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Motor activity of rats with peripheral nerve lesion

Dana Marešová[1], *Petr Kozler[1], Karla Kotková[2]
1.Institute of Physiology, First Faculty of Medicine, Charles University in Prague, Czech Republic
2.Department of Rehabilitation Medicine of the First Faculty of Medicine of Charles University and General Teaching Hospital in Prague, Czech Republic

ABSTRACT

The crucial factor for the locomotion of rats is the activity of forelimbs. Impairment of the control of a single forelimb brings alteration of motor activity of a degree that could be identified in the open field test. Three groups of adult Wistar rats were used: A Control group A (healthy animals), Marcain group B (blockade of the nerves of the right forelimb plexus by Marcain), Sham group C (Aqua for injection was used instead of Marcain). For the horizontal locomotion the average time spent in locomotion (s), average distance travelled (m) and average speed of locomotion (m/s) during one hour, at time intervals of ten minutes were recorded. Results confirm the role of forelimbs for locomotion of rats - inactivation of one forelimb brings reduction of all parameters of locomotion ($p < 0.001$). Paradoxically, no difference in any of the studied parameters of locomotion was found between the experimental groups B (Marcain) and C (Sham). The malfunction of the forelimb in sham group could be explained by volumetric effect of the fluid injection. Open field test is sufficiently sensitive method for registration of functional impairment of a single forelimb but it is not adequate to distinguish effect of local anaesthesia from the fluid compression.

Keywords: Peripheral nerve, locomotor activity, open field test, rat

*Corresponding Author Email: petr.kozler@lf1.cuni.cz
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INTRODUCTION

Study of motor activity is an important part of the experimental models of neural disorders of rats. From the currently used methods we employed the open field test, introduced into practice by Hall in 1934. The spectrum of parameters studied in the open field test horizontal locomotion, the effects of different drugs affect pain and neurological deficits and results of treatment methods for models various diseases and injuries [1-6].

For the present study, we have formulated this hypothesis: sensitivity and specificity of open-field test is sufficiently high to register and analyze malfunction of one of the front legs.

Our working hypothesis was based on the known fact that rats are primarily "front-wheel drive" – for the spontaneous locomotion front legs are dominant. If their forelimbs are lifted above the floor, the rats are unable to walk on hind legs only. In the position on both hind legs - the rat is able to initiate gait with one leg while the other rotates on the ground or does few small steps backward or sideward. However, if hind legs are lifted above the floor - the position on both front legs- the rat is able to walk immediately and cover a long distance in a position resembling driving the wheelbarrow ("wheelbarrow posture") [7]. To identify impaired function of the right forelimb, we therefore concentrated on testing the horizontal motor activities.

MATERIALS AND METHOD

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 carried out on adult male Wistar rats of our own breed (weight 413.7 ± 10.46 g). Animals were divided into three groups. The control group (A) consisted of 11 animals. In eight rats of the experimental group B the nerve blockade was performed by injection of local anaesthetic into the shoulder area of the right forelimb. In the group C (sham group), Water for Injection was used instead of the anaesthetic in eight rats.

Surgery

Application of the solutions in group B and C was performed in spontaneously breathing rats under inhalation anaesthesia by isoflurane (Florante ®, AbbVie Ltd.). In the supine position with abducted and fixed limbs a needle was introduced in the skin fold of the right axilla to the shoulder girdle and one third of the dose was injected within three minutes into the inner side of the girdle and the other two thirds were injected above and below the girdle. The nerve blockade was achieved by local anaesthetic Marcaine in concentration of 0.5%. Marcain (bupivacaine hydrochloride - Marcain ©, AstraZeneca plc) is a local anaesthetic of

the amide type with medium latency time which has a rapid onset of action and causes prolonged reversible blockade of the vegetative, sensitive and motor nerves and the cardiac conduction system. Marcaine blocks the flow of ions across the membrane of nerve fibers, thus blocking the generation and propagation of action potentials.

The dose of Marcaine used to block the nerves was determined according the recommended dose for adult humans used in clinical practice, which has the maximum effect without toxic side effects[8]. For rats used in the experiment (the average weight was 413.7g) the dose was 0.2 ml of 0.5% Marcaine solution per a single injection.

Instead of Marcain, animals in the experimental group C (sham) received the same volume of Aqua for injection (B. Braun Medical), solution used for parenteral administration of water-soluble drugs. After the injection, inhalation anaesthesia was terminated and animals were left to awaken spontaneously. Physical activity recording started after the resumption of righting reflex, and the recovery of spontaneous locomotion. The time interval between the end of inhalation anesthesia and the start of testing was consistently 25 to 30 minutes.

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 x 700 mm x 1000 mm x 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), average distance travelled (m) and average speed of locomotion (m/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 AND DISCUSSION

In all rats during spontaneous physical activity, injection of both Marcain and Aqua for injection into the vicinity of the right brachial nerve plexus brought about evident malfunction of the entire right front limb.

Average time spent in locomotion analyzed at time intervals of ten minutes was in groups B and C significantly shorter than in controls (A) in the first two intervals. Graph on the bottom represents the average time spent in locomotion calculated as an average value over six 10-minutes intervals (see Figure 1).

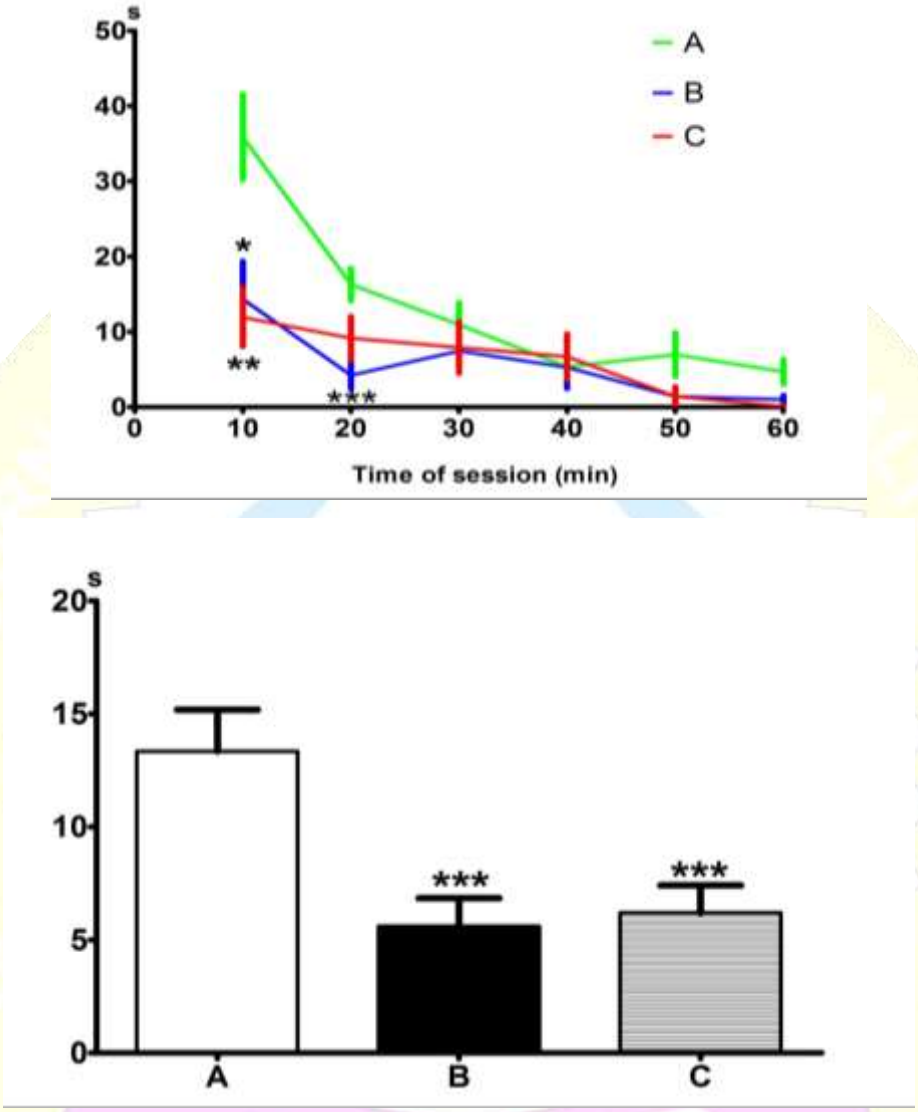

Figure 1 Average time spent in locomotion

A = Control group, B = Marcaine group, C = Sham group, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, error bars: ±SEM

Average distance travelled at time intervals of ten minutes was in groups B and C significantly shorter than in controls (A) in the first 20 min interval. Graph on the bottom represents the average distance travelled calculated as an average value over six 10-minutes intervals (see Figure 2).

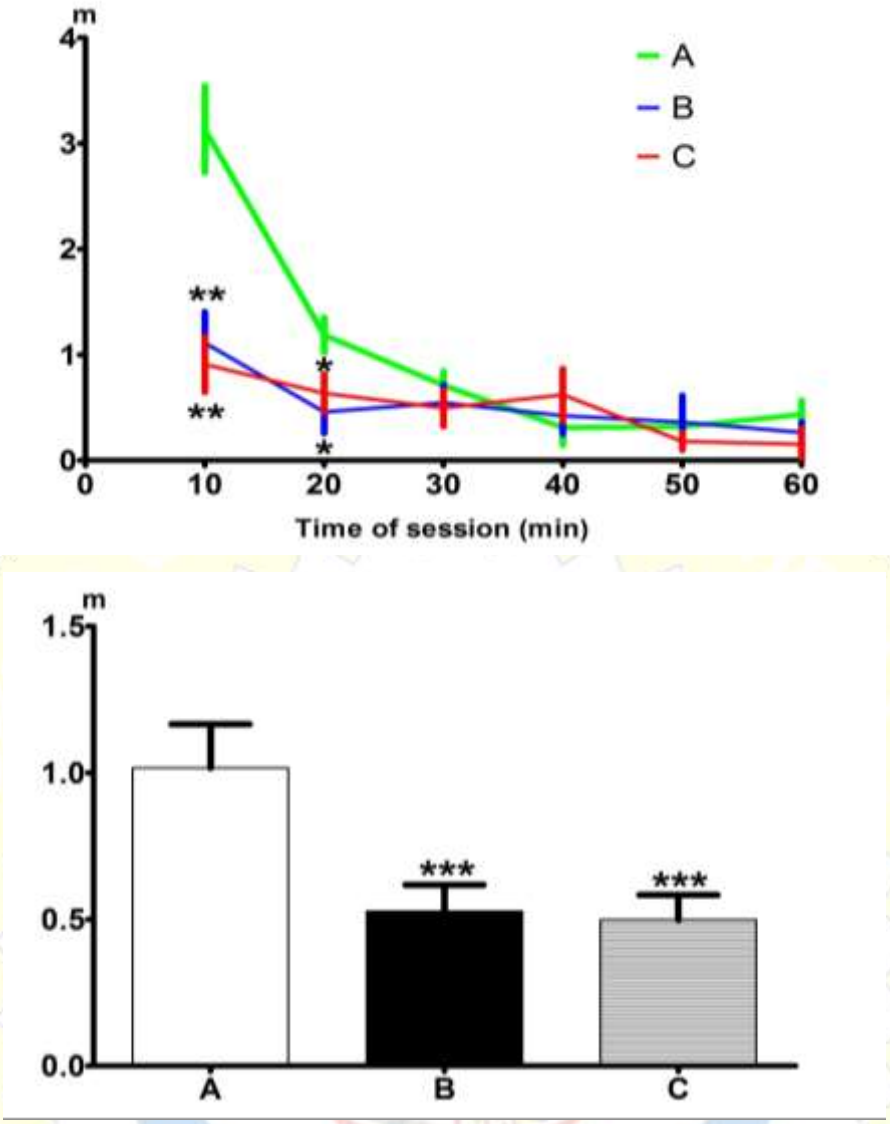

Figure 2: Average distance travelled

A = Control group, B = Marcaine group, C = Sham group, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, error bars: ±SEM

Average speed of locomotion at time intervals of ten minutes was in groups B and C significantly slower than in controls (A), both in the first and second ten minutes interval. Graph on the bottom represents the average speed of locomotion calculated as an average value over six 10-minutes interval (see Figure 3).

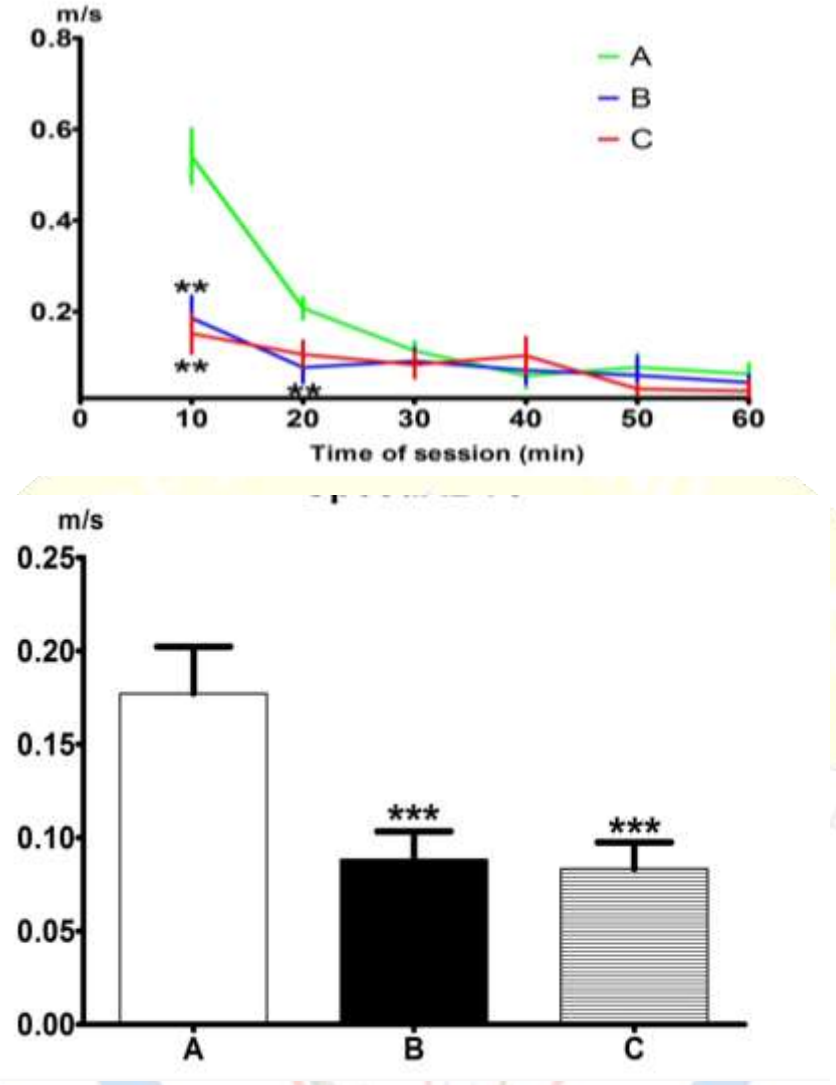

Figure 3: Average speed of locomotion

A = Control group, B = Marcaine group, C = Sham group, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, error bars: ±SEM

The crucial factor for the locomotion of rats is the activity of forelimbs. Impairment of the control of a single forelimb could bring alteration of motor activity of such intensity that it could be identified in the open field test.

When evaluating spontaneous motor activity we focused on the analysis of horizontal movement activities - average time spent in locomotion (s), average distance travelled (m) and average speed of locomotion (m/s). As we stated in the Introduction, the open field test is widely used in studies of motor activity aimed at various experimental models of the CNS impairment in rodents. Usage of open field test for examination of the peripheral nervous system lesions is much less frequent; in the PubMed database, only 7 papers dealing with this issue have been found. Most frequently (5 times), lesions of peripheral nerves of lower limbs were studied, 4 times the lesion of the sciatic nerve [9-12], one study was dealing with the lesions of peroneal nerve and tibial nerve [13]. In one paper, authors modelled facial pain by

induction of a compressive injury of n. trigeminus in order to study anxiety-related behaviours [14]. Only one paper was dealing with motor activity of forelimbs - experimental reconstruction of brachial plexus [15].

Our results showed that rats of the experimental groups B (Marcain) and C (Sham) moved within 60 minutes of testing period considerably less - only one third of the average locomotion in healthy rats with the maximum differences within the first 20 minutes of the period ($p<0.001$) (see Figure 1 - 3). Line graphs also showed inhibition of spontaneous locomotor activity in the second half of the period. It corresponds to the phenomenon of habituation, which explains the drop in activity after the rats get used to the new environment [16].

Our results confirm the crucial role of forelimbs for locomotion of rats - the functional impairment of one of the front legs brings about significant decrease of the motor activity [7]. However, our results also showed that the fluid injection into axilla, regardless Marcain or water were injected, brings the same motor impairment on all monitored categories.

Unlike spontaneous motion activity of healthy rats of the control group, locomotor activity of rats in experimental groups B (Marcain) and C (Sham) could be affected by certain common conditions of the experimental model. Marcaine blocks the conduction of nerve fibres of the forelimb plexus. Administration of Water for Injections in Group C (Sham), which is the common solvent for parenteral administration of water-soluble drugs and is also used as "sham solution" in rodent experimental models [17,18], can elicit local pressure effects on neighbouring nervous plexi, and it could also disbalance local osmolality conditions (19). As the dose of fluid applied to the soft tissues around the shoulder plexus was 0.2 ml, we believe that discussion on hypotonic effect is purely hypothetical and the local pressure effect was the dominant factor. Manifestations of compression neuropathies, so called entrapment syndromes such as carpal tunnel syndrome, are well known in the clinical practice [20]. Nerve compression can be also caused by a fluid, such as due to lymphedema [21] or cyst [22]. Compression neuropathies were proved in experimental models [23,24]. Malfunction of the right forelimb of rats in group C (Sham) can be therefore explained by the volumetric effect.

CONCLUSION

Open field test appears to be sufficiently sensitive method for registration and analysis of functional failure of a single forelimb but it is not specific enough to distinguish within the test period of 60 minutes the functional impairment induced by anaesthetic from the compression effect of the solvent. In order to eliminate the volumetric effect, the period of recording should be extended, enabling water to be absorbed and reveal only Marcaine

anesthetic effect. However, in our dose and concentration conditions it could lasts up to eight hours.

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