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Olfactory deficit as a result of clozapine withdrawal syndrome in an animal model of schizophrenia: preliminary results

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ABSTRACT

Clozapine is an antipsychotic that produces serious withdrawal effects in schizophrenic patients. Olfactory deficits are well known as part of negative symptoms, but it is not known whether antipsychotic use and/or withdrawal are implicated. Then, we tested clozapine withdrawal in association with two widely used schizophrenia models: Neonatal immune challenge by Polycitidilic-polyinosinic acid (polyI:C) and ketamine. PolyI:C (or saline) was injected subcutaneously in neonatal period, dose of 5 mg/kg from 2 to 6 Post Natal Days, and ketamine or saline at the dose 25mg/kg intraperitoneally (i.p.), daily for 7 days from 53 to 60 post natal day. Clozapine 10mg/kg (or saline) was administered i.p. from 46 to 60 post natal day. Olfactory discrimination test (sensorial and cognitive deficit) was performed at 61 post natal day, 24h after the last injections. The association of PolyI:C, ketamine and clozapine disrupted Olfactory Discrimination, equating time in familiar and non-familiar compartments. PolyI:C plus ketamine increased crossings between compartments. It was produced, for the first time, an olfactory deficit induced by clozapine withdrawal in Wistar rats subjected to schizophrenia animal models.

Key words: Olfactory deficit, clozapine, schizophrenia, animal model.

INTRODUCTION

Clozapine is an antipsychotic that produces serious withdrawal effects in schizophrenic patients (Wadekar and Syed 2010, Yeh et al. 2004). Olfactory deficits are well known in schizophrenic patients, but it is unclear whether such deficits are due to the condition itself, or a side effect of long term antipsychotic use (Ansoleaga et al. 2015, Kiparizoska and Ikuta 2017). Regarding rodents, seeking for the nest's odour is also a social skill, pivotal for survival (Scheider and Przewłocki 2005, Miller and Spear 2009). It is well known that rodents prefer staying in "familiar" ambient, with odours of urine, faeces and feromones from other rodents of its social groups, and the odours of its own body (de Souza et al. 2012). The present study aims, therefore, to verify the role of olfactory deficit as a symptom of clozapine withdrawal in SZ, using two well established SZ models: (Hida et al. 2014): neonatal immune challenge with polyI:C

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(Pearce 2001) and N-methyl-D-aspartate receptor hypofunction by subanesthetic doses of ketamine (Zugno et al. 2016).

MATERIALS AND METHODS

ANIMALS

Male Wistar rats were obtained from colonies kept in the vivarium of Universidade do Extremo Sul Catarinense (UNESC). The present work was submitted to the Ethics Committee on Animal Experimentation of Universidade do Extremo Sul Catarinense (UNESC-CEUA), and approved by the protocol number 061 / 2013-2.

DRUGS

Polycitidilic-poly-inosinic acid (PolyIC, Tocris Biosciences) 5 mg/kg subcutaneously (or saline in the volume of 1 ml / kg) from 2 to 6 PND (Hida et al. 2014).

Ketamine 25 mg/kg, 1ml/kg (CU ChemieUetikon, Germany), by intraperitoneal (i.p.) route, from 53 PND to 60 PND (Zugno et al. 2016).

Clozapine 10 mg/kg (Novartis, Switzerland) was administered i.p. between 46 and 60 PND, dissolved in acetic acid and diluted in saline in volume of 1 ml/kg (Chatterjee et al. 2011).

EXPERIMENTAL DESIGN

The experimental groups were:

1) Saline+saline+saline;
2) Saline+saline+ketamine;
3) Saline+saline+clozapine;
4) Saline+ketamine+clozapine;
5) PolyI:C+saline+saline;
6) PolyI:C+ketamine+saline;
7) PolyI:C+clozapine+saline;
8) PolyI:C+ketamine+clozapine.

The number of animals varied from 10 to 13 animals per group. This number varied because the administration of polyI:C (or saline) s.c. took place in the neonatal period, and it was not possible to separate the pups from their mothers, therefore, the number of subjects depended on the number of male rats born from each mother. The pups of each mother received the same treatment (polyI:C or saline), and only after 23 post natal days male rats were separated in the 8 experimental groups.

PolyI:C (or saline) was injected subcutaneously in neonatal period, dose of 5 mg/kg from 2 to 6 Post Natal Days, and ketamine or saline at the dose 25 mg/kg intraperitoneally (i.p.), daily for 7 days from 53 to 60 post natal day. Clozapine 10 mg/kg (or saline) was administered i.p. from 46 to 60 post natal day.

Olfactory discrimination test (sensorial and cognitive deficit) was performed at 61 post natal day, 24h after the last injections.

OLFACTORY DISCRIMINATION TASK

The present test was performed according to Castro et al. (2012). The animals, which were housed since the weaning 5 animals in each box, were transferred to an isolated box, in which they were kept for 3 days (from 1 day prior the last injections until the day of the task, 24h after the last injections). This period of isolation was necessary to the rat, so it soaks the wood shavings of the box with its odours of faeces, urine, and pheromones. The task was performed in a wooden box divided in two compartments by a wall, having an entrance between the compartments. On the day of the task, the wood shavings of the isolated rat were poured in one of the compartments (familiar compartment), and the other compartment was filled with fresh wood shavings (non-familiar compartment). The animal was put in the familiar compartment, and its activity was monitored for 5 minutes. Time in seconds of permanence in the familiar and non-familiar compartment was measured, as well as the number of crossings between the compartments. The animal that had the time in familiar compartment statistically longer

than in the time in the non-familiar compartment was considered to have normal OD. The animal that had the time in both compartments statistically equal was considered to have disrupted Olfactory Discrimination.

NUMBER OF CROSSINGS BETWEEN COMPARTMENTS

This measure had the aim to detect the degree of difficulty necessary for accomplishing the Olfactory Discrimination test (Castro et al. 2012).

STATISTICS

For time in the compartments in the Olfactory Discrimination test, we performed, after normality tests, Ratio paired t-tests within each treatment group (time in familiar compartment versus time in non-familiar compartment), and for the number of crossings, Two-Way ANOVA followed by Tukey post hoc test was performed. We utilized the software Graph Pad Prism 7.0 (GraphPad Software, La Jolla, Calif., USA).

RESULTS

For Olfactory Discrimination (Figure 1a), all the groups except for PolyI:C+Ketamine+Clozapine group fulfilled the task, spending more time in the familiar compartment than in the non-familiar compartment. Ratio paired t-test revealed p=0.0477 for saline+saline+saline; p=0.0024 for Saline+saline+ketamine; p=0.0043 for Saline+saline+clozapine; p=0.001 for Saline+ketamine+clozapine; p=0.0074 for PolyI:C+saline+saline; p=0.0199 for PolyI:C+saline+ketamine; p=0.0199 for PolyI:C+saline+clozapine and p=0.6014 for PolyI:C+ketamine+clozapine, meaning that rats of PolyI:C+ketamine+clozapine group had disrupted olfactory discrimination.

For Number of Crossings in the Discriminatory Olfaction test (Figure 1b), Two-way ANOVA revealed that PolyI:C had a main effect [$F(1,97) = 13.87$; $p=0.0003$]; but not ketamine and/or clozapine [$F(3,97) = 1.135$; $p=0.3387$] with no interaction either [$F(3,97) = 1.611$; $p=0.1918$]. Post hoc test revealed that the PolyI:C+ketamine+saline group had increased number of crossings during the task.

DISCUSSION

Our results suggest that clozapine withdrawal produced a sensorial deficit, suggestive of abstinence of this drug, only in the group PolyI:C+ketamine+clozapine, which was subjected both to a perinatal immune challenge and ketamine treatment (Figure 1a). PolyI:C+ketamine+saline group also had increased number of crossings between the compartments without altering the normal time in familiar compartment, suggesting that the task was accomplished, but with more difficulty by PolyI:C+ketamine+saline group (Figure 1b). Since the task was performed 24h after the last injections, and there is no evidence in previous literature of clozapine accumulation in the rat brain (Baldessarini et al. 1993), the effect in the olfactory discrimination in the PolyI:C+ketamine+clozapine group could only be mediated by alterations in the receptors which were subjected to clozapine action, mainly serotonergic, but also histaminergic, cholinergic, and others (Goudie et al. 1999), following the absence of the drug. The olfactory deficit in the present work represents a severe impairment of sensorial processes (Schneider and Przewłocki 2005, Niu et al. 2015). To our knowledge, olfactory deficits have not yet been tested in SZ patients in this specific situation (CLZ withdrawal). However, long term antipsychotic use has been associated with olfactory deficits in SZ, but it has been impossible to establish a causal relation (Ansoleaga et al. 2015, Kiparizoska and Ikuta 2017). Then, our study

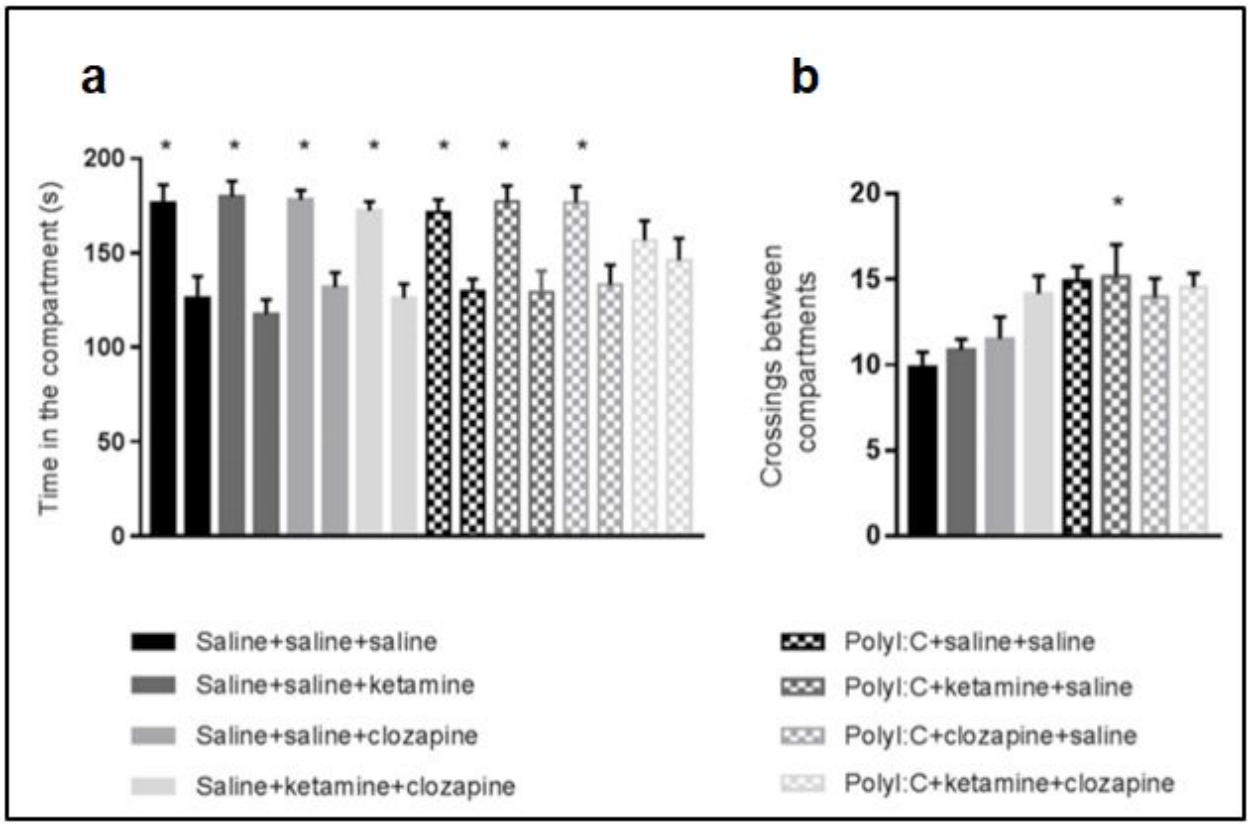

Figure 1 - Results for Olfactory Discrimination: time (in seconds) in familiar and non-familiar compartments (**a**) and number of crossings between the compartments (**b**). Results expressed in mean +/- Standard Error of the Mean (S.E.M). *statistically significant difference, $p<0.05$, among the time in compartments (**a**) and in comparision with Saline+saline+saline group (**b**).

raises the hypothesis that OD deficits in SZ may be linked with antipsychotics' withdrawal.

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