as commercial Zyprexa® IM (Lilly, Giessen, Germany) and MK801 (dizocilpine) was acquired from Research Biochemicals International (Natick, MA).

Clozapine, sulpiride and risperidone were solubilized in circa 100 μL of HCl (1N), the pH adjusted to 6.0 with NaOH 1N and the due volume complete with distilled water.

Chlorpromazine was diluted in saline (NaCl 0.9%). All other drugs were diluted in distilled water. All drugs were administered i.p. in a volume of 0.1 mL/10 g of body weight.

2.3 Locomotion

To determine the effects of antipsychotics on locomotion the method was adapted from de Moura Linck et al (2008). Activity cages (45×25×20 cm, Albarsch Electronic Equipments with three parallel and one perpendicular photocells) automatically recorded the number of crossings. Mice (n=8–11) were treated ip with saline or one of the antipsychotics (haloperidol 0.125 and 0.25 mg/kg; chlorpromazine 1.0 and 2.0 mg/kg; clozapine 2.0 mg/kg; sulpiride 10.0 mg/kg; risperidone 0.05; olanzapine 0.02, 0.025 and 1.5 mg/kg). 30 minutes after receiving the treatments, mice were individually placed in the activity cages and the number of crossings was recorded for 15 min – the first 5 minutes were considered as exploratory behavior and the other 10 minutes as locomotion.

2.4 Step down inhibitory avoidance

The test used was adapted from Netto and Izquierdo (1985), Maurice et al. (1994) and Barros et al. (2005). The inhibitory avoidance training apparatus was a plastic box with 40×25×25 cm, with a platform (5×5×4 cm) fixed in the center. The floor consists of a grid of parallel bronze bars with 1 mm in diameter with a gap of 1 cm between each bar, where a variable electric potential difference can be applied. In their home cage, mice were habituated to the dimly lit room for at least 30 min before the experiments. Each mouse was placed on the platform and the latency to step down (four paws on the grid) was observed in training and test sessions. In the training session, upon stepping down, the mouse received a 0.3 mA scrambled foot shock for 5 s. Animal training latencies shorter than 3 s or longer than 40 s were excluded from experiments. The test session was performed 10 s after training with the same procedure except that no shock was applied; an upper cut-off time of 300 s was set. The difference in latency between training and test sessions was taken as a measure of working memory. Only doses that did not affect locomotion were used in the step down inhibitory avoidance.

2.4.1 Effect of antipsychotics in step down working memory

Animals (n=9-25) were treated with saline, haloperidol 0.125 mg/kg, chlorpromazine 1.0 mg/kg, clozapine 2.0 mg/kg, sulpiride 10.0 mg/kg, risperidone 0.05 mg/kg or olanzapine 0.2 mg/kg 30 minutes before the training session.

2.4.2 Effects of antipsychotic in MK801-induced step down working memory deficit

Mice (n=11-20) were treated with saline or one of the antipsychotics (same doses as 2.4.1) 1 hour prior to the training session. Saline or MK801 0.05 mg/kg were given 30 min after the first treatment.

2.5 Statistical analysis

The results of locomotion are expressed as mean ± S.D. and were analyzed using ANOVA/Student Newman–Keuls. Data from step down are expressed as median ± interquartile range and were analyzed by Wilcoxon (to compare training and test latencies within the same treatment group) and Kruskal-Wallis/Mann-Whitney (to evaluate differences among treatments). $P<0.05$ was adopted as significance.

3. Results

3.1 Locomotor Activity

As can be seen at Fig 1B haloperidol 0.125 mg/kg, clorpromazine 1 mg/kg, clozapine 2 mg/kg, sulpiride 10 mg/kg, risperidone 0.05 mg/kg, olanzapine 0.2 mg/kg had no significant effects on locomotion ($p>0.05$; $F_{6,75}$ = 2,13), whereas higher doses of haloperidol, chlorpromazine and olanzapine significantly ($p<0.05$; $F_{7,60}$=5,56) diminished locomotion (Fig 1A).

3.2 Effect of antipsychotics in working memory

As can be seen in Fig 2, in control mice test sessions latencies are significantly higher than the training ones ($p<0.05$; Wilcoxon) indicating that working memory was properly accessed with the applied protocol. Kruskal-Wallis shows that there are significant differences in test ($p<0.05$) but not for training sessions ($p>0.05$). Only clozapine was prejudicial to working memory (comparable training and test sessions, Wilcoxon $p>0,05$), while test sessions were significantly ($p<0.05$; Wilcoxon) higher than training latencies for all other antipsychotics.

3.3 Effect of antipsychotics on MK801-indiced working memory deficit

MK801 was effective in inducing working memory deficit since contrary to the working memory revealed by significant ($p<0.05$, Wilcoxon) differences between test and training sessions latencies of control mice , no significant differences are found between test and training sessions latencies of mice treated with saline andMK801 ($p>0.05$, Wilcoxon). Haloperidol, chlopromazine, clozapine and olanzapine had no significant effects ($p>0.05$) on the MK801-induced working memory impairment, whereas sulpiride and risperidone were able to prevent ($p<0.05$) such deficit (Fig 3). Krusall-Wallis indicate no significant differences between training sessions, but a significant difference in test sessions. Mann Whitney test confirms that only sulpiride and risperidone test session latencies are significantly ($p<0,05$) different than control amnesic mice.

4. Discussion

NMDA receptor antagonists, such as MK801, induce behaviors that resemble positive (hyperlocomotion), negative (deficits in social interaction), and cognitive (deficits in working memory) symptoms in rodents, whereas models with dopamine agonists only mimic the positive symptoms of the disease (Powell and Miyakawa, 2006). Accordingly, this study confirms that an acute treatment with MK801 induces working memory deficit. It can be argued that MK801-induced working memory deficit has relevant construct value, since it is believed that the cognitive deficit in schizophrenia is associated with a glutamatergic hypofunction in the prefrontal cortex (Manahan-Vaughan et al., 2008; Rujescu et al 2006); nevertheless, the predictive value of this animal model is still unclear

(Large, 2007), especially given the paucity of clinical results regarding cognition in schizophrenics.

The aim of this study was to compare the efficacy of first and second generation antipsychotics in an NMDA antagonist-induced working memory deficit in mice. Both sulpiride and risperidone had a beneficial effect in the model, whereas chlorpromazine and haloperidol, two typical antipsychotics, as well olanzapine -- contrary to expectations -- did not show significant effects. Surprisingly enough, clozapine not only had no beneficial effects in the memory deficit, but was prejudicial to working memory in untreated mice.

Honey at al. (1999) reported that when treatment was exchanged from typical antipsychotic to risperidone an increased in frontal cortical activation was observed during working memory task. The researchers concluded that the increased regional cerebral blood/;oxygenation may reflect the ability of risperidone to increase cortical dopaminergic activity (most likely due its ability to block 5-HT_{2A} receptors) which could contribute to attenuate working memory defecits. Mehta et al. (2003) reported that sulpiride altered the blood flow, but no significant effects were seen in working memory tasks in healthy volunteers. Given the similarities with the results in mice here shown, we suggest that an increase in blood flow in relevant brain areas may be relevant and beneficial when deficit is present, although not noticeable in normal conditions. It is well established in literature that clozapine has anxiolytic properties, (Mead et al., 2008), and that anxiolitics drugs can affect memory by reducing the speed with which information is processed (Curran, 1999), a feature crucial to working memory. The acute amnesic effects of clozapine found in this study may be related to the anxiolytic property of this drug.

The introduction of second generation antipsychotics in the health market has created high expectations for greater efficiency and safety in the treatment of schizophrenia, compared with the gold standard treatment with typical antipsychotics then available. In spite of the claims of original studies, meta-analysis and systematic reviews have provided only limited support for the alleged superiority of these medications (Jones et al., 2006; Lieberman et al., 2005; Lewis and Lieberman, 2008). Considering the much higher cost of second-generation antipsychotics, such dispute over its actual advantages became even more significant (Leucht, 2009). Polsky et al. (2006) pointed out that most studies - but not all – agree with the claims that treatment with atypical antipsychotics are cost savers compared to the management of the disease with typical medication. Although methodological problems raised questions about the evidences showed in some of these

studies, it is obvious that considering the exponential increase in the cost of schizophrenia treatment, the quality of study design and sturdy results have additional meanings.

In Brazil, the amount spent to provide a year of quality life with typical antipsychotics and psychosocial care, ranges from U$ 1,743.00 to U$ 4,847.00 per patient; when atypical antipsychotics are used instead the figure increases to U$10,232.00 to U$14,481.00 (Aguiar, 2008). The results presented in this study are preliminary and should be complemented with other antipsychotic agents, dose ranges and chronic administration. Nevertheless, we argue that such studies are useful to support the above detailed discussion on the management of cognitive symptoms in schizophrenia and, ultimately, inform decision makers in public health on the cost-effectiveness of different antipsychotics usage.

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6. Figures

Figure1A

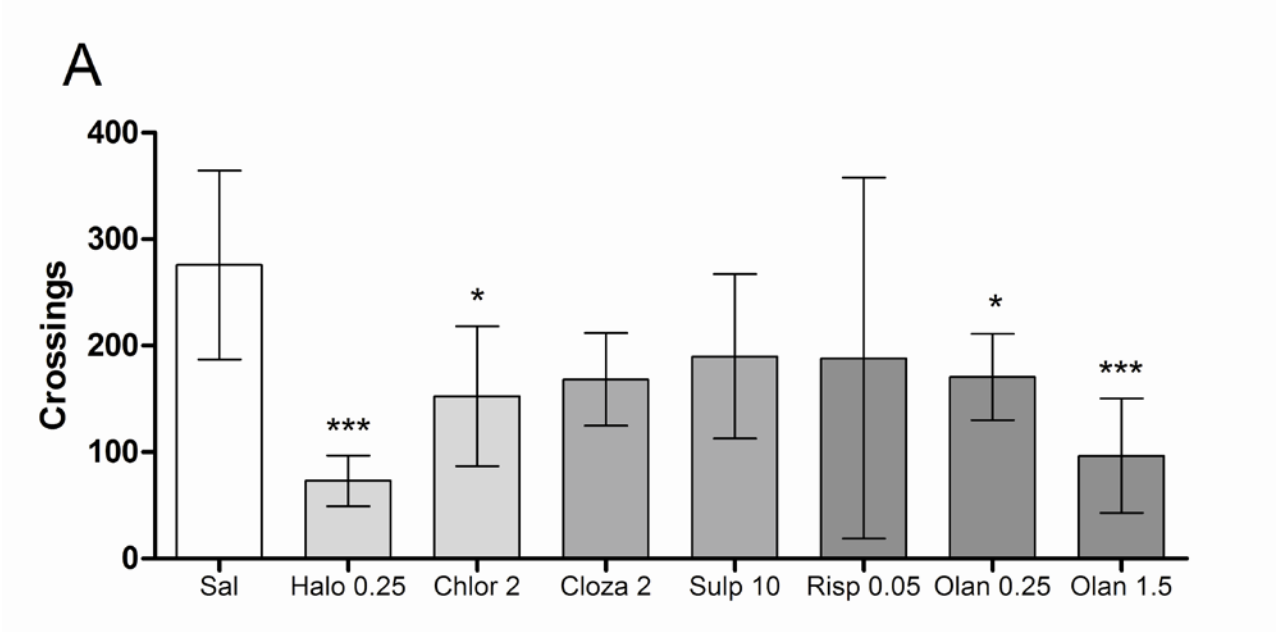


Figure 1B

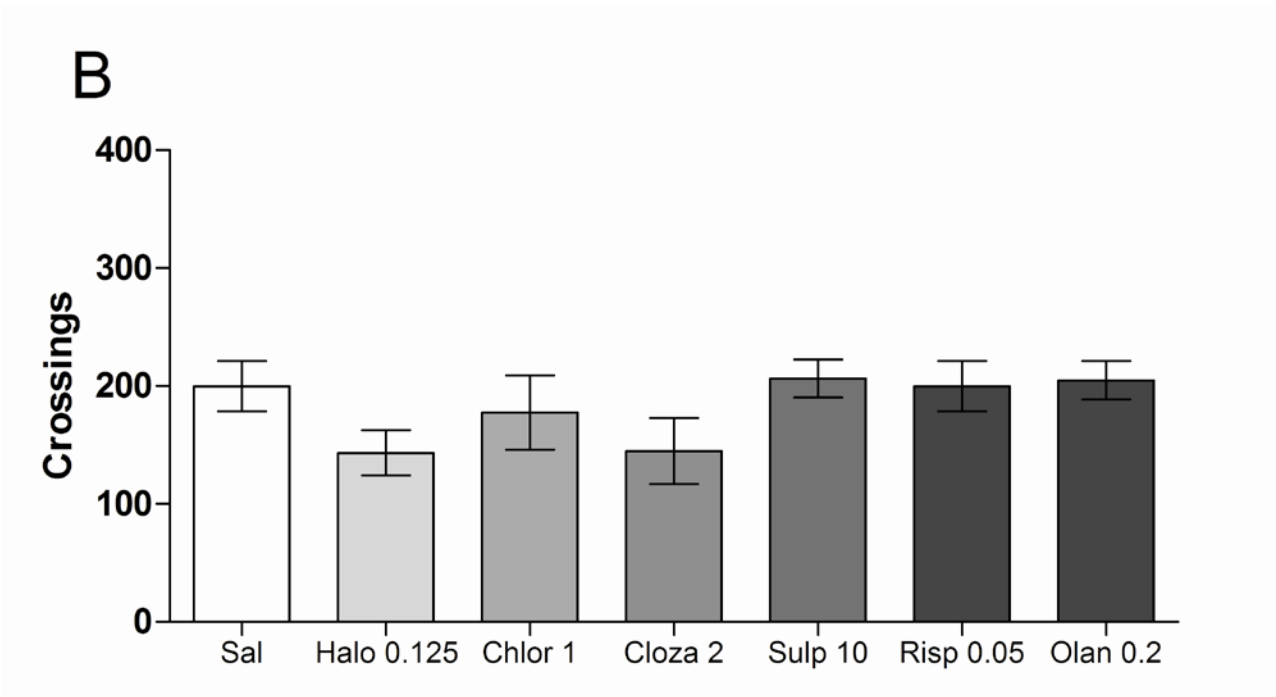


Figure 2

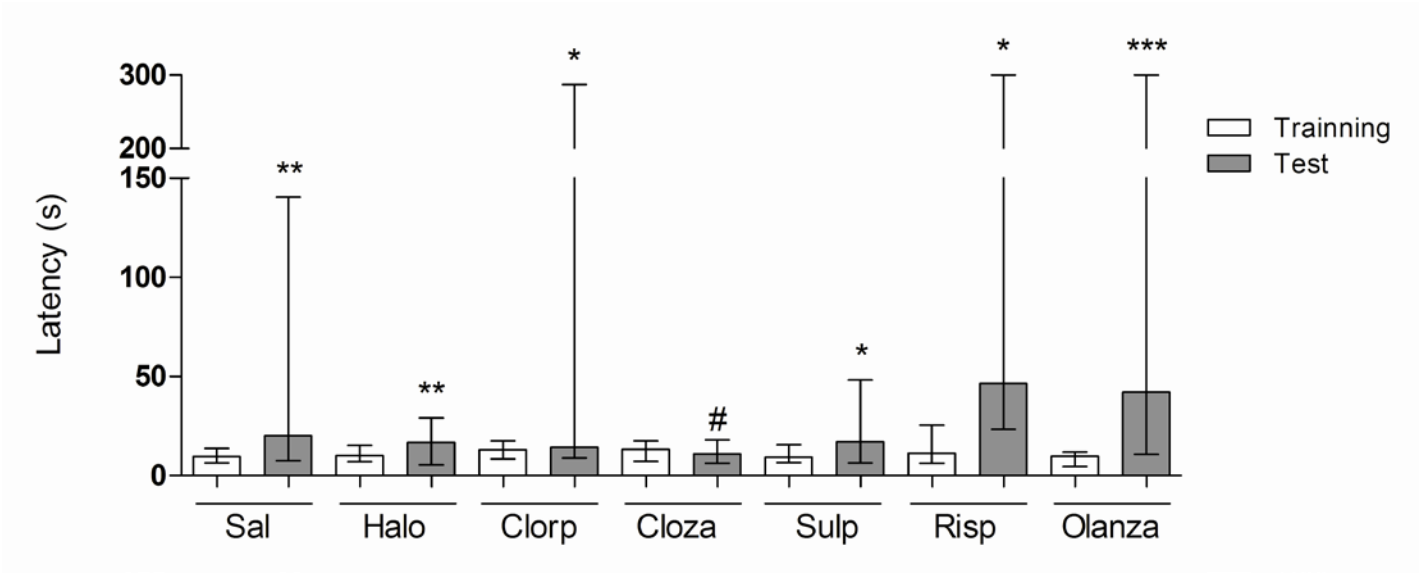
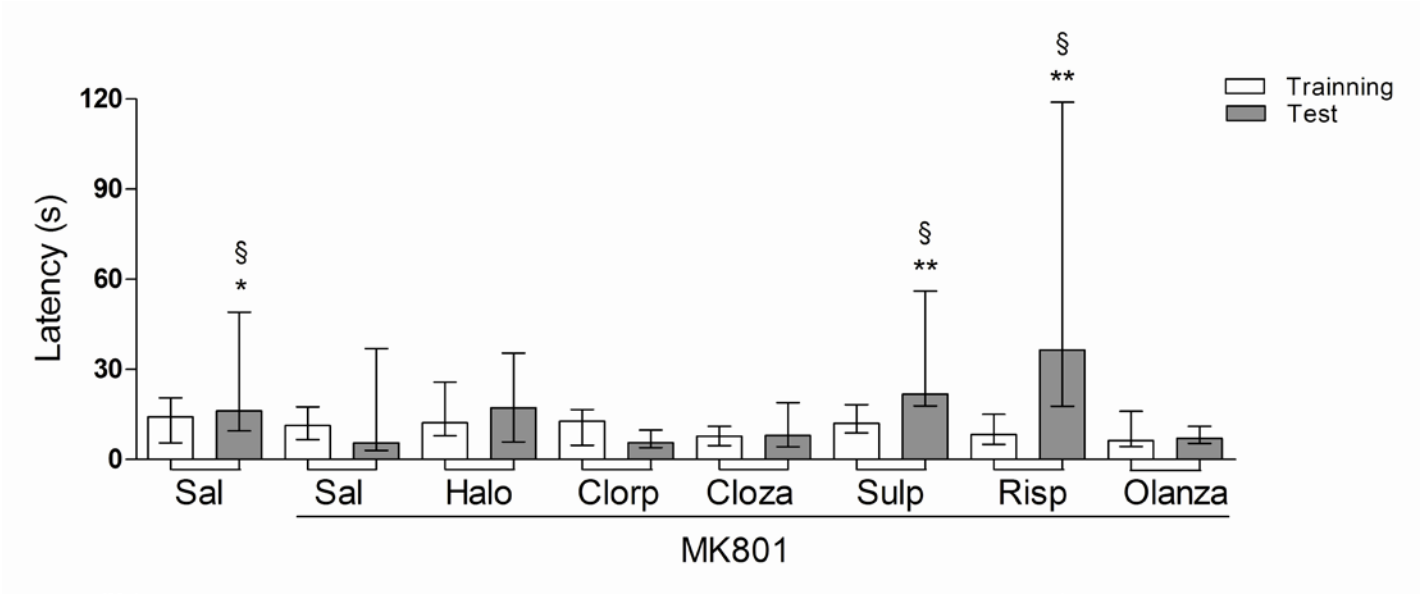


Figure 3



7. Figures Legends

Fig 1: Effect of antipsychotics on locomotor activity. A) Sal = NaCl 0.9%, halo 0.25 = haloperidol 0.25 mg/kg, chlor 2 = chlorpromazine 2.0 mg/kg, cloza 2 = clozapine 2.0 mg/kg, sulp 10 = sulpiride 10.0 mg/kg, risp 0.05 = risperidone 0.05 mg/kg, olan 0.25 = olanzapine 0.25 mg/kg and olan 1.5 = olanzapine 1.5 mg/kg. B) Experiments carried out at a different time of year from those at Fig 1A (higher baseline locomotor activity). All statistical analyses were done within same time-of-year groups; Sal = NaCl 0,9%, halo 0.125 = haloperidol 0.125 mg/kg, chlor 1 = chlorpromazine 1.0 mg/kg, cloza 2 = clozapine 2.0 mg/kg, sulp 10 = sulpiride 10.0 mg/kg, risp 0.05 = risperidone 0.05 mg/kg and olan 0.2 = olanzapine 0.2 mg/kg. Each column represents the Mean±SEM. *=$p<0.05$; ***=$p<0.001$ comparing with saline, ANOVA/SNK.

Fig 2: Effect of antipsychotics on working memory. Sal = NaCl 0.9%, halo = haloperidol 0.125 mg/kg, chlor = chlorpromazine 1.0 mg/kg, cloza = clozapine 2.0 mg/kg, sulp = sulpiride 10.0 mg/kg, risp = risperidone 0.05 mg/kg and olanza = olanzapine 0.2 mg/kg. Each column represents the latencies (sec) median±interquartile ranges of training (light columns) or test (dark columns). *=$p<0.05$ and **=$p<0.01$ comparing training and test latencies within the same treatment (Wilcoxon). #=$p<0.05$ comparing with the control test (Kruskal-Wallis/Mann-Whitney).

Fig 3: Effect of antipsychotics on MK801-induced working memory deficit. Sal = NaCl 0.9%, halo = haloperidol 0.125 mg/kg, chlor = chlorpromazine 1.0 mg/kg, cloza = clozapine 2.0 mg/kg, sulp = sulpiride 10.0 mg/kg, risp = risperidone 0.05 mg/kg, olan = olanzapine 0.2 mg/kg and MK801 = MK801 0.05 mg/kg. Each column represents the latencies (sec) median±interquartile ranges of training (light columns) or test (dark columns). *=$p<0.05$ and **=$p<0.01$ comparing training and test latencies within the same treatment (Wilcoxon); §$p<0.05$ comparing to amnesic control (Sal/MK801) test (Kruskal-Wallis/Mann-Whitney).

4. CONCLUSÃO E PERPECTIVAS

Nosso trabalho mostrou que apenas sulpirida e risperidona, entre os antipsicóticos e as doses testadas, foram capazes de prevenir o déficit de memória de trabalho modelado em camundongos por administração aguda de uma antagonista do receptor glutamatérgico NMDA.

Sulpirida, apesar de ser um bloqueador seletivo do receptor D_2 de dopamina (característica comum a antipsicóticos típicos), apresenta baixa incidência de efeitos extrapiramidais (atributo que o classifica como um antipsicóticos atípico). Propriedades tão distintas podem estar relacionadas com o efeito benéfico de sulpirida sobre o déficit de memória de trabalho. Risperidona também apresentou um perfil útil ao tratamento de déficit de memória de trabalho presente em esquizofrênicos. Possivelmente, essa qualidade está relacionada ao bloqueio de receptores 5-HT_{2A}. A ativação dos receptores 5-HT_{2A} diminui a liberação de dopamina, que no córtex pré-frontal desempenha um papel importante para o bom funcionamento da memória de trabalho.

Também mostramos que haloperidol, clorpromazina, antipsicóticos típicos; clozapina e olanzapina, antipsicóticos atípicos não foram capazes de prevenir o déficit cognitivo induzido por MK801.

Clozapina, a droga de escolha para pacientes refratários, não apenas causou prejuízo na memória de trabalho na ausência de MK801, como também deixou de prevenir o déficit causado pelo antagonista de NMDA. Talvez o prejuízo cognitivo causado pela clozapina esteja relacionada à sua propriedade ansiolítica, uma vez que ansiolíticos podem afetar a velocidade de processamento de informações, habilidade essa central para a memória de trabalho. Estes resultados dado que incita a discussão sobre se essa droga usada como sendo a referência para o tratamento de pacientes refratários a outras abordagens não poderia se acompanhada do uso de agentes adjuvantes com o intuito de prevenir o déficit de memória de trabalho aparentemente induzido por clozapina.

O uso de antagonista de NMDA como modelo de déficit de memória de trabalho apresenta valor de construto, já que acredita-se que o déficit cognitivo na esquizofrenia esteja relacionado com uma diminuição da função glutamatérgica no córtex pré-frontal. Tal modelo apresenta uma boa validade de face já que o tratamento com antagonistas de NMDA induz, em roedores, comportamentos que se assemelham a sintomas positivos, negativos e cognitivos vividos pelo paciente esquizofrênico. No entanto o valor preditivo

desse modelo animal ainda não está claro, apesar de estar intimamente relacionado à hipótese glutamatérgica da esquizofrenia.

Estudos adicionais se fazem necessários para uma melhor análise, com diferentes doses das drogas já testadas, a comparação com outros antipsicóticos presentes no mercado, bem como trabalhos com tratamentos crônicos. Seria também de interesse testar hipóteses sobre a eficácia destes compostos em outros modelos animais de déficit de memória de trabalho, bem como distintas tarefas cognitivas.

Assim, estimando o impacto econômico do elevado custo do uso de antipsicóticos atípicos para o paciente e as dúvidas quanto a sua eficácia em déficits cognitivos em esquizofrênicos, estudos como esses contribuem para uma melhor escolha do medicamento a ser adotado para tratamento, de acordo com o perfil do paciente. Em países onde a verba para saúde pública é limitada, tal perfil é especialmente importante para auxiliar um melhor direcionamento dos recursos.

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microatom	μat	liter (spell out in text)	l
Units of concentration		*Units of time*	
molar (moles/liter)	M	hour	hr
normal (EQ/liter)	N	minute	min
percent	%	second	sec
Units of electricity		*Miscellaneous*	
volt	V	degrees of temperature	°C
ampere	A	gravity	g
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		Optically isometric forms	d-, l-, d
Units of radioactivity		routes of drug administration	i.v., i.p., s.c. i.m.
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