

## Research Paper

## Age-related changes in deterministic behaviors of nociceptive firing of rat dorsal horn neurons

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**Abstract:** To demonstrate the age-related changes in the dynamics of the nociceptive discharge of dorsal horn nociceptive neurons, the nonlinear prediction method was used to quantify the degree of deterministic behavior within the interspike interval series of tissue injury-induced firing of spinal nociceptive neurons in anesthetized adult young (3~4 months) and aged (>22 months) rats. Subcutaneous bee venom injection induced long-term discharge of spinal wide dynamic range (WDR) neurons in both groups. However, the nociceptive discharge of single WDR neurons in the aged group showed higher determinism when compared with the adult young rats. This result suggests that the dynamics of single nociceptive neurons may not remain constant throughout the life span, and this age-associated change may be an underlying mechanism for various pain manifestations in the elderly population.

**Key words:** aging; spinal cord dorsal horn; wide dynamic range neuron; interspike interval series; nonlinear prediction; bee venom test

## 大鼠脊髓背角神经元痛放电确定性行为的年龄相关变化

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**摘要:** 为阐明脊髓背角神经元痛放电的年龄相关的动力学变化, 本研究采用非线性预报方法, 对两组不同年龄大鼠 (成年青龄鼠 3~4 月龄, 老年鼠 >22 月龄) 组织损伤诱发的脊髓背角神经元痛放电峰峰间期序列进行了确定性行为的定量分析。结果显示, 皮下注入蜜蜂毒, 在两组大鼠均诱发脊髓背角广动力域神经元长时程放电, 而老龄大鼠的痛放电峰峰间期序列表现出更高的可确定性。本研究表明, 单个神经元的痛放电动力学在整个生命过程中并不是恒定不变的, 伤害性神经元活动的年龄相关动力学变化可能是老年人群中多样化痛反应的内在机制之一。

**关键词:** 老龄化; 脊髓背角; 广动力域神经元; 峰峰间期序列; 非线性预报; 蜜蜂毒试验

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The process of aging is always accompanied by a progressive rearrangement of local neural circuits. To present a detailed demonstration on this age-related change, nonlinear dynamics methods were widely used to investigate neural activities under multiple physiological processes<sup>[1]</sup>. A general point of view obtained from these studies is that there is always a change in the dynamics feature in the neural activity with age. Furthermore, it was proposed that these changes may be an underlying mechanism for the

functional alteration of the nervous system in aged groups<sup>[1,2]</sup>. However, the neural activities investigated by most of these previous studies, were based on a generally accepted age-associated functional change in corresponding physiological process, such as cardiovascular control and pulsatile hormone release<sup>[1]</sup>. As for the process of nociceptive information, in contrast, accumulating evidence has proved that pain responses in aged animals are various and the age difference in pain may not be linear<sup>[3]</sup>. The age differences

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in nociception, to a large extent, may be dependent on the pain test employed. As a result, there is no firm conclusion about the age-related changes in nociception. However, these studies strongly indicate that the dynamics of nociceptive neurons may be different among the groups with different ages.

We used spinal wide dynamic range (WDR) neuron as the target to investigate the nociceptive activities. Spinal WDR neuron receives multiple kinds of sensory information including thermal, mechanical and chemical stimuli<sup>[4]</sup>. The response of spinal WDR neuron is directly proportional to the strength of the sensory input<sup>[4-6]</sup>. Moreover, the spontaneous activity can be characterized by a low dimensional dynamic structure<sup>[7]</sup>, and when noxious stimuli were applied, spinal WDR neuron may discriminate various kinds of noxious stimuli by different patterns of spike distribution<sup>[8]</sup>. These results suggest that WDR neuron may be an effective encoder and a good target to investigate the dynamics features of nociceptive activities of spinal cord sensory neurons. For the animal models we used bee venom (BV) test in the present study because BV model has been proved to be an ideal tissue injury model in the evaluation of inflammatory pain<sup>[5,6,9]</sup>. Electrophysiological studies show that s.c. BV injection could induce an immediate robust discharge of WDR neurons which last for about 1 h<sup>[5,6,10]</sup>, and the distribution of spike density and the time course well corresponded with the spontaneous pain behavior observed<sup>[9,10]</sup>. Furthermore, our recent study has confirmed that there exists deterministic behavior within the nociceptive discharge of spinal WDR neurons under BV-induced tissue injury<sup>[11]</sup>. Thus in the present study we tried to quantify, by using nonlinear prediction method, the content of deterministic behavior within the nociceptive discharge of the spinal WDR neurons in rats with different ages.

## 1 MATERIALS AND METHODS

Experiments were performed on 7 adult young (3~4 months old) and 9 aged (>22 months old) Sprague-Dawley albino rats. The experimental setup was described previously<sup>[6,11]</sup> and all procedures were conducted in accordance with the US Public Health Service Policy on Humane Care and Use of Laboratory Animals revised 1986. The rats were initially anesthetized by ketamine (1 mg/kg, i.p.) and general anesthesia was maintained with urethane-chloralose solution (urethane 125 mg/ml and 10 mg/ml) at 5 ml/kg. Absence of foot withdrawal to pinch indicated adequate anaesthesia. The rats were paralyzed by intravenous

pancuronium bromide (2~4 mg/kg·h<sup>-1</sup>). Under artificial ventilation with oxygen, a laminectomy was performed from the T13 to L2 vertebrae to expose the lumbosacral enlargement of the spinal cord. Extracellular single unit recordings were made from L4~5 with glass capillary microelectrodes (10~15 MW) filled with 0.5 mol/L sodium acetate. The dorsal horn neuron was identified as WDR unit on the basis of its characteristic responses to mechanical stimuli applied to the receptive field<sup>[4-6,11]</sup>. BV was lyophilized whole venom of *Apis mellifera* (Sigma, St. Louis, MO) dissolved in 0.9% sterile saline. A volume of 50  $\mu$ l of saline containing 0.2 mg of lyophilized whole venom was used during the whole experiment according to previous study<sup>[5,6,9-11]</sup>. After successful identification of a single WDR unit, BV was injected into the low threshold center of the receptive field and the spike trains were monitored with a memory oscilloscope. During the whole experiment core body temperature was monitored by a thermometer probe inserted into the rectum and maintained at 37.5 $\pm$ 0.5°C.

The approach used for detecting the deterministic structure within the interspike interval (ISI) of WDR neuronal discharge was nonlinear prediction method employed in the study of midbrain dopaminergic neurons<sup>[2,12]</sup>. We first embedded the ISI series into a set of delayed vectors  $x_t = \{x_t, x_{t+\tau}, x_{t+2\tau}, \dots, x_{t+(n-1)\tau}\}$  in state space, where  $n$  is called embedding dimension and  $\tau$  is embedding lag. Let  $x_q$  be an element of the set of lag vectors  $x_t$  generated from an ISI or from the surrogate data sets;  $y_q$  be a subset of  $x_t$  ( $t \neq q$ ) consisting of the  $k$  lag vectors nearest  $x_q$  in terms of Euclidean distance. A prediction of the future event,  $x_{q+n}$ , was calculated as the simple average of projections over the  $k$  lag vectors:

$$\text{pred}(x_{q+n}) = (1/k)(y_{1+n} + y_{2+n} + \dots + y_{k+n}).$$

Predictions of ISI or surrogate events immediately following each lag vector  $x_q$  were calculated, and were compared with the actual observed event,  $x_{t+n}$ . The number of lag vectors used to generate predictions was set to 2% of the total number of lag vectors in the data set<sup>[2,12]</sup>. For each sequence, the value of  $r_s$  (Spearman-rank correlation coefficient) was calculated between the pairs of predictions and the corresponding observed events. We then used a technique employed by Theiler *et al.*<sup>[13]</sup>, where for each  $r_s$  value of the experimental result, the standard deviation for the corresponding ten surrogate  $r_s$  values is calculated, and the number of standard deviations, “Sigmas”, separating the surrogate mean from each experimental value, are determined (nonlinear prediction S score). A higher S score indicates more deterministic behavior. For analysis we

selected 1500 ISI series about 20 min after BV injection. Moreover, the average ISI of the first and second half was statistically indistinguishable (*t*-test). With this evidence we can say the data was stationary over each window the analysis was applied<sup>[11]</sup>. All data were expressed as mean  $\pm$  SEM. Non-parametric Mann-Whitney *U*-test was used for comparative analysis.

## 2 RESULTS

S.c. BV injection induced an immediate, robust firing of spinal WDR neurons lasting for about 1 h. The time course of the scattered ISI graphs showed a significant difference in temporal series of spikes between the adult young and aged groups (Fig. 1). According to the random distribution of the ISI series, the BV-induced spinal WDR neuronal firing could be described as an irregular pattern.

Nonlinear prediction analysis showed deterministic behavior within the ISI series of the discharge of spinal WDR neurons. As can be seen from Fig. 2 (A and B), the correlation coefficient ( $r_s$ ) declined dramatically between the

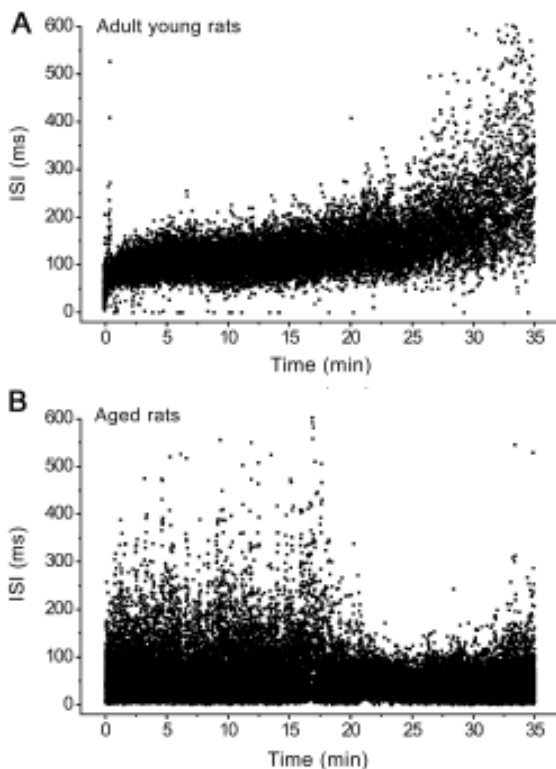


Fig. 1. Time course of the ISI series of BV-induced spinal WDR neuronal firing from the adult young (A) and the aged (B) rats. Note that the distributional pattern of temporal series of the BV-induced firing in spinal WDR neurons is different between the two groups. Zero in the abscissa is the starting time for subcutaneous BV injection.

second and third prediction steps. This change in dynamics implies that we may be able to predict what will happen within a short time, but a long-range prediction will be

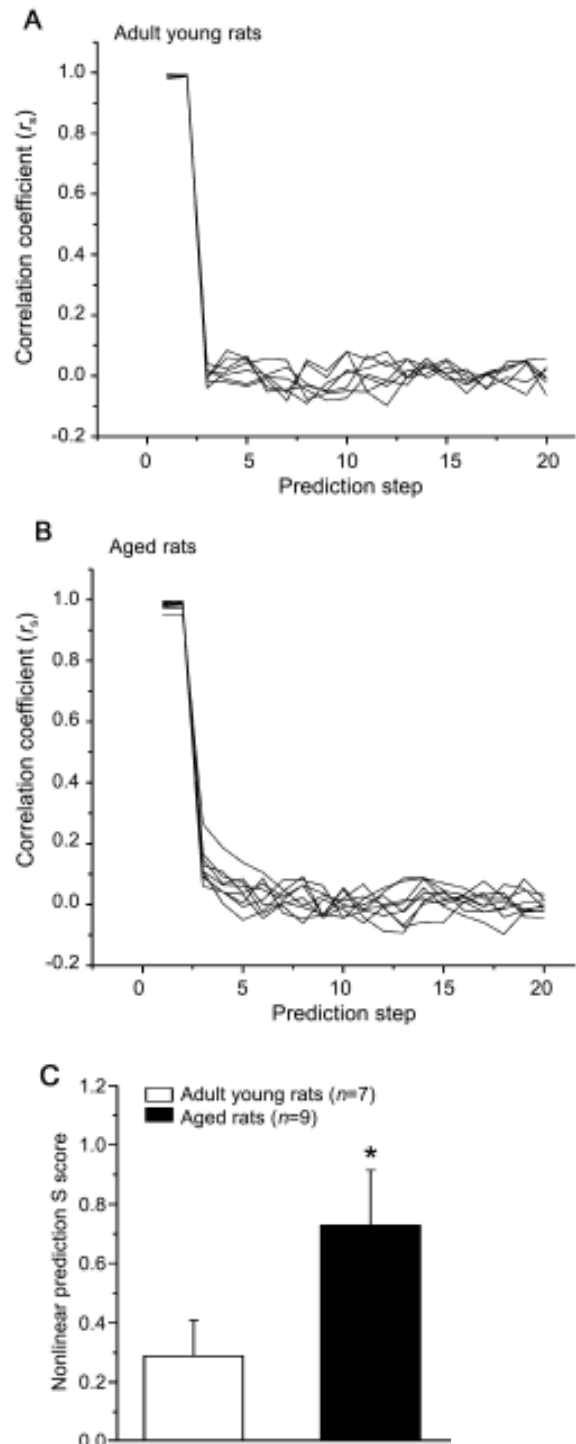


Fig. 2. Changes in nonlinear prediction correlation coefficient ( $r_s$ ) with the 20 prediction steps in the adult young group (A,  $n=7$ ) and the aged group (B,  $n=9$ ). C, Comparative analysis shows a significant higher nonlinear prediction S score in the aged rats than that of the adult young rats ( $0.73 \pm 0.17$  vs  $0.29 \pm 0.10$ , respectively). Vertical bars:  $\pm$ SEM, \* $P < 0.05$ .

impossible. This result is consistent with our previous study, which identified the deterministic behavior within the same firing series<sup>[11]</sup>, though the methods used were different. Furthermore, the analysis performed on the selected windowed data showed significant difference between the adult young and aged rats. The ISI series for the aged group possess higher predictability (Fig. 2B), according to the  $r_s$  value at the third step than the adult young group (Fig. 2A). The content of deterministic behavior, as judged by the nonlinear prediction S score (Fig. 2C), was significantly higher for the aged group ( $0.73 \pm 0.17$ ,  $n=9$ ) than that belonging to the adult young group ( $0.29 \pm 0.10$ ,  $n=7$ ,  $P < 0.05$ ).

### 3 DISCUSSION

Previous studies showed age-related changes in the dynamics feature of the multiple neural activities. However, when a specific function was taken into account, these changes were not universal according to different brain areas. As for the mechanism for this variety, it has been demonstrated that neurochemical and neuroanatomical changes showed diverse features for different brain areas<sup>[14]</sup>. In a nonlinear dynamics context, there can be a loss<sup>[1,2]</sup> or increase<sup>[15]</sup> of deterministic behavior in different physiological activities. As for the physiological meaning for these changes, it was proposed that it may represent the altered functional state of the biological system with age<sup>[1,2,15]</sup>. With this perspective, a particular physiological process was presumed to be characterized by a complex interaction of multiple control mechanisms, and the circuital deterministic structure may arise from the internal feedback loops of certain nonlinear systems<sup>[16]</sup>. As a consequence, rearrangement of the system, which accompanies the aging process, may lead to a remarkable change in the dynamics feature. For example, the spontaneous activity in the midbrain dopaminergic neurons showed aged-related loss of deterministic structure, and it might be a result of perturbed coordination of synaptic input that reflects an increase of synaptic noise<sup>[2]</sup>.

Our present study showed an increase in deterministic behavior within the nociceptive discharge of spinal WDR neurons under BV-induced tissue injury state in aged rats. This result was consistent with our previous behavioral observation in which the immediate peak time of the BV induced persistent spontaneous pain-related responses in aged rats was delayed for about 30 min when compared with adult young rats<sup>[17]</sup>. Since the BV induced persistent spontaneous pain-related response has been proved to

be mediated by the persistent firing of spinal WDR neurons<sup>[5,6]</sup>, it is therefore suggested that the decrease in the number of pain-related behavioral response in the initial period of 1 h time course in aged rats is due to the decrease in the number of spike discharges of dorsal horn WDR neurons as shown in the present study. In turn we propose that the behavioral and neuronal changes in pain perception observed in aged rats are likely to be determined by the intrinsic temporal changes in ISI which can only be detected by the nonlinear dynamic analysis. The reasons for the age-related change in pain are not clear, but the following issues should be taken into account: (1) unlike most physiological processes, pain responses in aged animals showed a diverse feature and the sensitivity to noxious stimuli and pain response might not be linear<sup>[3]</sup>; (2) the nociceptive WDR neuronal discharge in our present study is driven by persistent, strong sensory input, and it also receives the regulation of descending pain control system and local neural circuit<sup>[14]</sup>, which is important to determine the dynamic structure of dorsal horn sensory neurons<sup>[7]</sup>. In the aged animals, it has been found there is a remarkable degeneration of endogenous pain inhibition system<sup>[18]</sup> and a significant decrease in the number of dorsal root ganglia cells with aging<sup>[19]</sup>; and (3) furthermore, spinal neurotransmitters involved in the modulation of pain information, such as serotonin and norepinephrine, decreased significantly with age<sup>[20]</sup>. These changes may result in a new dynamic feature of the activity of nociceptive neurons. However, due to all these unparallel down regulation, the inherent physiological property of the WDR neuron itself may account for, to a greater extent, the outward dynamics features of its discharge. Though this temporal feature of single WDR neuronal activity may not be enough to explain the various pain behaviors in aged groups, it might reflect the degeneration of pain control system with age, and quantification of deterministic behavior within the nociceptive discharge may be an alternative method to monitor senescence.

In summary, our present study showed increased deterministic behavior within the nociceptive discharge of spinal WDR neuron under BV-induced tissue injury. This change in the dynamics of single nociceptive neuron, may result from degeneration of the pain control system with advancing age, and may be an underlying mechanism for the variety of pain manifestations in aged groups.

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