

# (-)-Epigallocatechin Gallate Attenuates Spinal Motoneuron Death Induced by Brachial Plexus Root Avulsion in Rats

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## Research Article

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# Abstract

**Background** EGCG benefits a variety of insults.

**Methods** Rats were randomized into: EGCG, Avulsion, and Sham.

**Results** P-c-Jun-positive motoneurons and the ratio of p-c-Jun/c-Jun were significantly lower in EGCG group at 3d and 7d after injury.

**Conclusions** EGCG protected motoneurons

## Introduction

The survival of spinal motoneurons is the key to the recovery of motor function [1]. we explored whether EGCG administration had any beneficial effects on the motoneurons.

## Materials And Methods

### Animal Preparation

Rats were randomized into: EGCG, Avulsion, and Sham . The animals in EGCG group received daily doses of 100 mg/kg EGCG (Hangzhou Gosun Biotechnologies Co., Ltd., China) from day 1 to day 7 as described in previous study [2].

### Surgical Procedures

The root avulsions of the left brachial plexus were performed according to the procedures described in previous publications [3-7].

At the end of each survival time, animals were deeply anesthetized with a lethal dose of ketamine and xylazine and perfused transcardially with normal saline followed by 4% paraformaldehyde in 0.1 M PB (pH 7.4). Every fifth section from each animal was used for Nissl staining and immunohistochemistry analysis.

### Nissl staining

After deparaffinization and rehydration, the sections were stained with warmed 0.5% cresyl violet solution (10 minutes) as described [8].

### Immunohistochemical staining

An avidin-biotin kit was used for the immunohistochemical (IHC) staining. Briefly, the sections were deparaffinized and treated with 3% H<sub>2</sub>O<sub>2</sub> for 15 min to block the endogenous peroxidase. The sections

were exposed to normal bovine serum for 30 min and then incubated with the primary antibodies overnight at 4°C.

## **Statistical Analyses**

The data are presented as the mean  $\pm$  SD and were analyzed using statistical software IBM SPSS version 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

# **Results**

## **Survival of the Injured Motoneurons**

Significant decreases of motoneurons in the spinal cords were observed from days 14 to 28 after the injury.

## **Apoptosis in Motoneurons**

The number of caspase-3-positive motoneurons was significantly increased in the Avulsion group compared to the Sham group at 3d, 7d, 14d and 28d after the injury.

## **Effects of EGCG on phospho-JNK expression**

The numbers of p-JNK-positive motoneurons were significantly increased in the Avulsion group compared to the Sham group at 3d and 7d after injury.

## **Effect of EGCG on phospho-c-Jun expression**

At 3d, 7d and 14d after injury, the numbers of p-c-Jun-positive motoneurons were significantly increased in the Avulsion group compared with the Sham group.

# **Discussion**

The data on astrocyte and microglia activity after brachial plexus root avulsion is described in previous publications. Although roots repair is unable to completely hampers neurodegeneration, reimplantation of avulsed roots is neuroprotective by itself [9]. Motoneuron survival after root repair is based on neurotrophic factor production by glial cells at the CNS/PNS interface [9]. This has also been connected to the breakage of the blood-brain barrier, allowing neurotrophic factor producing immune cells to enter spinal cord gray matter environment [10].

Clinically, patients with brachial plexus injuries suffer multiple injuries that delay treatments such as surgical repair or reimplantation [11]. Therefore, available neuroprotective treatments should be offered as early as it is safe to do so and the search for neuroprotective treatments must be expedited to prevent permanent loss of function among patients.

It has also been reported that EGCG exhibits neuroprotective actions against a variety of injuries [12-18]. Therefore, we chose a 100 mg/kg dose of EGCG for the treatment of brachial plexus avulsion in the current study. In the present study, we found that the motoneurons were protected by EGCG against the death induced by brachial plexus root avulsion as evidenced by our present data.

## Conclusions

We observed that the motoneurons were protected against death by EGCG.

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## Declarations

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### Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Contribution

Conceptualization: Zunpeng Liu Fatai Lu

Data curation: Fatai Lu Guodong Zhang

Formal analysis: Yingkang Zhu

Investigation: Fatai Lu Guodong Zhang

Methodology: Zunpeng Liu Fatai Lu

Project administration: Fatai Lu Yingkang Zhu

Resources: Zunpeng Liu

Software: Fatai Lu Yingkang Zhu

Supervision: Zunpeng Liu

Validation: Zunpeng Liu

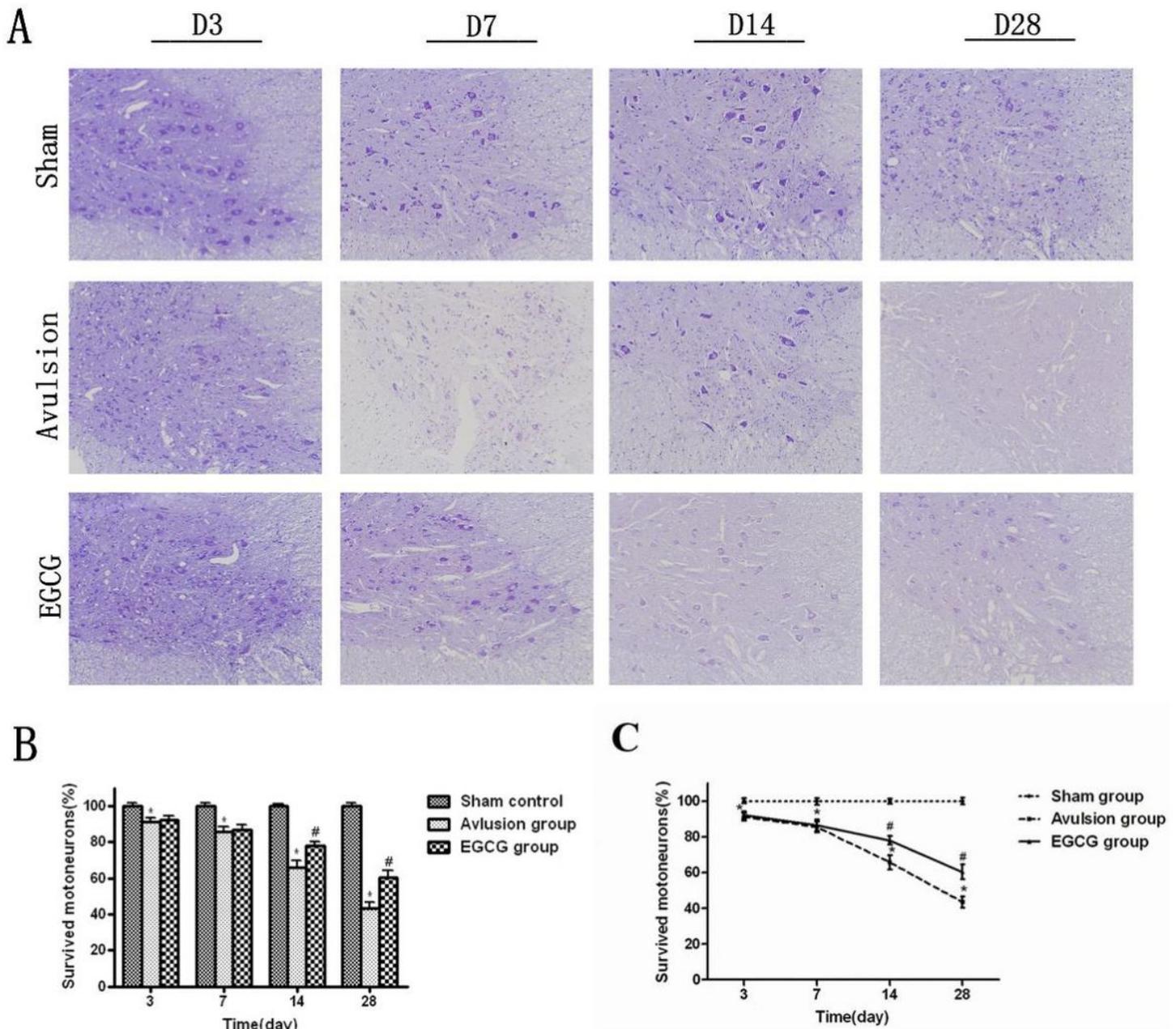
Visualization: Fatai Lu Guodong Zhang Yingkang Zhu

Writing - original draft: Fatai Lu Guodong Zhang

Writing-review editing: Zunpeng Liu

Zunpeng Liu proposed the concept and research methods, and supervised the experiment. Fatai Lu and Guodong Zhang completed the experiment and wrote the main manuscript text. Yingkang Zhu prepared figures 1-6. All authors reviewed the manuscript.

## Figures

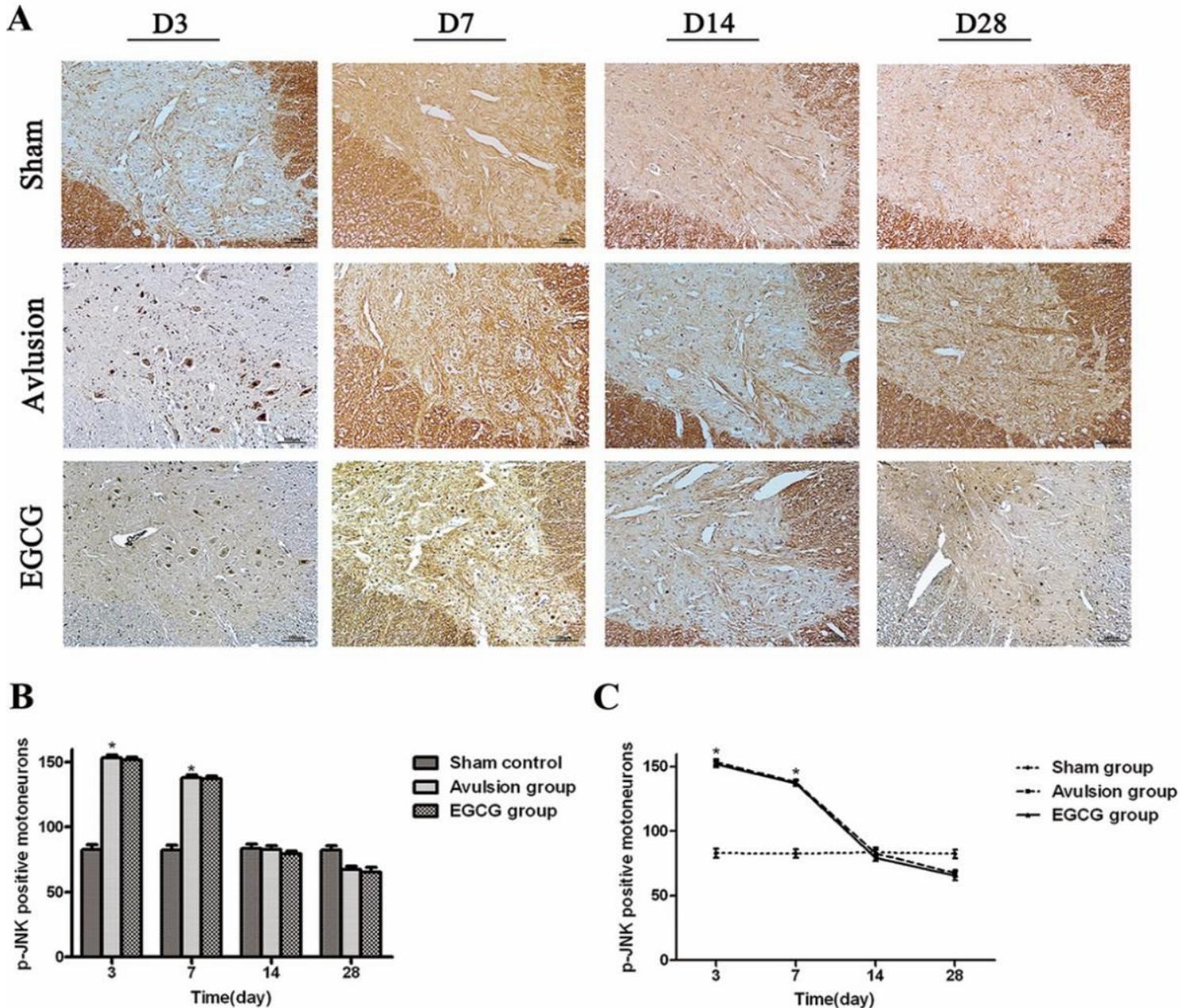


**Figure 1**

(A) Nissl staining of transverse sections of the spinal cord. (B,C) Percentages of surviving motoneurons in the spinal cord sections of the rats. The bars represent the means±the SDs (n=5 every group, \*P<0.05 between the Sham and Avulsion groups, #P<0.05 between the Avulsion and EGCG groups; D14, 14 days after injury; D28, 28 days after injury; Scale bar = 100µm).

**Figure 2**

(A) Immunohistochemical images of caspase-3-positive motoneurons. (B,C) Percentages of caspase-3-positive motoneurons in the spinal cord sections of the rats at the indicated times. The bars represent the means  $\pm$  the SDs ( $n=5$  every group,  $*P<0.05$  between the Sham and Avulsion groups,  $\#P<0.05$  between the Avulsion and EGCG groups; D3, 3 day after injury; D7, 7 days after injury; D14, 14 days after injury; D28, 28 days after injury; Scale bar = 100 $\mu$ m).



**Figure 3**

(A) Immunohistochemical images of phospho-JNK-positive motoneurons. (B,C) Percentages of phospho-JNK-positive motoneurons in the spinal cord sections of the rats at the indicated times. The bars represent the means  $\pm$  the SDs ( $n=5$  every group,  $*P<0.05$  between the Sham and Avulsion groups; D3, 3 day after injury; D7, 7 days after injury; D14, 14 days after injury; D28, 28 days after injury; Scale bar = 100 $\mu$ m).

## Figure 4

Western blot analyses of the JNK and phospho-JNK levels in the ipsilateral spinal segments following root avulsion of the left brachial plexuses of adult rats. (A) The samples were obtained from rats that were subjected to sham operations or root-avulsions at 3d, 7d, 14 d and 28d post-injury. The optical density(OD) of each protein was measured from the Western blot. (B,C) Semiquantitative changes in the level of phospho-JNK normalized to JNK expression after root-avulsion were determined by OD measurements. The data are presented as the means $\pm$ the SDs (n=5 every group, \*P<0.05 between the Sham and Avulsion groups; D3, 3 day after injury; D7, 7 days after injury; D14, 14 days after injury; D28, 28 days after injury).

## Figure 5

(A) Immunohistochemical images of phospho-c-jun-positive motoneurons. (B,C) Percentages of phospho-c-jun-positive motoneurons in the spinal cord sections of the rats at the indicated times. The bars represent the means $\pm$ the SDs (n=5 every group, \*P<0.05 between the Sham and Avulsion groups; D3, 3 day after injury; D7, 7 days after injury; D14, 14 days after injury; D28, 28 days after injury; Scale bar = 100 $\mu$ m).

## Figure 6

Western blot analyses of the c-jun and phospho-c-jun levels in the ipsilateral spinal segments following root avulsion of the left brachial plexuses of adult rats. (A) The samples were obtained from rats that were subjected to sham operations or root-avulsions at 3d, 7d, 14 d and 28d post-injury. The OD of each protein was measured from the Western blot. (B,C) Semiquantitative changes in the level of phospho-c-jun normalized to c-jun expression after root-avulsion were determined by OD measurements. The data are presented as the means $\pm$ the SDs (n=5 every group, \*P<0.05 between the Sham and Avulsion groups, #P<0.05 between the Avulsion and EGCG groups; D3, 3 day after injury; D7, 7 days after injury; D14, 14 days after injury; D28, 28 days after injury)