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Beneficial Effect of Nimodipine on Peripheral Nerve Function in Aged Rats

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VAN DER ZEE, C. E. E. M., T. SCHUURMAN, R. GERRITSEN VAN DER HOOP, J. TRABER AND W. H. GISPEN. *Beneficial effect of nimodipine on peripheral nerve function in aged rats*. NEUROBIOL AGING **11**(4) 451–456, 1990.—In aged rats neuromuscular function and motor coordination is gradually impaired. Major motor deficits were seen in rats of more than 2 years of age; with increasing age, the incidence of abnormal footprints increased sharply. Oral nimodipine, a Ca^{2+}-entry blocker of the dihydropyridine type, treatment suppressed and/or delayed the appearance of these abnormal footprints. In aged rats that already displayed a considerable amount of abnormal footprints in the free walking pattern, oral nimodipine treatment was similarly effective. Nimodipine not only delays the onset of age-related motor deficits, but also may counteract these deficits once already present. In aged rats the nerve conduction velocities were severely diminished. Nimodipine treatment resulted in an enhancement of the sciatic and caudal nerve conduction velocities. Histological analysis revealed a lower fiber density in aged rats compared to aged nimodipine-treated rats. Whether nimodipine acts directly on the peripheral nervous system is currently unclear. Nevertheless, the present study lends further support for the beneficial effects of nimodipine in age-related motor deficits in the rat.

Nimodipine Peripheral nerve function Motor deficits Abnormal footprints Nerve conduction velocity Fiber density

PROFOUND neuro- and psychopharmacological effects of dihydropyridine Ca^{2+}-entry blockers have been reported both in animals and in man (1). Nimodipine has been shown to exert anti-ischemic activity in brain and spinal cord following damage (10, 11, 14). Recently, evidence has emerged to suggest a beneficial effect of nimodipine on peripheral nerve regeneration (15). Oral administration of nimodipine in food pellets enhanced recovery of both sensory and motor function of the foot in rats bearing a unilateral crush lesion in the sciatic nerve, as assessed by a foot reflex withdrawal test, and the quality of the free walking pattern. Of the two doses used, 225 and 860 ppm, the latter dose was more effective (15).

It is well known that in aged rats neuromuscular function and motor coordination is greatly diminished [see for review (3)]. Using a battery of motor tests which varied in complexity and measured locomotion and balance, suspended hanging and climbing in a pool, major deficits were seen in rats of more than 2 years of age. With increasing age, from 24 months of age on, the incidence of abnormal footprints in the free walking pattern increased markedly (12).

As nimodipine was beneficial to postlesion repair mechanisms (see above) the effect of chronic treatment with nimodipine on the age-related deficit in neuromuscular function in aged rats was studied. It has been reported that 4 months oral treatment of aging rats markedly improved motor coordination and counteracted deterioration in the free walking pattern as evidenced by the reduction in abnormal footprints (12).

In the present study we used the incidence of abnormal footprints as a parameter to assess the neurotrophic action of oral nimodipine administration in aged rats, that were already troubled by a considerable motor deficit. The sciatic nerve and caudal nerve conduction velocities were measured and histological analysis of the sciatic nerves was performed, for evaluation of the nimodipine treatment.

METHOD

Animals

Male rats of an inbred Wistar strain (BOR WISW, Winkelmann, Borchen, FRG) were used. They were kept under SPF (specific pathogen free) conditions following birth until the age of 24 months. At 24 months of age they were group housed (n = 6) on sawdust in Macrolon cages. A strict dark-light cycle was main-

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tained, the lights being on from 7:00 a.m. till 7:00 p.m. Commercial rat chow and water were available ad lib. Body weight was measured weekly.

Experimental Design

Rats of 24 months of age were subjected to the footprint test (analysis of walking pattern) and selected for the experiment when footprint abnormalities were present. Depending on the severity of the displayed abnormalities, the animals were matched pairwise to either of two groups (25 rats per group). The groups were randomly allocated to nimodipine or control treatment (20 weeks duration). Motor function tests were performed at the start of the experiment, at 6, 10, 16 and 20 weeks. Following the final motor analysis the MNCV and SNCV of the sciatic nerve and the SNCV of the tail nerve was measured in 12 rats from each group, randomly chosen. Finally, from each group 6 rats were randomly taken and used for histological study.

Drug Treatment

From 24 months of age on, rats received either standard food pellets (Ssniff, Soest, FRG) or identical pellets to which nimodipine (800 ppm, Bayer, Leverkusen, FRG) was added. The dose was selected as recovery of function following peripheral nerve damage and was shown to be more effective than 225 ppm and this dose was also used in a previous study on nimodipine and motor function in the aged rat (15). Rats of this age eat approximately 15–20 g pelleted food per day, resulting in a dosing of nimodipine of approximately 25–30 mg/kg body weight (12). The experimenters performing the various measurements were unaware of the kind of treatment given. After statistical analysis had been performed the treatment code was broken.

Analysis of Walking Pattern

Motor function of the hind paws was assessed using the method first described by De Medinacelli *et al.* (5) and adapted by De Koning and Gispen (4). In short, rats walk over photographic paper (Ilford, 2.24 M, semimatt), after dipping the hind paws in developer (Eukobrom, Tetenal, FRG). The paper lies on the bottom of an illuminated confined corridor ($70 \times 12 \times 11.5$ cm), leading towards a dark goal box, with an upward inclination of 10° to the horizontal plane. Footprints show up immediately after the rat has passed. Footprints were considered abnormal when 1) there were additional small prints per footprint or 2) they were fuzzy as caused by external rotation of the foot or 3) they were fuzzy due to lack of elevation of the paw at the onset of a new step (12). If any of these abnormal footprints appeared during a test the animal was scored as displaying abnormal footprints. The intra- and interrater reliability of trained observers has a correlation coefficient over .9. An example of the walking pattern of a young rat and an old rat (24 months) as well as a control and a drug-treated rat at 28 months of age is shown in Fig. 1. Clearly the control fed rat of 28 months of age shows additional prints as well as fuzzy prints as a result of lack of elevation during walking.

Electrophysiology

Motor (MNCV) H-reflex related sensory nerve conduction (SNCV) velocities were obtained using the method described in detail by Stanley (13). Under general anaesthesia (Hypnorm, Duphar, Weesp, NL, containing fluanisone 10 mg/ml and phentanyl citrate 0.2 mg/ml, dose 0.8 ml/kg body wt., administered subcutaneously) and temperature-controlled conditions, tibial and sciatic nerves were stimulated at the ankle and at the hip,

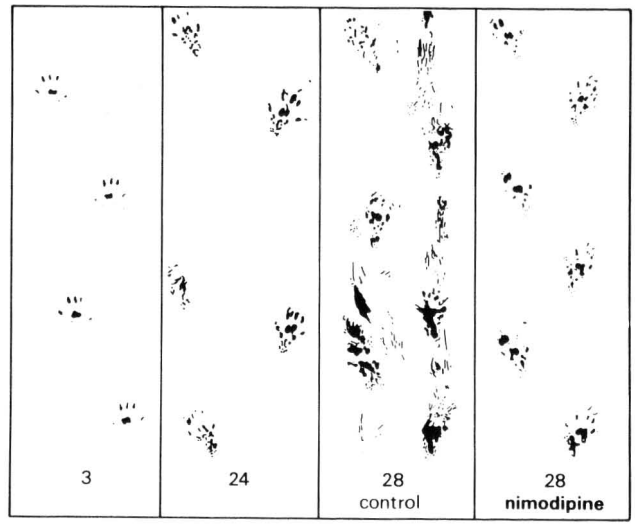

FIG. 1. An example of the walking pattern showing differences in footprints of a young rat (3 months of age; lane 1), an aged rat (24 months of age; lane 2), a control fed aged rat (28 months of age; lane 3) and a nimodipine fed aged rat (28 months of age; lane 4). The walking pattern of aged rats shows additional footprints and fuzzy prints (lanes 2 and 3).

respectively. The M- and H-responses were recorded from the plantar muscles of the foot, using surface electrodes. The distance between stimulation points and the differences in latencies obtained were used to calculate MNCV (motor nerve conduction velocity) and SNCV (sensory nerve conduction velocity). The SNCV of the tail nerve was measured by direct stimulation of the tail nerve with the stimulating electrodes placed at a site 6 cm distal to the base of the tail where the recording electrodes were situated.

Histology

Animals were sacrificed upon completion of the electrophysiological testing and the sciatic nerves were quickly removed, gently stretched on a board to prevent shrinkage and fixed in a solution containing 0.1 M cacodylate, 0.01 M $CaCl_2$ and 2% glutaraldehyde buffered at a pH of 7.3 for 4 hr. Subsequently the nerves were rinsed three times in an isotonic solution containing 0.1 M cacodylate, 0.01 M $CaCl_2$ and 3.6% glucose, pH 7.3. The tissue was postfixed with OsO_4 (1% OsO_4, 0.1 M cacodylate, 0.01 M $CaCl_2$, 2.9% glucose, pH 7.3) for 2 hr, dehydrated in graded acetones and embedded in epoxy resin (2,16). Thin (1 μm) transverse sections were cut using a microtome (LKB pyramitome, Bromme, Sweden) and stained with 1% para-phenylenediamine for myelin. Pictures were made both of the nerve as a whole and of 3 nonoverlapping parts of the nerve in detail. The number of myelinated axons were obtained by multiplying the scores of the three more detailed pictures with the total area of the nerve as measured with a light pen on a graphic tablet, connected to a PC loaded with a Digitizer program.

Statistical Analysis

The data of the motor function tests were processed in the following way. First, the proportion of distorted versus total prints was computed. Then, raw data were analyzed via mean-range plots to find the appropriate transformation. An arc-sin transformation for these proportional data seemed appropriate (17). In order to

TABLE 1

PERCENTAGE OF ANIMALS WITH NORMAL FOOTPRINTS, WITH ABNORMAL FOOTPRINTS AT ONE SIDE AND WITH ABNORMAL FOOTPRINTS AT BOTH SIDES

	Abnormal Footprints at *Both* Sides		Abnormal Footprints at *One* Side		Normal Footprints	
Weeks	Plac	Nimo	Plac	Nimo	Plac	Nimo
0	60	60	36	36	4	4
6	64	20.8	32	62.5	4	16.7
10	69.6	20.8	30.4	62.5	0	16.7
16	86.4	41.7	13.6	45.8	0	12.5
20	90.9	83.3	9.1	12.5	0	4.2

Placebo: Plac, n = 22; nimodipine: Nimo, n = 24.

assure the comparabilities of these ratios, the total number of prints from which these ratios were derived were analyzed via independent *t*-tests for each examination. Third, an analysis of covariance introducing prevalues as covariate was used employing a 2×4 repeated measures ANCOVA. Since there was no significant regression on prevalues and the prevalues themselves were not different, they were dropped from further analysis. Therefore a usual 2×4 repeated measures ANOVA was conducted using the arc-sin values as raw data. Nerve conduction velocities and histological data were analyzed using Student's *t*-test.

RESULTS

Effect of Nimodipine on Incidence of Abnormal Footprints

Fifty animals were matched for severity of motor function disturbances and selected randomly to receive nimodipine or placebo food pellets. Three animals out of 25 in the control group and one animal out of 25 in the nimodipine fed group died within the experimental period, those rats were excluded from the statistical analysis. The walking pattern analysis of all animals was performed at the start of the experiment (age of 24 months) and at 6, 10, 16 and 20 weeks following the onset of the treatment. There were no significant differences between the total number of footprints of the nimodipine and placebo group.

As can be seen in Table 1, at the onset of the experiment the percentage of animals in the nimodipine and placebo group, that showed abnormal footprints of both hindpaws, was approximately similar. In the placebo group the incidence of abnormal footprints at both sides increased, whereas in the nimodipine group this incidence first decreased with a concomitant rise in normal footprints at both sides or only one. There was a significant difference between the nimodipine and the placebo group, favouring nimodipine (Table 1; ANOVA, $p<0.001$). Moreover, there was a significant time-effect (ANOVA, $p<0.0001$) indicating that there were impressive changes in the ratios over the treatment course. However, during the last weeks of the experiment the index abnormal/normal footprints also increased sharply in the nimodipine group.

Effect of Nimodipine on the Nerve Conduction Velocity

At the end of the treatment period of 20 weeks, 12 rats from the nimodipine and 12 rats from the control fed group were selected randomly and the sciatic nerve and caudal nerve conduction velocities were determined, using the technique described before (4). The MNCV and SNCV in the sciatic nerve were determined by one experimentor, the caudal nerve conduction velocity by a second one. Both were unaware of the treatment a given rat had received and of the result that was obtained in the parallel nerve conduction velocity measurement.

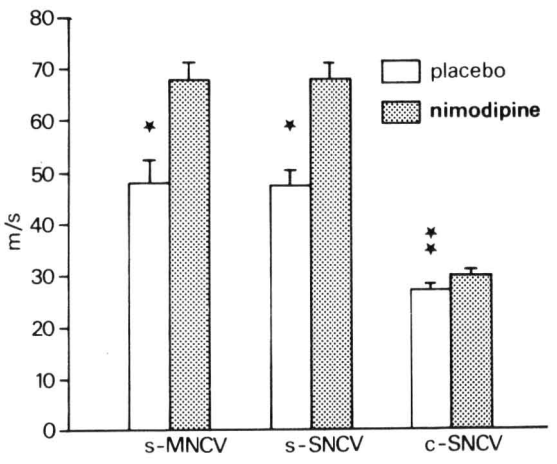

FIG. 2. Nerve conduction velocities in sciatic (both motor and sensory) and caudal (sensory) nerves of nimodipine-treated rats are significantly higher than in placebo-treated rats. (n = 12, each group; Student's *t*-test, $*p<0.002$; $**p<0.009$).

As illustrated in Fig. 2, the nimodipine treatment of aged rats that already displayed appreciable amounts of motor deterioration, resulted in faster NCVs in both sciatic and caudal nerves. The difference in NCV between the nimodipine- and the placebo-treated group was significant, as tested with the Student's *t*-test, for sciatic MNCV ($p<0.002$) and SNCV ($p<0.001$) and for caudal SNCV ($p<0.009$).

Fiber Density in Aged Control and Nimodipine-Treated Rats

Immediately following the NCV determination six of each group of 12 rats were sacrificed, their sciatic nerve excised and processed for histology (2). The histological data of the sciatic nerve fibers in control and nimodipine-treated rats are shown in Table 2. No difference was found between the two groups in mean total nerve area. However, the number of fibers counted per sciatic nerve showed less fibers present in control animals when compared with nimodipine-treated animals ($p<0.05$). This was also reflected in the fiber density (number of fibers/mm^2 area), with a significant lower fiber density for the control group compared to the nimodipine group ($p<0.05$).

TABLE 2

HISTOLOGICAL STUDY OF SCIATIC NERVE FIBERS IN PLACEBO- AND NIMODIPINE-TREATED RATS

	Placebo	Nimodipine	Significance
Nerve area (in mm^2)	1.73 (0.11)	1.67 (0.07)	n.s.
Number of fibers	9879 (764)	11676 (583)	$p<0.05$
Fiber density (in fibers/mm^2)	5744 (424)	6992 (376)	$p<0.05$

n = 6, each group; SEM in parentheses; *p* value of Student's *t*-test.

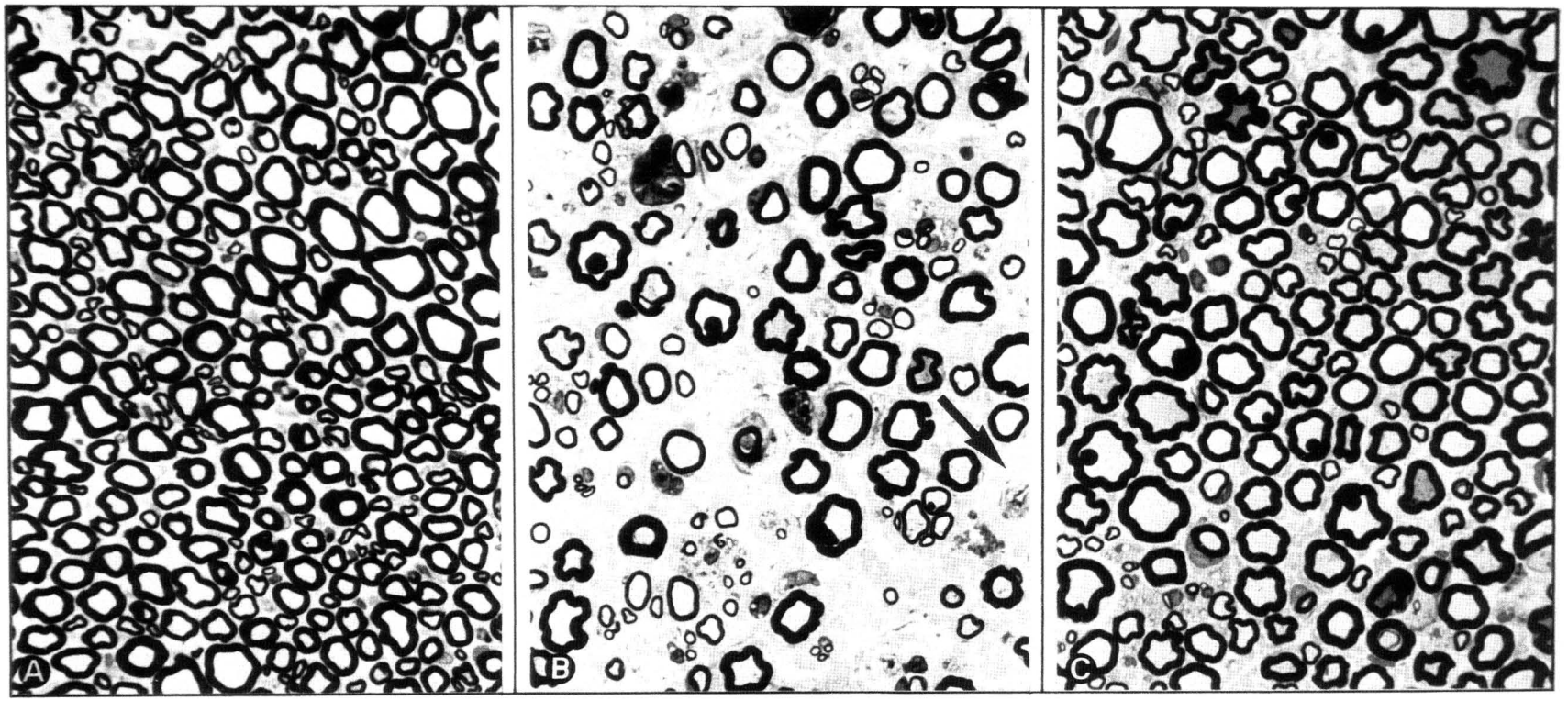

FIG. 3. Photomicrograph with a typical example of a cross-section of the sciatic nerve of a young adult rat (A), an aged placebo-treated rat (B) and an aged nimodipine fed rat (C). More nerve fibers are visible in (A) and (C) compared to (B). Arrow: the amyloid deposits around a blood vessel.

Figure 3 shows a photomicrograph with a typical example of a cross-section of the sciatic nerve of a young adult healthy rat (A), an aged placebo-treated rat (B) and an aged nimodipine fed rat (C). The fiber density in the aged control rat was much lower than that seen in the young adult rat. The compact aspect of the sciatic nerve had been replaced by an image of more dispersed nerve fibers situated in a large amount of connective tissue. Thick amyloid-like deposits were observed around the blood vessels (see arrow). In the aged nimodipine-treated rat the fiber density was larger than in the aged control rat and deposits around the blood vessels were rarely seen.

DISCUSSION

Aging in the rat is accompanied by a gradual impairment of locomotion. This deterioration with age occurs independently from other age-related deficits and cannot solely be attributed to the malfunctioning of the peripheral neuromuscular system (3). It has been suggested that the motor deficits are primarily due to a loss of coordination and a general slowing down of central control mechanisms (3).

Previously, we have shown that with increasing age (from 24 months of age on) the incidence of abnormal footprints increased sharply (12). Oral nimodipine treatment suppressed and/or delayed the appearance of these abnormal footprints (12). In the present study we showed that in aged rats that already displayed a considerable amount of abnormal footprints, oral nimodipine treatment exerted a beneficial effect at 6 weeks, by decreasing the incidence of abnormal footprints (Table 1). The rapid effect of nimodipine exerts a fast effect which lasted until 16 weeks following onset of the treatment (Table 1). Thus, nimodipine not only delays the onset of age-related motor deficits (12), but also may counteract these deficits once already present, albeit temporarily. Apparently all the improvement that was observed had already occurred during the first 6 weeks of treatment. Although we do not know the precise mechanism, the data are in line with the previous study in which it was shown that nimodipine already suppressed the occurrence of abnormal footprints during the first month of treatment (12). We assume that eventually age-related deterioration in motor performance is overcoming the beneficial effect of nimodipine resulting in a surprisingly rapid occurrence of motor deficits in nimodipine-treated animals during the last phase of the experiment.

In order to investigate the presence of changes in the condition of the peripheral nerve that might be responsible for the observed motor deficits, we measured the sciatic nerve and the caudal nerve conduction velocities. Adult rats of 9 months of age showed a motor conduction velocity of 70 m/sec and a sensory conduction velocity of 80–85 m/sec in the sciatic nerve (7). The caudal nerve conduction velocity is much lower (approximately 30 m/sec), due to the lower tail temperature (9). In the aged placebo-treated rats the nerve conduction velocities were considerably diminished. As Fig. 2 shows, the nimodipine treatment of aged rats that already displayed appreciable amounts of motor deterioration, resulted in an enhancement of all three NCVs measured.

Thus, oral nimodipine treatment was not only of benefit to the sciatic nerve of which the function presumably affects the footprint appearance, but also to the caudal nerve. This indicates a more general effect of nimodipine on the peripheral nervous system.

The histological analysis of the sciatic nerve disclosed the presence of a morphological substrate of the observed NCV changes, since the number of nerve fibers, counted in cross-sections of the sciatic nerve, in aged rats treated with nimodipine is higher than that in aged placebo-treated rats (Table 2). The observed fiber density in the aged control fed animals is considerably lower, when compared to values obtained in younger adult rats (6). In the aged nimodipine-treated rats the fiber density is less affected. A more compact picture of the sciatic nerve is seen in young adult rats, whereas a large amount of connective tissue, and thick amyloid deposits around the blood vessels, were observed in aged animals (see arrow, Fig. 3). The thick amyloid-like deposits were comparable to those reported for blood vessels in the aged rat brain (Luiten, personal communication). In contrast, in nimodipine-treated rats the deposits around blood vessels were rarely seen. Quantitative immunocytochemistry using specific amyloid antibodies should strengthen this observation.

Considering the different parameters, as the nerve conduction velocity, the number of myelinated axons and the amyloid-like deposits, all in favor of nimodipine, the sharply increased index abnormal/normal footprints in the last weeks cannot be easily explained. It may be that the effect of nimodipine on the walking pattern of aged rats is a combination of effects on both the central and peripheral component of the control of motor function [see (12)]. In view of the favorable condition of the nimodipine-treated peripheral nerve one might argue that the rapid deterioration during the last phase of the experiment is the result of failing central mechanisms in the control of locomotion.

The mechanism of action of nimodipine in postlesion repair is still largely unknown. It may be that nimodipine is affecting the peripheral nervous system and the motor performance in the aged rats, because in aged neural tissue there exists a dysregulation of calcium homeostasis (8). This speculation should be supported by experimental evidence indicating whether other calcium blockers have similar effects and whether calcium-channel activators worsen the condition of the peripheral nerve and the motor performance. Of course, nonneural aspects of nimodipine action, such as effects on the vascular system (1), may have contributed to the observed differences. Whatever the mechanism, the present data underscore the notion that nimodipine exerts a trophic influence on the peripheral nervous system in the aged rat (12), possibly underlying the observed improvement of the age-related deterioration in motor function and walking pattern [(12); this paper].

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