RESEARCH ARTICLE

End-to-end and end-to-side neurorrhaphy between thick donor nerves and thin recipient nerves: an axon regeneration study in a rat model

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Abstract

During nerve reconstruction, nerves of different thicknesses are often sutured together using end-to-side neurorrhaphy and end-to-end neurorrhaphy techniques. In this study, the effect of the type of neurorrhaphy on the number and diameter of regenerated axon fibers was studied in a rat facial nerve repair model. An inflow-type end-to-side and end-to-end neurorrhaphy model with nerve stumps of different thicknesses (2:1 diameter ratio) was created in the facial nerve of 14 adult male Sprague-Dawley rats. After 6 and 12 weeks, nerve regeneration was evaluated in the rats using the following outcomes: total number of myelinated axons, average minor axis diameter of the myelinated axons in the central and peripheral sections, and axon regeneration rate. End-to-end neurorrhaphy resulted in a significantly greater number of regenerated myelinated axons and rate of regeneration after 6 weeks than end-to-side neurorrhaphy; however, no such differences were observed at 12 weeks. While the regenerated axons were thicker at 12 weeks than at 6 weeks, no significant differences in axon fiber thickness were detected between end-to-end and end-to-side neurorrhaphy. Thus, end-to-end neurorrhaphy resulted in greater numbers of regenerated axons and increased axon regeneration rate during the early postoperative period. As rapid reinnervation is one of the most important factors influencing the restoration of target muscle function, we conclude that end-to-end neurorrhaphy is desirable when suturing thick nerves to thin nerves.

Key Words: *epineural window; transplantation; nerve reconstruction; suturing; facial nerve repair; axonal repair; neurosurgery; peripheral nerve; neural regeneration*

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doi: *10.4103/1673-5374.230296*

Accepted: *2018-01-31*

Introduction

Neurorrhaphy conducted under a surgical microscope is an established neurosurgical procedure (Viterbo et al., 1994; Yoleri et al., 2000; Hayashi et al., 2004; Frey et al., 2006; Ueda et al., 2007). However, the procedure can be challenging when nerve stumps of different cross-sectional areas need to be sutured together, and postoperative results from such procedures have considerable variation. Connecting a thick nerve to a thin nerve is particularly challenging. For example, a sural nerve jump graft to treat facial paralysis (May et al., 1991; Ueda et al., 2007) requires connecting the thick sural nerve with a considerably thinner buccal branch of the facial nerve on the paralyzed side. Similar challenges are also encountered in cross facial nerve grafting and surgical nerve transplantation following malignant craniofacial tumor resection.

In these scenarios, end-to-end neurorrhaphy leads to an excess of regenerated axons that extend uselessly outside of the suture surface, and the distal recipient axons undergo Wallerian degeneration within 3 days after injury. End-to-end neurorrhaphy is also associated with worse functional outcome when suturing a thick nerve to a thin nerve. For example, end-to-end neurorrhaphy between the nerve to the rectus femoris muscle and the thick buccal branch of the facial nerve showed significantly better muscle strength, axonal area, and axon number compared to suturing to the thin marginal mandibular branch (MacQuillan and Grobbelaar, 2008). End-to-side neurorrhaphy, which was originally described in the late 19th century (Letievant, 1873; Hoffman, 1884), has recently gained attention because of the finding that axon elongation occurs even if nerve stumps are not directly attached to each other (Viterbo et al., 1994). In particular, by suturing the end of the donor nerve to the side surface of the recipient nerve, an "inflow type" of nerve regeneration (regenerated axons extend through an epineural window into the windowed nerve) is established (Okouchi et al., 2008, 2009). In inflow type end-to-side neurorrhaphy, 52.8% of axon fibers regenerated, compared to 89% in outflow type end-to-side neurorrhaphy (regenerated axons extend in the opposite direction).

We hypothesized that the larger contact area between donor axons and recipient axons in end-to-side neurorrhaphy compared to end-to-end neurorrhaphy when suturing a thick nerve to a thin nerve reduces the number of regenerated axons that are misdirected or deviated and, as a result, achieves better functional recovery (**Figure 1**). In the current study, we created an inflow-type end-to-side and end-to-end neurorrhaphy model with nerve stumps of different thicknesses using a rat facial nerve, and we investigated axon regeneration by measuring the number and diameter of regenerated axons in the two models.

Materials and Methods

Neurorrhaphy model

Fourteen 9-week-old male Sprague-Dawley (SD) rats (Japan SLC, Inc., Hamamatsu, Japan), weighing 334 ± 15 g, were

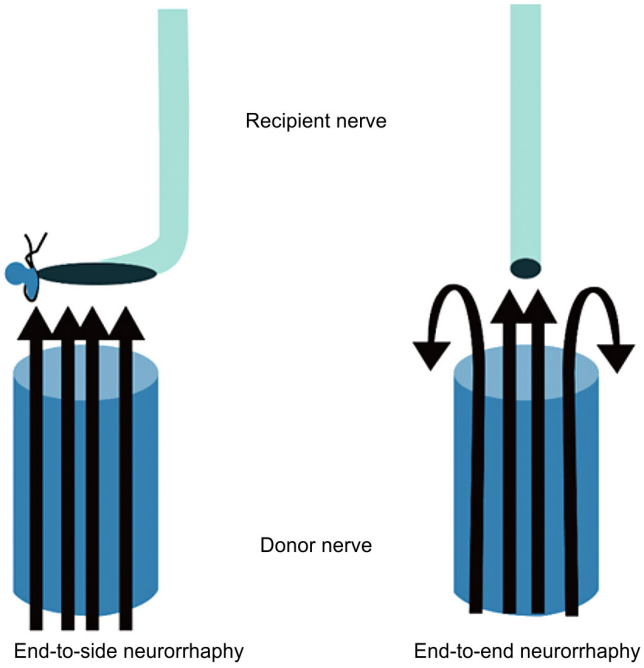

Figure 1 Schematic diagram of end-to-side and end-to-end neurorrhaphy showing a larger axon contact area between donor and recipient nerves in the end-to-side neurorrhaphy model.
Note that in end-to-side neurorrhaphy in this experimental model, an epineural window was opened in the recipient nerve.

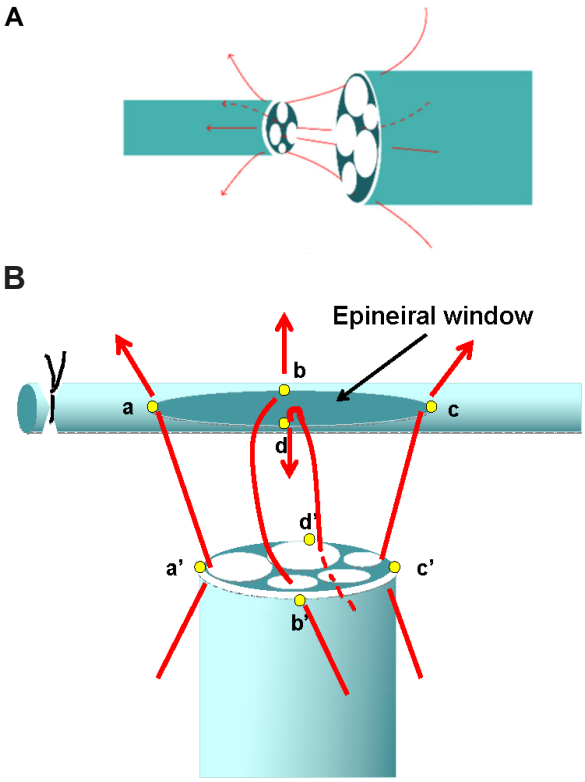

Figure 3 Schematic of surgical technique used in (A) end-to-end neurorrhaphy, and (B) end-to-side neurorrhaphy.
The nerve stumps were sutured with minimal axonal deviation in end-to-end neurorrhaphy and by opening an epineural window in end-to-side neurorrhaphy. a'–d' corresponds to a–d respectively when performing neurorrhaphy.

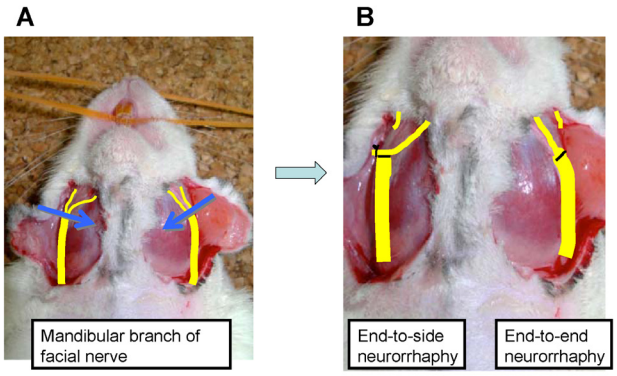

Figure 2 Experimental model showing (A) end-to-side and (B) end-to-end neurorrhaphy model in the rat facial nerve.
The mandibular branch was surgically detached until its bifurcation, and neurorrhaphy was performed under an operating microscope.

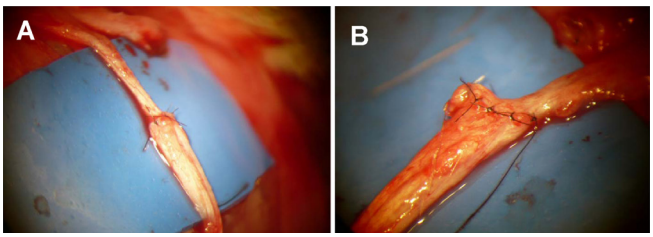

Figure 4 Appearance of nerve repair immediately following (A) end-to-end neurorrhaphy and (B) end-to-side neurorrhaphy.
The ratio of the diameters of the sutured nerves was approximately 1:2.

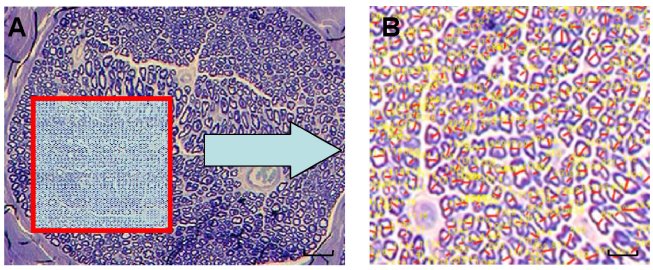

Figure 5 Histological appearance of cross-section of donor nerve at 12 weeks under (A) low and (B) high magnification (Toluidine Blue stain, original magnification 200×).
The total number of myelinated axons and their average minor axis diameter (traced in red) were measured using WinROOF image analysis software. Scale bars: 30 μm in A and 10 μm in B.

used to create an experimental neurorrhaphy model using the mandibular branch of the facial nerve. Anesthesia was induced using ether and maintained with intraperitoneal administration of Nembutal (Sumitomo Dainippon Pharma, Osaka, Japan). Next, the rat was fixed in a supine position, and the region extending from the face to the lower jaw was shaved, disinfected, and prepared for surgery.

A U-shaped incision was made, and the mandibular branch of the facial nerve was identified on both sides. The nerve was carefully dissected until its bifurcation into two branches. After carefully exposing the bifurcation region, two neurorrhaphy models were produced using an oper-

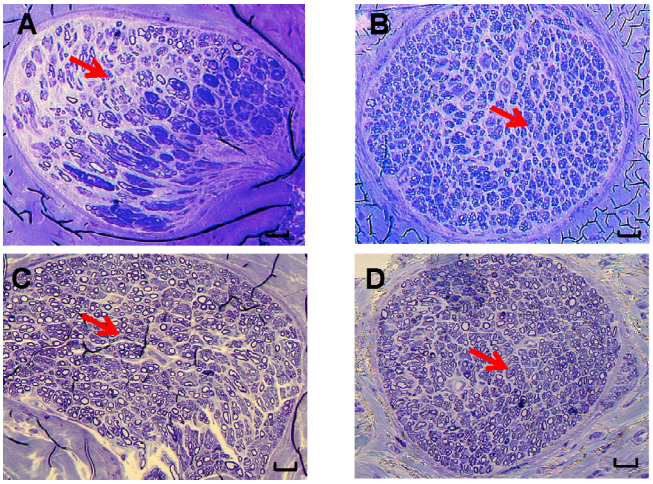

Figure 6 Histological appearance of cross-section of recipient nerves at (A, B) 6 weeks and (C, D) 12 weeks, following (A, C) end-to-side neurorrhaphy and (B, D) end-to-end neurorrhaphy (original magnification, 200×).
At 6 weeks, the total number of regenerated myelinated axons on the distal side was significantly higher for end-to-end neurorrhaphy (B) than for end-to-side neurorrhaphy (A). However, at 12 weeks, there were no significant differences between the two groups (C, D) (Toluidine Blue stain, scale bars: 30 μm). $n = 7$ for each time point.

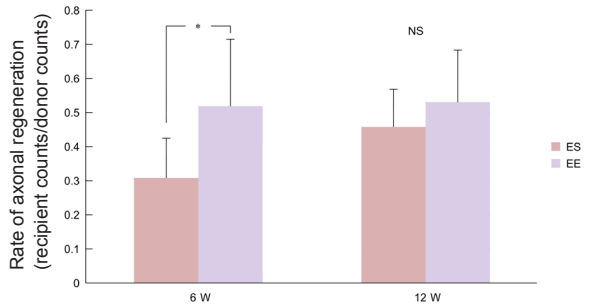

Figure 8 Rate of axonal regeneration.
At 6 weeks, the axon regeneration rate was significantly higher in end-to-end neurorrhaphy than in end-to-side neurorrhaphy (*$P < 0.05$); however, at 12 weeks, a significant difference was not observed. Data are expressed as the mean ± SD from $n = 7$ rats for each time point. ES: End-to-side neurorhaohy; EE: end-to-end neurorrhaphy.

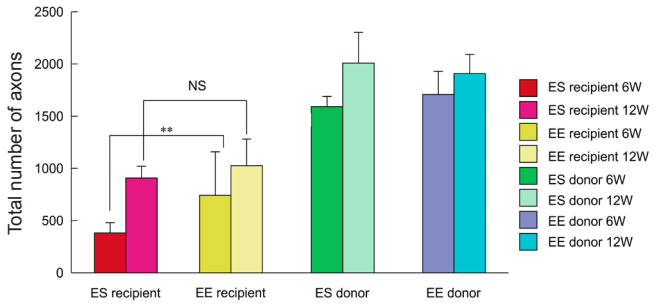

Figure 7 Total number of myelinated axons.
At 6 weeks, the total number of regenerated myleinated axons in the recipient side was significantly higher in end-to-end neurorrhaphy than in end-to-side neurorrhaphy (**$P < 0.01$); however, at 12 weeks, a significant difference was not observed. Data are expressed as the mean ± SD from $n = 7$ rats for each time point. ES: End-to-side neurorhaohy; EE: end-to-end neurorrhaphy.

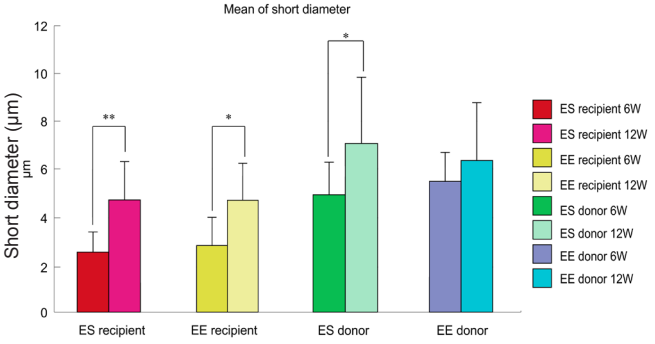

Figure 9 Minor axis diameter of regenerated axons.
A significant increase in axon diameter was observed between 6 and 12 weeks in both end-to-end and end-to-side neurorrhaphy groups (*$P < 0.05$, **$P < 0.01$); however, there were no significant differences between the two groups at either 6 or 12 weeks. Data are expressed as the mean ± SD from $n = 7$ rats for each time point. ES: End-to-side neurorhaohy; EE: end-to-end neurorrhaphy.

ating microscope (BX53, Olympus, Tokyo, Japan) (**Figure 2**). On each side, an approximately 1-mm wide region of the mandibular branch of the facial nerve that included the bifurcation point was excised. The central stump was confirmed to be approximately twice the diameter of the peripheral stumps. Next, the central stump (thicker donor nerve) and the medial strand of the peripheral stump (thinner recipient nerve) were stitched using an end-to-end or end-to side technique under the operating microscope.

(1) End-to-end neurorrhaphy group (EE): On the left side, the central and peripheral recipient nerve stumps were stitched end-to-end with a 10-0 black nylon suture. The nerve membrane and peripheral membrane were sutured with four stitches.

(2) End-to-side neurorrhaphy group (ES): On the right side, the central and peripheral recipient nerve stumps were stitched end-to-side with a 10-0 black nylon suture. The peripheral nerve stump was ligated with a 6-0 nylon suture, and an epineural window was created in the recipient nerve by completely excising the epineural membrane and exposing the perineural membrane. To the epineural window and four points of the central stump (a→a', b→b', c→c', d→d') (**Figure 3**), the nerve membrane and peripheral membrane were sutured.

In both models, 2–4 supplemental stitches were used so as not to expose the thicker nerve fiber bundles on the central stump (**Figure 3**). After the repairs were performed (**Figure 4**), the skin incision was then closed in layers. After 6–12 weeks, rats were evaluated for nerve regeneration. The study followed the guidelines of the Japanese Association for Laboratory Animal Science (JALAS) and was approved by the institutional animal ethics committee (Institute for Animal Experimentation, St Marianna University Graduate School of Medicine, No. 2013053004).

Evaluation of nerve regeneration

Nerve regeneration was assessed at 6 weeks (n = 7 rats) and 12 weeks (n = 7 rats) after neurorrhaphy. Rats were re-anesthetized, and the facial nerve repair regions were exposed. The repair region, including 10 mm of the peripheral nerve and 10 mm of the central nerve, was retrieved, and the rats were subsequently euthanized. Then, 3 mm samples from the retrieved nerves were Epon-embedded, sectioned, stained with Toluidine Blue (Sigma-Aldrich, St. Louis, MO, USA), and scanned (BX53 light miscroscope, Olympus, Tokyo, Japan).

The total number of myelinated axons and the average minor axis diameter of the myelinated axons in the central and peripheral sections of the end-to-side and end-to-end neurorrhaphy specimens were calculated using image analysis software (WinROOF ver. 5.2.1, Mitani Corp., Fukui, Japan) (**Figure 5**). The axon regeneration rate (peripheral section myelinated count/central section myelinated count) was also calculated.

Statistical analysis

The total number of myelinated axons, axon regeneration rate, and average minor axis diameter of the myelinated axons were compared between groups using the Wilcoxon (examining two related groups, non-parametric) test. Statistical comparisons were performed using WinROOF ver. 5.2.1 software, and differences were considered statistically significant at $P < 0.05$.

Results

Myelinated axon count

At 6 weeks, the total number of regenerated axons on the distal side was significantly higher for end-to-end neurorrhaphy than for end-to-side neurorrhaphy ($P < 0.01$) (**Figure 6A, B**). However, at 12 weeks, there were no significant differences between the two groups (**Figures 6C**, **D** and **7**).

Axon regeneration rate

At 6 weeks, the axon regeneration rate (distal counts/proximal counts) was significantly higher for end-to-end neurorrhaphy than for end-to-side neurorrhaphy ($P < 0.05$). However, at 12 weeks, there were no significant differences between the two groups (**Figure 8**).

Axon diameter

There was a significant increase in minor axis diameter of axons at 12 weeks in both end-to-end and end-to-side neurorrhaphy groups compared with at 6 weeks ($P < 0.05$ or $P < 0.01$). However, there were no significant differences in minor axis diameter of axons between the two groups at either time point (**Figure 9**).

Discussion

Our results show that compared to end-to-side neurorrhaphy, end-to-end neurorrhaphy between a thick donor nerve and a thin recipient nerve results in a significantly higher number of regenerated myelinated axons and axon regeneration rate at 6 weeks. While there were no differences in nerve count and axon regeneration rate at 12 weeks or axon diameter at either time point, the early improvement in axon regeneration in the end-to-end neurorrhaphy model suggests that end-to-end neurorrhaphy is more effective and favorable than end-to-side neurorrhaphy. Because faster axonal regeneration potentially reduces muscle atrophy in the target organ, end-to-end neurorrhaphy may be preferable when suturing a thick nerve to a thin nerve.

The absence of significant differences in axon regeneration between end-to-end and end-to-side neurorrhaphy at 12 weeks suggests that end-to-side neurorrhaphy might be slower but not completely inferior to end-to-end neurorrhaphy in terms of axonal regeneration ability, and equivalent results may be obtained given a prolonged duration of healing. It should be recognized that axon regeneration and wound healing abilities in rodent and human facial nerves may not be identical, and the absence of significant differences at 12 weeks could also be a result of the "blow through effect" commonly encountered in rodent models, wherein early significant differences become difficult to distinguish later on (Brenner et al., 2008). We speculate that slower axon regeneration in end-to-side neurorrhaphy is due to regenerated axons potentially deviating and getting misdirected towards the ligated end side, instead of being directed into the recipient nerve's target organ side.

Additionally, we observed increases in regenerated axon number and short diameter in both recipient and donor nerves in both end-to-side and end-to-end neurorrhaphy models. These increases are likely the result of collateral sprouting and proliferation of nerve fiber lateral buds from the traumatic neuroma that is formed by Schwann cells and connective tissues at the repair site. In outflow type end-to-side neurorrhaphy, lateral sprouting of regenerated axons occurs from the node of Ranvier proximal to the stump on the donor nerve and may traverse the epineurium and extend into the recipient nerve (Cao et al., 1997; Sterne et al., 1997; Zhao et al., 1997; Lutz et al., 1998; Tham and Morrison, 1998; Zhang et al., 1999, 2000, 2001; Hayashi et al., 2004). While the exact mechanisms underlying such collateral sprouting after end-to-side neurorrhaphy are unclear, growth factors and neurotrophic factors such as insulin-like growth factor and neurotrophin-3 at the nerve connection site likely play an important role (Sterne et al., 1997) and need additional investigation.

The study has several limitations. First, we investigated nerve regeneration by measuring nerve counts and axon diameters, but we did not investigate underlying mechanisms or changes in neuronal and muscle function. Future studies that investigate local changes in neurotrophic and growth factors, as well as evaluate neuromuscular junction characteristics (Cheever et al., 2011), would help in clarifying the precise mechanisms and effects of axonal regeneration in the two models. In addition, muscle size and function could be evaluated by assessing vibrissal movement and eye blink reflex (Beahrs et al., 2010). Second, we used a normalized

outcome axon regeneration rate (distal counts/proximal counts) to adjust for size differences in proximal donor and distal recipient nerves in the two models. Additional parameters, such as the g-ratio (ratio of the inner axonal diameter to the total outer diameter), could also be utilized as functional and structural indexes of optimal axonal myelination (Perrot et al., 2007). Lastly, the study lacked an uninjured control model that would have allowed comparisons among native injured and repaired scenarios.

To conclude, end-to-end suturing of a larger diameter nerve to a smaller diameter nerve (ratio of sutured nerve diameter, 2:1) resulted in a significantly higher number of regenerated axons and rate of regeneration than end-to-side suturing at 6 weeks following neurorrhaphy; however, there were no significant differences between the two groups at 12 weeks. There were also no differences in axon diameter at 6 or 12 weeks. Though end-to-side suture and end-to-end suturing appear to result in equivalent axon regeneration at 12 weeks, earlier regeneration in end-to-end suturing may reduce target organ muscle atrophy. End-to-end suturing may therefore be more desirable when suturing a thick nerve to a thin nerve.

Author contributions: *TT was responsible for data acquisition, analysis and interpretation and manuscript writing. KU was responsible for conceptualization and design of experiments, data interpretation, and manuscript writing. AK were responsible for data interpretation and manuscript writing. All authors approved the final version of this paper.*
Conflicts of interest: *There are no conflicts of interests to declare.*
Financial support: *This research received no specific funding agency in the public, commercial, or not-for-profit sectors.*
Research ethics: *The study followed the guidelines of the Japanese Association for Laboratory Animal Science (JALAS) and was approved by the institutional animal ethics committee (Institute for Animal Experimentation, St. Marianna University Graduate School of Medicine No. 2013053004).*
Data sharing statement: *Datasets analyzed during the current study are available from the corresponding author on reasonable request.*
Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*

Open peer reviewers: *Jayant P. Agarwal, University of Utah, USA; Donald Albert Godfrey, University of Toledo College of Medicine and Life Sciences, USA.*
Additional file: *Open peer reviewer reports 1 and 2.*

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