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# The CNTF-derived peptide mimetic Cintrofin attenuates spatial-learning deficits in a rat post-status epilepticus model

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## HIGHLIGHTS

- We report first *in vivo* efficacy data for the novel CNTF peptide mimetic Cintrofin.
- Impact of Cintrofin was evaluated in a rat post-status epilepticus model.
- Cintrofin prevented long-term alterations in number of neuronal progenitor cells.
- Cintrofin significantly attenuated the persistence of basal dendrites.
- Cintrofin exerted beneficial effects on disease-associated cognitive impairment.

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## ABSTRACT

Ciliary neurotrophic growth factor is considered a potential therapeutic agent for central nervous system diseases. We report first *in vivo* data of the ciliary neurotrophic growth factor peptide mimetic Cintrofin in a rat post-status epilepticus model. Cintrofin prevented long-term alterations in the number of doublecortin-positive neuronal progenitor cells and attenuated the persistence of basal dendrites. In contrast, Cintrofin did neither affect acute status epilepticus-associated alterations in hippocampal cell proliferation and neurogenesis nor reveal any relevant effect on seizure activity. Whereas status epilepticus caused a significant disturbance in spatial learning in reversed peptide-treated rats, the performance of Cintrofin-treated rats did not differ from controls. The study confirms that Cintrofin comprises an active sequence mimicking effects of its parent molecule. While the data argue against an antiepileptogenic effect, they indicate a putative disease-modifying impact of Cintrofin.



## 1. Introduction

The ciliary neurotrophic factor (CNTF) possesses various beneficial effects demonstrated in experimental models and clinical trials. It proved to promote neurite outgrowth, ameliorate learning and memory deficits, enhance glial glutamate uptake and thereby protect from excitotoxic cell damage [1–3,6,7,9,11]. Considering that neuronal plasticity and network reorganization as well as neuronal cell loss characterize the process of epilepsy development following an initial brain injury [12,15], treatment with CNTF might be an attractive prophylactic compound to interfere with epileptogenesis. In support of this hypothesis intrahippocampal injections of CNTF significantly attenuated kainate mediated cell death [8]. Translational development of respective non-invasive approaches is hampered by limitations in stability and brain distribution of large complex molecules [1]. Therefore, the most important work in this context relates to the development of small molecules that mimic or potentiate the activity of neurotrophic factors [1]. The region corresponding to the A helix–AB loop, and CD loop–D helix of CNTF has been shown to be important for high affinity interactions of CNTF with leukemia inhibitory factor receptor (LIFR) [10]. Recently, a peptide derived from this region termed Cintrofin has been demonstrated to induce STAT3, Akt and ERK phosphorylation in primary neurons, induce neuronal differentiation and promote neuronal survival *in vitro* [17]. Based on its promising *in vitro* effects, we decided to evaluate the disease-modifying and

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antiepileptogenic potential of Cintrofin in a rat post-status epilepticus (SE) model.

## 2. Materials and methods

### 2.1. Animals

Female Sprague Dawley rats (200–224 g) were purchased from Harlan Winkelmann, An Venray, The Netherlands, or Charles River, Sulzfeld, Germany. All experiments were done in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize pain or discomfort of the animals used.

### 2.2. Determination of Cintrofin concentration in plasma and CSF

Biotinylated Cintrofin (Schafer-N, Copenhagen, Denmark) was administered to 18 rats (Charles River, Sulzfeld, Germany; 10 mg/kg s.c.). At 15 min, 30 min, 1 h, 2 h, 4 h, and 8 h after injection blood samples were taken under fentanyl/droperidol/midazolam anesthesia. Cerebrospinal fluid (CSF) was collected 1 h after administration as described previously [18]. Cintrofin concentrations were measured using a competitive ELISA described recently [13] (for details see Data S1). Peptide concentration peaked in plasma 1 h after administration and was detected in CSF with ratio 8 (plasma) to 1 (CSF). Thus, the pharmacokinetic analyses confirmed that Cintrofin crosses the BBB (Supplementary Fig. 1).

### 2.3. Post-status epilepticus model

Electrodes were stereotactically implanted into the right basolateral amygdala (BLA) of 60 rats (Harlan Winkelmann, An Venray, Netherlands) as described previously [14] (stereotactic coordinates in mm relative to Bregma AP – 2.2; L – 4.7; DV – 8.5). Bupivacain 0.5% was used for local anesthesia. For induction of a SSSE, rats ($n = 34$) were electrically stimulated *via* the BLA electrode as described previously [19].

### 2.4. Treatment with the CNTF-derived peptide mimetic Cintrofin and BrdU labeling

The sequence of Cintrofin was derived from human CNTF (148-DGGLFEKKLWGLKV-161; UniProtKB entry P26441). Cintrofin and a respective control peptide (amino acids order reversed) were synthesized by GL Biochem Ltd. (Shanghai, China). Rats were treated with either reversed peptide or Cintrofin (10 mg/kg s.c.). Five minutes following SSSE animals received the first administration, which was repeated at days 1–4 following SSSE. Furthermore, rats received a total of ten i.p. injections of 50 mg/kg 5′Bromodeoxyuridine (BrdU; Sigma–Aldrich, Taufkirchen, Germany), which was injected twice daily at days 5–9 following SSSE (interval 8 h).

### 2.5. Monitoring and behavioral evaluation

Seven weeks after SSSE, animals were monitored for 19 days as described elsewhere [4,14]. Since only 16 rats could be EEG-monitored at the same time, we randomly chose rats from each group ($n = 8$/treatment group) for monitoring of spontaneous seizures. For rating severity of seizures, Racine's scale was used [16]. Eleven weeks after SSSE, behavior of the animals was evaluated in groups of $n = 10$ in open field (OF), elevated plus maze (EPM), Black-white box (BWB) and Morris Water Maze (MWM). During the MWM testing phase one animal was euthanized reducing the number of Cintrofin-treated SE rats to $n = 9$. Behavioral paradigms were performed as described previously [4,14,19].

### 2.6. Histological and immunohistological evaluation

Following behavioral analyses, rats were perfused as reported previously [19]. Nissl staining and immunohistochemistry was performed as described earlier [14,20] (for details see Data S2). One series was Nissl-stained with thionin to verify the electrode localization and to visualize neurodegeneration. Cell proliferation in the early phase following SE was assessed by BrdU staining, whereby neurons were identified by BrdU/NeuN double-staining. Neuronal progenitor cells and early postmitotic neurons were evaluated based on doublecortin expression. Activated microglia were identified based on expression of the surface marker ED1. Evaluation of neurodegeneration was performed in CA1, CA2, CA3a, CA3c, hilus, dentate gyrus, parietal and piriform cortex of the hippocampal formation. Severity of neuronal damage was assessed by a grading system used in previous studies [4,5]. The number of doublecortin-labeled cells and hilar basal dendrites was quantified by unbiased stereological counting as described previously [21]. In the CA1, CA3a, CA3c, hilus and dentate gyrus activated microglia were assessed by a grading system described previously [19]. Double-labeled cells were counted in at least six sections per animal as reported in former studies [19,20]. Due to damage of some brain sections we were not able to analyze data from each rat. BrdU-, BrdU/NeuN-, and ED1-labeling were analyzed in reversed peptide-treated control rats ($n = 10$), reversed peptide-treated SE rats ($n = 10$), Cintrofin-treated control rats ($n = 10$), and Cintrofin-treated SE rats ($n = 9$). Doublecortin-labeled cells were analyzed in reversed peptide-treated control rats ($n = 10$), reversed peptide-treated SE rats ($n = 10$), Cintrofin-treated control rats ($n = 10$), and Cintrofin-treated SE rats ($n = 8$).

### 2.7. Statistics

Seizure frequency and duration were analyzed by Mann–Whitney *U*-test. Kruskal–Wallis test followed by Mann–Whitney *U*-test was used for the analysis of neurodegeneration and microglia activation. Behavioral parameters and differences in proliferation rates and neurogenesis were analyzed by two-way analysis of variance, followed by *post hoc* comparison with Bonferroni. All tests were used two-sided and $p < 0.05$ was considered significant.

## 3. Results

### 3.1. Impact of Cintrofin on neurogenesis, hippocampal neurodegeneration and microglia activation

In the granule cell layer and the hilus of reversed peptide-treated rats with SE the number of BrdU-labeled cells significantly exceeded that in reversed peptide-treated control rats without SE by 86% (Fig. 1A). In Cintrofin-treated rats the SE-associated increase in the cell proliferation rate reached comparable levels to reversed peptide-treated SE rats exceeding that in control rats without SE by 88% ($p < 0.0001$).

In rats treated with the reversed control peptide analysis revealed the typical SE-associated increase in hippocampal neurogenesis, which was assessed based on BrdU/NeuN double-labeling ($p < 0.0001$) (Fig. 1B). Cintrofin did not alter this effect of SE.

In the reversed peptide-treated group, SE resulted in long-term effects on the number of doublecortin-expressing neuronal progenitor cells with a significant increase as compared to control animals without SE ($p < 0.05$; Fig. 1D). The number of doublecortin-positive cells with basal dendrites was