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Chronic prostatitis/chronic pelvic pain syndrome increases susceptibility to seizures in rats and alters brain levels of IL-1β and IL-6

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ABSTRACT

Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) is a result of interplay between psychological, immune, neurological and genetic factors, manifested by variety of urological, as well as brain-related symptoms. However, its relation with brain excitability has not been addressed. herefore, our aim was to assess susceptibility to seizures in rats with CP/CPPS.

We induced CP/CPPS in adult rats by intraprostatic injection of 3% λ-carrageenan. Sham operated rats served as controls (0.9% NaCl, Sham). On day 7 upon injection, rats were treated with lindane (4 mg/kg) and observed for convulsive behavior (seizure incidence, latency and severity) and EEG manifestations (number and duration of ictal periods). Interleukin levels (IL-1β and IL-6) were measured in prostate, hippocampus, thalamus and cerebral cortex. Scrotal skin mechanical pain thresholds were determined and prostates were histologically evaluated. Animals with CP/CPPS showed significantly higher incidence, decreased latency time and augmented severity of lindane-induced seizures compared with Sham group. EEG revealed increased number of ictal periods in CP/CPPS rats. Higher levels of IL-1β and IL-6 were determined in the thalamus and cortex in CP/CPPS animals vs. Sham. IL-1β level was higher and IL-6 was lower in prostates from CP/CPPS animals comparing to Sham. CP/CPPS development was verified by histological findings of nonbacterial inflammation in the prostates, as well as by significantly decreased scrotal pain threshold in CP/CPPS animals. On the basis of this research, we concluded that CP/CPPS increases susceptibility to lindane-induced seizures in rats associated with increased level of IL-1β and IL-6 in the cortex and thalamus.

1. Introduction

Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS), also known as NIH Category III prostatitis, is a multifactorial and non-specific clinical syndrome. It is characterized by genitourinary pain, urinary and ejaculatory symptoms in the absence of uropathogenic bacteria and frequently is accompanied by psychosocial disorders (Brünahl et al., 2017; Krsmanovic et al., 2014; Passavanti et al., 2017). Riegel et al. (Riegel et al., 2014) spotted connection between CP/CPPS and brain disorders, like depression, anxiety and mood alterations. Antiepileptic drugs, like gabapentin and pregabalin, are commonly used in the CP/CPPS treatment (Strauss and Dimitrakov, 2010). Namely, urinary system diseases are also frequent comorbidity of epilepsy, which is complex disorder having not only psychological and neurological, but also somatic comorbidities (Tellez-Zenteno et al., 2005; Yuen et al., 2018).

It is believed nowadays that CP/CPPS is a result of an interplay between genetic, psychological and behavioral factors, immune and endocrine dysfunction, as well as neurogenic inflammation (Arora et al., 2017; Nickel et al., 2015). Inflammatory and neuropathic pain may play a central role in the pathogenesis of CP/CPPS (Belanger and VerLee, 2016; Potts, 2016). Tissue injury or stressful events which trigger chronic peripheral inflammation or nerve injury (Breser et al., 2017) result in high production of inflammatory mediators and neurotransmitters (Silva et al., 2017), which lower the threshold of primary nociceptive neuron activation (peripheral sensitization) and lead to the development of inflammatory/neuropathic pain. Clinical evidence showed increased synthesis of proinflammatory cytokines (IL-1β, TNF-

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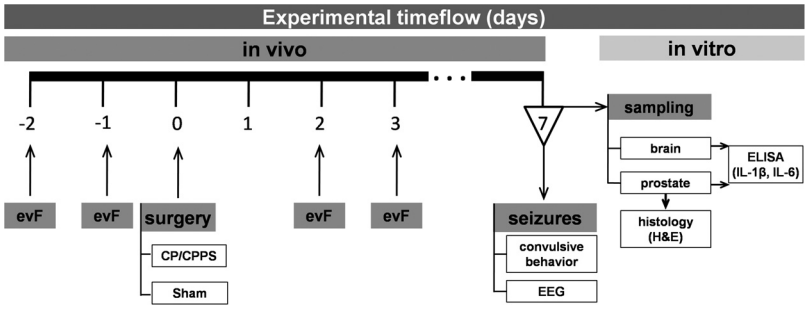

Fig. 1. Experimental time flow.
Pain threshold measurement, using electronic von Fray (evF) esthesiometer, was performed day -2 and day -1 prior to, as well as on day 2, day 3 and day 7 upon surgery (intraprostatic injection, day 0).
Challenging animals from CP/CPPS and Sham groups to lindane (seizures) was performed 7th day upon surgery. Sham and CP/CPPS animals, which have not been exposed to lindane, were sacrificed for prostates sampling for histology examination (H&E) and brain structures isolation for IL-1β and IL-6 measurement by ELISA.

α, IL-6 and IL-8) in CP/CPPS indicating their predominant involvement in the pathogenesis of this syndrome (Han et al., 2016; Pontari and Ruggieri, 2008). At the same time, the central nervous system (CNS) is responsible for continuing pain even without ongoing inputs from the peripheral nerves (Sadler and Kolber, 2016) due to the central sensitization, which results in allodynia, hyperalgesia and spontaneous pain (Potts and Payne, 2007). Pathological changes in different brain structures (like thalamus, cerebral cortex, hypothalamus and many others) could be involved in CP/CPPS development, but could also be a consequence of CP/CPPS itself (Sadler and Kolber, 2016).

Among other associated diseases, epilepsy has been reported as a frequent comorbidity in different inflammatory disorders (Tellez-Zenteno et al., 2005). Neuroinflammation has been reported as a novel mechanism of epileptogenesis (Li et al., 2011; Xu et al., 2013) that can lead to hyperexcitability, a ground base of seizures (Heise et al., 2016). The CNS is not an immunoprivileged organ (Hrncic et al., 2018), so the peripheral inflammation, followed by an increase of proinflammatory cytokines IL-1β, TNF-α, IL-6 may contribute to the activation of glial cells (Murta et al., 2015; Silverman et al., 2015). Peripheral inflammation present in CP/CPPS could affect the brain, possibly through an action of various cytokines (Rao et al., 2008), endogenous opioids and prostaglandins (Vezzani et al., 2013). However, the influence of CP/CPPS on brain excitability and propensity to seizures is not known. We hypothesized that CP/CPPS is associated with higher seizure susceptibility possibly due to chronic prostatitis evoked neuroinflammation, i.e. altered proinflammatory cytokines profile. This relationship, as well as its mechanisms, has not been previously investigated experimentally, to the best of our knowledge.

Therefore, the objective of this study was to assess the influence of experimentally induced CP/CPPS on rat susceptibility to lindane-induced seizures, a model that has been fully characterized both behaviorally and electroencephalographically (EEG), as well as brain levels of neuroinflammatory mediators IL-1β and IL-6.

2. Materials and methods

2.1. Ethical statement

All experimental procedures were in full compliance with Directive of the European Parliament and of the Council (2010/63/EU) and approved by The Ethical Committee of the University of Belgrade (Permission No. 323-07-01339/2017-05/3).

2.2. Animals and housing

Adult (three-month old, weighted 250–350 g, n = 28) male *Wistar albino* rats, obtained from the Military Medical Academy breeding laboratory (Belgrade, Serbia), were used in the experiments. The animals were housed individually in transparent plexiglas cages (55 × 35 x 30 cm) with soft bedding and access to food and water *ad libitum* during the entire experiment. They were kept under controlled ambient conditions (22–24 °C, 50 ± 5% relative humidity, 12/12 h light: dark cycle with light turned on from 8:00 to 20:00 h). Animals were used only once during the experiment. Acclimatization period to the laboratory ambient lasted for 7 days.

2.3. Experimental design and test protocol

Based on our preliminary experiments and literature data (Radhakrishnan and Nallu, 2009; Zeng et al., 2014), rats were randomly divided into control, sham operated (Sham, n = 14 in total) and experimental group with CP/CPPS (CP/CPPS, n = 14 in total). Randomization has been achieved by thawing a coin. In experimental group CP/CPPS was induced by single intraprostatic injection of 3% λ- carrageenan (mucopolysaccharides from the cell walls of the red algae) on day 0 of the experiment. In order to assess the development of pelvic pain syndrome, we evaluated mechanical pain thresholds in the scrotal skin by electronic von Frey aesthesiometer (evF). Three days before intraprostatic injection, rats were adapted to eVF aesthesiometer for pain threshold measurement. Mechanical pain thresholds were determined at different time points, 48 h (day -2) and 24 h (day-1) prior to intraprostatic injection, as well as 48 h (day 2), 72 h (day 3) and on day 7 upon intraprostatic injection. On day 7 one cohort from both groups (n = 8 per group) was used to assess seizure susceptibility by administration of subconvulsive dose of lindane (4 mg/kg i.p., CD_{50} = 5.5 mg/kg, unpublished data) forming Sham + L4 and CP/CPPS + L4 groups. Seizure susceptibility was assessed by analysis of convulsive behavior and EEG recordings as described below (Fig. 1). Another cohort of the rats from Sham and CP/CPPS groups (n = 6 per group) was sacrificed on day 7 of the experiment and prostate and brain samples were collected for determination of IL-1β and IL-6 levels. Additionally, prostate was collected for pathohistological analysis.

All experiments were performed in blinded fashion. Animals were coded. It was not possible to recognized sham and CP/CPPS rats visually.

The time course of the experiment is presented schematically on Fig. 1.

2.4. CP/CPPS model induction

Induction of CP/CPPS was performed in the accordance with described procedures (Radhakrishnan and Nallu, 2009; Zeng et al., 2014). Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and the skin of lower abdomen and scrotum was shaved before the surgery. After this procedure, rats were fixed in a supine position on a water-circulating heating pad, and the skin in the area of surgery was cleaned using three repeated applications of 70% v/v ethanol and one application of 10% povidone-iodine solution. Local anesthetic (2% lidocaine) was applied in order to reduce postoperative pain and minimize sensitization of the wound surrounding area. Hereupon, a small midline incision (1.5 cm) was made in the sterile area of abdominal wall and the ventral lobes of the prostate gland were exposed. Using a disposable, sterile 30 G needle and sterilized Hamilton syringe, sterile suspension of 3% λ-carrageenan in a volume of 50 µl was injected in both ventral lobes of the prostate gland (CP/CPPS group).

Control animals were treated by intraprostatic injection of the equal

volume of sterile 0.9% saline (Sham group). After the injection, a 2% lidocaine solution was applied to the wound again. Wound was closed in layers using #4.0 sterilized absorbable suture materials.

2.5. Pelvic pain syndrome assessment

At the beginning of the measurement, rats were placed in plexiglas cubicles on a wire mesh platform for 30 min to acclimatize. Electronic von Frey aesthesiometer (IITC Life Sciences, CA) with rigid filaments was used. Stimulation was performed when the rat remained quiet with the scrotum resting on bottom of cage. Hereupon, evF filament was applied to the scrotal skin with gradual increase of the pressure, until the animal responded by moving from the original position.

The average value of three stimulations was used for analysis. After the measurement of the pain behavior, animals were returned to their respective cages.

2.6. Prostate histology

Prostates were removed from the rat body instantaneously upon sacrifice, fixed in 10% buffered formalin, dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Five μm thick sections were subjected to routine staining with hematoxylin and eosin (HE) and examined under the Olympus BX41 light microscope with Olympus C5060A-ADU digital camera.

2.7. Assessment of convulsive behavior

Convulsive behavior was monitored during 30 min upon lindane administration.

Convulsive behavior was assessed by the following parameters: incidence, latency period and seizure severity. Incidence was expressed as a percentage of total number of animals per group with evident convulsions. Latency period was defined as a time between lindane administration and first seizure sign. For rats without seizures 30 min latency time was scored. Seizure severity was assessed by modified descriptive scale with grades from 0 to 4, defined as follows: 0 - no seizure; grade 1- head nodding and lower jaw twitching; grade 2 - myoclonic body jerks (hot plate reaction) and bilateral forelimb clonus with full rearing (Kangaroo position); grade 3 - progression to generalized clonic convulsions followed by tonic extension of fore and hind limbs and tale; grade 4 - status epilepticus (Stanojlović et al., 2009). Status epilepticus was defined as prolonged severe tonic-clonic convulsions lasting over 20 s or frequent repeated episodes of clonic convulsions for an extended period of time (over 5 min).

2.8. EEG registration and analysis

EEG electrodes were implanted 7 days before the induction of CP/CPPS or sham operation. Animals were anesthetized (pentobarbital sodium 50 mg/kg, i.p.) and three gold-plated recording electrodes were implanted in a stereotaxic apparatus. Electrodes were implanted over frontal (2 mm rostrally to bregma and 2 mm from the median line), parietal (2 mm rostrally to lambda and 2 mm laterally to median line) and occipital (2 mm caudally to lambda) cortices for further EEG recordings. The electrodes were fixed to the skull using dental acrylic cement. One-week recovery period was allowed prior to further experiments. Also, 24-hour-habituation to the recording environment was allowed to all animals.

An 8-channel EEG apparatus (RIZ, Zagreb, Croatia) with sampling frequency of 512 Hz/channel and 16-bit A/D conversion was used for EEG recording in freely moving rats. The signals were digitized using a SCB-68 data acquisition card (National Instruments Co, Austin, Texas, USA). Ambient noise was removed using a 50 Hz notch filter and the cutoff frequencies for were set at 0.3 Hz and 100 Hz for the high-pass and low-pass filters, respectively. Data acquisition and signal processing were performed with LabVIEW platform software developed in the Laboratory (NeuroSciLaBG).

All EEG recordings were visually monitored and screened for ictal activity and stored on disk for subsequent analysis. The rats were removed from the recording chamber and returned to its home cage upon completion of the 30 min recording sessions.

Ictal periods in EEG were defined as follow:

a) spontaneous and generalized spiking activity; b) lasting > 1 s and c) amplitude of at least twice the background EEG activity (Hrnčić et al., 2011). The number and duration of ictal periods were calculated during 30 min period after lindane administration. All ictal periods were detected visually.

2.9. Determination of interleukin levels

Brain and prostate levels of IL-1 beta and IL-6 were determined in rats from Sham and CP/CPPS groups that were not included in the seizure induction. Brains were dissected and the hippocampus, thalamus and frontoparietal cerebral cortex were isolated according to atlas (Paxinos and Watson, 2007). All tissue samples were homogenized on ice in commercial RIPA buffer (#R0278, Sigma-Aldrich, USA) supplemented with protease inhibitory cocktail (#P8340, Sigma-Aldrich, USA) and stored at -80 C until further usage. The IL-1β and IL-6 concentrations were assayed in prostate and above-mentioned brain structures using commercial ELISA kit (Rat IL-1 beta ELISA kit, Rat IL-6 ELISA Kit) according to manufacturer's instructions which did not require any specific protein isolation procedure apart from the above-mentioned homogenization. Briefly, sandwich assay procedure included addition of samples/standards into antibody pre-coated wells with an overnight at 4 °C incubation after which biotin labeled detection antibody was added. Concentrations of IL-1 beta and IL-6 were calculated based on standard curve plot.

2.10. Substances

Lindane, λ-carrageenan, and Rat IL-1β ELISA kit (#RAB0278-1KT), Rat IL-6 ELISA kit (#RAB0312-1KT) were products of Sigma Aldrich (St. Louis, MO, USA).

2.11. Data analysis

The difference in the incidence of lindane-induced seizures was determined by using Fisher's exact probability test. Since the normal distribution of the data regarding latency period and their severity, as well as number and duration of ictal periods in EEG, has not been determined by Kolmogorov-Smirnov test, the non-parametric Mann Whitney U tests were applied in further data analyses for the assessment of the difference between groups. On the other hand, the normal distribution of the data regarding pain thresholds and interleukin levels has been proven by Kolmogorov-Smirnov test. The difference of pain thresholds for mechanical stimuli between groups was determined using Student *t*-test, while within each group the difference between various time points was determined by one-way ANOVA with LSD *post hoc* test. *T*-test was used to evaluate differences in interleukin levels between groups. For parametric variables, data were presented with means ± standard error, while non-parametric variables were presented as medians with 25th and 75th percentiles. Criteria for the significance of statistical differences were $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3. Results

3.1. Development of pelvic pain syndrome

There were no differences in scrotal pain threshold between experimental and control animals (Sham *vs.* CP/CPPS, $p > 0.05$, Fig. 2)

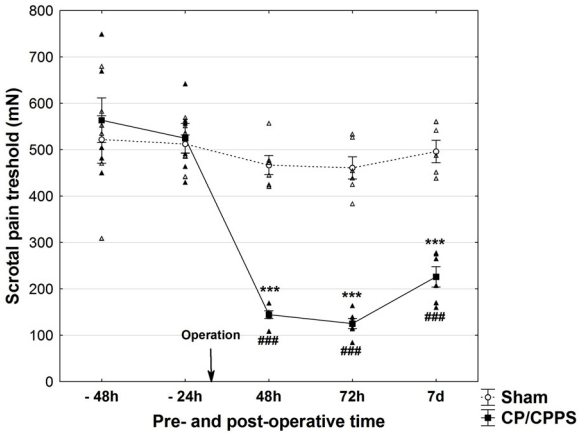

Fig. 2. Scrotal pain threshold in Sham and CP/CPPS rats.
Animals were tested by evF 24 h and 48 h prior to, as well as 48 h, 72 h and 7 days upon operation. Depending on the intraprostatic treatment during the operation rats were divided into two groups: Sham– injection of 0.9% NaCl and CP/CPPS–injection of 3% λ-carrageenan.
Between group differences in the scrotal pain threshold were estimated by *t*-test (***p < 0.001, *vs.* Sham), while within group differences were estimated by one-way ANOVA with LSD post hoc test (###p < 0.001, *vs.* -24 h).
Open triangles denote individual data for Sham, while filled triangles denote individual data for CP/CPPS animals.
For details see caption to Fig. 1.

in basal conditions, *i.e.* in measurement taken at 48th and 24th hours prior to surgery. The same hold true with Sham rats at 48th and 72nd hours, as well as at 7th day upon the operation in comparison to basal values.

Scrotal pain thresholds in CP/CPPS rats were highly significantly reduced ($p < 0.001$) compared to the threshold in Sham rats 48 and 72 h and the same holds true at 7th day upon intraprostatic injection (Fig. 2). Also, there is a high significant reduction ($p < 0.001$) of pain threshold 48 and 72 h as well as on day 7 upon intraprostatic injection in the CP/CPPS rats, in comparison to their basal pain threshold values (Fig. 2).

3.2. Histological analysis of prostates

Rat prostates injected with 0.9% sterile saline (Sham group) showed uniformly normal histology without leukocyte infiltration of the lumina and stroma (Fig. 3A, B, C). On the other hand, intraprostatic injection of 3% λ-carrageenan led to the inflammation of prostate (CP/CPPS group) (Fig. 3). Inflammatory changes were also uniform in all animals in CP/CPPS group, and they included: enhanced interstitial proliferation with predominant mononuclear cell infiltration (Fig. 3D), cell desquamation and leukocyte infiltration in tubulo-alveolar glands (Fig. 3E) with sporadic interstitial necrosis (Fig. 3F), suggesting existence of chronic prostatitis.

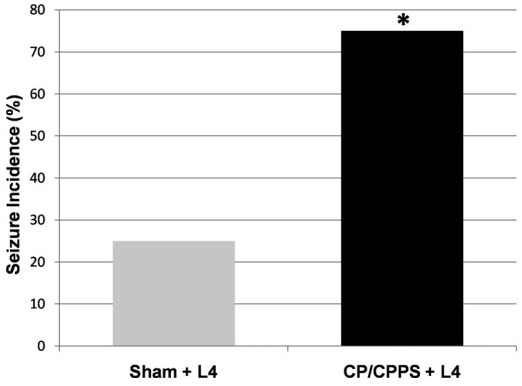

Fig. 4. Incidence of lindane-induced seizures in rats in Sham and CP/CPPS rats.
Incidence was defined as a number of convulsing rats in group, expressed as percentage.
Sham and CP/CPPS groups were treated 7 days after CP/CPPS with sub-convulsive (4 mg/kg, i.p.) dose of lindane (L4) and groups were formed: Sham + L4 and CP/CPPS + L4 (n = 8 per group).
The statistical significance of the difference between the groups was estimated by Fisher's exact probability test (*p < 0.05 vs. Sham + L4).
For details see caption to Figs. 1 and 2.

3.3. Convulsive behavior analysis

The incidence of seizures in Sham + L_4 group was 25%. On the other hand, seizure incidence was significantly higher in CP/CPPS (75%) by comparison with Sham + L_4 group ($p < 0.05$, Fig. 4).

Furthermore, the duration of a latency period to the first seizure sign was significantly shortened in CP/CPPS + L_4 when compared with Sham + L_4 group ($p < 0.05$, Fig. 5A).

When seizure intensity was analyzed, it has been determined that the severity of lindane-induced seizures was significantly higher in CP/CPPS + L_4 rats compared to Sham + L_4 rats ($p < 0.05$, Fig. 5B). Actually, maximal seizure intensity in group of CP/CPPS rats treated with lindane (CP/CPPS + L_4) was grade 4, while the highest grade found in Sham + L_4 group was 2.

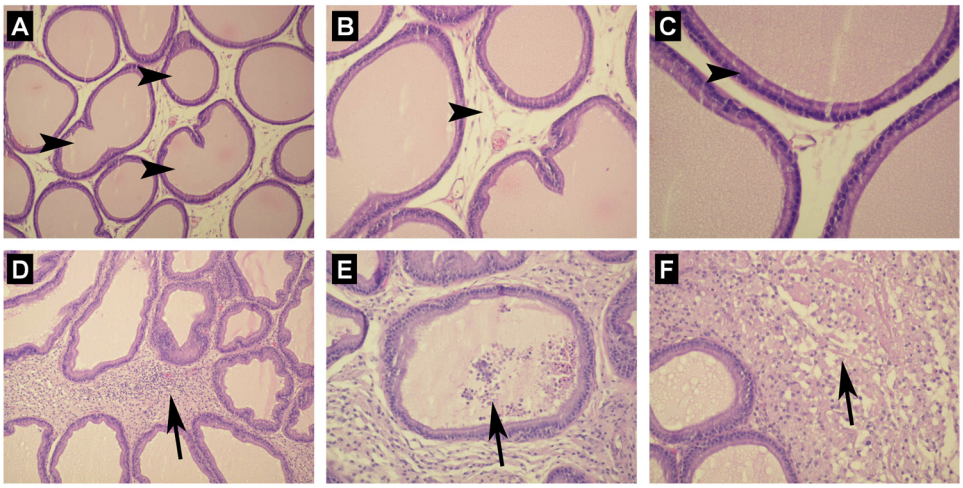

Fig. 3. Representative micrographs of histological examination of the prostates in Sham (A, B, C) and CP/CPPS (D, E, F) rats.
Preserved histological structure of prostates without leukocyte infiltration and destruction in SHAM group, magnifications ×100, ×200, ×400, respectively (A, B and C). Arrowheads indicate normal appearance of prostatic glands (A) and interstitium (B) with preserved glandular epithelium (C). Prostates in rats from CP/CPPS group show: interstitial proliferation with leukocyte infiltration (arrow), magnification ×100 (D), cell desquamation and leukocyte infiltration in tubulo-aleveolar glands (arrow), magnification ×200 (E), interstitial necrosis (arrow), magnification ×200 (F).

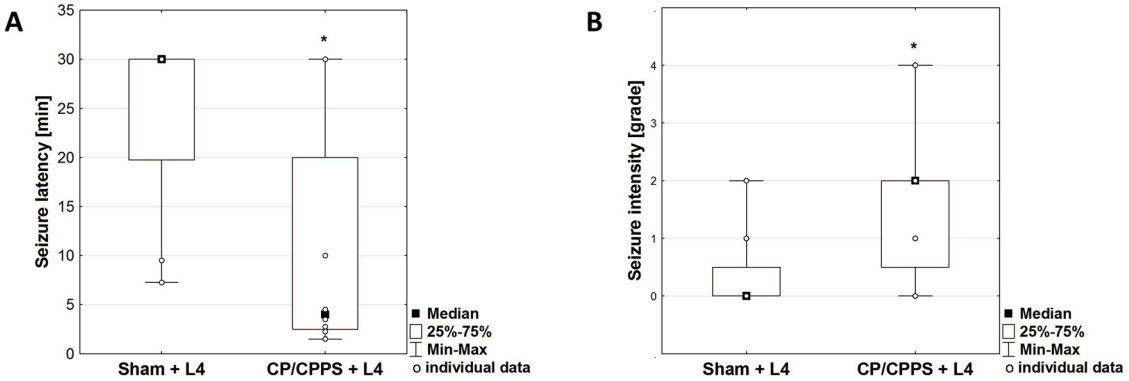

Fig. 5. Latency period of lindane-induced seizures (A) and their severity (B) in Sham operated and CP/CPPS rats.
Latency period is defined as a time between lindane administration and the first seizure sign. For rats without seizures 30 min latency time was scored.
Severity of seizure episodes was determined by a descriptive – rating scale with following grades: 1 – head nodding, lower jaw twitching; 2 – myoclonic body jerks (hot plate reaction), bilateral forelimb clonus with full rearing (Kangaroo position); 3- progression to generalized clonic convulsions followed by tonic extension of fore and hind limbs and tail; 4- prolonged severe tonic-clonic convulsions lasting over 20 s (status epilepticus) or frequent repeated episodes of clonic convulsions for an extended period of time (over 5 min).
The significance of the differences between the groups was estimated by Mann - Whitney U test (*$p < 0.05$ vs. Sham + L4).
For details see caption to Fig. 4.

3.4. EEG analysis

Bioelectrical EEG brain activity in rats from Sham and CP/CPPS rats showed no signs of ictal activity. During registration procedure rats were quiet, but awake, corresponding with baseline EEG recordings (Fig. 6A, B).

Injection of lindane induced the appearance of ictal periods consisting of series of high-amplitude spikes (EEG ictal period, Fig. 6C). Number and duration of these ictal periods were quantitatively analyzed.

Off-line analysis of ictal periods exposed the differences between Sham + L_4 and CP/CPPS + L_4 groups. Number of ictal periods per rat was significantly higher in CP/CPPS + L_4 rats by comparison with Sham + L_4 ($p < 0.05$, Fig. 6D). In contrast, no statistical significance was found in the duration of ictal periods between these two groups ($p > 0.05$, Fig. 6E).

3.5. Interleukin profile in brain and prostate

Intraprostatic injection of 3% λ-carrageenan led to a significant increase ($p < 0.05$) in IL-1β level in the cerebral cortex in CP/CPPS group, compared to Sham group (Fig. 7A). Also, CP/CPPS animals showed a significant increase in the thalamic IL-1β level by comparison with Sham group ($p < 0.01$; Fig. **7 A**). In contrast, no statistical significance was found in the hippocampal IL-1β level between these two groups ($p > 0.05$, Fig. 7A). Notably, λ-carrageenan-treatment caused a highly significant increase in the thalamic IL-6 level in CP/CPPS group, compared to corresponding Sham group ($p < 0.01$, Fig. 7B). Additionally, IL-6 level in the cerebral cortex was also significantly higher in CP/CPPS group by comparison with Sham group ($p < 0.05$ vs. Sham, Fig. 7B). No statistical significance was found in the hippocampal IL-6 levels between these two groups ($p > 0.05$, Fig. 7B).

Analysis of cytokine profile in the prostate showed a significant increase in IL-1β level in CP/CPPS group when compared to control animals from Sham group ($p < 0.05$, Fig. 7C). On the other hand, CP/CPPS animals showed a significant decrease in the prostatic IL-6 level by comparison with Sham group ($p < 0.05$, Fig. 7C).

4. Discussion

Assessment of convulsive behavior and EEG analysis revealed that CP/CPPS rats administered with subconvulsive dose of lindane (4 mg/kg; L4) demonstrated enhanced susceptibility to seizures. That has been manifested as higher seizure incidence, shorter seizure latency, higher seizure intensity, as well as higher number of EEG ictal periods in CP/CPPS + L4 rats compared to their Sham + L4 mates.

The CP /CPPS model used in the present study has been widely validated as a suitable model of non-infective prostatitis with no large and permanent tissue damage (Öztekin et al., 2015; Radhakrishnan et al., 2003; Radhakrishnan and Nallu, 2009; Zeng et al., 2014; Zhang et al., 2017). Carrageenans is used in many cosmetic products and dairy products as a food additive. λ carrageenan is extensively used for the induction of numerous experimental pain models (Necas and Bartosikova, 2013). Carrageenans have very low toxicity, and have been shown not to be teratogenic (Necas and Bartosikova, 2013). In vivo studies in laboratory animals showed that carrageenan has no potential to provoke organ pathology, hematology alterations, or to disturb normal health (Weiner, 2014). Additionally, this method of prostatitis induction excludes generation of autoimmunity as a potential mechanism of inflammation, the usage of irritant agents, stress stimuli, drug or hormone administration or dietary modulation. The chronicity of the inflammatory process in our study has been histologically verified.

Prostatic inflammation in the present study was further confirmed by increased prostatic level of IL-1β, one of the major proinflammatory cytokines, 7 days upon prostatitis induction. Increased IL-1β synthesis has also been found in clinical studies of CP/CPPS (Desireddi et al., 2008; Quick et al., 2012), as well as in many different animal prostatitis models (Asakawa et al., 2001; Chen et al., 2013, 2014; Hu et al., 2016). On the other hand, decreased prostatic IL-6 level in our research is opposite to experimental studies suggesting its elevation (Asakawa et al., 2001; Yoon et al., 2013). This discrepancy can be explained by different models of prostatitis used in these studies and by different duration of the experiments. There are evidence that dynamic fluctuations in IL-6 level correlate with severity of prostatitis signs and symptoms, *i.e.* its higher levels in acute phase of inflammation and lower levels in chronic prostatitis (Boehm et al., 2012; John et al., 2001).

Similar to other previous reports (Chen et al., 2013; Radhakrishnan and Nallu, 2009; Zeng et al., 2014), our study also clearly revealed lowering of the threshold for pain sensation after mechanical stimulation of the scrotal region (mechanical allodynia) in λ-carrageenan-induced prostatitis. This is not surprising, since IL-1β in combination with other cytokines and proinflammatory mediators evoke inflammatory pain through peripheral and central sensitization of the nociceptive pathway (Cohen and Mao, 2014). Peripheral sensitization by IL-1β can

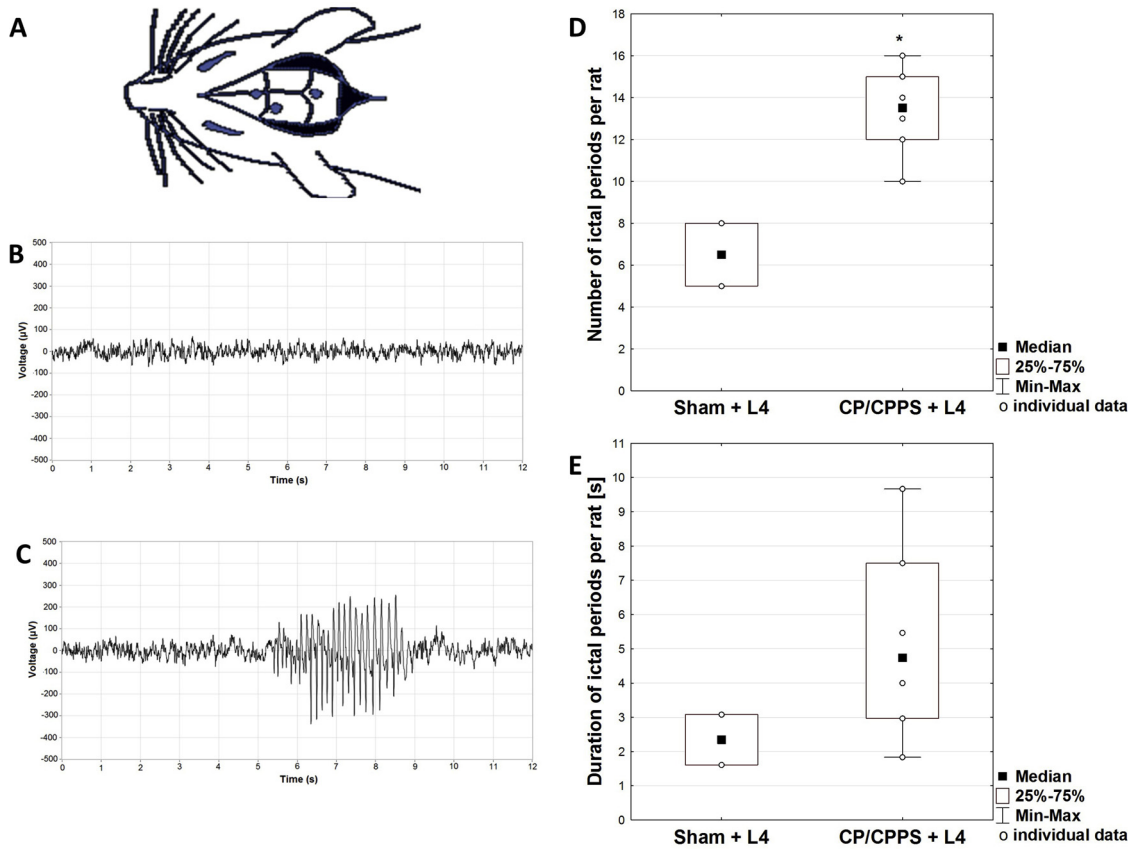

Fig. 6. The scalp electrode placement location (A), representative EEG tracings recorded in (B) Sham + L4 group 15 min after lindane treatment and (C) in CP/CPPS + L4 group 15 min after lindane administration. The number (D) and duration (E) of ictal periods per rat during EEG recordings of 30 min upon lindane administration.

Electrodes implantation spots are schematically presented (A).

Note baseline activity without signs of epileptiform discharges in (B) and ictal pattern in (C). Ictal period was characterized by high voltage spikes with amplitude more 200–300 μV and dominant frequency 7–8 Hz, in alpha range.

Lead: left frontal – right parietal cortex.

The number (D) and duration (E) of ictal periods per rat during EEG recordings of 30 min upon lindane administration. The significance of the differences between the groups was estimated by Mann - Whitney U test (*$p < 0.05$ vs. Sham + L4).

For details see caption to Figs. 2 and 4.

be evoked by direct and indirect mechanisms. Direct mechanism includes the activation of p38 mitogen-activated protein (MAP) kinase with subsequent activation of two types of tetrodotoxin-resistant sodium currents on nociceptive terminals (Binshtok et al., 2008). Indirect mechanisms include induction of nerve growth factor (NGF) (Safieh-Garabedian et al., 1995) and stimulation of prostaglandin E2 (PGE2) synthesis (Maier et al., 1990), which then activates protein kinase A, ultimately resulting in the phosphorylation and activation of sodium channels (Baker, 2005).

Central sensitization is partly mediated by microglia- and astrocyte-derived cytokines, including IL-1β and IL-6 (Vallejo et al., 2010), which activate protein kinase C and protein kinase A in CNS (Barkhudaryan and Dunn, 1999; Cohen and Mao, 2014). These mechanisms have been most extensively studied in dorsal horn of the spinal cord and they contribute to the increased excitability of the secondary nociceptive neurons. The role of cytokines in pain modulation at supraspinal levels is less known, but increased thalamic and cortical levels of IL-1β and IL-6 in prostatitis found in the present study may contribute to mechanical allodynia in CP/CPPS. Thalamus is an important relay structure in pain transmission, while cortex is essential for pain perception, attention to pain, emotional responses, behaviors, and memory retrieval related to pain (Cohen and Mao, 2014).

We found in the present study that chronic prostatitis increased rat brain susceptibility to lindane-induced seizures, evident as higher seizure incidence and severity, shorter seizure latency, and increased number of ictal periods on EEG after prostatitis induction. Although it is believed that pro-convulsive effect of lindane is primarily the result of GABAA receptor blockage (Vale et al., 2003), other mechanisms have been proposed to contribute to these effects, including calcium ion mobilization, excitatory amino acid-related phenomena, as well as NO-mediated signaling (Hrnčić et al., 2011). Lindane has been shown as a good model for generalized seizures and suitable model in a group of chemically induced seizures. It enables to assess both behavioral and EEG manifestations in fast, reliable and reproducible manner (Blaszczak and Turski, 1998; Hrnčić et al., 2011; Mladenović et al., 2007; Stanojlović et al., 2013). Results regarding seizure activity in Sham + L4 group were similar to expected one (Hrnčić et al., 2011; Stanojlović et al., 2013). Having in mind procedure for CP/CPPS induction we opted for model of seizures that will not require extensive manipulations, as well as to be a model of generalized seizures. Therefore, we used lindane-induced seizures in this study.

To our knowledge this is the first study that demonstrated brain hyperexcitability with increased propensity to seizure development in CP/CPPS. Mechanisms of this hyperexcitability are probably diverse, but one of the possible mechanisms could be the generation of proinflammatory microenvironment in the cerebral cortex and thalamus due to chronic pain.

The role of immunity alterations and cytokines in the

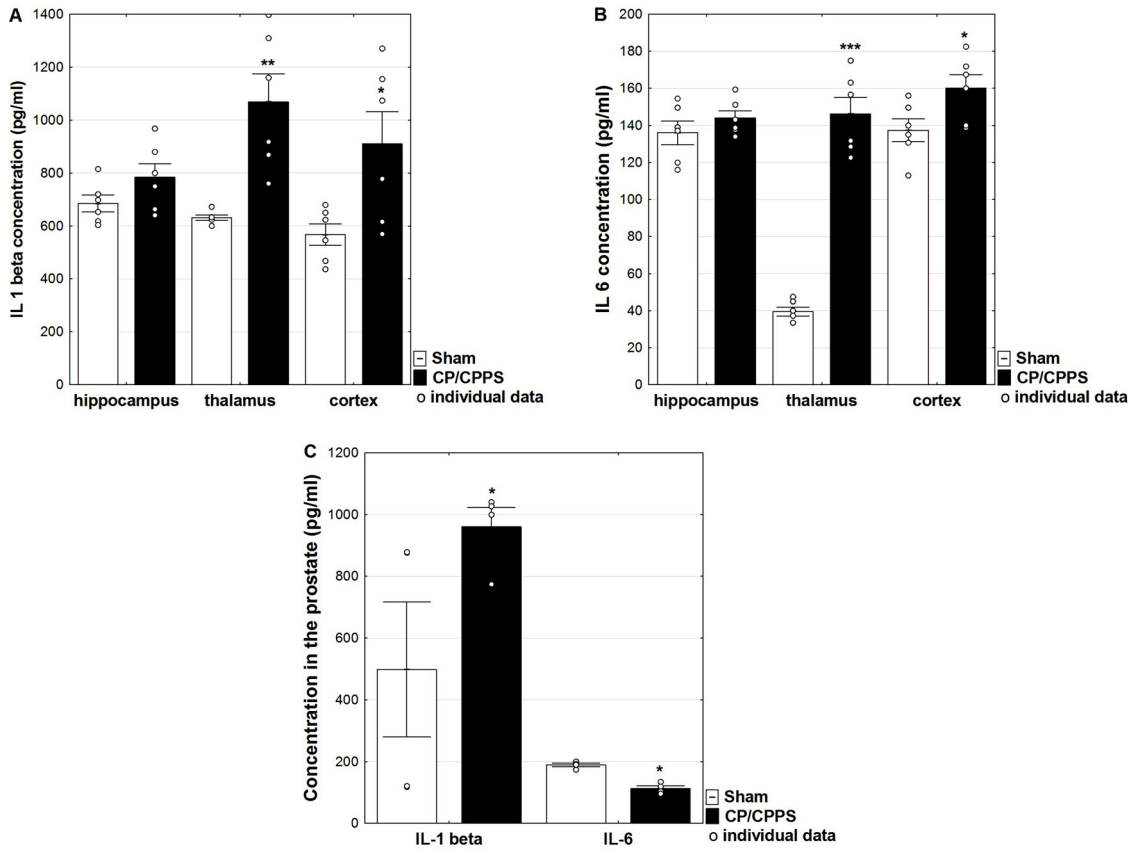

Fig. 7. Interleukin profile in the brain structures and prostate: IL-1β (A) and IL-6(B) concentrations in different brain structures; prostatic concentration of IL-1β and IL-6 (C).

The rat cohort form Sham and CP/CPPS groups (n = 6 per group) that were not included in the seizure induction were sacrificed seven days upon Sham operation or CP/CPPS induction and used for analysis of IL-1β and IL-6 level in the prostate and different brain structures (hippocampus, cerebral cortex and thalamus). IL-1β and IL-6 concentrations were measured by using ELISA kit.

The significance of the difference between the groups was estimated by Student's *t*-test (*p < 0.05, **p < 0.01 vs. Sham).

epileptogenesis and seizure generation has been studied extensively (Galic et al., 2012; Ravizza et al., 2008; Ravizza and Vezzani, 2006). Majority of studies proved higher production of different cytokines in activated astrocytes and microglia during epileptogenesis, especially IL-1β, TNF-α and IL-6 (Ravizza et al., 2008; Ravizza and Vezzani, 2006). Increased cytokines production is not the only mechanism which is responsible for altered brain excitability. Namely, experimental studies also showed that increase in the CNS excitability in a model of inflammatory bowel disease, could be mediated by increased microglia-dependent TNFα production (Riazi et al., 2008). Also, synaptic changes caused by microglia-mediated inflammatory response in hippocampus may underlie the behavioral changes seen in peripheral inflammation (Riazi et al., 2015). Clinical data suggests that in humans epilepsy is also associated with changes in immunological profile (Lehtimäki et al., 2007; Ravizza and Vezzani, 2006; Rosa et al., 2016; Vezzani et al., 2002). The proconvulsive effect of IL-1β has been reported by Vezzani et al. (Vezzani et al., 2002, 1999) on limbic seizures in mice caused by bicuculine, kainic acid, and electrical stimulation. Administration of IL-1 receptor antagonist (IL-1Ra) reduces the severity of bicucucline-induced seizures (Vezzani et al., 2002).

Mechanisms of proconvulsive effect of IL-1β are diverse and still not fully established. Some of the proposed mechanisms include: induction of an intracellular Ca^{2+} ion surge, modifications of voltage-dependent ion channels with reduction of seizure threshold (Xu et al., 2013), and stimulation of NO production in the brain (Meini et al., 2000). Additionally, IL-1β stimulates chronic release of excitatory neurotransmitters, activates NMDA-R and evokes neuronal hyperexcitability (Zhu et al., 2006). At the same time IL-1β may negatively modulate GABA (A) receptors (Wang et al., 2000), inhibit K^+ efflux (Meini et al., 2000), uptake of excitatory neurotransmitters by the glial population, and the recycling of GABA receptors (Hu et al., 2000). These data support the hypothesis that high levels of IL-1β found in the cortex and thalamus due to CP/CPPS in the present study may contribute to the lower threshold for lindane-induced seizures.

Apart from IL-1β, our experimental findings showed elevated levels of IL-6 in the thalamus and cortex of CPPS rats. The role of IL-6 in seizure generation is less known than of IL-1. Plenty of clinical studies demonstrated rapid and transient post-ictal increase in serum and cerebrospinal fluid IL-6 concentration, which peaked at 12 h and remained elevated for 24 h after seizures, but some patients suffering from epilepsy also had higher levels of IL-6 under basal conditions (Lehtimäki et al., 2004, 2007; Liimatainen et al., 2009). Experimental studies in various animal models of seizures also reported increased IL-6 synthesis in different parts of the brain and meninges in a time-dependent manner (Li et al., 2011; Rosell et al., 2003; Vezzani et al., 2002). Exogenous IL-6 is capable to aggravate experimentally induced seizures (Kalueff et al., 2004). Interestingly, IL-6 level was found to be increased in temporal lobe, but not extra-temporal lobe epilepsy (Liimatainen et al., 2009). Multiple factors have been found to influence IL-6 synthesis in epilepsy: the type and duration of seizures, epilepsy type, the frequency of seizures (Alapirtti et al., 2018). It should be mentioned that further blocking studies using anti-inflamatory agents could additionally prove our findings.

According to the known data and our results, we can propose the following association between CP/CPPS, proinflamatory cytokines as neuroinflamatory mediators and susceptibility to seizures. Chronic

prostatitis induces inflammatory pain and mechanical allodynia in the scrotal region, associated with altered proinflamatory cytokines profile and increased susceptibility to lindane-induced seizures. Proinflammatory cytokines IL-1β and IL-6 may be potential mediators of brain hyperexcitability in CP/CPPS and may contribute to the enhancement of proconvulsive effect of lindane. Elevated levels of IL-1β and IL-6 were found in the cortex, which is always involved in epileptic discharge, and in thalamus, which in pathophysiological conditions through thalamocortical circuits could be intricate in initiation and propagation of epileptiform brain activity (Meeren et al., 2002). Additionally, maladaptive alterations in the CNS pain neural networks, especially those involving thalamus, could contribute to increased herein observed seizure susceptibility. Namely, pain is frequently underling centralization through maladaptive responses within the CNS that can profoundly alter brain systems and thereby behavior, regardless of its origin (Borsook, 2012).

However, this interpretation has some limitations. Namely, other mechanisms may be also involved in the development of brain hyperexcitability, that were not investigated in the present study. Proton magnetic resonance spectroscopy in female patients has revealed increased choline and reduced GABA level in anterior cingulated cortex in patients with urologic chronic pain syndrome (Harper et al., 2018). In addition, neuropathic and probably inflammatory pain are associated with altered descending modulation of nociceptive signals by adrenergic and serotoninergic pathways (Cohen and Mao, 2014; Wei et al., 2010). This indicates that neurotransmitter alterations may also be involved in the generation of hyperexcitability. We focused herein on the two interleukins (IL-1β and IL-6) the most frequently reported to be involved in the regulation of neuronal excitability with high significance, although they are not the only cytokines involved in regulation of neuronal excitability. Administration of IL-1 and IL-6 antagonists as well as modifications of IL-1 and IL-6 genes could clarify the role of this cytokines in the generation of seizures.

Based on our results, it can be concluded that experimental λ-carrageenan-induced CP/CPPS increases the susceptibility of rats to lindane-induced seizures, associated with increased level of IL-1β and IL-6 in the cerebral cortex and thalamus.

Conflict of interest

The authors declare that they have no conflict of interest.

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