Altered Sleep and Behavioral Patterns of Arthritic Rats

Monica L. Andersen and Sergio Tufik

Department of Psychobiology, Universidade Federal de São Paulo

The present study sought to evaluate concomitant alterations of behavioral and sleep patterns of arthritic rats. Rats were implanted with electrodes for polysomnographic recordings and submitted to the model of arthritis by a subcutaneous (s.c.) administration of Freund adjuvant in the posterior right paw and saline in the posterior left paw. The SHAM group was injected with saline in both paws, whereas the control group (CTL) was not submitted to any manipulation. Behavioral tests were carried out twice before induction of arthritis, on the second day of arthritis, and once a week afterwards until the eighth week. Body weight, colonic temperature, and measurements of the injured paw were carried out on the same days. Arthritic rats presented a reduction of total sleep time, increased latency to synchronized sleep, augmented number of episodes of synchronized sleep, reduction of sleep efficiency, more stage shifts, and increased total alert time. Moreover, these animals presented a lower pain threshold than control and SHAM animals. This reduction was observed on the second day of arthritis and remained so reduced until the end of the study. The data appear to indicate a relationship between altered sleep pattern and increased pain sensitivity in arthritic rats.

CURRENT CLAIM: Arthritic rats present sleep fragmentation.

Experimental models of polyarthritic rats have been extensively used to study long-lasting chronic pain processes and to evaluate the potential analgesic and anti-inflammatory effects of drugs (Besson and Guilbaud, 1988; Butler et al., 1992). The model of adjuvant-induced arthritis (AIA) is similar to the rheumatoid polyarthritis that exists in humans, and is induced by a subcutaneous administration of Freund adjuvant. This procedure results predominantly in arthritis in the joints of the hind limbs, promoting a considerable reduction of the motor activity and an increase of itching and scratching behaviors (Costa et al., 1981; Calvino et al., 1987). Moreover, the animals present increased sensitivity to pressure on the affected paw and to flexion and extension of the inflamed joints, weight loss and hyperventilation (Besson and Guilbaud, 1988). Augmented diameter of both fore- and hind limbs due to the inflammation and the edema (Cain et al., 1997), as well as inflammation accompanied or not by lesions on the skin, eyes, and gastric- and genital-urinary tracts are also observed (Gendimenico and Mezick, 1995).

The arthritic rats in this study were used 10 days after the induction of arthritis. Arthritic rats present sleep fragmentation, manifested by an increased number of sleep episodes of shorter duration, an augmented number of alert episodes, and a decrease in the number of episodes of high amplitude sleep (synchronized sleep–SS). In addition, the reduction of desynchronized sleep (DS) and the inability of arthritic rats to maintain long sleep periods have also been reported.

The purpose of the present study was to carry out a concomitant evaluation of the sleep pattern and the behavioral alterations in rats submitted to the AIA.

METHODS

Subjects: All experimental procedures were submitted to and approved by the Ethics Committee of the Universidade Federal de São Paulo and followed the recommendations of the Committee for Research and Ethical Issues of IASP (1983). Thirty Wistar male rats, aged approximately 90 days at the beginning of the study, were used. The entire study was conducted under a controlled 12-h light/dark cycle (light on at 7 a.m.) and room temperature (23±2°C). The animals were singly housed in plastic cages covered with soft sawdust, with food and water available ad libitum.

Surgical Preparation: Under chloral hydrate anesthesia (0.4 ml/100 g of body weight, administered i.p.), eight pairs of electrodes were implanted in the cortical (A1 and A10), and subcortical (CA1 and CA3) hippocampal fields and the ventral-lateral thalamic nucleus (VLT) areas and in the muscles (vibrissae, eyes and neck). The electrodes were soldered to a socket containing 16 pins and covered with dental acrylic. The animals were allowed seven days to recover from the surgery.

Immediately after surgery, the animals were placed in round, transparent cages (32 cm in diameter and 30 cm in height) where they remained for the entire period of the study. The sawdust in the cages was changed according to the laboratory routine.

Adjuvant-induced Arthritis: After administration of the anesthetic, arthritis was induced in nine animals by a s.c. injection of 0.1 ml of Freund adjuvant (complete fraction of denatured Mycobacterium butyricum suspended in mineral oil, Sigma Chemical Co., USA) in the right hind limb. In the

Correspondence: Sergio Tufik, M.D., Ph.D., Department of Psychobiology, Universidade Federal de São Paulo, Rua Botucatu, 862 - 1° andar, Vila Clementino, SP 04023-062, São Paulo, Brazil, Tel: 55-11-539-0155, Fax: 55-11-572-5092, E-mail: stufik@psicobio.epm.br.
counterpart paw, 0.1 ml of saline was injected. The initial inflammatory response developed in a few hours, but the more severe clinical signs appeared from the 10th post-inoculation day on, and the alterations remained for several weeks (Colpaert et al., 1980, 1982; Costa et al., 1981).

Groups: The animals were divided into three groups: AIA (N=9), Non-handled controls (CTL, N=11) and SHAM control (N=10). The SHAM group was injected with saline in both paws, whereas CTL animals were not manipulated, except for cage cleaning, according to the animal room routine.

Polysomnographic Recording: On the morning preceding the beginning of the study, the animals were adapted to the recording chambers and the cables by placing their home cages inside the Faraday chambers for about 3 h. On the following day, the recording was carried out in the morning, for a period of 2 h (between approximately 9:00 a.m and noon). During the recording, food and water were provided ad libitum. The recording cages were kept in a soundproof room, whereas the recording equipment was placed in an adjacent room containing a one-way window through which the experimenter could observe and record the animals' behaviors.

The acquisition of digital signal was performed on a Nihon Kohden model QP 223 (Nihon Kohden Corporation, Tokyo, Japan), using eight channels: five for electroencephalogram (EEG), two of which were destined to electrocorticogram and electrooculogram; one channel for the snout; and one channel containing a one-way window through which the experimenter could observe and record the animals' behaviors.

The rats were inspected daily, carefully and in detail, in regard to the affected paw and their general well being.

Sleep Parameters
Table 1 shows the results of sleep parameters obtained in four recording sessions for each group.

TST: A main effect of group was detected (F(2.27)=7.58; p<0.01). Animals from the AIA group showed reduced TST, compared to both CTL and SHAM groups.
Table 1. Sleep Parameters Assessed in Four Polysomnographic Recordings: Basal (1), Second Day of Arthritis (2), First Week of Arthritis (3) and Second Week of Arthritis (4).

<table>
<thead>
<tr>
<th>Sleep Parameters</th>
<th>CTL</th>
<th>SHAM</th>
<th>AIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST 1</td>
<td>68.18±14.93</td>
<td>68.10±11.56</td>
<td>64.83±19.33</td>
</tr>
<tr>
<td>TST 2</td>
<td>70.90±20.10</td>
<td>70.05±16.92</td>
<td>42.72±18.53*</td>
</tr>
<tr>
<td>TST 3</td>
<td>73.72±21.06</td>
<td>81.26±11.98</td>
<td>48.77±12.00*</td>
</tr>
<tr>
<td>TST 4</td>
<td>76.54±26.07</td>
<td>73.65±15.33</td>
<td>59.16±23.44*</td>
</tr>
<tr>
<td>LATSS 1</td>
<td>31.86±17.14</td>
<td>22.45±11.31</td>
<td>27.77±20.17</td>
</tr>
<tr>
<td>LATSS 2</td>
<td>28.18±11.68</td>
<td>29.70±16.64</td>
<td>52.22±25.08‡</td>
</tr>
<tr>
<td>LATSS 3</td>
<td>24.31±12.12</td>
<td>18.90±7.63</td>
<td>47.22±12.62‡</td>
</tr>
<tr>
<td>LATSS 4</td>
<td>22.18±15.9</td>
<td>25.05±10.51</td>
<td>30.16±15.95</td>
</tr>
<tr>
<td>NSSE 1</td>
<td>4.18±1.66</td>
<td>6.50±2.36</td>
<td>6.44±2.24</td>
</tr>
<tr>
<td>NSSE 2</td>
<td>5.36±2.37</td>
<td>5.40±3.09</td>
<td>7.55±3.64</td>
</tr>
<tr>
<td>NSSE 3</td>
<td>6.45±3.85</td>
<td>8.40±2.59</td>
<td>8.00±4.00</td>
</tr>
<tr>
<td>NSSE 4</td>
<td>6.18±3.12</td>
<td>6.00±3.05</td>
<td>10.77±5.11‡</td>
</tr>
<tr>
<td>TTDS 1</td>
<td>1.77±2.53</td>
<td>5.25±5.28</td>
<td>2.94±3.31</td>
</tr>
<tr>
<td>TTDS 2</td>
<td>3.40±3.81</td>
<td>3.40±5.05</td>
<td>6.16±6.64</td>
</tr>
<tr>
<td>TTDS 3</td>
<td>5.40±8.70‡</td>
<td>9.00±4.98‡</td>
<td>7.66±6.01‡</td>
</tr>
<tr>
<td>TTDS 4</td>
<td>3.72±4.02‡</td>
<td>6.85±7.41‡</td>
<td>10.38±9.58‡</td>
</tr>
<tr>
<td>LATDS 1</td>
<td>99.54±33.23</td>
<td>73.85±27.37</td>
<td>100.44±25.15</td>
</tr>
<tr>
<td>LATDS 2</td>
<td>80.09±38.99</td>
<td>100.25±33.44</td>
<td>73.88±36.98</td>
</tr>
<tr>
<td>LATDS 3</td>
<td>92.86±33.61</td>
<td>62.60±34.97</td>
<td>82.22±30.88</td>
</tr>
<tr>
<td>LATDS 4</td>
<td>83.31±29.18</td>
<td>61.95±33.71</td>
<td>70.55±38.95</td>
</tr>
<tr>
<td>EFFIC 1</td>
<td>56.46±12.57</td>
<td>59.91±11.17</td>
<td>53.27±14.44*</td>
</tr>
<tr>
<td>EFFIC 2</td>
<td>57.75±15.85</td>
<td>58.17±14.79</td>
<td>35.22±15.12*</td>
</tr>
<tr>
<td>EFFIC 3</td>
<td>62.46±16.15</td>
<td>64.60±10.12</td>
<td>39.70±10.10*</td>
</tr>
<tr>
<td>EFFIC 4</td>
<td>63.50±21.68</td>
<td>60.81±12.53</td>
<td>48.88±19.38*</td>
</tr>
<tr>
<td>StS 1</td>
<td>9.36±4.47</td>
<td>14.00±4.94</td>
<td>14.33±4.71#</td>
</tr>
<tr>
<td>StS 2</td>
<td>11.54±5.52</td>
<td>12.10±7.21</td>
<td>18.00±8.73#</td>
</tr>
<tr>
<td>StS 3</td>
<td>13.90±8.82</td>
<td>17.90±5.43</td>
<td>18.55±8.80‡</td>
</tr>
<tr>
<td>StS 4</td>
<td>13.36±6.87</td>
<td>13.50±7.41</td>
<td>24.55±11.95‡</td>
</tr>
<tr>
<td>TWT 1</td>
<td>51.86±16.36</td>
<td>46.80±15.92</td>
<td>57.66±22.12*</td>
</tr>
<tr>
<td>TWT 2</td>
<td>51.45±18.37</td>
<td>53.20±18.79</td>
<td>78.44±18.24*</td>
</tr>
<tr>
<td>TWT 3</td>
<td>45.45±21.76</td>
<td>45.35±16.69</td>
<td>74.38±13.50*</td>
</tr>
<tr>
<td>TWT 4</td>
<td>44.04±26.18</td>
<td>47.40±15.00</td>
<td>61.88±23.55*</td>
</tr>
</tbody>
</table>

* = Different from CTL and SHAM values; ‡ = different from CTL values; # = different from basal recording. TST = total sleep time; LATSS = latency to synchronized sleep; NSSE = number of synchronized sleep episodes; TTDS = total time of desynchronized sleep; LATDS = latency to desynchronized sleep; EFFIC = sleep efficiency; StS = stage shifts; TWT = total wake time. Values are given in min and are expressed as mean ± s.d. For further differences, refer to Results section.

Latency to Synchronized Sleep: Main effects of group (F(2,27)=7.19; p<0.01) and time (F(2,81)=3.66; p<0.003) were observed. In addition, a significant interaction between the factors was detected (F(6,81)=3.02; p<0.01). Post hoc analysis of this interaction showed that on Day 2 and Week 1 after induction of arthritis, AIA animals took longer to initiate synchronized sleep than CTL and SHAM animals (p<0.001). Moreover, latency to synchronized sleep was augmented in the AIA group on Day 2 and Week 1 of arthritis, compared to both Basal (p<0.05) and Week 2 of arthritis (p<0.01).

Number of Episodes of Synchronized Sleep: ANOVA revealed main effects of group (F(2,27)=3.37; p<0.05) and time (F(3,81)=4.58; p<0.0005) and an interaction between these factors (F(6,81)=2.19; p<0.05). Analysis of this interaction showed that on Week 2 of arthritis, the number of episodes of synchronized sleep was increased in the AIA group, compared to CTL and SHAM animals (p<0.0006) and that AIA animals presented more synchronized sleep episodes on Week 2 of arthritis than in all other time points (p<0.02).

Total Time of Desynchronized Sleep: A main effect of time was detected (F(3,81)=28.28; p<0.01). Total time of desynchronized sleep increased on Week 1, compared to Basal and Day 2 of arthritis (p<0.05) and on Week 2, compared to Basal (p<0.02).

Latency to Desynchronized Sleep: An interaction between group and time was shown (F(6,81)=2.26; p<0.05). SHAM animals presented an increased latency on Day 2 after administration of the adjuvant, compared to Weeks 1 and 2 (p<0.02).

EFFIC: There was a main effect of group (F(2,27)=8.72; p<0.002), where animals of the AIA groups showed lower sleep efficiency than animals from CTL and SHAM groups (p<0.002).

Stage Shifts: The number of awakenings were altered as a function of group (F(2,27)=4.54; p<0.02) and of time (F(1,81)=4.16; p<0.009). The group AIA presented more shifts than group CTL (p<0.008). Furthermore, the stage shifts were augmented on Week 1, compared to Basal (p<0.01) and on Week 2, compared to Basal and Day 2 of arthritis (p<0.05).

TWT: A main effect of group was observed (F(2,27)=8.22; p<0.002), and once again AIA showed longer TWT than the other groups (p<0.002).

The only parameter that was not altered by any manipulation on any recording day was the number of desynchronized sleep episodes.

Behavioral Parameters

Body Weight: ANOVA detected a main effect of testing (F(10,260)=33.25; p<0.01) and an interaction between the variables (F(20,260)=1.90; p<0.01). The Duncan Multiple Range Test indicated that the weight of CTL animals was augmented over basal values from Week 4 on, whereas that of SHAM animals was augmented from Week 2 on, and that of AIA group was different from basal from Week 3 until the end of the study (Figure 1A).

Colonic Temperature: A main effect of testing was observed (F(10,260)=3.87; p<0.01). Post hoc analysis showed that the temperature on B2 (basal following electrode implant) was lower than on D2 (second day of arthritis) and from Week 3 to Week 8 of the study. These results can be seen in Figure 1B.

Measurements of the Affected Paw: Main effects of group (F(2,27)=93.29; p<0.01) and of testing (F(10,260)=16.10; p<0.01) were observed. A significant interaction between these factors was also shown (F(20,260)=16.31; p<0.01). Post hoc analysis of this interaction showed that animals of the AIA group presented augmented measures of the right paw, compared to CTL and SHAM animals from D2 until the end of the study (Figures 2A, 2B and 2C). No differences were observed on the left paw.

Mechanic Stimulation:

a) Analgesimeter: The results are presented in Figure 3A. The threshold to withdraw the right paw in the analgesimeter test was altered as a function of group (F(2,27)=28.99; p<0.00001) and testing (F(10,260)=4.07; p<0.0001). An...
interaction between these factors was also revealed ($F_{(20,260)}=3.62; p<0.00001$). Post hoc comparison showed that from D2 on, the threshold of AIA animals was lower than that of CTL (except on Week 5 of pain) and SHAM rats ($p<0.03$). Moreover, these animals presented a reduction of pain threshold on D2 until the end of the study, compared to B1 and B2 ($p<0.04$).

In regard to the left paw there was also a change on the withdrawal threshold. Thus, main effects of group ($F_{(2,26)}=12.26; p<0.0002$) and testing ($F_{(10,260)}=3.46; p<0.0003$) and an interaction between the factors ($F_{(20,260)}=2.58; p<0.0004$) were detected.

Analysis of the interaction showed a shorter threshold for group AIA than groups CTL and SHAM on B2, D2 and W1 ($p<0.01$).

b) Paw-pinching: ANOVA revealed a main effect of testing ($F_{(10,260)}=4.21; p<0.00002$) and an interaction between the factors ($F_{(20,260)}=1.58; p<0.05$). Comparison among the tests showed that withdrawal of the right paw was the longest on B2 ($p<0.007$). The results are shown in Figure 3B.

**Thermal Stimulation:** No differences were observed on the hot and cold plates.

---

**Figure 1.** **Panel A:** Body weight (g) of groups CTL (N=11), SHAM (N=10) and AIA (N=9) measured throughout an 8-week period. (B1) Basal test; (B2) Post-electrode implant test; (D2) Second day; (W1) Week 1; (W2) Week 2; (W3) Week 3; (W4) Week 4; (W5) Week 5; (W6) Week 6; (W7) Week 7; (W8) Week 8 after AIA. Values are presented as mean ± s.d. (*1 – CTL group: different from W4 to W8; *2 – SHAM group: different from W1 to W8; *3 – AIA group: different from W3 to W8). **Panel B:** Colonic temperature (°C) of CTL (N=11), SHAM (N=10) and AIA (N=9) groups throughout an 8-week period. For testing definition, refer to legend of Figure 2. Values are presented as mean ± s.d. (* – significantly different from D2 and W3 to W8).

**Figure 2.** Panels A, B and C represent the different measurements in mm–paw width, paw height and joint width–of CTL (N=11), SHAM (N=10) and AIA (N=9) groups throughout an 8-week period. Values are expressed as mean ± s.d. (* – differs from CTL and SHAM groups from D2 until the end of the study).
Behavioral Observation: The observation of AIA animals indicated behavioral alterations, such as avoidance to support the affected paw on the ground, thus sparing the limb. Moreover, these animals showed reduced motility from Weeks 2 to 4 of pain, returning to normal levels thereafter. Finally, AIA animals displayed a characteristic behavior of licking certain body parts, biting the skin and/or the anterior or posterior limbs. This behavior was augmented during the acute phase, and increased progressively from Week 2 to Week 3, when it then gradually disappeared.

DISCUSSION

Adjuvant-induced arthritis in rats has been widely used as an animal model of inflammation (Pearson, 1956; Glenn and Gray, 1965; Jones and Ward, 1966). It is reasonable to assume that AIA, in addition to causing inflammation, also causes pain. Colpaert and coworkers (1982) validated AIA as a model of chronic pain and characterized the evolution of the disease.

In the present study, the follow-up of arthritic animals allowed the assessment and establishment of some relations between sleep and pain. During the 67 days of animal observation, of which 56 were with arthritis, some relevant results were obtained. For instance, induction of arthritis disrupted the sleep of rats. Thus, the animals showed shorter TST, increased latency to synchronized and desynchronized sleep, reduced sleep efficiency, more change of stages, longer TWT, and a higher number of alert periods. These results are in accordance to those reported by Landis et al. (1989), and similar to these authors, we believe that the explanation for such abnormal sleep patterns is due to the incapability of arthritic rats to maintain long periods of sleep.

The reduction of TST on the 2nd (2nd day after AIA), 3rd (1st week after AIA) and 4th (2nd week after AIA) recordings, as well as an increased sleep latency on the 2nd and 3rd recordings and reduced sleep efficiency, compared to the other two groups, are interconnected with the behavioral results. This was the case with the threshold for paw withdrawal on both analgesimeter and pinching tests that were reduced from the second day of arthritis until the end of the study. Significant alterations on sleep parameters were found on Week 1 (total time of desynchronized sleep and stage shifts) and Week 2 after AIA (number of episodes of synchronized sleep, total time of desynchronized sleep, and stage shifts). We believe that the reduced threshold to withdraw the affected paw is the result of an augmented pain sensitivity, which would be sufficient to produce more changes of stage, which reflects a more fragmented sleep probably due to the pain, in addition to an augmented number of synchronized sleep episodes. Although the total time spent in desynchronized sleep was not different among the groups, it was augmented on the last two recordings, suggesting that the sleep fragmentation observed in AIA animals resulted in a rebound of desynchronized sleep.

Several epidemiological studies (Pilowsky and Basset, 1982; Pilowsky et al., 1985; Gislason and Almquist, 1987; Moffitt et al., 1991; Drewes et al., 1994) report a pathological sleep pattern in patients suffering from various types of pain, and although the reason for pain-induced sleep problems is quite logical, sleep problems can also exacerbate pain (Moldofsky and Scarisbrick, 1976; Phillips and Cousins, 1986; Kryger and Shapiro, 1992).

The sleep microstructure of fibromyalgic and rheumatoid arthritis patients present abnormalities such as intrusion of alpha waves during non-REM sleep, a pattern known as alpha-delta sleep (Drewes et al., 1994; Mahowald et al., 1994).
The possibility that pain may disrupt the sleep of arthritic rats is suggested not only because these animals present signs of chronic pain, but also because a blockade of pain reduces the signs of morbidity (Dardick et al., 1989). Many groups have evaluated hyperalgesia in arthritic rats in different ways, using the analgesimeter, tail flick, hot plate, rotorod tests, and spontaneous vocalization when in contact with other animals.

In the present study, we used the analgesimeter, paw-pinching, and hot and cold plate tests. The results showed that hyperalgesia had already begun on the second day following administration of Freund's adjuvant. Calvino and coworkers (1987) believe that these alterations occur in the pre-clinical period, when the clinical signs are marginal, although biological and biochemical alterations are also taking place.

Abbadie and Besson (1994) and Calvino et al. (1994) reported that three weeks after inoculation, the clinical and behavioral signs of the disease reached a peak level of hyperalgesia. Our results with mechanical tests revealed similar effects to those observed by the above mentioned authors. The paw-pinching test showed a reduced pain threshold, reflected by a short latency to withdraw the paw, on the third week of pain, which gradually increased thereafter. According to Colpaert (1987), the hypersensitivity decreases after the third week.

Hargreaves and coworkers (1988) affirm that thermal stimulation provides a quantifiable measurement of hyperalgesia-related behaviors. Previous studies demonstrate that diminution of the latency to withdraw the paw corresponds to a threshold reduction that is observed in the course of hyperalgesia. Although no differences were observed in the hot and cold plate tests, we observed a shorter latency to paw withdrawal on the second day of pain in the AIA animals. It is likely this may have been due to the hyperalgesia displayed by these animals and coincided with the period in which these animals displayed a longer latency to synchronized sleep, spent more time in alertness, displayed a shorter total sleep time and reduced sleep efficiency.

Behavioral evidence of pain in arthritic rats has been described and includes weight loss, hyperventilation, and reduced motor activity (Costa et al., 1981; Calvino et al., 1987; Dardick et al., 1989). The weight loss, reported by many authors as an indication of an abnormal condition (Colpaert et al., 1980, 1982; Costa et al., 1981; Calvino et al., 1987; Dardick et al., 1989), was not observed in our study. Animals of the AIA group put on weight similarly to CTL and SHAM groups. This result is in accordance with that reported by Butler and colleagues (1992).

Although some studies explain, at least in part, the sleep fragmentation by the temperature elevation (Kadlecova et al., 1972; Kales and Kales, 1984), our results are not in accordance with them, since temperature differences among the groups were not found, but there was a clear sleep disruption in AIA animals. Landis and coworkers (1988) suggest that the small elevation of body temperature observed in arthritic animals has minimal effects on sleep.

In conclusion, AIA animals presented sleep fragmentation, being incapable to maintain long periods of sleep. In particular, there was a reduction of total sleep time, increased latency to synchronized sleep, reduced sleep efficiency, more change of sleep stages, augmented total alert time, and number of synchronized sleep episodes. The results of behavioral tests indicated a reduction of the pain threshold in AIA animals beginning on the second day after induction of arthritis and remaining so reduced until the end of the study. These results suggest an interrelationship between altered sleep and behavioral patterns observed in rats with adjuvant-induced arthritis.

ACKNOWLEDGMENTS

This work was supported by grants from Associação Fundo de Incentivo à Psicofarmacologia (AFIP) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Monica L. Andersen is the recipient of a fellowship from FAPESP #96/08878-8.

REFERENCES

1. Abbadie C, Besson J-M. Chronic treatments with aspirin or acetaminophen reduce both the development of polyarthritids and c-fos immunoreactivity in rat lumbar spinal cord. Pain 1994; 57: 45-54.


