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Study of locomotion, rearing and grooming activity after single and/or concomitant lesions of central and peripheral nervous system in rats

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Abstract

OBJECTIVE: Locomotion, rearing and grooming represent different forms of behaviour and motor activity in rats. In this study, changes in these activities were analysed in relation to impaired function of the nervous system by single and/or concomitant lesions representing an experimental model of the dual diagnosis.

METHODS: 32 rats were divided into 4 groups of 8 rats: intact rats, rats with single lesion of peripheral nervous system (PNS) – Marcaine neuropathy, rats with single CNS lesion – cellular brain edema induced by water intoxication, and the concomitant lesions (combination of CNS and PNS lesion in one rat). Water intoxication was performed in a standard way by fractionated hyperhydration. The average time spent by locomotion, rearing and grooming was registered and analyzed using an open field test.

RESULTS: All activities of the rats after water intoxication became inhibited due to the generally suppressive effect of brain edema. Lesion of PNS reduced activity in locomotion only, because for rearing and grooming activities, the function of the forelimb is not dominant. Combination of lesions (dual diagnosis) reduced locomotion and rearing activity more than single lesions, and enhances the stressogenic effect, which was manifested by a long periods of grooming.

CONCLUSION: Results of our study confirmed the physiological and pathophysiological differences in the movement stereotype between locomotion, rearing and grooming caused by the characteristics and algorithms of the movements, which are inborn to rats – the dominant role of the forelimbs in locomotion, the dominant exploratory activity in rearing, and the precise syntactic movement pattern in grooming.

Abbreviations:

L	- locomotion
R	- rearing
G	- grooming
MN	- Marcaine neuropathy
DD	- dual diagnosis
CNS	- central nervous system
PNS	- peripheral nervous system
WI	- water intoxication
SEM	- standard error of mean
i.p.	- intraperitoneally
DW	- distil water
deg C	- degrees Celsius
AQP	- aquaporin
ADH	- antidiuretic hormone

ORIGINAL ARTICLE

INTRODUCTION

The concept of dual diagnosis was originally introduced into psychiatric clinical practice for the simultaneous occurrence of mental illness and alcohol or drug addiction (Ridgely *et al.* 1990). Since the mid-1980s it has been used also for simultaneous brain and spinal cord injuries, in order to develop new treatment and rehabilitation protocols for adequate and optimal procedures that would respect the mutual interaction of the consequences of the two different injuries. In the present literature, attention in this field is given to the issue of clinical studies, but also to the presentation of experimental models (Arzaga *et al.* 2003; Macciocchi *et al.* 2008; Inoue *et al.* 2013; Kokotilo *et al.* 2009; Schallert & Woodlee 2003; Strong *et al.* 2009).

In our laboratory, an experimental dual diagnosis model was developed on the bases of concomitant lesions – cellular brain edema induced by water intoxication (WI) – (CNS lesion) plus brachial plexus blockade induced by application of a local anaesthetic – (PNS lesion) and their effects were tested according to motor activity assessed with the open field test (Hall 1934; Prut & Belzung 2003; Aragão *et al.* 2011; Russell *et al.* 2011; Jandová *et al.* 2014; Kozler *et al.* 2013; Šlamberová *et al.* 2013).

In the present study the elementary spontaneous motor activities were recorded and analyzed: horizontal movement – locomotion, vertical movement (rearing) – exploratory behaviour and grooming – comfort behaviour (Schaller t& Woodlee 2003; Sousa *et al.* 2006; Kalueff & Tuohimaa 2005). The aim of this study was to find out how the elementary spontaneous movements of adult rats are influenced by a single lesion (induced brain edema or blocking of the brachial plexus) and by a concomitant one (combined CNS and PNS lesions in one rat).

MATERIAL AND METHODS

All experiments were approved by the Ethical Committee of the First Faculty of Medicine (Charles University in Prague) and were in agreement with the Guidelines of the Animal Protection Law of the Czech Republic and Guidelines for the treatment of laboratory animals EU Guidelines 86/609/EEC. Experiments were done using male Wistar strain rats of our own breed.

The first group of experimental animals (16 rats) was not influenced by water intoxication. One half of them (eight animals) formed group A (control, intact rats), the second half was group B (MN). Another16 rats were intoxicated with water (WI) and they were divided in a similar way: group D (WI only), group E (WI + MN) (see Table 1).

The sham groups to groups B and E were performed (sham blockade – instead of Marcaine, Aqua pro injectione was administered, groups C and F) but the results are not mentioned in the present paper; they were discussed in our previous paper (Kozler *et al.* 2017, in press).

Tab. 1. Groups of rats.

Rats without water intoxication	A Control group	B Marcaine group
No	8	8
Body weight (Mean ± SEM) g	415.5 ± 9.413	417.0 ± 11.62
Rats after water intoxication	**D WI group**	**E WI + MN group**
No	8	8
Body weight (Mean ± SEM) g	409.8 ± 7.762	409.4 ± 7.250

Surgery

Injection of solutions in groups B, C, E and F was performed in spontaneously ventilating rats in inhalation anaesthesia with isoflurane (Florane ®, AbbVie s.r.o.). In the back position with abducted and fixed limbs, a needle was introduced through the skin into shoulder of the right forelimb and within three minutes one third of the total dose was injected to the inner part of the brachial plexus, another one third was applied below and the remaining one above the plexus. To block the brachial plexus, the local anaesthetic Marcaine in a 0.5% solution was used. Marcaine, 0.5% (bupivacaine hydrochloride solution for injection, MARCAINE©, AstraZeneca plc) is a local amide type anaesthetic with a rapid onset and prolonged action. It brings a long-lasting reversible blockage of the vegetative, sensory and motor nerves as well as the cardiac conductive system. Marcaine blocks flow of the ions across the membrane of the nerve fibers, thereby blocking the development and propagation of action potentials.The size of the dose used for blocking the brachial plexus was determined according to the recommended dose for an adult human (70 kg), used in clinical practice that is maximally effective and safe yet nontoxic (www.medicines.org.uk/emc/medicine/23926). For the blockade of brachial plexus in rats, the dose of 0.2 ml of Marcaine 0.5% solution was used (see average weight of the rats used in the experiment – Table 1). For animals in experimental groups C and F (sham), 0.2 ml of Agua pro injectione was administered instead of Marcaine.

After administration, the inhalation anaesthesia was completed and rats were let to awake spontaneously on the sideway position. After the rat awakened (return of the righting reflex and spontaneous movements), the open field test was performed. The time interval between the end of inhalation anaesthesia and the beginning of the test was 25–30 minutes, depending on and the duration of wakening from anaesthesia.

Water intoxication

Water intoxication was achieved by fractionized hyperhydration combined with administration of an

antidiuretic drug desmopressin. Distil water (DW) in the total amount corresponding to 20% of the animal's body weight was administered intraperitoneally (i.p.) in three consecutive doses within 24 hours.To prolong effect of water intoxication, antidiuretic drug desmopressin was administered along with each water injection (1/3 of the total dose). Desmopressin (1-desamino-8-D-arginine vasopressin) is an analogue of the human hormone arginine vasopressin (the antidiuretic hormone, or ADH).

Desmopressin (OCTOSTIM®, Ferring) was administered at a dose of 0.03 μg/kg (www.rxmed.com/b.main/b2.pharmaceutical/OCTOSTIM.html,www.drugs.com/mmx/octostim.html#citec00119503).

The hyperhydration procedure was standard and corresponded to literary data (Olson *et al.* 1994; Vajda *et al.* 2000; Manley *et al.* 2000; Yamaguchi *et al.* 1997; Silver *et al.* 1999).

Open field test

To test the motor activity of rats, we used the system Laboras (Metris, B.V., Netherland) for continuous registration and analysis of physical activity. It consists of triangular shaped sensing platform (carbon fiber plate 700 mm × 700 mm × 1000 mm × 30 mm), positioned on two orthogonally placed sensor-transducers and third fixed point attached to bottom plate. Makrolon cage (type III, 840 cm^2) is placed on this platform. Any mechanical vibrations caused by the movement of the animal are converted into electrical signals, which are then evaluated using software Laboras. Animals were tested in a darkened room at a constant room temperature 22 to 23 deg C, always in the same time, between 9:00 and 12:00. Horizontal locomotor activity – average time spent in locomotion (s) and vertical activities – rearing (exploratory behaviour) – average duration of rearing (s), grooming (comfort behaviour) – average duration of grooming (s) during one hour at time intervals of ten minutes were recorded and analyzed.

Statistical evaluation

The results of all measurements were statistically evaluated using the tests of the GraphPad Prism program (parametric ANOVA and nonparametric Kruskal-Wallis test, the statistical significance was set at 5%).

RESULTS

Motor activity of the individual experimental groups is given in the following graphs: average time spent in locomotion (s), average duration of rearing (s) and average duration of grooming (s) during one hour of the observation.

From Figures 1 and 2, the following results can be summarized: motor activities when compared to control group A, locomotion was significantly decreased in all experimental groups. Rearing and grooming was significantly decreased in all groups except for group B. When motor activities were compared in groups with a single lesion (group B, D) and the group with concomitant lesions (group E), locomotion in group E was significantly lower than that of groups B and D, rearing in group E was significantly decreased only against group B, grooming in groups E and D was significantly higher than that in group B.

DISCUSSION

To study the spontaneous behaviour of rats, three basic movement stereotypes were selected for our work according to recent published data (Sousa *et al.* 2006). Using an open field test, interval of one hour, divided in ten minute periods was used to register and analyze activity of experimental and control rats. The

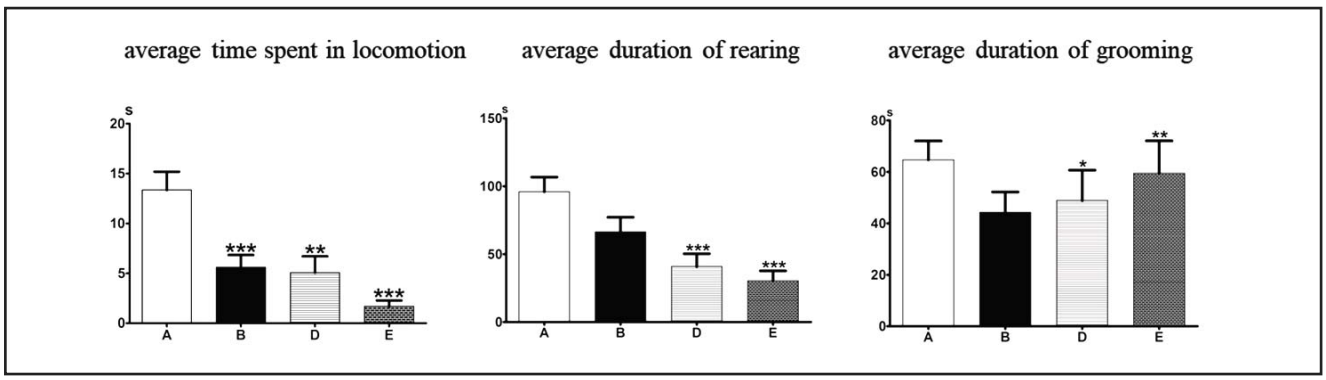

Fig. 1. Comparison of the patterns of motor activity in control and experimental groups. Horizontal axis: groups of animals (see Tab. 1), vertical axis: duration of the motor activity in the studied categories given in seconds (s), experimental groups were compared to control group A (*p<0.05, **p<0.01, ***p<0.001); results are given in averages ±SEM. In locomotion (left), activity in group B (Marcaine only) was significantly lower compared to activity in control group A. In rearing (middle) and grooming (right) activity in group B vs. activity in control group A was not suppressed. In rats after water intoxication (D) all activity was significantly smaller than in animals of the control group A. In rats with concomitant lesions (E) the activity was significantly lower in locomotion and rearing (left, middle) while in grooming the activity was higher (right) than in animals of the control group A.

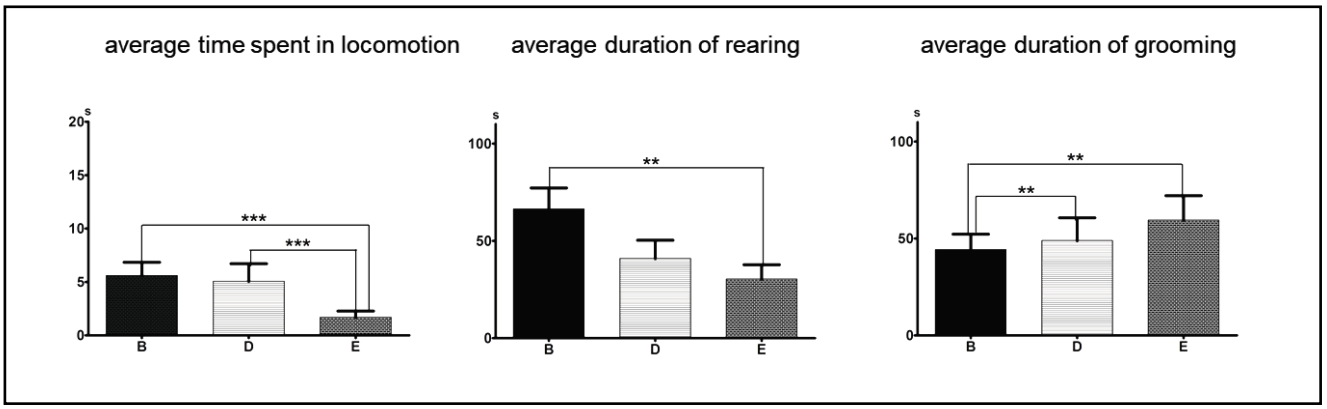

Fig. 2. Comparison of the patterns of motor activity in animals with different type of lesion. Horizontal axis: Group B – PNS single lesion (MN), group D – CNS single lesion (WI), and group E – concomitant lesions (MN+WI), vertical axis: duration of the motor activity in the studied categories given in seconds (s), (*p<0.05, **p<0.01, ***p<0.001); results are given in averages ±SEM. When locomotion was studied (average time spent by locomotion) (left), a significant decrease in locomotor activity was found in group E over groups B and D. When rearing behaviour was considered (middle), a significant decrease in activity in group E compared to group B was found. The activity of the animals in group D did not differ from the activities in groups B and E. When the time of grooming was recorded (right), a significant decrease in activity in group B was found in comparison with activities in groups D and E. The activity of rats between groups D and E was not different.

first pattern of behaviour studied was the locomotion, expressed as the average time spent in locomotion (s), represents a horizontal movement activity. The other two monitored activities were vertical: rearing – which in addition to motor activity represents also the exploratory behaviour, and grooming – which is included into the category of comfort behaviour. Both of these last-mentioned vertical activities can be expressed not only as a time spent on the activity but also as a frequency of this activity. In order to keep homogeneity of the results, also the vertical activities were recorded and analyzed only over the time as an average duration of rearing (s) and average duration of grooming (s).

In the study of locomotion it is necessary to consider that the rat is primarily "front-wheel drive" – their forelimbs are dominant for their spontaneous locomotion (Schallert & Woodlee 2003). For the normal functioning of forelimbs the corticospinal pathway supported with the extrapyramidal motor systems are essential (Whishaw *et al.* 1993).

Rearing is included among vertical motion activities, but unlike to locomotion, the movement is performed in a position on the hind legs with the forelegs leaning against the walls of the cage. The aim of the movement is to familiarize with the environment and usually to look for a source of food (Sousa *et al.* 2006). Beside the role of motor pathways, rearing is generated from the medial prefrontal cortex, amygdala and hippocampus (Levine *et al.* 2008;Vitalo *et al.* 2009; Alves *et al.* 2005).

Grooming represents a motor activity performed exclusively by the front limbs in the resting position on the hind limbs (comfort behaviour) (Sousa *et al.* 2006). Grooming in the rat consists of 4–5 sequences routinely, up to 100x repeated movements coordinated into the craniocaudal syntactic pattern. This "no stress comfort grooming" is an inborn pattern and represents the main activity of a rat when it is not sleeping (Jolles *et al.* 1979). A completely different type of grooming is stress-induced "displacement grooming," in which the syntactic pattern of the movement is completely disintegrated and rat is producing chaotic, uncoordinated movements of the upper limbs. This difference in motion pattern is used to study responses to stress situations (Kalueff & Tuohimaa 2005). The anatomical structure generating syntactic grooming is in the striatum along with a number of connections with the brain stem (Berridge 1989; Berridge & Whishaw 1992).

For the induction of lesions that influenced the locomotor activity, effect of the local anaesthetic Marcaine was used (see the method in detail). For central nervous system lesion, water intoxication was chosen (water intoxication by fractionated hyperhydration along with desmopressin administration – see method in detail). This method induces cytotoxic, cellular brain edema, whose development, timing and consequences have been described by Liang *et al.* (2007). In particular, the authors emphasize that cytotoxic edema, defined as a premorbid cellular process irrespective of the mode of induction (oncotic, ischemic, traumatic), may lead to some functional and anatomical abnormalities (Marešová *et al.* 2014; Kozler & Pokorný 2012; Creed *et al.* 2011; Onaya 2002). Besides, the cellular cytotoxic brain edema inhibits all brain activities, including spontaneous motor activity, because the centers and pathways that control this activity are affected by brain edema as well (Inoue *et al.* 2013).

As stated in the Introduction, this work should answer two questions – whether the lesions of the peripheral or/and central nervous system affect rat's motor activities (first question) and whether the concomitant lesions lead to a higher degree of functional impairment than the single one (second question).

The answer to the first question is given in Figure 1. In locomotion, activity in group B (MN) was significantly lower compared to activity in control group A. In rearing and grooming activity in group B vs. activity in control group A was not suppressed. In rearing or grooming the dominant role of the forelimbs is not typical and therefore no significant difference between the intact rats (A) and rats with the blockade of the brachial plexus (B-MN) was observed in contrast to decreased locomotion where the normal function of the forelimbs plays a crucial role (Schallert & Woodlee 2003).

The second type of single lesion by the induction of brain cellular edema (D) brought about inhibition of all the motor activity. Significantly decreased activity in all categories of motor behaviour in water intoxicated rats corresponds to the general decrease of brain activity caused by edema, regardless of the area from which the activity is generated (Whishaw *et al.* 1993; Levine *et al.* 2008; Vitalo *et al.* 2009; Alves *et al.* 2005; Sousa *et al.* 2006; Jolles *et al.* 1979; Kalueff & Tuohimaa 2005; Whishaw *et al.* 1999; Cromwell & Berridge 1996; Kruk *et al.* 1998).

The answer to the second question can be found in Figure 2 which presents results comparing the locomotor activity in isolated lesion groups (B, D) in relation to the combined CNS and PNS lesions (E group).

In locomotion, E group activity was significantly lower than that of B group (MN only) and D group (WI only). The combination of lesions resulted in more intensive motor deficit than the single lesions. This result is fully in agreement with the published finding that contralateral (left) cortex damage along with spinal cord injury at the root level C 5 on the right leads to significantly more severe right anterior limb damage than isolated spinal cord injury (Inoue *et al.* 2013). From this perspective, our result represent a contribution to the discussion of dual lesions in the nervous system (Kokotilo *et al.* 2009; Schallert & Woodlee 2003; Strong *et al.* 2009).

In rearing no difference between groups D and E (after WI – Figure 2) was found, as well as between groups A and B (without WI – Figure 1), indicating that for the vertical movement at the hind limb position during exploration the function of the forelimbs is not dominant. These results indicate that induced cellular edema (D, E) generally inhibits brain activity but does not affect the basic stereotype of the vertical activity of the rat. This finding supports the comparison of the mean duration of rearing in rats with isolated lesions in groups B (MN) and D (WI) versus the group E with both lesions (Figure 2 middle), which showed a statistically significant decrease in the activity of the rearing only in the group E with a concomitant lesions where the stereotype of the vertical activity of the rearing is affected by both lesions of the nervous system (Sousa *et al.* 2006; Levine *et al.* 2008; Vitalo *et al.* 2009; Alves *et al.* 2005). In isolated lesions in groups D (WI) and B (MN), both locomotion and rearing was lower compared to controls; the lowest activity, however, was always in rats with concomitant lesions (group E).

For the grooming the situation was different(see Figure 2 right). The equally long grooming time for groups D and E goes parallel to the rearing, and reflects the fact that in vertical motion activities the rat behaviour is affected more by the cellular brain edema than by peripheral lesions of the forelimb. The grooming time for D and E groups was significantly longer than that of Marcaine only. The result can be considered as evidence that brain edema (D) and edema of the brain accompanied with a peripheral lesion (E) is highly stressful and rat responds spontaneously to this situation by producing the movements that are inborn, automatical and most frequently performed when awaken. While the duration of grooming in group D (WI) was still significantly lower comparing with intact rats (A) which reflects the general inhibition of brain activity due to induced cellular edema, the duration of grooming in group E (concomitant lesions) was even longer than that in group A.

This finding underlined the evidence that grooming represents more "behaviour" activity than the "locomotor" one (Sousa *et al.* 2006).

In our experiment we observed the movement activity during grooming – described in details by Sousa *et al.* (2006) and by Kalueff & Tuohimaa (2005) – of rats in group A and group E. While the intact rats exhibit the classical syntactic movement pattern, the rats with induced cellular edema plus peripheral lesion produced chaotic, uncoordinated movements of the upper limbs. Our observation proved that grooming of rats in group A was a stress-free comfort behaviour and the activity of rats in group E was a stress-based "displacement grooming" (Sousa *et al.* 2006; Kalueff & Tuohimaa 2005; Jolles *et al.* 1979; Whishaw *et al.* 1999; Cromwell *et al.* 1996; Kruk *et al.* 1998).

CONCLUSION

Results of our study confirmed the physiological and pathophysiological differences in the movement stereotype between locomotion, rearing and grooming. These differences arise from various anatomical structures that generate individual activities – the corticospinal pathway with the support of extrapyramidal motor systems (locomotion), medial prefrontal cortex, amygdala and hippocampus (rearing), striatum and brain stem (grooming). Furthermore, the differences are caused by the characteristics and algorithms of the movements, which are inborn to rats – the dominant role of the forelimbs in locomotion, the dominant exploratory activity in rearing, the precise syntactic movement pattern in grooming. Despite these differences, all rat activities after induction of cellular brain edema by water intoxication became inhibited. This was due to the generally suppressive effect of brain edema without any isolated damage in the structures generating individual activi-

ties. Induced peripheral lesions reduced activity in locomotion only, because for the rearing and grooming activities, the function of the forelimb is not dominant. Dual diagnosis of concomitantly induced lesions (cellular edema + peripheral lesions) reduced locomotion and rearing activity more than isolated lesions (cellular edema or peripheral lesions). This finding confirms the literary data on the higher degree of functional impairment in dual diagnoses of CNS injuries (brain and spinal cord). In our study, the combined impairment of the central and peripheral nervous system were used the first time. Dual diagnosis enhances the stressogenic effect, which was manifested by a long periods of grooming, which lack the typical syntactic pattern.

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