Self-mutilation in young rats after dorsal rhizotomy

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Abstract

OBJECTIVES: The aim of the study was to describe the development of self-mutilation after extensive dorsal rhizotomy of the brachial plexus performed during early ontogeny in rats.

SETTINGS AND DESIGN: The rhizotomy was performed in three groups of rats according to the central nervous system maturation: infant, young, and adult. After the surgery the occurrence of self-mutilation behavior was compared. Rats from the infant group and non-mutilating deafferentated rats from the adult group underwent extracellular recordings from intralaminar thalamic neurons. Interspikes intervals of the records were compared by means of chaodynamic methods.

RESULTS: In the infant group self-mutilation did not develop at all. Among the young group self-mutilation developed in 40% of rats and consisted of superficial wounds in all cases. In adult self-mutilation appeared in 80% rats and consisted of both superficial wounds (75%) and amputation (25%). In the newborn group and the deafferentated adult group without any signs of self-mutilation means of the parameters were not significantly different and were significantly lower than those of intact adult rats.

MAIN FINDINGS: 1. Self-mutilation does not develop after the rhizotomy in the infant rats. 2. Neurons behave in chaotic way in adult as well as in young animals. 3. Chaodynamic parameters do not differ between infant and adult rats without any signs of self-mutilation.

CONCLUSIONS: The results suggest that development of self-mutilation behavior in rats strongly depends on the ontogenetical period of nervous system injury, and that mature nervous system is required for the development of described pathological behavior.

ORIGINAL ARTICLE

Abbreviations:
ANOVA – analysis of variance
BPL – brachial plexus lesion
C – cervical
ISIs – interspike intervals
LYAP – Lyapunov exponents
MUT – mutual information
SEM – standard error of the mean
SHANN – Shannon entropy
Th – thoracical

Introduction

The brachial plexus is the most common site involved in upper extremity neuropathies in human neonates. The incidence of the brachial plexus lesion (BPL) in neonates varies between 0.03 and 1%. The BPL in neonates has been traditionally divided into three groups according to the affected roots as follows: Erb's (C5-C6, ev. C7; most common), Klumpke (C8-Th1; the lowest incidence) and total [7]. The BPL in neonates impairs sensory and motor functions, sometimes evokes self-mutilation which is severe in patients with total lesion and usually consists of biting the tips of the digits [2,15]. The pathological mechanics of these injuries remain unclear, however high birth weigh, shoulder dystocia, forceps-assisted deliveries have been identified as risk factors.

The model of brachial plexus avulsion – the multiple dorsal rhizotomy in rats – was introduced in 1974 by Basbaum [5]. Various changes following the unilateral section of 3 to 9 dorsal roots at the cervicothoracical level implicate the development of chronic pain syndrome localized in the ipsilateral limb. The development of pain syndrome is affected by the number of impaired roots, gender, season, genetics, used anesthesia and prior noxious stimulation [20].

The aim of the present study was to describe the development of self-mutilation during early ontogeny in rats, and to establish an animal model of total type of neonatal BPL. Additionally, single unit activities of thalamic intralaminar nuclei after the deafferentation performed in infant rats were compared to those of non-mutilated rats deafferentated in adulthood. Results of our previous papers show that firing patterns of non-bursting intralaminar thalamic neurons after the deafferentation depend on the occurrence of self-mutilation, when investigated by chaodynamic method [18].

Methods

The deafferentation was performed during postnatal period (up to 60 days postnatally) in male Wistar rats. Dorsal rhizotomy at the cervicothoracical level (C5-Th1) was performed under pentobarbital anaesthesia (50 mg/kg; 2% solution) in 20 rats divided into three groups: infant (immature nervous system before massive reorganization, younger than 22 days), young (around NMDA receptors maturation, 23–35 days) and adult (mature nervous system, older than 36 days).

After the surgery animals were caged separately with free access to food and water, and were observed daily for a maximum of 80 days. The occurrence of self-mutilation behavior was compared after the dorsal rhizotomy between the groups. Self-mutilation was evaluated at the daily basis. Three-grade scale based on qualitative pattern was used for the evaluation of self-mutilation [18]. Immediately after the onset of self-mutilation and depending on its gravity, the rats were excluded from the group within 2 days.

Only rats from the infant group and non-mutilating deafferentated rats from the adult group were included in the electrophysiological study. At post-surgery day 80, single unit activities of intralaminar thalamic neurons were recorded in both groups. As control numbers we used records of intralaminar thalamic neurons from intact adult animals. For the recordings, animals were anaesthetized by intramuscular injection of a combination of ketamine (100 mg/kg) and xylazine (16 mg/kg). According to the Swanson atlas the stereotaxic coordinates for the intralaminar thalamic nuclei were as follows: AP –4.2 mm, LL 1.5 mm and DV 3.6–6.0 mm to the bregma as a coordination point. At the end of the recording the recording sites were iontophoretically signed by pontamine blue for further histological verification. Extracellular single neurons activities were recorded using standard technique. The data were digitized at a sampling rate of 6000 Hz. Microcal Origin 6.0 Labtalk was used for THE calculation of interspike intervals (ISIs). Then ISIs were counted and those of non-bursting cells were analyzed using chaodynamic methods, namely Lyapunov exponents, Shannon entropy and mutual information. The ISIs were used as a new time mapping to characterize the dynamics of firing neurons. For the above mentioned calculations we used the software package for the methods of chaodynamics worked out as M-tool-boxes under MATLAB at the Institute of Computer Science, Academy of Sciences, Prague and partly also TISEAN Package of Max Planck Institute for Physics of Complex System, Dresden.

The data in figures below represent the mean ± SEM. The Two by Two Tables Fischer's Exact Test was used to evaluate levels of statistical significance of the number of self-mutilating animals in the groups. To determine the statistical significance between the chaodynamic parameters, the ANOVA test (Statistica 6.0, StatSoft Inc.) was used. Differences between means were considered statistically significant if $p < 0.05$.

The experiments were permitted by the Expert Committee for Animal Care and Use of 3rd Faculty of Medicine, Charles University, Prague, and conducted according to the guidelines of the Ethics Committee of the International Association for the Study of Pain [21].

Results

Animals were observed during 80 days after the deafferentation. In their movements only occasional misplacement was noticeable. Sitting down on the hind legs, the rat kept the deafferentated forelimb in extension, but when the forelimb was to be used, again, no visible differences could be noticed. Programmed behavior (grooming) was identical with that of intact animals. The complete insensitivity of the deafferentated limb was approved by the absence of defense reflexes

to mechanical and thermal stimulation. After the onset of self-mutilation animals were euthanized or underwent other experiments in 2 days.

When rhizotomy was performed during the early postnatal period (5 rats, average age 18 days, range 16–20 days) self-mutilation did not develop at all. In five rats of the young group undergoing rhizotomy at average age 29 days (range 23–35) self-mutilation consisted of superficial wounds and its appearance (40%) was lower if compared to that in adults. Furthermore, the serious type of self-mutilation consisting of amputation of distal part of a forelimb has not been found in this group.When rhizotomy was performed in ten rats older than 36 days (average age 50 days, range 38–60) self-mutilation appeared in 80% rats and consisted of both superficial wounds (75%) and amputation (25%). In the control group used for the thalamic recordings self-mutilation was not present at all (Fig. 1).

Significant difference in the number of self-mutilating animals was found between infant rats (younger than 22 days) and adults (older than 36 days). In the infant group self-mutilation did not appear at all. Young adults group seems to be similar to the adult group, however only one type of self-mutilation appeared.

Lyapunov exponents calculated from ISIs series were positive values in all investigated neurons. In the control group mean Lyapunov exponent was 1.582 ± 0.016, while in both the newborn group and the rhizotomized adult group without any signs of self-mutilation mean Lyapunov exponents were significantly lower, 1.094 ± 0.362 and 1.159 ± 0.120, respectively. The decrease in chaodynamic parameters in the experimental groups were similar for both Shannon entropy and mutual information. Shannon entropy and mutual information average values in the control group were as follows: 1.624 ± 0.131 and 0.170 ± 0.062, respectively. In the newborn group the above-mentioned parameters were 1.263 ± 0.100, and 0,036 ± 0.010 respectively. In the rhizotomized adult animals without any signs of self-mutilation the means of Shannon entropy and mutual information were: 1.038 ± 0.146 and 0.039 ± 0.008, respectively (Fig. 2).

Histograms of ISIs are shown in the Fig. 3.

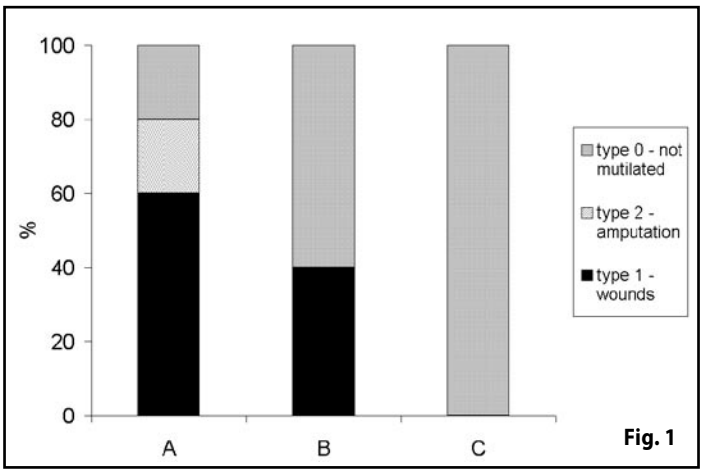

Fig. 1

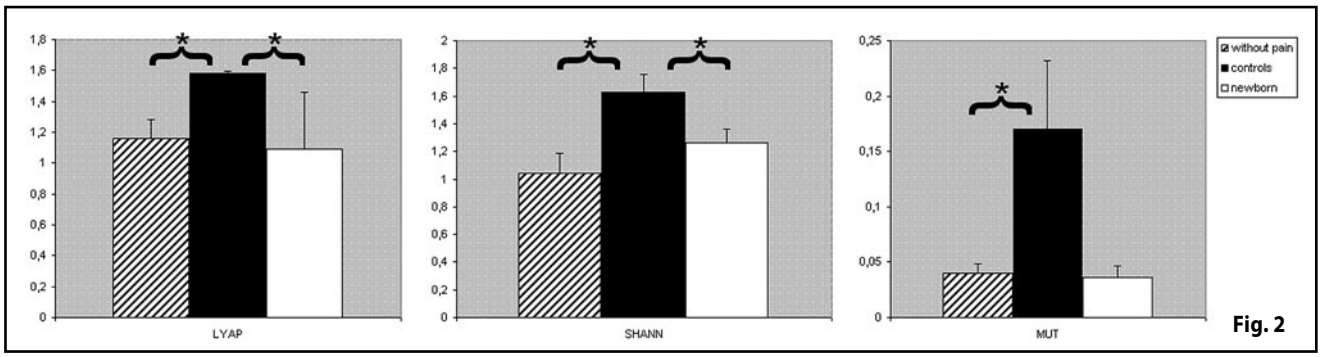

Fig. 2

Figure 1: The occurrence of self-mutilation after the deafferentation in three age groups and its types. A – adult rats, B – young rats, C – infant rats.

Figure 2: Comparison of Lyapunov exponents, Shanon entropy and mutual information of ISIs in two experimental groups (newborn: deafferentation performed in infant rats; without pain: non-mutilating deafferentated rats from the adult group) and in control animals (mean ± SEM; n = 5–6). * $p < 0.05$, ANOVA test.

Figure 3: Histogram of interspikes intervals in two experimental groups (newborn: deafferentation performed in infant rats; without pain: non-mutilating deafferentated rats from the adult group) and in control animals. Distributions of ISIs is almost the same in two experimental groups suggesting functionally similar neural network in both groups.

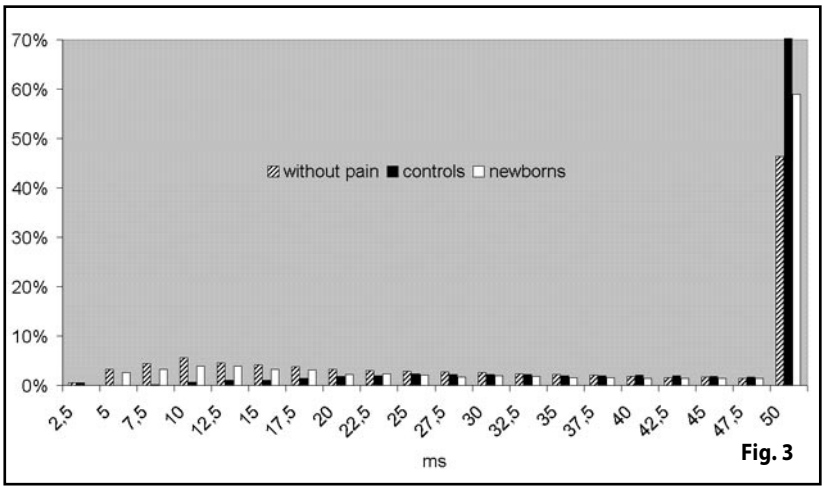

Fig. 3

Discussion

Unilateral dorsal rhizotomy of brachial plexus nerves (C5-T1 deafferentation) is known to induce self-mutilation in rats. There are conflicting views about the correlation between self-mutilation and pain sensation in animal models [14]. However, evidence would point rather to the theory of self-mutilation as a behavioral correlate of pain sensation [10,11,16,20].

There was high mortality rate in the infant group.In spite of special attention and care applied after the dorsal rhizotomy, successful recovery was not achieved in rats younger than 16 days.

In terms of motor function no difference between the groups with the deafferentation was found. When feedback sensation from proprio~ and exteroreceptors is not required for precise movement, no visible difference in the movements was observed. It is especially so in the programmed behavior like grooming.

In the electrophysiological experiments we focused on non-bursting cell because it was shown that bursts correlate rather to deafferentation than to self-mutilation [17,19]. Chaotic behavior has been found in all investigated neurons. This is confirmed by positive values of Lyapunov exponents calculated from all ISIs series. It corresponds well with previous findings [1,4,12,13,18]. When comparing dynamics of chaotic firings of recorded units by means of ISIs maps of the mentioned three groups (Fig. 2), it was found out that the deafferentation in adults without any signs of self-mutilation (as well as in the infant group) results in significantly more regular firing than that in control animals, except for the comparison of means of mutual informations between the control and the newborn groups ($p = 0.1$). The results from the adult deafferentated group have confirmed our previous findings [18]. When comparing dynamics of chaotic firings between the newborn and the adult non-mutilating group, no difference is found by means of chaodynamics.

Absence of self-mutilation in the infant group may be explained due to the immaturity of neural system at the moment of deafferentation. Central nervous system in rat matures around 3rd postnatal week and the maturation and rearrangement of NMDA receptors occurs around the 30th postnatal day [3,8,9]. Second possible explanation is based on the fact that in newborn rat spinal cord neurones receive inputs from wider fields than in adults [6]. It means that in newborns finally no neuron is left without inputs from periphery after the extensive dorsal rhizotomy. In our previous study [20] it was shown that self-mutilation in adults is not likely to develop when NMDA receptors had been blocked before the deafferentation performed in adults. Since the chaodynamic parameters of the firing of the intralaminar nuclei were almost the same either when the deafferentation had been performed in early postnatal period, or in adulthood, the results suggest rather the possibility of NMDA receptors immaturity. Then, in the young group, the self-mutilation developed likely due to the progressive maturation and specialization of NMDA receptors at that time. Serious type of self-mutilation might be based on pain experiences – amputation has been observed only in the oldest, adult group.

The results suggest that development of self-mutilation behavior in rats strongly depends on the period of nervous system injury, and that mature nervous system is required for the development of described pathological behavior. Despite the absence of self-mutilation this design might be used as a model of total type of BPL.

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