

PRELIMINARY DATA ON SOME BEHAVIORAL CHANGES INDUCED BY SHORT-TERM INTRAPERITONEAL OXYTOCIN ADMINISTRATION IN AGED RATS

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received: 9.7.2017;

revised: 13.12.2017;

accepted: 21.2.2018

SUMMARY

Introduction: Oxytocin (OT) is a well-known neuropeptides which together with vasopressin, melatonin, insulin and other hormones can alter both behavior and physiological or neuronal functions. This growing interest on OT roles is also based on the demonstrated beneficial effects as a stress reliever and a social bonding agent. The association between old age and OT was only vaguely studied. Little or few is known on the effect of the OT hormone on the old body. Hereby, we present our preliminary results in the research on behavioral changes regarding the intraperitoneal administration of OT in aged rats.

Subjects and methods: OT was administered for 8 days in Wistar aged rats in parallel with saline administration for control group. Behavioral markers were assessed in some specific behavioral tasks, such as the Y-Maze test for short-term working memory, Open Field test, Elevated Plus Maze, and Forced Swim test for anxious and depressive behavior assessment, and Three-chambered Maze test for sociability assessment.

Results: Increased mobility and decreased anxiety behaviors were reported for the aged intraperitoneal OT-treated animals, as compared with controls, during FST and OFT, and respectively FST, EPM, and OFT. Also, decreased depressive-like behaviors were observed in the same animal group during FST and ST. Moreover, a decrease in anxiolytic behavior was observed as exposed to stressful stimuli (such as grooming behavior in OFT, and forced grooming behavior in ST), and as exposed to social stimuli (such as grooming behavior in TCT). Similarly, significant differences were obtained regarding the social behavior of the intraperitoneal OT-treated animal as compared to control group, the animals showing increased sociability and social preference for the stranger animal in TCT. However, no significant effects on the working memory (assessed as spontaneous alternation in YMT) were observed.

Conclusions: Intraperitoneal administration of OT in aged rats has clear effects on anxious and depressive behavior, but no significant effects on the working memory. Also, several beneficial effects of OT on social preferences and sociability were observed.

Key words: ageing - animal models - oxytocin - sociability - behavioral testing - anxiolytic - anti-depressant

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INTRODUCTION

Lately, increased interest in understanding the roles of oxytocin (OT) in the main neuropsychiatric disorders, such as Alzheimer's disease, anxiety, depression, schizophrenia or autism and the variety of behaviors exhibited by both the administration of intranasal or peripheral OT on the developed animal models for the aforementioned disorders (Kis et al. 2015, Guastella & Hickie 2016) was noted.

OT is a well-known neuropeptides which together with vasopressin, melatonin, insulin and other hormones can alter both behavior and physiological or neuronal functions (Lischke et al. 2012). This growing interest on OT roles is also based on the demonstrated beneficial effects as a stress reliever and a social bonding agent. Several studies actually review these psychosocial roles of OT (Meyer-Lindenberg et al. 2011, Striepens et al. 2011) and conclude that these findings encourage the therapy application of OT in many psychiatric disorders such as the affective disorders and the autistic disorders.

The main effect of OT on the brain is the alteration of several limbic regions activity patterns. In this way, OT may induce both amygdala activation and inactivation when exposed to adverse social stimuli (Lischke et al. 2012). This is the reason why, it was concluded that the OT roles very much depend on the various factors with which the OT administration is accompanied. Thus, the nature of the stimuli, the gender and the genotype, have been demonstrated to be influencing the OT pattern of action (Striepens et al. 2011).

Several studies also show different OT activities, and ways of transportation. In this way, it has been shown that the administration method can crucially influence the OT penetration of blood-brain barrier. In this way, many studies showed significant cerebrospinal fluid OT levels after intranasal administration both in humans (Striepens et al. 2013) and rats (as reviewed by Veening et al. 2010), but also in macaques (Dal Monte et al. 2014). By contrast, animal-based studies showed that less than 0.1% of the administered OT can cross the blood-brain barrier by intravenous mean (Kendrick et al.

1991). Moreover, very brief half-life of OT is noted especially when administered peripherally (Mens et al. 1983). In spite that, there are several studies that suggest several correlations between intraperitoneal administration of OT and social affiliation increase (Mooney et al. 2014), low anxiety relief (Crine et al. 1983), but strong effects on nerve recovery following nerve damage (Gümüs et al. 2015). Moreover, several studies show strong correlations between OT plasma levels and cognition and behaviour (Lancaster et al. 2015, Valstad et al. 2016, Zhong et al. 2012).

The association between old age and OT was only vaguely studied. Little or few is known on the effect of the OT hormone on the old body. For instance, OT beneficial effect has been demonstrated in old age context regarding the reparatory yield improvement of old muscles exposed to OT. In this way, Elabd et al. (2014) shows that plasma OT and receptors' levels of OT in muscle dramatically decline during aging and demonstrate that muscle tissue regeneration and homeostatic maintenance is dependent on the OT levels. A similar mechanism was suggested regarding the effect of OT in the brain. In this way, Parker et al. (2010) show that CSF OT levels in female Rhesus monkeys may be associated with central nervous system alterations due to old age and, moreover, may be used as a lifespan developmental biomarker for age-related central nervous system changes.

Keeping that in mind, we aimed to examine the OT effects on several cognitive functions in old age individuals. In this way, here we present some of our preliminary research regarding the intraperitoneal administration of OT for 8 days in some specific behavioral tasks, such as the Y-Maze test for short-term working memory, Open Field test, Elevated Plus Maze, and Forced Swim test for anxious and depressive behavior assessment, and Three-chamber Maze test for sociability assessment, used to assess animal behavior in aged Wistar rats.

SUBJECTS AND METHODS

Experimental design

Two groups of male Wistar rats were used for this study, divided based on their age: control group (n=5) consisted in aged male rats (two years old) which received intraperitoneal injection with saline solution (Aged + Saline), and treatment group (n=5) consisted in aged male rats which received intraperitoneal injection with oxytocin solution (Aged + i.p. OT).

Animal housing and habituation

The animals were housed in polyacrylic cages (5 animals/cage) containing woodchip bedding, and maintained in a temperature and environment-controlled room (22±2°C, a 12-h cycle, relative humidity of 40-60%). The animals were acclimatized to laboratory condition

for 10 days before the beginning of the experiment. Rats were fed a standard laboratory diet of rat chow pellets, according to the McCollum diet requirements. Except for the periods of the behavioral tests which require food limitation and two hours before OT administration, food and water were available ad libitum. Rats were treated in accordance with the current national and European Regulations regarding the scientific research using animals and in accordance with NIH- Care and Use of Laboratory Animals Manual (8th Edition) and in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). This study was approved by the local Faculty Ethics Committee and also efforts were made to minimize animal suffering and to reduce the number of animals used.

Reagents and treatments

OT (Sigma-Aldrich Co. LLC., Darmstadt, Germany) was intraperitoneally injected in the OT group in a dose of 10 mg/kg/body weight for 8 days consecutively. Control animals received intraperitoneal administration of the same volume of OT vehicle. The treatment began 6 days before the behavioral testing.

Behavioral testing

Rats were subjected to a battery of behavioral tests in the following order: Y maze test, elevated-plus maze, open field test, forced swim test, splash test, and Tri-chamber sociability test performed from the last 2 days of treatment to the second day past the final treatment day.

Y-maze test

A reliable, non-invasive short-time memory test, the Y-maze test (YMT) was used to determine the cognitive changes in Wistar rats occurred in the spontaneous alternation task as a behavioral marker for the short term memory function. The maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. The animals were tested following the standard protocol described by Kokkinidis et al. (1976).

Open Field test

Locomotor activity and anxiety-like behavior in open area were evaluated using the open field test (OFT). An open field rectangular apparatus consisting in an empty open area with white floor (plastic-covered flooring) and white walls was placed in a low illuminated room, far from the room walls and furniture. The animals were observed for 5 minutes and evaluated against the standard test markers described and adapted by Stanford (2007).

Elevated Plus Maze test

Anxiety-like behavior was also assessed in the elevated plus maze paradigm (EPM). The apparatus consisting in two open arms black Plexiglas arms, and two enclosed black Plexiglas arms with black lateral walls and open roof, opposite to each other and elevated from the ground at the height of 50 centimeters, was placed in a illuminated room, far from the room walls and furniture. The behavior markers were assessed following the standard protocol methodology described by Pellow et al. (1985).

Forced Swim test

Behavioral despair as a marker for depressive behavior was assessed using the forced swim test (FST). The test apparatus consisted in a transparent glass cylinder containing water (23–25°C, 30 cm depth). The animals were placed in the water for 15 minutes. The next day, the rats were re-exposed to the swim arena for a 5 minute period. Using time-sampling method, the animal behaviors were assessed as following: immobility time (floating), swimming time (horizontal movements) and climbing (vigorous upward movements) (Detke et al. 1995).

Splash test

The splash test (ST) was performed by spraying a 10% sucrose solution on the dorsal part of the rats in their home cage which induces grooming behavior. The latency to the first groom, the number of grooms, and total duration of the grooming were recorded for 5 minutes (Zou et al. 2015). Depressive behavior is characterized by increased latency time, and decreased time spent grooming.

Three-chamber sociability test

In order to assess sociability and social behavior expressing species such as mice and rats, Crawley et al. (2004) suggests using a device consisting in three chambers (central compartment, and lateral compartments, so that it is given the choice of whether to interact or not with an unknown animal) (TCT). The 30 minutes test consists in three 10 minutes phases: the first phase in which the evaluated animal is placed in the central compartment and allowed to explore the apparatus; in the next phase, a new animal is placed in one of the lateral chambers; and in the last phase, another one is added to the other lateral chamber. The time spent exploring the chambers, the phase I animal, the number and duration of contacts, the preference for the phase II animal were evaluated. Sociability indicates the preference for the novel mouse compared to the empty compartment or known animal.

Statistical analysis

All data collected during the behavioral testing of the animals were statistically analyzed, P-values below 0.05 being considered statistically significant. Statistical analysis of the data was carried out using Minitab 17 (Minitab Inc., 2013) application. Standard one way and two ways ANOVA were applied. All data are shown as means \pm S.E.M. Post-hoc analysis was also performed for covariance analysis (Tukey's honesty test) and correlation between the behavioral parameters (Pearson's correlation and Spearman's correlation).

RESULTS

Y maze test

While analyzing the behavioral parameters obtained in YMT, we obtained a mild increase in spontaneous alteration in OT group (55.82 \pm 2.68%), as compared to control group (49.33 \pm 4.94%) (Figure 1) suggesting a mild influence on short-term spatial memory (F(1,6)=1.32; p=0.29).

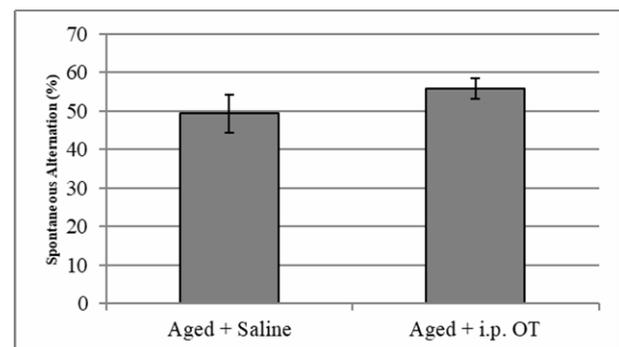


Figure 1. The effects of intraperitoneal OT administration in the Y-maze test, as showed by the spontaneous alternation behavior. The values are expressed as mean \pm S.E.M. (n=5, per group)

Forced swim test

In the FST, we evaluated several parameters such as general mobility, struggling duration, and floating duration. In this way, regarding the general mobility, we obtained considerably higher mobility times in OT group (203.0 \pm 7.15 s) as compared to control group (173.75 \pm 12.09 s) in the same experimental conditions, but no statistical significance was obtained during post-hoc analysis (Fmobility (1,6)=4.33; p=0.082). On the contrary, statistical significant differences were obtained while analyzing data regarding struggling time and floating time. We observed that struggling time decreases in OT treated animals (8.0 \pm 2.08 s), as compared to controls (21.5 \pm 3.77 s), in a statistical significant manner (F(1,6)=9.80; p=0.02). Similarly, we obtained several differences while comparing the floating time between the OT group (37.0 \pm 7.15 s) and the control group (66.25 \pm 12.09 s), but no statistical variation was registered (F(1,6)=4.33; p=0.08) (Figure 2).

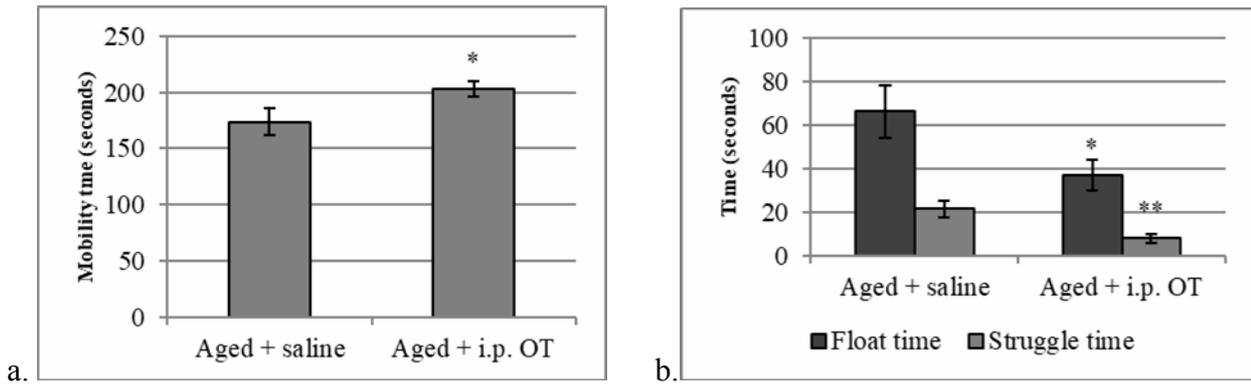


Figure 2. Effects of intraperitoneal OT administration on the parameters evaluated in forced swim test: a. mobility time (seconds); b. floating time and struggling time (seconds). The values are expressed as mean \pm S.E.M. (n=5, per group) (* p=0.08 vs. saline; ** p=0.02 vs. saline)

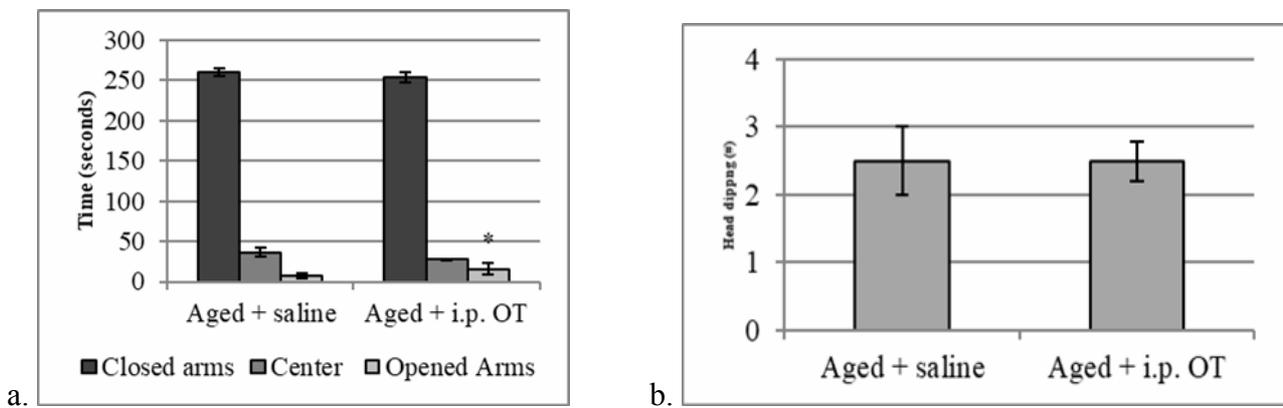


Figure 3. Effects of intraperitoneal OT administration on the parameters evaluated in elevated plus maze: a. time spent in the closed arms, center, and opened arms; b. number of head dipping behavior. The values are expressed as mean \pm S.E.M. (n=5, per group) (*p=0.12 vs. saline)

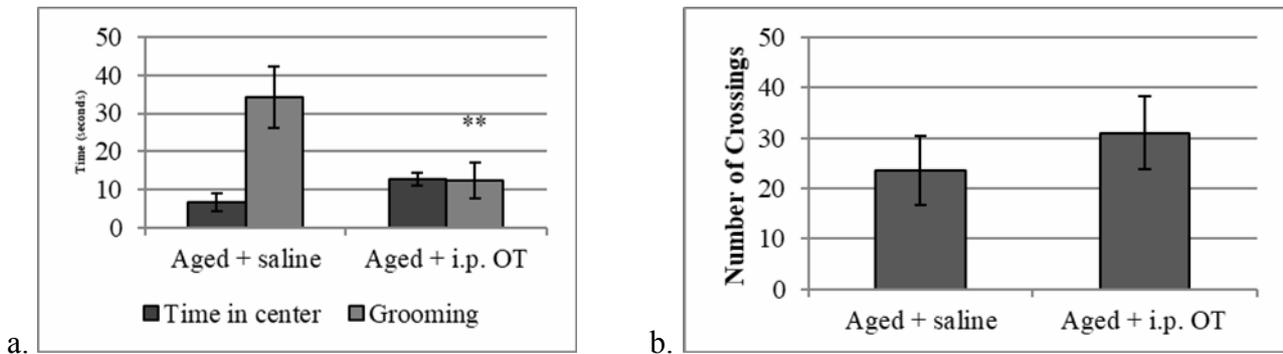


Figure 4. The effects of intraperitoneal OT administration on the evaluated open field test parameters: a. time spent in the center of the OFT apparatus and grooming behaviors; b. number of crossings. The values are expressed as mean \pm S.E.M. (n=5, per group) (**p=0.03 vs. saline)

Elevated plus maze

During EPM administration, we observed that the OT-treated animals tendency to spend more time in the open arms (25.33 ± 6.35 s) was significantly higher, as compared to control group (9.66 ± 4.91 s) ($F(1,6)=3.80$; $p=0.12$). Also we observed that the time spent in the closed arms and center of the apparatus decreases after the OT administration, but not in a statistical significant manner ($F_{\text{closed arms}}(1,6)=0.52$; $p=0.49$; $F_{\text{center}}(1,6)=2.4$; $p=0.17$). Although the time spent in the open arms

was significantly different between the experimental groups, we observed that the head dipping behavior was practically of the same frequency (2.55 ± 0.5) (Figure 3).

Open field test

In the typical test for assessing anxious behavior, the OFT, we mainly obtained differences regarding the time spent in the center of the open field paradigm apparatus, and the frequency and duration of the grooming behavior. In this way, we observed that the OT-treated

animal spent more time exploring the center of the apparatus (12.75 ± 1.66 s), as compared to the control group (6.75 ± 2.33 s) ($F(1,6)=1.47$; $p=0.27$). Also, we observed that the grooming behavior was significantly lower in frequency in the OT group (12.5 ± 4.72 s) than in the control group (34.25 ± 8.02 s) ($F(1,6)=7.86$; $p=0.03$) (Figure 4).

Splash test

During the ST, we observed that the OT-treated animals exhibited a lower delay time until first grooming behavior (37.3 ± 7.6 s), as compared to the control group (46.3 ± 6.4 s), in the same experimental conditions. Also, we observed that the forced induction of grooming behavior resulted in decreased grooming behavior duration in OT group (58.5 ± 27.8 s, as compared to 65.75 ± 14.1 s in controls) (Figure 5). However, none of the presented variations were statistically significant ($F_{\text{delay}}(1,6)=1.32$; $p=0.29$; $F_{\text{grooming time}}(1,6)=0.05$; $p=0.82$).

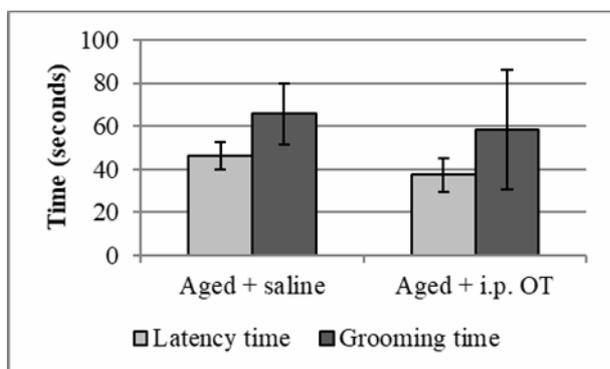


Figure 5. The effects of intraperitoneal OT administration on the evaluated Splash test parameters > latency time until first grooming, and grooming time. The values are expressed as mean \pm S.E.M. ($n=5$, per group)

Three-chambered sociability test

During the TCT, we observed several differences regarding the time spent in the inhabited room (during first test phase), preferences for the stranger animal (contact duration with the familiar animal versus stranger animal), and anxiety behaviors when exposed to social stimuli (grooming and freezing behaviors). While Crawley's test offers reliable data on sociability and social preference, it is important to consider that the measured social behavior is independent of general mobility (during all three phases), since the evaluation is made based on the contact duration rather the number of contacts with the animals quantified by other sociability tests (Takeuchi et al. 2011).

In this way, in the first test phase (sociability test), we observed that the OT-treated animals preferred to spent more time in the inhabited room (317.5 ± 21.5 s) as compared to control group (195.0 ± 10.2 s) ($F(1,8)=15.42$; $p=0.007$). Regarding the social preference, we observed

also that OT group recorded higher contact duration with the stranger animals (56.75 ± 10.3 s), than with the familiar ones (18.0 ± 366 s) ($F(1,8)=17.61$; $p=0.003$). Similarly, the sociability and social preference in the control group were increased, but not in a statistical significant manner. Post-hoc analysis revealed significant differences regarding the mentioned parameters.

In the same paradigm, we also measured the anxiety markers associated to the social stimuli. Therefore, we observed that the grooming behavior was less frequent in OT-treated animal, as compared to the control group ($F(1,6)=2.12$; $p=0.2$). Also, differences were observed in regarding the freezing behavior, but no statistical significance was obtained ($F(1,6)=1.19$; $p=0.33$) (Figure 6).

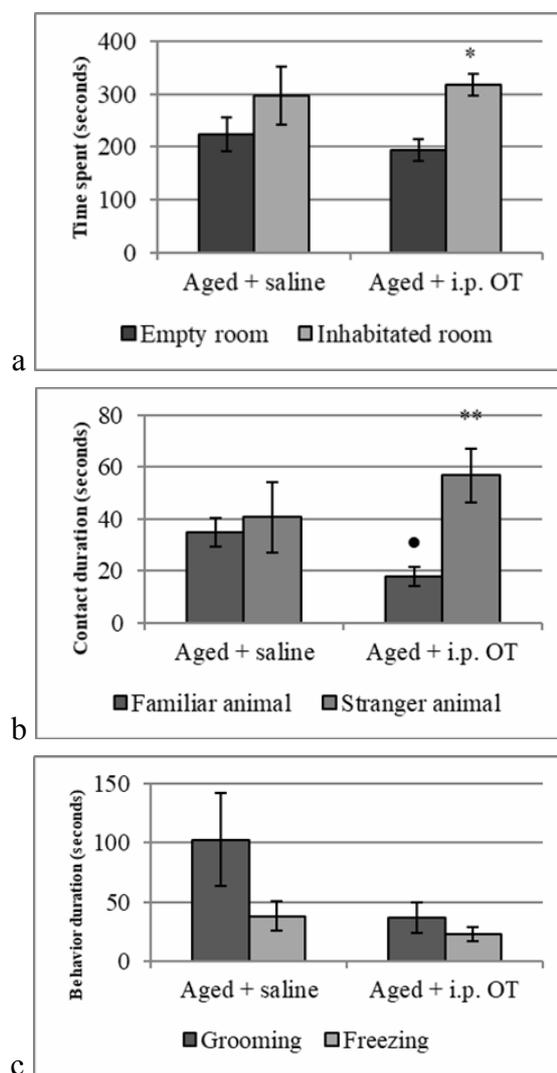


Figure 6. The effects of intraperitoneal OT administration on the evaluated three-chambered sociability test parameters: a. time spent in the empty and inhabited rooms (during phase I); b. time spent in the presence of the familiar and stranger animals (during phase II); c. grooming and freezing behaviors durations. The values are expressed as mean \pm S.E.M. ($n=5$, per group) (* $p=0.007$, inhabited room vs. empty room; ** $p=0.01$, familiar animal vs. stranger animal; • $p=0.06$ vs. saline)

DISCUSSION

The present study investigated the effects of intraperitoneal administration of OT on the behavior of old animals, as compared to age and sex-matched control group. Now it is generally known that OT possesses high potential for social behavior enhancement (Lukas et al. 2011), but few information is available on its effect in memory, learning, and affective behavior of aged individuals. As stated before, the main known OT effects based on its hormone and neuropeptide properties include several physiological reproductive processes modulation and some behavioral implications such as maternal instinct, social bonding and mating, empathy, and mood enhancement (van Leengoed et al. 1987, Bick & Dozier 2011, Sheng et al. 2013).

Our results provide evidence regarding the memory, mood and social behavior changes induced by intraperitoneal OT administration. The fact that peripheral OT administration leads to observable changes at central levels suggests that intraperitoneal route of administration may also be as effective as the intranasal route. However, several changes in pharma kinetics were observed by comparing OT levels and peaks in brains and plasma of OT-treated rats (Neumann et al. 2013). In this way, although intranasal OT administration was proved more efficient, intraperitoneal OT administration could cause rapid peak levels in brain and plasma during the first 30 min after treatment. In spite that, we showed that intraperitoneal OT administration also provides sufficient transport and interactions so that behavior changes are obtained.

Moreover, following OT administration, we observed a mild increase of the memory related behavioral parameter from the YMT leading to the suggestion that it may cause some memory changes. Kunchiulia et al. (2010) showed that OT administration before stress exposing does not cause memory impairment prevention. Also, Sun-Young Lee et al. (2015) reports decreased cumulative search error, higher swim speed, and increased time in target annulus in OT treated rats, as compared with control group in the Morris water maze memory test. These findings together with our results suggest that OT may influence hippocampal activity and memory acquisition in rats. However, our data showed no significant modification regarding the immediate working memory, as compared to our control group (Figure 1).

Even in that condition, we observed that OT administration resulted increased total mobility time in FST. Also regarding general mobility stand the results for number of crossings in the OFT paradigm (Figure 4.b) which exhibit a similar trending. These results show that OT may be involved in an anxiolytic pathway of spontaneous stress exposure relief (caused by exposure to water in FST or open field in OFT). Also, Yan et al. (2014), while modeling depression in rats via intra cerebro-ventricular OT receptor antagonist administra-

tion, obtained low immobility times in OT-treated rats, as compared with controls.

Moreover, the fact that OT administration decreases struggling behavior duration (in FST), and increases the time spent in the open arms of the EPM clearly suggests that OT exhibits a strong anxiolytic effect. However, no difference was observed in regarding the head dipping behavior, also considered an important behavior in assessing anxiety levels. Even in that circumstance, it seems that OT had a more important effect on anxiety-like behaviors reduce since freezing behavior was not observed almost in any of the OT-treated group (data not shown). Moreover, we observed that the time spent in the center of the EPM was prolonged in OT-treated animals as compared to controls meaning that the OT-treated animals were prone to exploration (a non-anxious behavior). Also, we observed that the grooming behavior, considered an anxiolytic behavior, used to relief stress, was reduced in duration in OT-treated animals in both EPM and TCT as a result to environmental and social stress exposure. Similarly, the freezing behavior in social environment losses its intensity after OT administration leading to the suggestion that OT may also relief stress in social stimuli conditions.

Another interesting aspect was observed while observing grooming behavior in ST. By contrast to non-induced grooming, which is an anxiolytic behavior, induced-grooming stands for depressive behavior evaluation. In this way, by observing that delay time until first grooming in ST was considerably lower in OT-treated animals leads to the conclusion that OT also exhibits anti-depressant effects. These effects were also demonstrated by the low floating times observed in OT-treated animals meaning that the renunciation tendency was considerably decreased when exposed to OT.

As we expected, we observed important improvements in social behavior after OT administration. In this way, we observed that the animals which received OT were prone to explore the inhabited room of the TCT during the first test phase meaning that the animals chose to spent time in a social environment rather than in isolation. Furthermore, during the second phase of the test, we observed that the OT-treated animals expressed the same social curiosity as compared to the first phase tendencies towards the stranger animals in the test. This leads to the conclusion that OT pays important role in sociability and also in social preferences.

CONCLUSIONS

Short-term intraperitoneal OT administration as studied in aged rats shows important effects on anxiety and depressive behavior, and also in social interactions. In this way, increased mobility and decreased anxiety behaviors were reported for the aged intraperitoneally OT-treated animals, as compared with controls, during FST and OFT, and respectively FST, EPM, and OFT. Also, decreased depressive-like behaviors were observed

in the same animal group during FST and ST. Moreover, a decrease in anxiolytic behavior was observed as exposed to stressful stimuli (such as grooming behavior in OFT, and forced grooming behavior in ST), and as exposed to social stimuli (such as grooming behavior in TCT). Similarly, significant differences were obtained regarding the social behavior of the intraperitoneal OT-treated animal as compared to control group, the animals showing increased sociability and social preference for the stranger animal in TCT. However, no significant effects on the working memory (assessed as spontaneous alternation in YMT) were observed.

Acknowledgements:

This work was supported by the PN-II-RU-TE-2014-4-1886 grant called "A complex study regarding the relevance of oxytocin administration in some animal models of neuropsychiatric disorders", number 120 from 01/10/2015.

Conflict of interest: None to declare.

Contribution of individual authors:

Ioana Miruna Balmus: wrote the draft paper, did statistical analysis, helped with behavioral tasks, searched literature, interpreted the biological significance of behavioral data;

Radu Lefter: did most of the behavioral tasks, some preliminary data acquisition of the behavioral tasks and final statistics;

Alin Ciobica: designed the study, corrected the draft, wrote the oxytocin ip vs. intranasal part, interpreted the biological significance of behavioral data, did final statistics;

Iulia Antioch: did splash test, corrected draft, helped with the acquisition of behavioral data, searched literature;

Daniela Ababei: did open field and some preliminary data acquisition of the behavioral tasks, searched literature;

Romeo Dobrin: designed the study and wrote part of the discussion section.

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