UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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EFEITOS DA N-ACETILCISTEÍNA EM MODELOS EXPERIMENTAIS DE ANSIEDADE EM ROEDORES.

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GUILHERME NOETZOLD

Local: Laboratório de Etnofarmacologia
Departamento de Farmacologia
Instituto de Ciências Básicas da Saúde

Orientadora: ELAINE ELISABETSKY
Co-orientadora: ANA PAULA HERRMANN

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ÍNDICE GERAL

RESUMO

Transtornos de ansiedade determinam condições de importante morbidade neuropsicológica, afetando parte significativa da população. Estima-se que mais de 15 milhões de pessoas sejam acometidas anualmente nos Estados Unidos por algum tipo de transtorno de humor relacionado à ansiedade. A resposta ansiosa é uma condição normal, uma vez que estímulos ameaçadores desencadeiam comportamentos de defesa; já estados de ansiedade patológica são caracterizados por uma resposta precipitada ou excessiva a estímulos aversivos ou potencialmente perigosos, de forma que passam a prejudicar o desempenho e a qualidade de vida dos pacientes. O tratamento das diversas modalidades de patologias ligadas à ansiedade é preferencialmente baseado em abordagens psicológicas e/ou farmacológicas, sendo que cerca de 40-50% dos pacientes são refratários a esses tratamentos. Fica assim evidente o interesse no desenvolvimento de novos fármacos que sejam mais eficazes, preferencialmente com mecanismo de ação inovador. Os fármacos utilizados no seu tratamento são, em geral, também utilizados como antidepressivos e hipnóticos; seus objetivos clínicos são distintos e, portanto, no tratamento de ansiedade são apresentados efeitos colaterais indesejáveis, como sonolência ou sedação, diminuição de reflexos e coordenação motora. Dessa forma, esses medicamentos estão longe de ser o tratamento ideal. Nesse contexto, o foco atual é o sistema glutamatérgico, cuja importância em transtornos psiquiátricos tem sido melhor caracterizada a partir da década de 80. Um modulador do sistema do sistema glutamatérgico que mostra interessante ação em várias desordens psiquiátricas é a N-acetilcisteína (NAC), com efeitos positivos tanto em modelos pré-clínicos como em testes clínicos para condições tais como depressão e esquizofrenia. O objetivo deste trabalho é examinar o efeito ansiolítico NAC, caracterizando-os numa bateria de testes comportamentais em roedores.

Palavras-chave: ansiedade, N-acetilcisteína, campo aberto, claro-escuro, interação social, hole board.

1 INTRODUÇÃO

1.1 Ansiedade

Transtornos de ansiedade induzem importante morbidade neuropsicológica, afetando parte significativa da população. Estima-se que mais de 15 milhões de pessoas sejam acometidas anualmente nos Estados Unidos por algum tipo de transtorno de humor relacionado à ansiedade (Lepine et al., 2002). Além disso, a ansiedade é um sintoma comum a várias condições psiquiátricas e, curiosamente, nem sempre mostra um curso de melhora, ou piora paralelo à condição primária.

A resposta ao medo é uma condição normal, uma vez que estímulos ameaçadores desencadeiam comportamentos de defesa necessários à sobrevivência das espécies. Estados de ansiedade patológica são caracterizados por respostas precipitadas ou excessivas a estímulos aversivos ou potencialmente perigosos, de tal forma que prejudiquem a qualidade de vida dos pacientes ansiosos (Rosen & Schulkin, 1998). De acordo com a quarta edição do Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-IV), existem seis principais doenças relacionadas a ansiedade: transtorno de ansiedade generalizada (TAG), transtorno de pânico, transtorno de ansiedade social (TAS), transtorno de estresse pós-traumático (TEPT), fobias específicas e transtorno obsessivo-compulsivo (TOC).

O diagnóstico de transtornos relacionados a ansiedade é feito, de acordo com International Statistical Classification of Diseases and Related Health Problems (ICD-10), a partir de sintomas presentes diariamente e por pelo menos seis meses, tais como: tensão, preocupação e medo, seguidos de manifestações vegetativas, como taquicardia, diaforese, tremores e boca seca. Além desses incluem-se dores, desconforto ou aperto no peito ou abdominal, e sintomas mentais recorrentes como tontura, alteração de percepção, medo de perda de controle ou morte. Também são comuns perda de concentração, irritabilidade excessiva, agitação, tensão muscular e parestesia, entre outros.

Uma das hipóteses de transtornos de ansiedade mais aceitas é a da ansiedade por incerteza e antecipação recentemente ampliada por Grupe & Nitschke (2013). Segundo os autores a ansiedade patogênica envolve cinco processos principais envolvidos na adaptação inadequada a condições ansiogênicas aversivas: estimativa aumentada do custo da ameaça, hipervigilância, deficiência na percepção de segurança, fuga cognitiva

e comportamental ao problema a ser enfrentado e reação exagerada a situações de incerteza. Outro ponto considerado relevante, são os custos e a probabilidade de desfecho de uma situação ansiogênica: evidências mostram que situações que levariam a um maior dano ou prejuízo são mais causadoras de ansiedade patológica do que as situações com uma maior probabilidade de real acontecimento (Mitte, 2007).

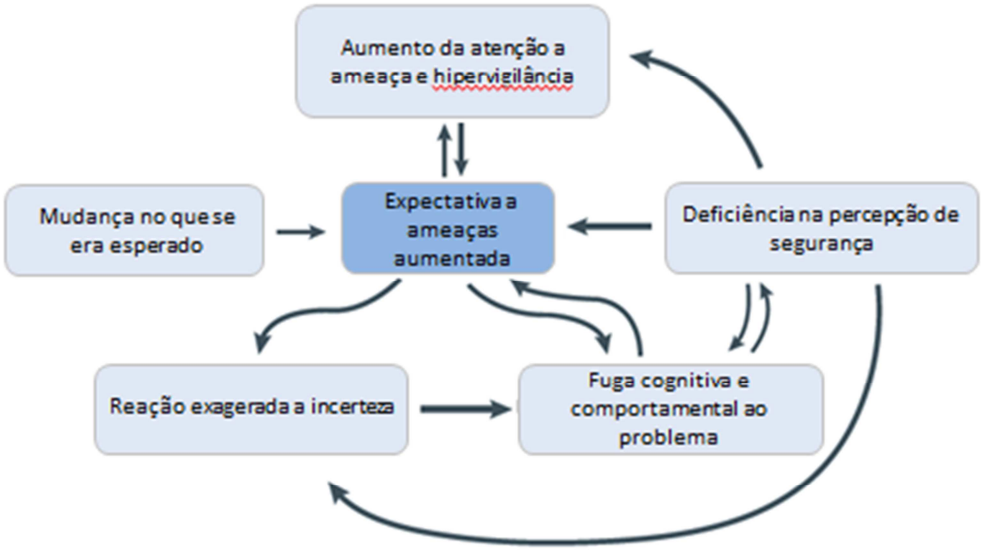

Figura 1. Processos antecipatórios alterados em resposta a ameaça e incerteza na ansiedade (Adaptado de Grupe & Nitschke 2013).

1.1.1 Circuitos neurais da ansiedade

As áreas cerebrais envolvidas com a ansiedade incluem a amígdala, núcleo leito da estria terminal, córtex pré-frontal ventromedial, córtex orbitofrontal, córtex cingulado médio anterior e a ínsula anterior. A amígdala é uma região de extrema importância no controle da ansiedade patológica por estar muito ativada de forma tônica e indiscriminada (Fox et al., 2008)(Furmark et al., 2002). O núcleo central da amígdala possui projeções colinérgicas para estruturas do prosencéfalo basal, as quais podem modular seletivamente processos sensoriais e de aprendizado através de projeções colinérgicas ascendentes para regiões corticais ativados após situações que causem surpresa (Pessoa, 2010).

Na estimativa aumentada do custo da ameaça estão envolvidas as seguintes áreas corticais: córtex pré-frontal dorsomedial, giro do cíngulo rostral e córtex orbitofrontal;

além dessas, também há participação do estriado ventral e da ínsula anterior. No aumento da atenção a ameaça e hipervigilância há aumento da atividade da amígdala, estimulando o prosencéfalo basal, levando a um aumento da sensibilidade visual e outros estímulos sensoriais, resultando em aumento da atenção ao perigo. A deficiência na percepção de segurança ocorre pela inibição da amígdala pelo córtex pré-frontal ventromedial (Phelps et al., 2004)(Mobbs et al., 2010). A fuga cognitiva e comportamental ao problema reflete-se na interação da amígdala com áreas importantes quanto a tomada de decisão e concomitante ação, incluindo córtex orbitofrontal, córtex pré-frontal dorsolateral, estriado, córtex cingulado médio anterior e ínsula anterior (Aupperle & Paulus, 2010)(Delgado et al., 2009). O aumento na reação exagerada a incerteza se deve à hiperatividade do núcleo leito da estria terminal e da amígdala em resposta a ameaças imprevisíveis. Esse aumento de atividade leva à resposta defensiva, também mediada pelo hipotálamo, substância cinzenta periaquedutal e outras regiões do mesencéfalo e tronco encefálico. A ínsula anterior também está associada ao aumento da intolerância ao incerto e contribui para a hiperatividade do núcleo do leito da estria terminal e da amígdala.

Disfunção no córtex cingulado médio anterior ou danos estruturais a essa área e suas conexões levam a prejuízo na identificação e execução de respostas adaptativas ao desconhecido e contribui para os desfechos patológicos controlados pelas vias citadas acima. (Schiller et al., 2008)(Grupe & Nitschke, 2013).

1.1.2 Tratamento de transtorno de ansiedade

No tratamento farmacológico de transtornos de ansiedade, as drogas de primeira escolha são os inibidores seletivos da recaptação de serotonina (ISRSs) (escitalopram, paroxetina e sertralina, por exemplo), os inibidores da recaptação de serotonina e noradrenalina (IRSNs) (duloxetina e venlafaxina, por exemplo) ou benzodiazepínicos (como a pregabalina). Medicamentos de segunda escolha incluem a buspirona (agonista parcial serotonérgico), imipramina e opipramol (antidepressivos tricíclicos).

Em caso de ausência de resposta em quatro a seis semanas de tratamento, é recomendável que se aumente a dose do fármaco, troque-se a medicação para outro de primeira escolha, uso de outro fármaco de segunda escolha, uso de medicamentos *off-label* ou combinação de fármacos. É muito comum o acompanhamento psicológico e

utilização de terapia cognitiva comportamental ou psicanalítica concomitante ao tratamento farmacológico. (Bandelow et al., 2013).

Os fármacos utilizados no tratamento farmacológico são também utilizados como antidepressivos e hipnóticos; seus objetivos clínicos são distintos e, portanto, no tratamento de ansiedade são apresentados efeitos colaterais indesejáveis, como sonolência ou sedação, diminuição de reflexos e coordenação motora. Dessa forma, esses medicamentos estão longe de ser o tratamento ideal.

1.2 O sistema glutamatérgico em transtornos psiquiátricos

A partir da década de 80 o sistema glutamatérgico passou a ser importante alvo de estudos e vem ganhando crescente interesse na pesquisa relacionada a distúrbios psiquiátricos e comportamentais. Evidência do papel do glutamato foi marcadamente destacada pela ação antidepressiva imediata do antagonista de receptores NMDA cetamina quando administrada em doses subanestésicas (Zarate et al., 2006). Sendo o glutamato uma molécula presente em todo o sistema nervoso central, cuja transmissão está sujeita a uma fina regulação e que, ao mesmo tempo em que é modulado, também modula vários outros sistemas de neurotransmissores, vem se tornando um alvo farmacológico importante na área de psiquiatria (Hashimoto, 2011).

Recentemente, foi verificada a ação de fármacos que modulam o sistema glutamatérgico em transtornos relacionados a ansiedade. O uso de acamprosato (um antagonista de receptores NMDA) foi capaz de reverter a ansiedade causada pela retirada da administração de anfetamina em camundongos (Koltunowska et al., 2013). Em outro estudo, LY354740 (um agonista de receptores $mGlu_{2/3}$) teve efeito ansiolítico no teste de labirinto em cruz elevado. Essas evidências chamam atenção para o glutamato como alvo potencial para fármacos ansiolíticos.

1.3 N-acetilcisteína

A N-acetilcisteína (NAC) é um precursor de cisteína que age no trocador cistina-glutamato presente em astrócitos, aumentando a quantidade de glutamato extrassináptico (Baker et al., 2008). Essa elevação promove uma maior ativação de receptores metabotrópicos $mGlu_{2/3}$ localizados no espaço extra sináptico, resultando em diminuição da liberação sináptica de glutamato e consequente redução na ativação de

receptores glutamatérgicos pós sinápticos, especialmente do tipo NMDA (Dean et al., 2010). A utilidade de NAC em transtornos psiquiátricos, tanto em modelos pré-clínicos (Linck et al., 2012) como em estudos clínicos (Berk et al., 2008; Berk et al., 2013) tem sido documentadas. O uso de NAC também já foi preliminarmente documentado em transtornos de ansiedade, como por exemplo, na tricotilomania (Grant et al., 2009). Em modelos animais, recentemente foi feito um pequeno estudo utilizando NAC no teste de marble-burying (Egashira, et al. 2012). Neste estudo verificou-se ação ansiolítica de NAC.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar o possível efeito ansiolítico da N-acetilcisteína, numa bateria de modelos animais reconhecidos como relevantes para identificar e caracterizar atividade ansiolítica.

2.2 Objetivos específicos

(i) Avaliar os efeitos de N-acetilcisteína no teste de campo aberto em camundongos, com tratamento agudo e subagudo.

(ii) Avaliar os efeitos de N-acetilcisteína no teste de hole board em camundongos, com tratamento agudo e subagudo.

(iii) Avaliar os efeitos de N-acetilcisteína no teste de claro-escuro em camundongos, com tratamento agudo e subagudo

(iv) Avaliar os efeitos de N-acetilcisteína (três doses) no teste de interação social em camundongos, com tratamento agudo e subagudo

3 ARTIGO CIENTÍFICO

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EFFECTS OF N-ACETYLCYSTEINE IN EXPERIMENTAL MOUSE MODELS OF ANXIETY

Guilherme Noetzold[1,*], Ana Paula Herrmann[1,2] and Elaine Elisabetsky[1,2].

[1] *Laboratório de Etnofarmacologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Avenida Sarmento Leite 500/202, Porto Alegre, RS, 90050-170, Brazil*.

[2] *Programa de Pós Graduação em Ciências Biológicas - Bioquímica, Universidade Federal do Rio Grande do Sul, Avenida Sarmento Leite 500/202, Porto Alegre, RS, 90050-170, Brazil*.

[*]Corresponding author:
Guilherme Noetzold
Universidade Federal do Rio Grande do Sul, ICBS
Rua Sarmento Leite, 500/202, 90050-170, Porto Alegre, RS, Brazil
Phone/Fax: 55 51 33083121
guilhermenoetzold@gmail.com

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Abstract

Anxiety disorders cause neuropsychological conditions with significant morbidity, and affects an important part of the population. It is estimated that over 15 million people are affected annually in the United States by some sort of mood disorder related to anxiety. Though anxious response is a normal condition in coping with threatening stimuli that should trigger defensive behaviors, pathological anxiety states are characterized by a hasty or excessive response to aversive or potentially dangerous stimuli. The maintenance of such overactive state undermines professional performance and the overall quality of life of pathologically anxious patients. The treatment of the different types of pathologies linked to anxiety is preferably based on psychological and/or pharmacological approaches, but it is estimated that 40-50% of patients are refractory to these treatments. Therefore there is great interest in the development of new and more effective drugs, preferably with innovative mechanism of action. The purpose of this study is to evaluate N-acetylcysteine (NAC) in a battery of mice tests useful to identify anxiolytic properties. The effects of NAC were evaluated (single or 4 days ip treatment) in the open field, hole board, light-dark and social interaction mice models. Models were validated with Diazepam. The data was analyzed by ANOVA/SNK. N-acetylcysteine (NAC) is modulator of the glutamatergic system shown to possess interesting effects in various psychiatric disorders, in both preclinical models and clinical trials, including depression and schizophrenia. This study shows that NAC was able to reduce anxiety-like behaviors in mice models. The effective acute dose is higher than those active with repeated administration. The study suggests that NAC has anxiolytic properties potentially of interest for the clinical effects already identified for this drug, and deserves a more comprehensive evaluation for treating anxiety disorders. .

Keywords: anxiety, N-acetylcysteine, open field, light-dark, social interaction, hole board.

1. Introduction

Anxiety disorders induce important neuropsychological morbidity, affecting a significant portion of the population. It is estimated that over 15 million people are affected annually in the United States by some sort of mood disorder related to anxiety (Lepine et al., 2002). Furthermore, anxiety is a common symptom of various psychiatric conditions and, interestingly, does not always show a course of improvement or worsening parallel to the primary condition. The anxious answer to fear is a normal and necessary condition, since threatening stimuli trigger defensive behaviors necessary for survival of the species. However, pathological anxiety states are characterized by a hurried and/or excessive response to aversive or potentially dangerous stimuli, so that anxiety rather than being protective undermines professional performance and the quality of life of these patients. Among the variable spectrum of disorders, obsessive compulsive disorder (OCD) and trichotillomania are to be highlighted for its prevalence. Anxiety disorders therapy includes psychological and/or pharmacological, means, but it is estimated that approximately 40-50% of patients are refractory to treatments (Wu et al. 2012).

The primary drug class used in the treatment of anxiety disorders is the selective serotonin reuptake inhibitors and benzodiazepines, also widely used as antidepressives and hypnotics. Though clinical goals are distinct, these drugs possess various side effects such as drowsiness or sedation, decreased motor reflexes and coordination. It is thus of great interest to explore new drugs, especially those with innovative mechanism of action that might be better effective and/or present fewer adverse effects than those currently available.

Since the 80s the glutamatergic system has become an important subject of studies and gained greater interest in research related to psychiatric and behavioral disorders. Long known to be off normal in epileptic and neurodegenerative disorders, it is now considered central to most neural and mental disorder. Evidence of the importance of glutamate in mediating behavior is highlighted by immediate antidepressant action of the NMDA receptor antagonist ketamine administered in subanesthetic doses (Zarate et al., 2006). Glutamate is an ubiquous molecule present in the central nervous system, where it represents the primary excitatory transmission through ionotropic and metabotropic receptors. Glutamate transmission is subjected to fine adjustment, by the inhibitory gama-amino butyric acid (GABA) as well as other neurotransmitters. At the

same time that glutamate is modulated, it is now accepted that glutamate also regulates several neurotransmitter systems, and has increasingly become an important target for drug development in the field of psychiatry (Hashimoto, 2011).

N-acetylcysteine (NAC) is a cysteine precursor that acts on the cystine-glutamate exchanger present in astrocytes, increasing the amount of extrassinaptic glutamate (Baker et al., 2008). This elevation of glutamate promotes a greater activation of the metabotropic receptors $mGlu_{2/3}$ located in the extrassynaptic space, resulting in decreased synaptic release of glutamate and consequent reduction in the activation of post synaptic glutamatergic receptors, especially the NMDA type (Dean et al., 2011). The usefulness of NAC in psychiatric disorders, both in preclinical models (Linck et al., 2012) as in clinical trials (Berk et al., 2008; Berk et al., 2013) has been increasingly documented. Specifically for anxiety disorders, the usefulness of NAC has been documented in a small clinical trial for trichotillomania (Grant et al., 2009). Reinforcing the potential use of NAC in anxiety-related conditions, a short communication reported the effect of N-acetylcysteine in marble-burying behavior, considered a model of OCD in rodents, (Egashira et al., 2012).

However, the anxiolytic profile of NAC is still not well characterized in experimental models or human patients. In translational research it is accepted that animal models can be instrumental to identify specific uses, as well as potential adverse effects. In this context, the present study is a starting point on the warranted evaluating of the potential anxiolytic effect of N-acetylcysteine.

2. Methods

Animals: Adult albino CF1 mice from Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS) were used. Mice were maintained at the Department of Pharmacology animal facility, in 30 x 19 x 13 cm cages, 3-5 animals/cage, under controlled environment (22 ± 2 ° C, 12-h light/dark cycle, food and water ad libitum). The animals were kept in our animal facility sector for at least 10 days before experiments. Cleaning and maintenance are the responsibility of the university staff and meet the standards of this university.The project was approved by the Ethics Committee (#22308).

Treatments: N-acetylcysteine and other reagents were purchased from Sigma. Diazepam is used from injectable ampoules (Diazepam, Teuto, Brazil). The animals were divided into the following treatment groups (n = 10): saline, diazepam 2 mg/kg,

NAC (60, 100 or 150 mg/kg) for acute tests; saline, diazepam 2 mg/kg, NAC (60 or 100 mg/kg) for subchronic (four days) tests. N-acetylcysteine is dissolved in saline and diazepam in distilled water. All solutions were administered intraperitonially in a volume of 1 ml/100 g of body weight. NAC was administered 60 min before experiments; diazepam was administered 30 min before experiments. When given for four days, experiments were done right after the last drug administration. Mice were habituated to the experimental room for at least 30 min before the experiments.

Behavioral Tests

Open Field: To discard non-specific reactions of pharmacological treatments or changes in locomotion, mice were tested in a gray wooden apparatus (40 x 40 x 40 cm). In addition to the locomotor activity (locomotion), the time spent in the central squares (as opposed to peripherals) will be assessed as a measure of anxiety (Saitoh et al. 2004). The experiment was video taped by a digital camera installed above the arena for 15 minutes, considering the first five minutes as exploratory behavior and the last ten minutes as locomotion,. Videos were analyzed using Any-maze software (Stoelting Co., Wood Dale, IL, USA).

Hole Board: The hole board apparatus consists of a gray plate (40 x 40 x 2.2 cm thick) with 16 holes equally spaced (3 cm diameter), each containing photocells that count the number of head-dips. The plate is positioned 15 cm above the table and it has been divided into 9 squares of 10 x 10 cm. Animals were observed for 5 minutes and the following parameters assessed: number of head-dips, latency to the first head-dip, number of rearings and spontaneous ambulation (the number squares crossed with all four paws) (Costa-Campos et al. 2004).

Light-Dark: The light-dark apparatus consists of a rectangular wooden box (46 x 27 x 30 cm) divided into a smaller area (18 x 27 cm) and a larger area (27 x 27 cm) with a door-like opening (7.5 x 7.5 cm) between them. The smaller compartment is painted black and not lighted, while the bigger is painted in white and lightened by a 60 W lamp. Mice were individually placed in the dark compartment facing the door-like opening. The experiment was video taped for five minutes by a digital camera installed above the arenas for 5 minutes and the time spent in the light compartment was later noted (Piato et al., 2010).

Social Interaction: Mice were tested in a wooden gray apparatus (40 x 40 x 40 cm), containing two small metal barred cages placed in opposite corners of the apparatus. Pairs of mice unknown to each other were placed in the apparatus, one inside one of the

barred cages. The animal under observation was placed in the apparatus facing the caged animal and the experiment was video taped for ten minutes by a digital camera installed above the arenas. Social interaction defined as sniffing and grooming the partner and was analyzed using the software The Observer ® XT 5.0 (Noldus Information Technology, Wageningen, The Netherlands). (Linck et al. 2010).

Statistical analysis: Data were analyzed by one-way analysis of variance (ANOVA) followed by the Newman–Keuls post hoc. The program used in the analysis was Graph Pad Prism 5.0 and the statistical significance was set at 0.05. Values were expressed as mean and standard error of mean

3. Results

Figure 1 and 2 show results from the open field test. With acute administration, there were no significant differences in the time ($F_{4,41}$ = 2.598, p>0.05) (Fig. 1A) or distance traveled ($F_{4,41}$ = 2.311, p>0.05) (Fig. 1B) in the center squares among the various treatment groups. The experiment shows that none of the treatments altered locomotion ($F_{4,41}$ = 3.124, p<0.05) (Fig. 1C). With subchronic administration, NAC 60 mg/kg significantly increased the time ($F_{3,36}$ = 4.543, p<0.01) (Fig. 2A) and distance traveled ($F_{3,36}$ = 3.799 p>0.05) (Fig. 2B) in the central area of the arena. There was no statistical difference in the locomotor activity of sub chronically treated animals ($F_{3,36}$ = 1.752, P>0.05) (Fig. 2C). Of note, diazepam did not increase the time spent in the central zone of the arena after acute or sub chronic administration.

Figures 3 and 4 show data from the hole board test. With acute administration, only NAC 150mg/kg and diazepam were able to increase the number of head-dips ($F_{4,50}$ = 3.722 p<0.05) (Fig. 3A); no differences were found in latency to the first head-dip ($_{F4,49}$ = 2.141, p>0.05) (Fig. 3B). With subchronic administration significant increase in the number of head-dips were observed with 60 and 100 mg/kg NAC ($F_{3,36}$ = 6.991, P<0.01) and diazepam (Fig. 4A), accompanied by significantly reduced latency to first head-dip ($F_{3,36}$ = 3.250, p>0.05) (Fig. 4B). No differences were seen in the number of crossings with acute (3C) or subchronic (4C) treatments.

Figure 5 shows data from the light-dark test. With acute administration (5A), only NAC at 150mg/kg and diazepam significantly increased the time spent in the illuminated part of the box ($F_{4,45}$ = 6.214, p<0.01). With subchronic administration (5B)

NAC 100 mg/kg and diazepam increased the time spent on the lit box ($F_{3,36}$ = 5.366, $p<0.01$).

Figure 6 shows the effects of NAC in the social interaction test. With acute administration (6A), only NAC 150 mg/kg and diazepam were able to increase interaction time with an unfamiliar mice ($F_{4,42}$ = 7.383, $p<0.01$); with sub chronic treatment (6B) only diazepam ($F_{3,36}$ = 4.165, $p<0.05$) was active.

4. Discussion

The main finding of this study is that NAC shows anxiolytic properties in various mice models. After a single administration the data suggest that NAC shows anxiolytic properties only in higher doses. When the administration is repeated, in this case for four consecutive days, doses ineffective acutely shows anxiolytic effects. This pattern of dose-effect in acute versus repeated administration was observed for the open-field, hole-board and light-dark tests. Further experiments and neurochemical data is needed to clarify the factors that underline the changes observed with repeated administration, that can include a mobilization of the cystine-glutamate antiporters resulting in increased expression in astrocytes membranes, in synaptic and/or extrassynaptic sites.

. The classic validity values applicable to behavioral pre clinical models are predictive, face and construct validities (Willner, 1997). One of the limitations in this study is that the tests used have good predictive value, but do not contemplate ethiological factors or the course of this disorder. A potential strategy to complement the data is the use of stressor (such as restraint or isolation), to promote anxious states against which the effects of NAC could be assessed.

The hypothesis that modulating glutamate can result in clinically relevant anxiolytic effect is reinforced by studies using glutamate ligands: Koltunowska and collaborators (2013) tested acamprosate (an antagonist of NMDA receptors) in amphetamine-evoked withdrawal anxiety with positive results. Moreover, Linden and collaborators (2004) tested LY354740 (an agonist of $mGlu_{2/3}$ receptors) in the elevated plus maze test, again with positive results.

Of note, NAC has established uses in medicine (as mucolytic and antidote for acetaminophen intoxications) and possess low toxicity and is nearly devoid of side effects. Additionally, inexpensive formulations are available in the market.

5. Conclusion

This study shows positive effects of N-acetylcysteine in mice tested in established models that predict anxiolytic properties. As a starting point it reveals different dose-effects relationships when acutely or repeatedly administered, other studies are obviously necessary to better characterize this effect and ultimately a confirmation is needed by clinical trials. Experiments with other doses and time of administration need to be completed, preferably testing the effect of NAC administered before and after the anxiety-induced scenarios.

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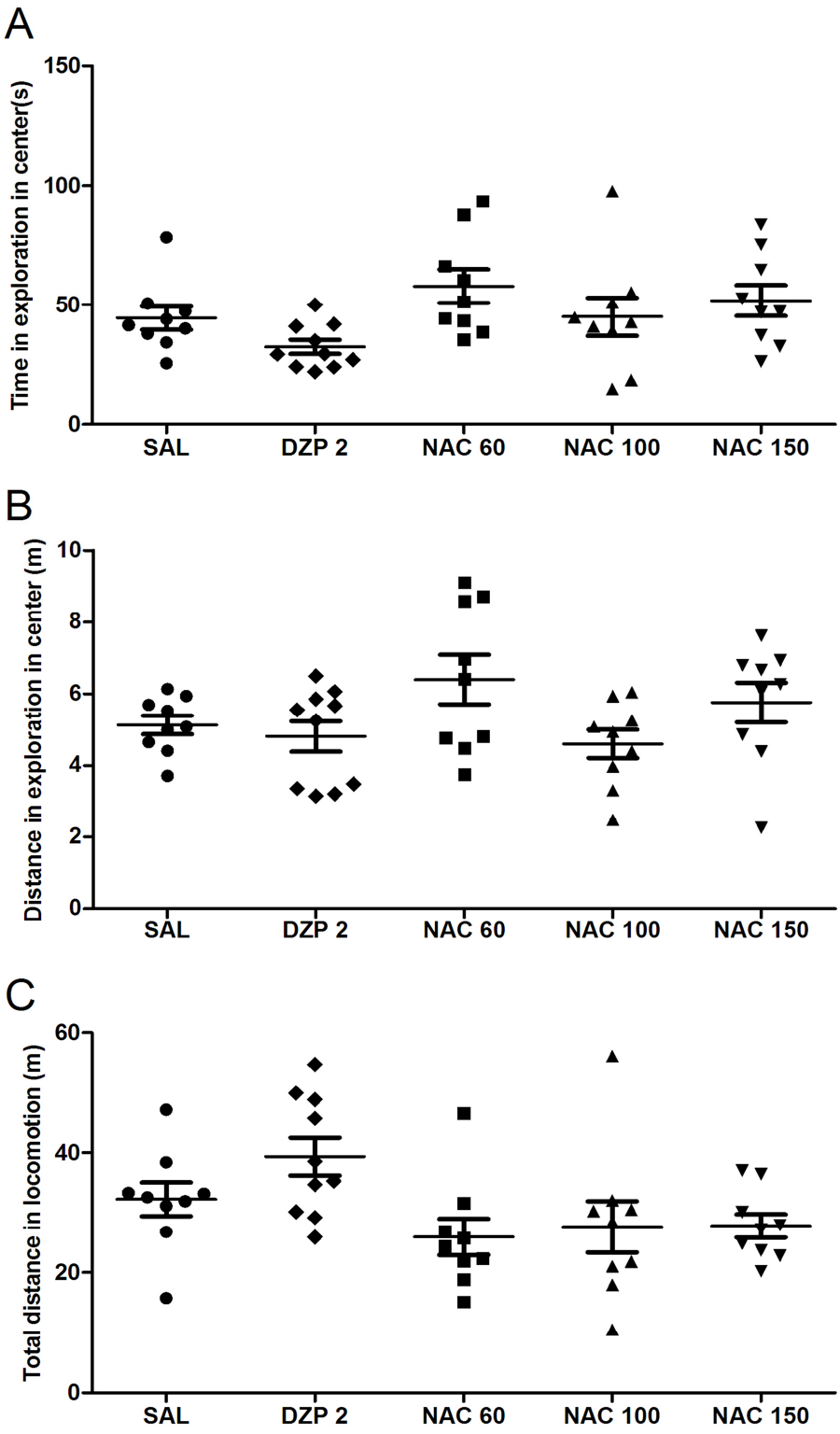

Figure 1. Effects of acute N-acetylcysteine in the open field test. (A) Exploration time in the central zone, (B) distance travelled in the central zone, and (C) Total distance (locomotion). (n = 9-10). NAC = N-acetylcysteine (60, 100 and 150 mg/kg), and DPZ 2 = diazepam 2 mg/kg. ANOVA/SNK.

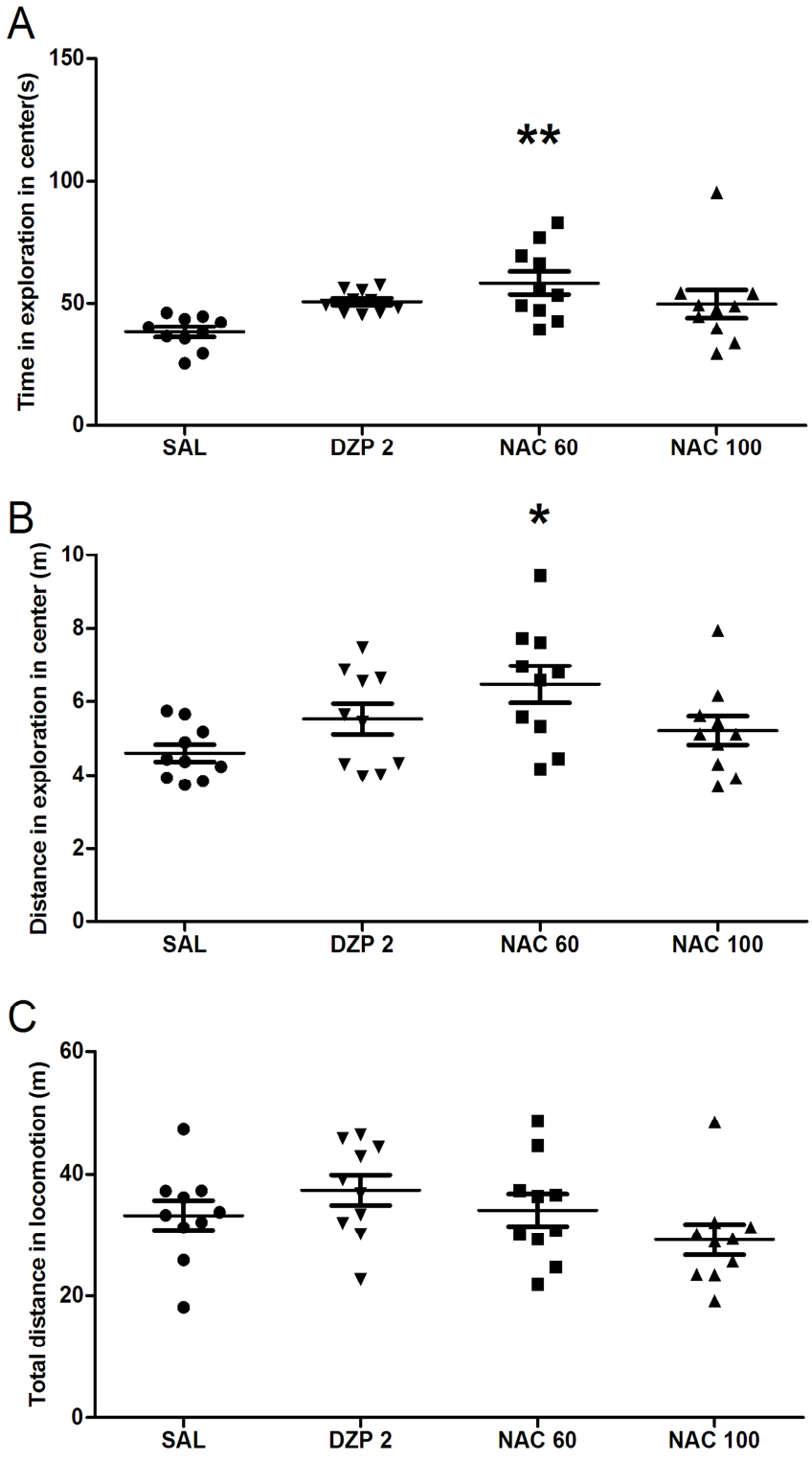

Figure 2. Effects of subchronic N-acetylcysteine in the open field test. (A) Exploration time in the central zone, (B) distance travelled in the central zone, and (C) Total distance (locomotion). (n = 9-10). NAC = N-acetylcysteine (60, 100 and 150 mg/kg), and DPZ 2 = diazepam 2 mg/kg. *=p<0.05 and, **=p<0.01 vs SAL, ANOVA/SNK.

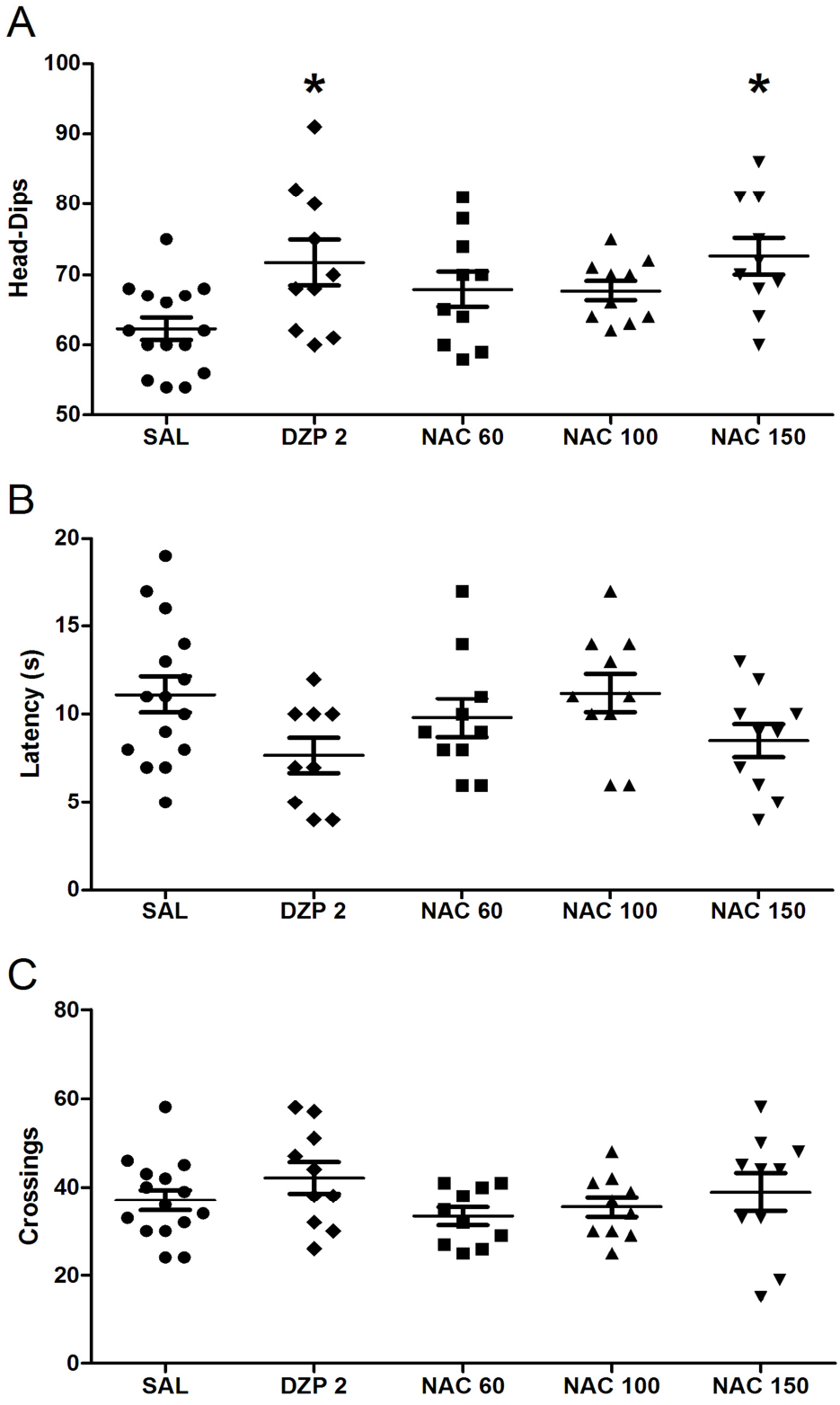

Figure 3. Effects of acute N-acetylcysteine in the hole board test. (A) Number of head-dips. (B) Latency to first head-dip. (C) Number of crossings. (n = 9-16). NAC = N-acetylcysteine (60, 100 and 150 mg/kg), and DPZ 2 = diazepam 2 mg/kg. *=$p<0.05$ vs SAL, ANOVA/SNK.

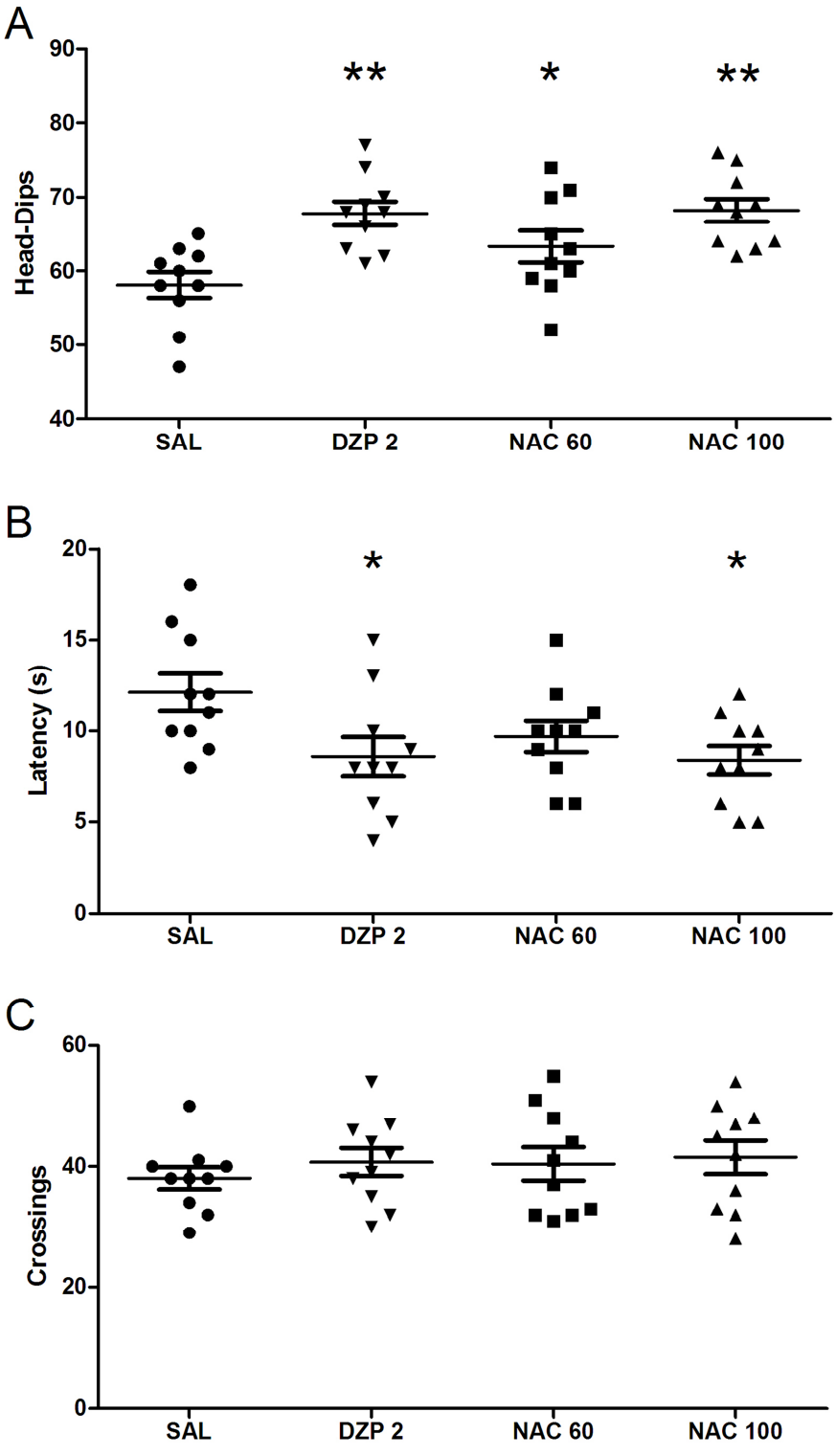

Figure 4. Effects of sub chronic N-acetylcysteine in the hole board test. (A) Number of head-dips. (B) Latency to first head-dip. (C) Number of crossings. (n = 10) NAC = N-acetylcysteine (60, 100 and 150 mg/kg), and DPZ 2 = diazepam 2 mg/kg. *=$p<0.05$ and ** =$p<0.01$ vs SAL, ANOVA/SNK.

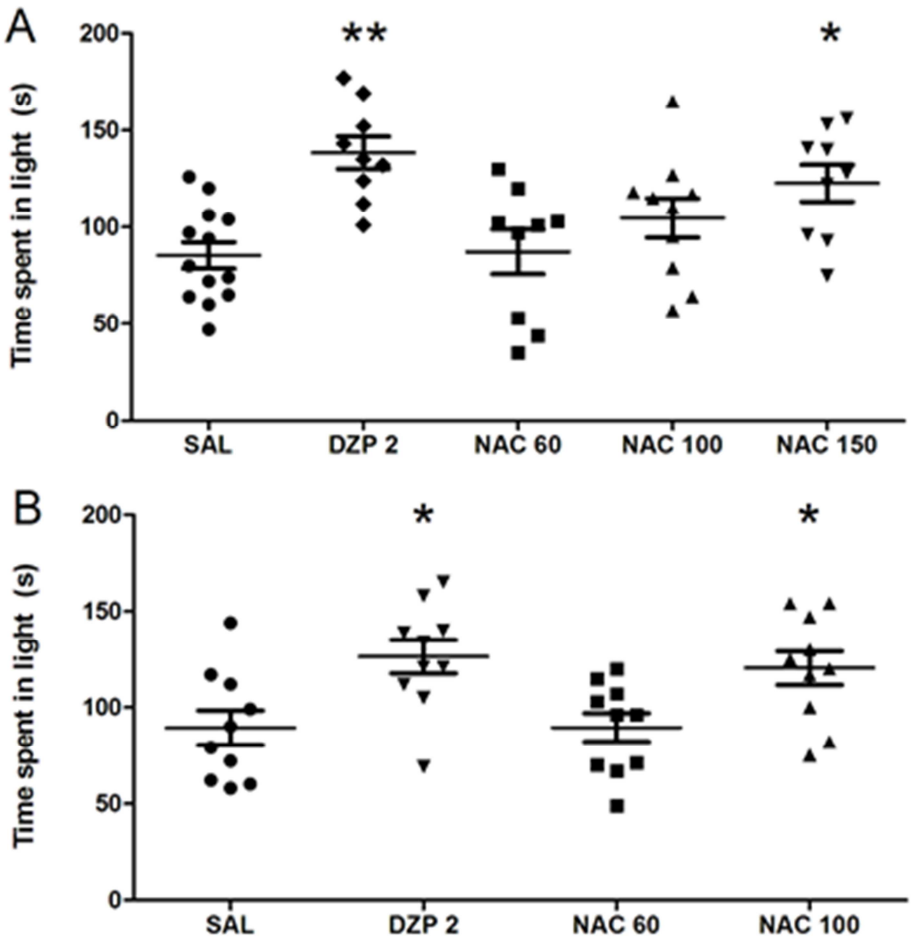

Figure 5. Effect of acute (A) and subchronic (B) N-acetylcysteine in the light-dark test. (n = 9-15). NAC = N-acetylcysteine (60, 100 and 150 mg/kg), and DPZ 2 = diazepam 2 mg/kg.*=$p<0.05$ and ** =$p<0.01$ vs SAL, ANOVA/SNK.

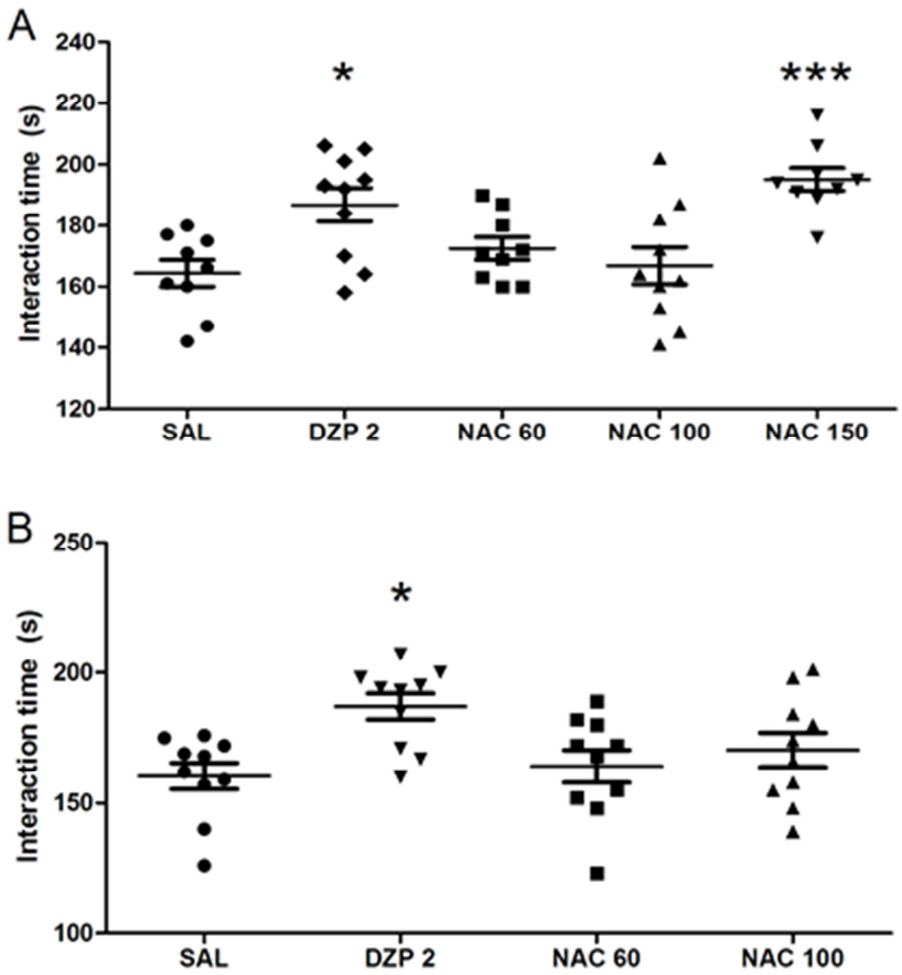

Figure 6. Effect of acute (A) and subchronic (B) N-acetylcysteine in the social interaction test. (n = 9-1o). NAC = N-acetylcysteine (60, 100 and 150 mg/kg), and DPZ 2 = diazepam 2 mg/kg. *=p<0.05 and ** =p<0.001 vs SAL, ANOVA/SNK**.**

4 CONCLUSÃO E PERSPECTIVAS

Esse estudo mostrou efeitos ansiolítcos de NAC em camundongos, verificado em vários testes experimentias com valor preditivo para atividade ansiolítica. Ainda que esses dados sejam iniciais e devem ser complementados com mais estudos para melhor caracterizar esse efeito e confirmá-lo, o estudo cumpriu o objetivo de dar um primeiro passo na avaliação da potencial atividade ansiolitica de NAC.

Pretende-se realizar testes utilizando outras doses e tempo de administração, bem como outros modelos que permitam a indução de ansiedade exacerbada, como estresse por contenção ou isolamento, que poderão fornecer dados importantes sobre o efeito de NAC na prevenção ou reversão de estados ansiosos provocados por circunstâncias específicas.

Além disso, pretende-se realizar testes bioquímicos, como dosagens de corticosterona, neurotransmissores ou marcação de receptores e transportadores celulares a fim de esclarecer as bases neuroquímicas da atividade ansiolítica identificada. Dessa forma, teremos uma base de dados mais robusta para subsidiar provas clínicas que podem verificar a validade de um potencial novo tratamento transtornos relacionados a ansiedade.

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