ORIGINAL ARTICLE

# Oxytocin and carbetocin ameliorating effects on restraint stress-induced short- and long-term behavioral changes in rats

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**Abstract**

**OBJECTIVES:** Carbetocin (CBT), an oxytocin (OXY) analog, was designed to exert prolonged peripheral actions. It has also been proposed as potential therapeutic mean in certain psychiatric disorders where OXY role has been implicated. This study examined the effects of both peptides on behavior of naive and restraint stress exposed rats in the open field (OF) and elevated plus maze (EPM) tests.
**METHODS:** Spontaneous behavior in the OF and EPM was measured in Wistar rats after intraperitoneal (i.p.) application of OXY or CBT and/or repeated restraint stress. Behavioral parameters were recorded and subsequently elaborated by an automated activity monitoring system (AnyMaze, Stoelting, U.S.A.). Changes in the total movement distance (TMD) and movement in the center area (CMD) were postulated as indicators of the anxiety level.
**RESULTS:** OXY (0.05 mg/kg) and CBT (0.3 mg/kg) increased TMD but not CMD 60 min after the i.p. treatment; the increased locomotion/exploration indicate participation of arousal/vigilance. Daily stress exposures for three consecutive days, followed by behavioral tests, reduced locomotion of rats in OF and EPM tests; OXY and CBT partly prevented these effects. Five days after the last stress, rats exhibited an increase of both TMD and CMD in the OF. CBT but not OXY prevented these long-term post-stress changes. In the EPM the stressed rats exhibited an increase in time spent in open arms; CBT accelerated this time development. Similar prevention of stress behavioral sequel in OF were obtained in study when stress and peptides were applied for three consecutive days but behavioral testing was postponed for several days to determine the long-lasting effects. CBT reduced the developed locomotor enhancement (6–11 days post-stress) irrespectively whether injected before or after stress.
**CONCLUSIONS:** Repeated restraint stress exposure produced acute and persisting effects on Wistar rat behavior in the OF and EPM tests. CBT either injected before or after stress practically abolished the developed changes in the mobility parameters. The CBT effectiveness to ameliorate the late post-stress behavioral alteration supports the notion of its therapeutic potential in psychiatric disorders in which the role of OXY has been implicated.

**Abbreviations:**

CBT - Carbetocin
CMD - Movement in the center area
EPM - Elevated plus maze
OF - Open field
OXY - Oxytocin
IS - Restraint/immobilization stress
TMD - Total movement distance
TTM - Total time mobile

## INTRODUCTION

Oxytocin (OXY) is a well acknowledged neuromodulator/neurotransmitter in the brain regulating a diverse range of central nervous functions. A growing body of evidence indicates that OXY modulates neuroendocrine, behavioral and autonomic responses to stress (Neumann 2002; Neumann *et al.* 2000; Peterson *et al.* 1996; Peterson & Uvnäs-Moberg 2007; Uvnäs-Moberg 1997, 1998b; Gimpl & Fahrenholz 2001). In studies with laboratory rodents, the behavioral effects of centrally or systemically administered OXY observed in the open field, elevated plus or zero maze, or as punished crossing in the four plate test, have been interpreted as an anxiolytic-like action (Uvnäs-Moberg 1998b; Windle *et al.* 1997; 2006; Waldherr & Neumann 2007; Ring *et al.* 2006). Furthermore, OXY has been found to be critically involved in mammalian affiliative behaviors including sexual bonding, mother-infant and adult-adult pair-bond formation (McCarthy & Altemus 1997; Uvnäs-Moberg *et al.* 2005; Uvnäs-Moberg 1998a). Based on the evidence from animal studies demonstrating that OXY is implicated in the regulation of species-typical social behavior, this peptide has been proposed as a potential factor in certain psychiatric disorders. For example, OXY neurotransmission may account for several features of autism or obsessive compulsive disorder (Insel *et al.* 1999; Insel & Young 2000; Modahl *et al.* 1998; Hollander *et al.* 2007; Leckman *et al.* 1994).

A number of OXY analogs have been designed with the aim to prepare possible substitute agent with prolonged uterotonic and milk-let down activities. Deamino-1-monocarba-(2-O-methyltyrosine)-oxytocin (carbetocin, CBT) was prepared to be protected from aminopeptidase and disulfidase cleavage (Barth *et al.* 1974). These changes of the molecule resulted in prolongation of uterotonic activity and the analog is used to prevent or treat postpartum hemorrhage (Barth *et al.* 1974; Hunter *et al.* 1992; Engstrem *et al.* 1998). In search for new biological agents for treatment of psychiatric disorders, in which the role of OXY has been implicated, CBT was suggested as a potential candidate (US Patent 2005).

In previous studies we found that one hour lasting restraint stress alone or combined with cold water immersion reduced spontaneous locomotion and rearing in Wistar, Sprague-Dawley and Lewis male rats tested in the open field one hour after stress termination. On the contrary, several days after stress exposure, rats displayed an increase of horizontal and vertical activities (Klenerová *et al.* 2006; 2007). The purpose of the present study was to examine the effects of CBT in comparison with OXY on long-term behavioral consequences of repeated immobilization. The experiments also attempted to reveal to what extent the found acute and long-term behavioral changes are related to the anxiety level, and on the other hand to general activity related to arousal, vigilance, alertness. To measure these behavioral components we employed the open field (OF) and elevated plus maze (EPM) tests. Exposure of rats to the OF represents by itself a stressogenic event and the increase of mobile activity has been widely used as a test for drugs with anxiolytic-like action (Prut & Belzung 2003). EPM test relies upon rodents´ proclivity towards dark, enclosed spaces, and on unconditioned fear of heights/open spaces. For rats and mice the exposure to EPM represents a stressogenic event similar to the OF and has been validated as test of anxiety (Pellow & File 1986; Rodgers & Cole 1994; Ruarte & Alvarez 1999; Hlinak *et al.* 2009).

For the potential use in the treatment of the psychiatric disorder symptoms, CBT may be administered by several administration modes, including intramuscular, intravenous or parenteral injections (US Patent 2005). Based on our previous experiments we employed the intraperitoneal administration.

## MATERIALS AND METHODS

### *Animals*

Wistar male rats with starting body weight 250–280 g (VELAZ, Czech Republic) were used. They were housed by four in cages (42×26×25 cm, plexi-glass) in a breeding room at constant temperature (21 ± 1 °C) with a 12L/12D schedule, the onset of the light phase being at 6.00 hr a.m. The animals were daily handled by the same person and allowed at least 10 days of recovery before experimental schedule. Food and water were supplied ad libitum except during the testing. Treatment of animals was in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals (DHEW Publication, NHI 80-23).

### *Drugs*

The following drugs were used: oxytocin and carbetocin [deamino-1-monocarba-(2-0-methyl tyrosine)-oxytocin] from Polypeptide Laboratories, A/S, Czech Republic. Both drugs were dissolved in saline and injected intraperitoneally (i.p.) in a volume of 2 ml/kg and were administered immediately after termination of stressor exposure or 60 min before behavioral testing. Control animals received saline. Doses of drugs were chosen according to the results of previous studies. OXY has been shown to have dual effect in the OF: in smaller doses it slightly increased or did not change

while the higher doses (1 mg/kg) suppressed locomotion and rearing (Klenerova *et al.* 2009a; 2009b; Uvnäs-Moberg *et al.* 1994). CBT increased locomotor activity up to the 1mg/kg. Therefore, we selected the 0.05 mg/kg OXY and 0.3 mg CBT doses**.**

*Stress procedure*

Restraint/immobilization stress (IS) was applied by fixing front and hind legs of the rat with adhesive plaster; then the animal was restrained in a snug-fitting plastic-mesh. The mesh was bent to conform to the size of the individual animal and a bandage fixed this shape of the mesh. During the stress, the animals were kept in a vertical position (Klenerová *et al.* 2007). Stress exposure lasted 60 min. Immediately after stress termination rats received drugs or saline and were returned to the home cage. For the stress application rats were transferred into a room separated from a testing one. Two different persons performed the stress procedure and the behavioral testing.

*Behavioral testing*

Elevated plus maze (EPM) behavior was tested in the apparatus consisting of four arms. The opposite-facing open arms were 40 cm long and 10 cm wide with 0.2 mm edges; the opposite-closed arms were surrounded by a 40 cm wall. Central area measured 10 × 10 cm. The apparatus was placed on the floor and the arms were elevated at a height of 50 cm. Always, rats were taken from their home cage and placed in the central area of the maze facing an open arm, and allowed to explore the maze for 5 min. A four-paw criterion validated an entrance into an arm. The behavioral parameters collected were the following: total movement distance (TMD, in m), total time mobile (TTM, in s), total distance traveled in open and closed arms (m).Time spent in the open arms is presented as the percentage of total time.

Open field (OF) behavior was tested in a circular arena with the diameter of 150 cm, the walls being 50 cm high. The arena was divided into two concentric circles, the inner one defined by 130 cm diameter, the outer one by 20 cm wide area. Each animal was placed on the same position in the outer part of the arena. The test lasted for 5 min. The following behaviors were automatically recorded: total movement distance (TMD), total time mobile (TTM), center movement distance (CMD). The experimenter recorded rearing and grooming numbers as well as the time spent in both behavioral elements. Further, the ratio of CMD/TMD was calculated.

All behavior tests were conducted between 08:00 and 12:00 h during the light phase in the experimental room separated from the breeding room. Experimental room was illuminated by two 60 W lamps located on the opposite walls. At the end of observation period each rat was returned to its home cage. Before using next animal both EPM and OF arenas were cleaned and dried. Behavioral parameters were recorded and subsequently elaborated by an automated activity monitoring system (AnyMaze, Stoelting, U.S.A.).

*Experimental design*

**Study 1:**
**Effects of OXY and CBT on behavior of rats in the OF**
Animals were assigned to three groups (N = 8). OXY (0.05 mg/kg) and CBT (0.3 mg/kg) were administered i.p. 60 min before testing; animals in control groups received saline.

**Study 2:**
**Effects of OXY and CBT administered after stress exposure on behavior of rats in EPM and OF**
Animals were assigned to 4 groups (N = 8): CO – saline controls, IS – exposed to stress and receiving saline, IS+OXY – exposed to stress and receiving OXY (0.05 mg/kg), IS+CBT – exposed to stress and receiving CBT (0.3 mg/kg). Drugs and saline were injected i.p. immediately after the stress termination. The procedure was repeated on Day 2 and Day 3.

Always, behavioral testing started 1 hr after stress and treatment. On Day 1 and 3, animals were tested in the EPM, on Day 2 in the OF. To evaluate possible persisting behavioral changes, testing continued on Day 7 in the EPM and on Day 8 in the OF without additional stress and drug treatment.

**Study 3:**
**Effects of CBT administered before or after stress exposure on behavior of rats in OF**
Animals were assigned to 4 groups (N = 8–10): CO – saline controls, IS – exposed to stress and receiving saline after stress, IS+CBT – exposed to stress and receiving CBT (0.3 mg/kg) immediately after stress exposure, CBT+IS – receiving CBT (0.3 mg/kg) 15 min before the stress exposure. Stress and treatment were applied for three consecutive days. OF testing started five days later (Day 8) and was repeated on Day 9 and 14.

*Statistics*

Data in all studies were analyzed with the one way ANOVA followed by the Student-Newman-Keuls test for multiple comparisons. For study 1 df 2, 21; for study 2 df 3, 28 and for study 3 df 3, 31.

## RESULTS

The results describe the behavioral effects of OXY and CBT in OF and EPM under three experimental conditions described in the experimental design. In Study 1 both peptides were tested in OF 1 hour after drug application. In study 2 stress and peptides were applied for 3 consecutive days and testing followed 60 min after termination of treatment. In Study 3 stress and peptides were applied for three consecutive days but behavioral

testing was postponed for several days to determine the long-lasting effects.

**Study 1:**
**Effects of OXY and CBT on behavior of rats in the OF**
The data are summarized in Table 1. Statistical analysis revealed a significant effect of experimental condition for TMD (F=4.77, $p$=0.019) and TTM (F=5.75, $p$=0.01). As compared with the controls both locomotor parameters were significantly increased in OXY and CBT treated animals. No significant differences were found in behavioral parameters such as the center movement distance, rearing number and grooming time due to the treatment.

**Study 2:**
**Effects of OXY and CBT in stressed rats on behavior in the EPM and OF**
Day 1: The data received in the EPM are summarized in Table 2. Statistical analysis revealed a significant effect of experimental conditions for TMD (F=2.99, $p$=0.048), TTM (F=3.59, $p$=0.026) and total distance traveled in closed arms (F=9.48, $p$<0.001). Follow-up comparisons showed significantly lower values for these three behavioral parameters in IS group as compared with the controls. A significantly reduced TMD traveled in closed arms was also found in rats exposed to IS and given OXY or CBT.

Day 2: The data received in the OF are summarized in Table 3. Statistical analysis revealed no significant effect of experimental conditions for both TMD (F=1.16, $p$=0.35) and CMD (F=0.92, $p$=0.44) and TTM (F=0.78, $p$=0.51). There was a significant difference in rearing time (F=4.30, $p$=0.014) and in the speed of movement (F=4.70, $p$=0.009). As compared with controls, behavioral level in both parameters was suppressed in stressed rats. CBT prevented both IS caused changes, OXY prevented only the lower rearing time.

Day 3: Testing in the EPM following threefold IS or IS+OXY or IS+CBT treatment (see Table 2) revealed no significant differences in measured behavioral parameters except for the total distance traveled in closed arms (F=3.72, $p$=0.02). Rats exposed to IS as well as those stressed rats given OXY or CBT had significantly decreased distance values as compared with the controls. Further, there was a significant difference in the percentage of time spent in open arms (F=3.79, $p$=0.021); specifically, IS rats given CBT spent significantly more time in open arms as compared with animals in other three groups.

Day 7: Testing in the EPM on the 4th day following the last IS, IS+OXY or IS+CBT treatment (see Table 2) revealed no significant changes in measured behavioral parameters except for the percentage of the time spent in open arms (F=8.18, $p$<0.001). Follow-up comparisons showed that IS exposed rats as well as those given OXY or CBT had significantly higher (approximately twofold) percentage of time spent in open arms than the controls.

Day 8: The data received in the OF on the 5th day after stress plus peptides treatment are shown in Table 3. Statistical analysis of data revealed significant differences in TMD (F=7.03, $p$=0.001), CMD (F=5.36, $p$=0.005), TTM (F=7.03, $p$=0.001) and the percentage of CMD/TMD (F=4.19, $p$=0.014). In fact, the parallel pattern of changes in these four parameters shows that the increase of locomotion in the center might be related to the enhanced general mobility, specifically to the TMD in IS and IS+OXY treated animals; on the contrary, in originally IS+CBT treated animals behavioral performance did not differ from the controls.

**Study 3:**
**Effects of CBT administered before or after stress exposure on behavior of rats in OF**
Day 8: Testing on the 5th day following threefold IS plus CBT given before or after IS revealed no significant changes in measured parameters. Data from Study 3 are summarized in Table 4.

Day 9: Statistical analysis revealed significant differences in TMD (F = 4.91, $p$=0.007) and in TTM (F=4.45, $p$=0.010); IS increased TDM and TTM compared with CO and also with both groups treated with CBT.

Day 14. Statistical analysis of data showed significant difference in TMD (F=3.19, $p$=0.037), CMD (F=3.35, $p$=0.031). Continuing trend to enhanced activity in IS group was found in the increased TMD compared to controls, whereas the increased CMD in stressed rats differed also in comparison with both CBT treated groups.

**Tab. 1.** Effects of oxytocin (OXY) and carbetocin (CBT) on behavior of rats in the open field recorded 60 min after drug administration.

| Measured parameters | Experimental groups | | |
|---|---|---|---|
| | CO | OXY | CBT |
| Total movement distance | 13.95±2.27 | 23.23±3.24* | 24.35±2.20* |
| Total time mobile | 75.80±10.9 | 117.00±14.1* | 130.86±10.5* |
| Center movement distance | 1.22±0.72 | 2.14±0.69 | 1.29±0.25 |
| Rearing number | 25.86±5.77 | 25.71±2.71 | 26.00±1.43 |
| Grooming time | 3.50±1.67 | 12.31±9.43 | 11.66±4.77 |

For the details see Experimental design (Study 1). Given means ± SEM; *$p$<0.05 vs CO.

## DISCUSSION

The aim of the study was to examine the effects of OXY and CBT on the short- and long-term sequel of the restraint stressor (Study 2 and 3). OXY has been widely acknowledged to attenuate the behavioral response to stress in laboratory rodents. Most of the published

**Tab. 2.** Effects of OXY and CBT on behavior of restraint stressed (IS) rats in elevated plus maze. IS and peptides were applied in three consecutive days and testing followed 1 hr after stress termination and OXY or CBT treatment (Day 1 and Day 3); on Day 7 testing was performed without the actual treatment.

| **Measured parameters** | **Experimental groups** | | | |
|---|---|---|---|---|
| ***Day 1*** | **CO** | **IS** | **IS+OXY** | **IS+CBT** |
| Total movement distance | 10.80±1.11 | 6.41±0.99* | 8.17±0.86 | 8.66±1.18 |
| Total dist. in open arms | 2.18±0.51 | 1.98±0.61 | 2.54±0.52 | 2.67±0.60 |
| Total dist. in closed arms | 7.11±0.65 | 3.54±0.49* | 4.52±0.40* | 4.72±0.17* |
| Total time mobile | 140.75±11.03 | 89.90±10.66* | 117.24±9.27 | 119.60±12.75 |
| Percent time in open arms | 22.57±3.54 | 21.25±5.00 | 30.45±4.29 | 30.11±4.52 |
| ***Day 3*** | **CO** | **IS** | **IS+OXY** | **IS+CBT** |
| Total movement distance | 9.68±1.08 | 6.86±0.38 | 7.97±0.80 | 8.92±0.80 |
| Total dist. in open arms | 1.78±0.37 | 1.53±0.31 | 2.23±0.27 | 2.72±0.60 |
| Total dist. in closed arms | 6.42±0.70 | 4.44±0.23* | 4.60±0.52* | 5.00±0.25* |
| Total time mobile | 117.01±11.20 | 97.46±8.72 | 111.46±9.80 | 123.47±9.66 |
| Percent time in open arms | 17.02±2.56 | 17.16±1.86 | 18.85±1.99 | 30.74±5.63*+x |
| ***Day 7*** | **CO** | **IS** | **IS+OXY** | **IS+CBT** |
| Total movement distance | 8.62±1.12 | 8.91±1.17 | 10.14±0.67 | 10.95±1.28 |
| Total dist. in open arms | 1.87±0.46 | 2.55±0.70 | 2.91±0.38 | 3.42±0.65 |
| Total dist. in closed arms | 5.38±0.55 | 4.99±0.41 | 5.68±0.42 | 5.85±0.64 |
| Total time mobile | 109.88±12.68 | 110.96±13.64 | 128.31±6.04 | 133.14±15.51 |
| Percent time in open arm | 15.71±1.16 | 28.40±2.11* | 28.30±1.94* | 33.69±4.07* |

For the details see Experimental design (Study 3). Given means + SEM. $p<0.05$: *vs CO; $^{+}$ vs IS; $^{x}$ vs IS+OXY.

**Tab. 3.** Effects of OXY and CBT on behavior of restraint stressed (IS) rats in the open field. IS and peptides were applied in three consecutive days and testing followed 1 hr after stress termination and OXY or CBT treatment (Day 2); on Day 8 testing was performed without the actual treatment.

| **Measured parameters** | **Experimental groups** | | | |
|---|---|---|---|---|
| ***Day 2*** | **CO** | **IS** | **IS+OXY** | **IS+CBT** |
| Total movement distance | 17.87±3.51 | 11.95±1.55 | 17.19±2.74 | 18.04±2.83 |
| Center movement distance | 2.99 ±1.27 | 1.05±0.33 | 2.37±0.78 | 2.53±0.88 |
| Percent CMD/TMD | 15.74±3.20 | 7.5±2.03 | 13.04±4.19 | 11.57±3.01 |
| Rearing number | 15.00±3.25 | 9.63±2.43 | 13.43±2.29 | 20.00±2.99 |
| Rearing time | 16.70±3.85 | 5.86±1.41* | 9.61±2.13 | 16.27±2.00$^{+}$ |
| Total time mobile | 76.97±14.81 | 63.50±7.17 | 87.50±12.54 | 81.20±10.09 |
| Speed of movement (m/s) | 0.23± 0.01 | 0.18±0.01* | 0.19±0.01* | 0.22±0.01 |
| ***Day 8*** | **CO** | **IS** | **IS+OXY** | **IS+CBT** |
| Total movement distance | 20.87±4.14 | 39.48±2.26* | 36.84±2.17* | 26.34±4.06$^{+x}$ |
| Central movement distance | 4.60±1.33 | 10.94±1.98* | 9.79±1.16* | 4.43±1.23$^{+x}$ |
| Percent CMD/TMD | 17.0±2.5 | 26.60±4.00 | 25.8±2.0 | 14.00±3.00$^{+x}$ |
| Rearing number | 19.88±3.04 | 27.88±2.21 | 25.63±2.62 | 20.38±3.95 |
| Rearing time | 23.89±4.99 | 23.95±4.42 | 18.44±2.99 | 15.37±3.15 |
| Total time mobile | 85.49±14.97 | 146.76±8.78* | 146.26±5.44* | 100.70±13.31$^{+x}$ |
| Speed of movement (m/s) | 0.24±0.01 | 0.27±0.01 | 0.26±0.01 | 0.25±0.01 |

For details see Experimental design (Study 2). Given means + SEM. $p<0.05$: * vs CO, $^{+}$ vs IS, $^{x}$ vs IS+OXY.

**Tab. 4.** Effects of CBT on behavior of restraint stressed (IS) rats in the open field. IS and CBT were applied in three consecutive days and testing was performed on Days 8, 9 and 14 without any actual treatment.

| Measured parameters | Experimental groups | | | |
|---|---|---|---|---|
| ***Day 8*** | **CO** | **IS** | **CBT+IS** | **IS+CBT** |
| Total movement distance | 25.15±2.66 | 34.20±1.85 | 30.37±3.40 | 27.71±2.20 |
| Center movement distance | 1.64±0.42 | 1.81±0.35 | 1.91±0.57 | 1.81±0.35 |
| Rearing number | 16.80±1.51 | 22.00±3.06 | 26.25±4.30 | 23.38±2.88 |
| Total time mobile | 123.90±11.0 | 157.07±8.19 | 141.32±12.6 | 137.00±8.97 |
| Speed of movement (m/s) | 0.20±0.01 | 0.22±0.01 | 0.21±0.01 | 0.20±0.01 |
| ***Day 9*** | **CO** | **IS** | **CBT+IS** | **IS+CBT** |
| Total movement distance | 16.95±1.70+ | 31.81±2.20* | 19.55±4.90$^{+}$ | 22.70±2.86$^{+}$ |
| Center movement distance | 0,85±0.20 | 2.74±0.93 | 1.56±0.81 | 1.87±0.61 |
| Rearing number | 12.90±2.00 | 20.33±3.06 | 17.88±3.40 | 24.20±3.50 |
| Total time mobile | 84.21±7.80$^{+}$ | 134.48±7.60* | 85.53±18.0$^{+}$ | 102.24±9.43$^{+}$ |
| Speed of movement (m/s) | 0.20±0.01 | 0.23±0.01 | 0.21±0.01 | 0.22±0.01 |
| ***Day 14*** | **CO** | **IS** | **CBT+IS** | **IS+CBT** |
| Total movement distance | 24.88±2.20 | 38.29±3.12* | 34.25±4.80 | 27.72±3.90 |
| Center movement distance | 7.19±1.23 | 13.81±2.00* | 9.20±1.69$^{+}$ | 7.96±1.64$^{+}$ |
| Rearing number | 18.4±1.00 | 26.89±3.21 | 29.50±3.88 | 21.61±4.42 |
| Total time mobile | 107.62±7.3 | 145.71±7.60* | 126.00±10.80 | 113.90±13.60 |
| Speed of movement (m/s) | 0.23±0.01 | 0.26±0.01 | 0.26±0.02 | 0.23±0.01 |

For the details see Experimental design (Study 3). Given means ± SEM. $p<0.05$: * vs CO, $^{+}$ vs IS.

results were obtained with OXY either centrally released or administered. In addition, the behavioral response to stress was estimated largely in the EPM test, which implies the anxiety/fear reaction to the stressogenic environment and where the OXY effect is interpreted as an anxiolytic-like action (Waldherr & Neumann 2007; Windle *et al.* 1997, 2006). Therefore, we first verified the purported anxiolytic-like action of the peptides in intact rats tested in the used comparatively large OF arena (Study 1).

The OF test measures the emotion/anxiety reaction of rats and mice to their forcefully placement into an aversive, frightening and consequently stressogenic space (Prut & Belzung 2003; Ramos & Mormede 1998; Genaro & Schmidek 2000). It has been widely accepted that in the OF anxiolytic drugs (like benzodiazepines) increase locomotion by reducing inhibition of exploratory behavior induced by the enviromental stress and anxiety. An additional criterion suggested for assessing anxiety attenuation utilizes an important feature of rat behavior in the OF, namely the preference to walk or stay close to the walls, which indicates an anxiety-relieving body contact (thigmotaxis) (Eilam 2003; Genaro & Schmidek 2000). Accordingly, the increase of movement in the inner part of the OF arena without the increase in total distance traveled, expressed as increase of the ratio central/total locomotion (CMD/TMD), are considered to reflect anxiety attenuation (Prut & Belzung 2003).

In the present experiment, in accordance with our previous results (Klenerová *et al.* 2009b) a single low dose of OXY and CBT increased locomotion/exploration and time spent moving (TTM) when observed 1 hr after the administration (Table 1). However, movement or time spent in center remained unchanged. Among other actions, intracerebrally given OXY has been shown to influence sleep-wake behavior and to induce the state of arousal (Lancel *et al.* 2003). We may speculate that under the used experimental conditions, simultaneous anxiety attenuation and arousal, vigilance induction caused the OXY elicited locomotion enhancement. Another parameter reflecting the level of exploratory motivation, rearing frequency, was not affected by the used peptides. Grooming, an inherent feature of OXY effect increased, but the difference did not reach significance.

The purpose of Study 2 ad 3 was to evaluate the capability of both peptides to cope with the behavioral consequences of the restraint stress exposure. In our previous studies employing the OF, the acute restraint stress decreased both horizontal and vertical activity, although the difference did not always attain significance (Klenerová *et al.* 2006; 2008; 2009a). The deficits in exploratory activity produced by stressors like

restraint or inescapable foot-shock have been interpreted as being related to increased fear and/or anxiety (Ramos & Mormede 1998; Carli *et al.* 1989; Pijlman *et al.* 2003a; Shinba *et al.* 2001; Tsuji *et al.* 2000; Van Dijken *et al.* 1992; Nosek *et al.* 2008). However, also an initial and gradually developing increase or bidirectional changes in mobility measures were displayed in response to physical or psychogenic type of stressors (Pijlman *et al.* 2003b; El-Hage & Belzoni 2002; Pynoos *et al.* 1996; Sawamura *et al.* 2004). In the present experiment (Study 2) we found reduced movement parameters in rats tested one hour after the IS presentation, but only deficit in time spent in rearing, and decrease in speed of movement reached significance (Study 2, Day 2). The decrease in these energy as well as motor coordination demanding activities indicates that besides the commonly presumed alteration of psychological functions (arousal, emotion, anxiety, exploratory motivation) also physical impairment may play a role in the behavioral response to acute stress exposure. CBT and partly OXY restored these motor deficits (Table 2).

Testing repeated on Day 8 revealed an increase of overall traveled distance, with higher proportion of center locomotion in stressed rats. Due to simultaneous enhancement of both parameters, the increase of CMD/TMD ratio did not attain significant difference compared to control group. The increase in both locomotion parameters as well as in total activity measure, TTM, have been prevented by CBT, but not by OXY. In this experiment, stress and treatment preceded testing by 1 hour. Although the interval appears to be long enough to prevent potential impact of contextual association between stress and the OF exposure, we postponed testing for 5 days in Study 3. The results again showed post-stress developing behavioral changes characterized by increased locomotion in the whole space and also in the OF inner part 11 days after stress. CBT given before or after stress attenuated the behavioral consequences.

The EPM like the OF represents for laboratory rodents a fear-inducing experimental environment, in which they always will prefer the sites where the possible "danger" is minimal, concretely the closed arms (Pellow & File 1986; Rodgers & Cole 1994; Ruarte & Alvares 1999; Martinez *et al.* 2002). As shown in Table 2, this prediction was confirmed in control rats: the movement in closed arms as well as time spent there were in principle higher than those measured in open arms. Rats tested shortly after the first stress exposure (Study 2, Day 1) showed a decrease in activity parameters: lower general mobility, lower distance traveled in all, specifically in closed arms.This alteration may be, similarly as in the OF, to some extent attributed to physical impairment. OXY and CBT repaired the deficit of the distance traveled in all arms. Neither treatment influenced parameters indexing the anxiety level, most importantly time spent in open arms. Repeated stressor exposure (Day 3) led to a decreased locomotion only in closed arms and not prevented either by either peptide. However, rats treated with CBT displayed almost twofold increase in percentage of time spent in open arms. Increase in this variable appeared also four days later in all stressed animals. These results show that the used restraint stressor produced a long-term modification of behavior in the EPM that can be interpreted as an alteration of the sense of danger, in another words a certain loss of fear (anxiolytic effect). The finding in mice can support this idea: an increase in open arms entries developed 18 days post-stress (El-Hage & Belzung 2002).

In the present study the late behavioral consequences of the stressor exposure were detected as an increased level of exploratory activity in both OF test. Altered psychological functions leading to changed behavior could involve increased arousal, lower anxiety level, a change in motivation or numbing (Korte 2001; Ramos & Mormede 1998; Steckler 2001; Yehuda & Antelman 1993). In previous identical experiments, we found enhancement of behavioral response to a low dose of amphetamine, concretely of locomotion and rearing 20 days after stress exposure (Klenerova *et al.* 2006). Long lasting sensitization to amphetamine has been observed in rats stressed with repeated mild tail pressure and proposed as being related to sensitization of brain dopamine mechanisms (Antelman *et al.* 1980). In the present experiments we also found an increase of OF inner part movement, reflecting anxiety attenuation (Study 2, Day 8; Study 3, Day 14), however, this variable appeared to be partly dependent on the increase of the total locomotion as revealed by not-significant increase of CMD/TMD ratios. The results might be influenced by the large size of the OF arena: voles, the social burrow-dwelling rodents, traveled in the small arenas both in the center and along the perimeter, whereas in the large arenas they mainly moved close to the walls (Eilam 2003). We deem it is plausible to contemplate that under the used experimental conditions the overall movement also express lower anxiety level.

## CONCLUSION

Repeated restraint stress exposure produced acute and persisting effects on rat behavior in the OF and EPM tests. The long-term alterations in the OF performance indicate influence of factors like anxiety attenuation and vigilance, motivation enhancement. Changes found in the EPM behavior suggest an altered perception and responsiveness to strange, noxious stimuli. Both the short and long-term alterations of behavior in the OF could be prevented or modified by the used peptides injected intraperitoneally shortly after the stress termination: OXY was effective in preventing the acute locomotor inhibition in the OF, but it failed to attenuate the persisting sequel of the stress. CBT injected either before or after stress practically abolished the developed changes in the mobility parameters. In addi-

tion, the earlier onset of the increase in the EPM open arms stay exhibited by CBT treated rats indicates the influence of the analog on the post-stress developing changes in emotion/anxiety level. The CBT effectiveness to ameliorate the late post-stress behavioral alteration supports the notion of its therapeutic potential in psychiatric disorders in which the role of OXY has been implicated.

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