of whirring as an expression of Zugunruhe. To demonstrate this problem the example of the spotted flycatcher given here has been chosen because of the big difference it showed between whirring and hopping in the night. Out of the ten birds half of them showed a pattern similar to the example in the figure, whereas the other half showed less difference between whirring and hopping. The spotted flycatcher may be exceptional in its high levels of whirring. In addition, whirring may also have been facilitated by the fact that the cages used were larger than those used in most other experiments[4].
But despite these facts recording Zugunruhe by the perch-microswitch method only may yield too low values for Zugunruhe in other passerines which express part of their Zugunruhe as whirring. Special attention should be given to this problem if different bird species or different physiological stages are compared.

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Glia maturation factor influences recovery from injury in neonatal rat brains[1]

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Summary. Newborn rats were injured with a puncture wound in one cerebral hemisphere. Experimental animals were treated with three i.p. injections of Glia Maturation Factor (GMF) at daily intervals starting from the time of injury, whereas control littermates were treated with equivalent amounts of bovine serum albumin. At 25 days old the size of the cerebral cortex at the plane of injury was measured on representative brain sections. In control rats the injured side was 18% smaller than the normal side whereas in GMF-treated animals the difference was only 1%. The results suggest a possible regulatory role of GMF in promoting tissue recovery from brain damage.
Key words. Growth factor; brain injury; brain regeneration; development.

Glia maturation factor (GMF) stimulates astrocytes in culture to proliferate and to undergo morphological and chemical differentiation[2–12]. The chemical changes in these cells include a sequential increase in cyclic GMP[8] and cyclic AMP[7], a rise in the neuroectodermal marker S-100 protein[7], and an increase in the astrocytic marker glial fibrillary acidic protein[10]. The factor also promotes contact inhibition in certain glial tumor cell cultures[9]. Although ample data exist on the in vitro effect of GMF, information on its in vivo function has been lacking. We now present evidence of a biological effect of GMF in the whole animal.
Materials and methods. For this project we used a 10,000-fold purified sample of GMF obtained from beef brains[10,13]. Five litters of 50 newborn rats (Sprague-Dawley) were used for the injury experiment. Animals from each litter were randomly divided into experimental and control groups. Within 24 h after birth, all animals were inflicted with a puncture wound in one cerebral hemisphere by piercing an 18-gauge needle through the cranium in the parietal region, at a point 2.5 mm lateral and 2 mm anterior to the bregma. The needle was directed perpendicular to the surface of the skull, with the bevel facing laterally. The depth of the wound was controlled with a polyethylene sleeve fitted to the needle, exposing a length of 4 mm to the tip. Immediately following the injury, the experimental group was injected i.p. (through a 25-gauge needle) with 50 ng of GMF in 10 µl of 0.2 M potassium phosphate buffer, pH 7.4, while the control group was similarly injected with 50 ng of bovine serum albumin in 10 µl of the same buffer. Thereafter, all rats were returned to their respective mothers. Two additional i.p. injections were given in the two subsequent days without further manipulation of the wound. All animals were sacrificed when 25 days old.
The brains were fixed for 17 h in 10% formalin (buffered at pH 7.0) and trimmed. After dehydration and paraffin embedding, serial coronal sections 10 µm thick were made. Alternate sections were stained with hematoxylene-eosin (H & E) or immunostained for glial fibrillary acidic protein (GFAP), a specific marker for fibrous astrocytes. The latter procedure employed the 'DAKO' PAP kit (Accurate Chemicals, Westbury, N.Y.) using the amino-ethylcarbazole (AEC) chromogen. One representative H & E section, cutting through the area of maximal injury, was selected from each brain and photographed. The image from the negative was projected onto a piece of paper. The area of the projected image outlined by the

Table 1. Summary of means for injured rats not treated with GMF

Group	N	Normal side (n)	Injured side (i)	Difference (n − i)	Ratio (i/n)
Litter 1	2	32.71 ± 0.39	17.22 ± 3.96	15.48 ± 3.58	0.53
Litter 2	6 (3, 3)	30.90 ± 1.83	24.43 ± 3.42	6.48 ± 3.97	0.79
Litter 3	6 (1, 5)	32.82 ± 1.79	27.49 ± 1.58	5.34 ± 1.45	0.84
Litter 4	6 (0, 6)	29.12 ± 1.08	25.27 ± 2.08	3.85 ± 1.05	0.87
Litter 5	4 (2, 2)	29.91 ± 0.86	26.82 ± 2.05	3.09 ± 2.37	0.90
All males	6	30.69 ± 1.35	26.03 ± 3.03	4.66 ± 3.82*	0.85
All females	16	30.79 ± 2.25	25.89 ± 2.49	4.89 ± 2.22**	0.84
All rats	24	30.92 ± 2.00	25.20 ± 3.57	5.72 ± 4.00***	0.82

Values in mm^2 ± SD. Animals in tables 1 and 2 were littermates. Except for litter 1, the sex of individual animals was determined. Numbers of males and females, respectively, from each litter are in parenthesis following N values. *$p < 0.0305$; **$p < 0.0001$; ***$p < 0.0001$ (paired t-test).

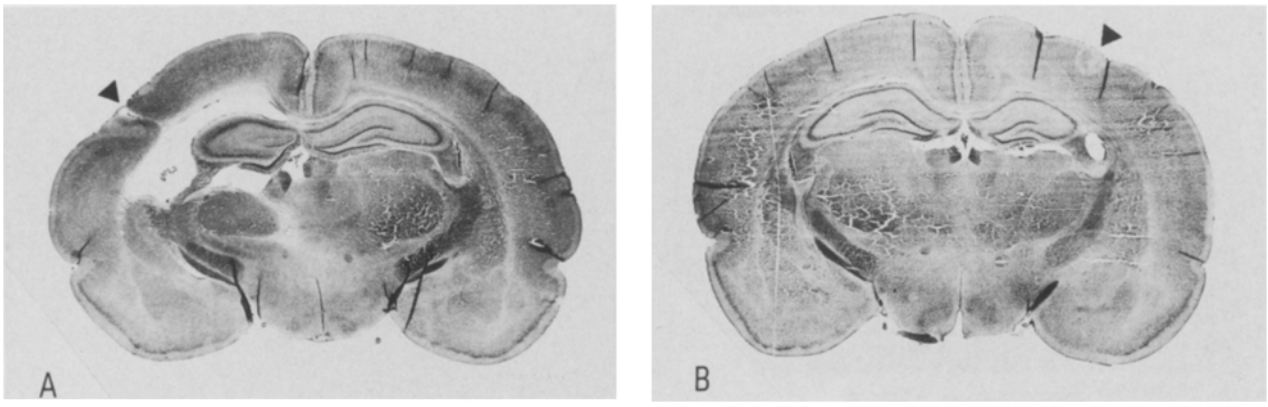

Figure 1. Coronal sections of injured rat brains. *A* Not treated with GMF; *B* treated with GMF. Arrow indicates site of injury. H & E stain. × 4.7.

cerebral hemisphere (excluding the hippocampus) was measured for each hemisphere using a digitized planimeter (Zeiss MOP-3). All values presented have been corrected for magnification factors.

Results and discussion. Figure 1 shows the typical appearance of the brain sections taken from control and GMF-treated animals. Without GMF, the injured side was considerably smaller than the normal side. With GMF treatment, the two sides were approximately equal. The measurement data taken from all 50 injured rats (littermates) are presented in tables 1 and 2. In control animals, the injured side was 18% smaller than the normal side (paired $t = 7.01$, $p < 0.0001$), whereas in GMF treated rats, the injured side was only 1% smaller than the normal side (paired $t = 0.64$, $p < 0.5301$). Only the former value is statistically significant. Analysis of variance for all the 50 rats using the difference data (normal minus the injured side) revealed that GMF treatment was the only significant variable ($F = 37.15$, $p < 0.0001$). A t-test also confirmed this result ($t = 5.87$, $p < 0.0001$). Sex and litter were not significant variables, as indicated by the small F-values for sex ($F = 0.10$, $p < 0.75$) and for litter ($F = 0.57$, $p < 0.63$). Litter still did not represent a significant variable even after separation into male and female groups, as reflected in a nonsignificant F-value on the interaction of litter and sex ($F = 0.83$, $p < 0.48$).

When the brain sections were immunostained for glial fibrillary acidic protein (GFAP), no GFAP-positive cell was seen along the track of injury, indicating the absence of gliosis (figs. 2 and 3). For comparison, figure 4 shows the GFAP-positive, naturally occurring fibrous astrocytes in the hippocampus of the same brains but far from the injury site.

In a separate experiment, we measured the size of rat brains not inflicted with injury. 34 newborn rats from four litters were given 3 i.p. injections of bovine serum albumin. At 25 days old, coronal brain sections were prepared, and one section from each brain, chosen from the level corresponding to the site of injury in the wounded group, was measured. As depicted in table 3, we failed to detect a significant difference between the right and the left cerebral hemispheres. Analysis of variance of the difference data revealed that neither sex nor litter, nor the interaction of litter and sex, constitute a significant variable, as indicated by the small F-values for sex ($F = 0.01$, $p < 0.91$), for litter ($F = 0.91$, $p < 0.44$), and for litter versus sex interaction ($F = 0.66$, $p < 0.59$).

These experiments were undertaken to evaluate the role of

Table 2. Summary of means for injured rats treated with GMF

Group	N	Normal side (n)	Injured side (i)	Difference (n − i)	Ratio (i/n)
Litter 1	2	26.71 ± 3.64	27.32 ± 2.78	−0.61 ± 0.86	1.03
Litter 2	8 (3, 5)	29.95 ± 3.42	29.77 ± 2.91	0.17 ± 1.95	0.98
Litter 3	6 (3, 3)	32.17 ± 2.09	31.92 ± 2.01	0.24 ± 2.40	0.99
Litter 4	6 (1, 5)	28.78 ± 3.13	27.79 ± 0.92	0.98 ± 3.54	0.97
Litter 5	4 (2, 2)	27.76 ± 1.31	27.83 ± 1.47	−0.07 ± 0.57	1.00
All males	9	30.96 ± 3.04	30.23 ± 2.62	0.73 ± 1.48*	0.98
All females	15	29.17 ± 2.96	29.05 ± 2.56	0.13 ± 2.71**	1.00
All rats	26	29.60 ± 3.14	29.33 ± 2.61	0.28 ± 2.24***	0.99

Values in $mm^2 \pm SD$. Animals in tables 1 and 2 were littermates. Except for litter 1, the sex of individual animals was determined. Numbers of males and females, respectively, from each litter are in parenthesis following N values. *$p < 0.1777$; **$p < 0.8584$; ***$p < 0.5301$ (paired t-test).

Table 3. Summary of means for non-injured rats

Group	N	Left side (l)	Right side (r)	Difference (l − r)	Ratio (r/l)
Litter 1	6 (2, 4)	34.97 ± 1.13	33.95 ± 1.23	1.02 ± 0.54	0.97
Litter 2	10 (4, 6)	36.14 ± 2.72	36.32 ± 2.03	−0.18 ± 1.87	1.01
Litter 3	8 (3, 5)	40.64 ± 1.16	39.99 ± 1.69	0.66 ± 1.91	0.98
Litter 4	10 (5, 5)	35.74 ± 1.58	35.04 ± 1.21	0.70 ± 1.08	0.98
All males	14	36.51 ± 2.78	36.06 ± 2.46	0.45 ± 1.24*	0.99
All females	20	37.14 ± 2.86	36.51 ± 2.78	0.51 ± 1.71**	0.98
All rats	34	36.88 ± 2.80	36.39 ± 2.67	0.49 ± 1.52***	0.99

Values in $mm^2 \pm SD$. The sex of all individual animals was determined. Number of males and females, respectively, from each litter are in parenthesis following N values. *$p < 0.1983$; **$p < 0.1948$; ***$p < 0.0694$ (paired t-test).

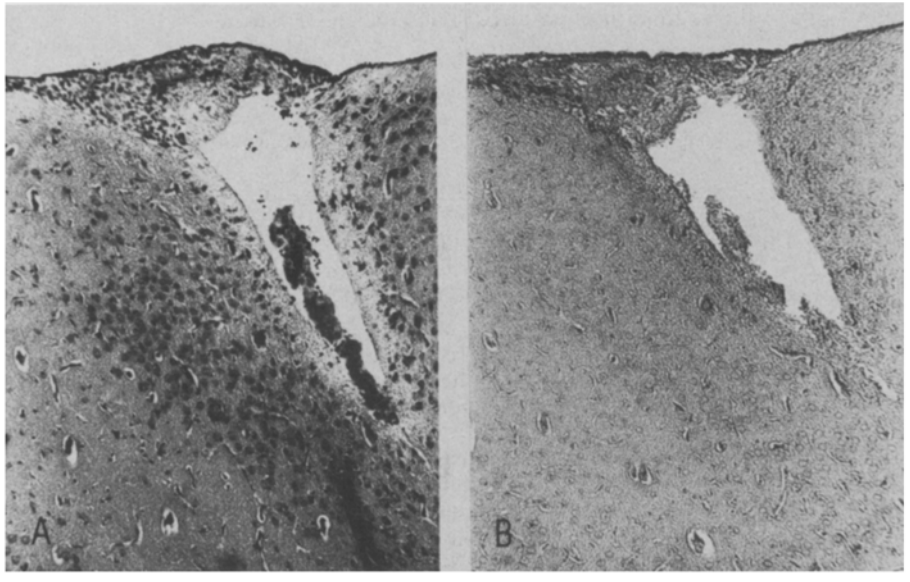

Figure 2. High power view of figure 1A at site of injury. A: H & E stain; B: GFAP immunostain. Note absence of GFAP-positive cells. × 73.

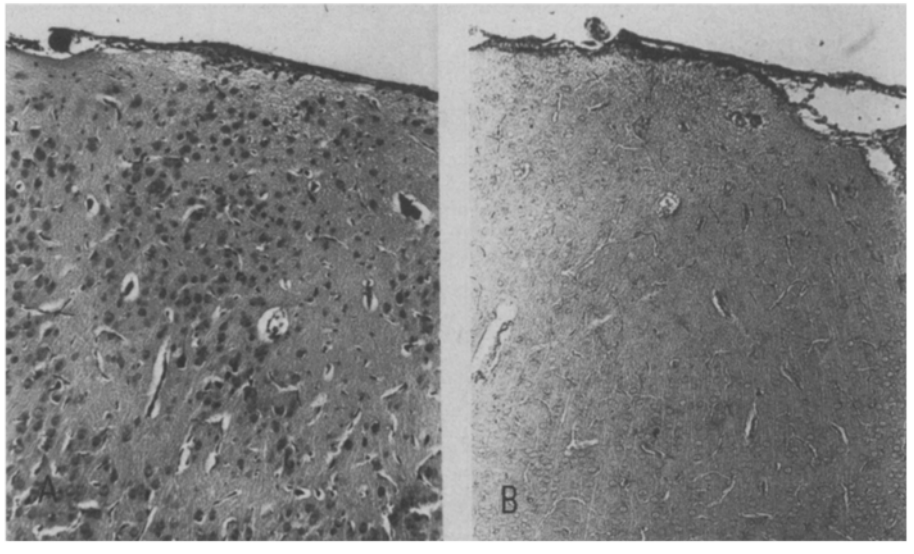

Figure 3. High power view of figure 1B at site of injury. A: H & E stain; B: GFAP immunostain. Note absence of GFAP-positive cells. × 73.

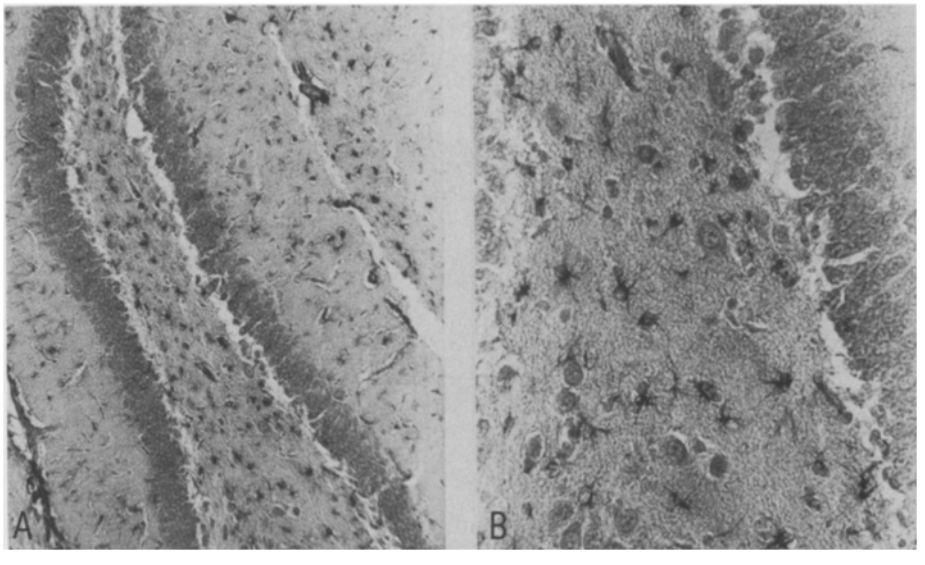

Figure 4. Positive control for GFAP. GFAP immunostaining showing the natural occurrence of fibrous astrocytes (GFAP-positive) in the hippocampus of the same brains at a site not related to injury. A, × 73; B, × 154.

GMF in wound healing in the brain. We previously applied GMF onto adult rat brains inflicted with a stab wound and observed marked gliosis in the wound area (Troy, Lim and Eng, unpublished). However, the interpretation was complicated by the presence of large amounts of endogenous GMF in the mature animal, which minimized the differential response between the GMF-treated and the untreated animals. In the current report, the experiment was conducted in neonatal rats, where the endogenous GMF level is low[14]. Since in newborn rats the blood-brain barrier is poorly developed, we chose to administer GMF by the i.p. route. In addition, the wound itself disrupted the barrier locally, making the site accessible from circulation. I.p. injection avoided the possibility of extending the size of the wound, which could have happened with local application of GMF sample to the wound.

At first glance, it may be difficult to reconcile the ability of GMF to minimize posttraumatic atrophy and the fact that GMF did not enhance the formation of glial scar in neonatal brains. The difficulty can be resolved, however, if we consider that the brain responds to injury in two major ways: the regenerative attempts of the cells to regain the normal histotypic organization, and the formation of a scar (gliosis) to fill up the wound. It is logical to assume that regeneration, which is possible in young animals, is the repair mechanism of choice. It is only when the regenerative ability is impaired, as in the adult brain, that a glial scar is inevitable.

Our data provided no clues as to whether this regenerative process involved neurons or glia, or the interaction between the two. Nor did it necessarily imply axonal regrowth or sprouting. Inasmuch as no direct effect of GMF on neurons has been reported so far, one can only postulate an indirect influence of GMF on neurons through glia. In view of our recent evidence that GMF stimulates astrocytes to secrete other growth factors[15,16], such a hypothesis may not be far off.

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Synkinesis in hemifacial spasm: results of recording intracranially from the facial nerve

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Summary. We show evidence that the motonucleus of the facial nerve is involved in producing the synkinesis in patients with hemifacial spasm. These results were obtained by recording from the intracranial portion of the facial nerve and from the orbicularis oculi muscle in patients operated upon for hemifacial spasm during electrical stimulation of the mandibular branch of the facial nerve. Also, the electromyographic response from the same muscle was recorded when the facial nerve was electrically stimulated at a location near the brainstem. The results show that it is unlikely that the symptoms of patients with hemifacial spasm can be explained on the basis of ephaptic transmission at the site of lesion of the facial nerve.
Key words. Facial nerve; hemifacial spasm; synkinesis; intraoperative recordings.

Hemifacial spasm (HFS) is a rare disorder that causes involuntary contractions of the mimic muscles of one side of the head. The contractions usually begin in the orbicularis oculi muscle, and unless treated, progress to all mimic muscles, including the platysma[1,2]. It has been shown in several studies that, in addition to the spasm, patients with HFS experience synkinesis, which implies that an attempt to contract one muscle group also involves other muscles[3,4]. In the great majority of patients with HFS, an artery or a vein is found to be compressing the root entry zone (REZ) of the facial nerve. Decompression of the nerve using microvascular techniques is an effective treatment with a rate of cure of over 95%[1,2]. There is evidence that vascular compression of the nerve causes damage to the myelin of the axons at the REZ,[5] and it has been hypothesized that the symptoms of HFS are the result of the formation of artificial synapses between nerve fibers of that local damage to the nerve causes sprouting, degeneration, and rerouting of connections in the nucleus[6]. Other hypotheses state that HFS is a disorder of the facial motonucleus[7,8].

Recent studies have demonstrated that ephaptic transmission, indeed, occurs between abnormally myelinated axons[8-10], as well as development of 'trigger zones' in the injured portion of a peripheral nerve causing spontaneous (ectopic) activation[11], and there is an increase in the nerve's sensitivity to mechanical deformation[12,13]. These abnormalities occur while the nerve maintains its ability to conduct nerve impulses.

Attempts to determine experimentally which of these hypotheses about HFS is valid has been made by Auger[3], who found that the synkinesis observed when the blink reflex was elicited disappeared after microvascular decompression surgery to