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Functional outcome after facial allograft transplantation in rats☆,☆☆,☆☆☆,☆☆☆☆

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Summary *Background:* Full face, hemiface and facial subunit transplants have been reported before. However, the functional recovery of the face transplant largely remains unknown. The mystacial pad (also known as the vibrissal or whiskers region) is the main sensorimotor unit in rats' faces. We included the mystacial region in the hemifacial flap of the rat, and our aim was to study its functional recovery after transplant.
Methods: Hemifacial flaps were transplanted from Brown-Norway RT^n to Wistar-Lewis RT^l rats, under tapered doses of tacrolimus immunosuppression monotherapy (8 mg/Kg/day to 2 mg/Kg/day after 4 weeks). Group I ($n = 12$) was the anatomic study group, in which the harvesting technique of the flap was trial run and angiographies of the flap were obtained. In group II ($n = 12$), non-vascularized hemifacial allografts were transplanted. Group III ($n = 24$) was the vascularized hemiface allotransplant group. This was divided into two subgroups relating to nerve repairs. In subgroup III_a ($n = 12$) no nerve repairs were performed, while in subgroup III_b ($n = 12$) the zygomaticoorbital, bucolabial and upper marginal mandibular branches of the facial nerve, and the infraorbital branch of the trigeminal nerve were repaired. Clinical, neurophysiological and histological studies were performed to evaluate the recovery of the mystacial region after six weeks.

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Results: In group I the hemifacial flap harvesting technique to include the mystacial region was established, and angiographies confirmed a rich axial vascular network in the flaps. All grafts in group II necrosed. In group III, each procedure required an average of 7 h (range 5–11). Of this group, 75% of the rats survived for 8 weeks. In subgroup III_a no signs of recovery were noted, whilst in subgroup III_b clinical, neurophysiological and histological recovery were found in face transplant recipients after 6 weeks.

Conclusions: The hemifacial flap including the mystacial region could be transplanted successfully in the rat model. Face allotransplants in which nerves were repaired showed clinical, neurophysiological and histological signs of recovery.

Development of immunosuppressant drugs has opened the field of non-vital organ transplantation. Reports from reconstructive centres worldwide confirmed feasibility of face[1,2] and hand[3–7] transplantation from a donor cadaver, and composite tissue allotransplants are slowly being introduced in the reconstructive surgery armamentarium.[8–13]

Face and scalp are specialised units important for social life. Burn and trauma on these units represent an enormous challenge to plastic and reconstructive surgeons. Numerous face reconstruction techniques have been described, although full functional and aesthetic reconstruction has remained elusive.[14] An extensively disfigured patient lacking any functioning facial/expression muscles, with loss of nose and/or ears, and with complex defects in the periorbital and perioral regions, could be reconstructed using a facial allotransplant. Face allografting would be a major transplant–reconstruction procedure to protect the eyeball and recover oral competence.

A number of groups worldwide have performed facial transplant research.[15–28] To date, most face transplants have been done in the rat model, reporting more than 330 days of survival.[17] Surgical viability,[15] tolerance induction,[16,17] technical aspects of the full-face[17] and the hemifacial transplants,[16] and facial subunit transplant[19] in rats have been reported. Facial coverage without function was the main objective of these reports and, to the best of our knowledge, there is no evidence of functional recovery after a face transplant in research models. Functional outcome of the face transplant is important for the success of this procedure in humans, and it should be first demonstrated in animal models.[29,30]

The focus of our work is the mystacial region, also known as the whiskers or vibrissal region. Rodent's whiskers are known to be critical for environment exploration. These vibrissae are long tactile hairs that originate from follicles arranged in an orderly manner within a specialised facial structure, the *mystacial pad*.[31] The rat uses its vibrissae to acquire tactile sensory information by sweeping them in a coordinated, rhythmic fashion. Innervation to the mystacial region is provided by the infraorbital nerve (IoN), a branch of the trigeminal (fifth) nerve, which is the main sensory nerve in rodents. Motor innervation comes from the facial (seventh) nerve through several branches.[32] According to Dörfl's classic nomenclature, these branches are the bucolabial (BL) branch, the upper marginal mandibular (uMM) branch, and the zygomaticoorbital (ZyO) branch.[33] Movement of the vibrissal follicles is controlled by the facial motor nerve, which innervates two classes of muscles. One class, the intrinsic muscles,[32] has its points of attachment completely within the mystacial pad and forms a sling around each follicle. The BL and uMM branches control the contraction of the intrinsic muscles, which is seen to correlate with the protraction of the vibrissae.[34] A second class of muscles, the extrinsic muscles (levator labii superioris), forms bridges from the surface of the pad to anchors that lie external to the mystacial pad. The ZyO branch controls the contraction of the extrinsic muscles, shifting the position of the orifice of the follicle relative to the underlying plate, and thus providing a force that shifts the pivot points of the vibrissae.

We modified the hemifacial flap[16] to include the mystacial region in it. The hemifacial–mystacial flap would be an appropriate model for the evaluation of the functional recovery after a face transplant, because sensitivity and movement can be studied in the flap without jeopardising the eyes or mouth. Henceforth, we will refer to the hemifacial–mystacial flap as the 'hemifacial flap'.

Tacrolimus is a calcineurin-inhibitor drug that has an immunosuppressive effect, largely documented both in experimental and clinical transplant research.[35–40] The rationale for such a use is the enhancing effect on axonal growth after axotomy, as compared to cyclosporine.[41]

Our investigation was designed to clarify the recovery of function by means of clinical, neurophysiological, and histological examination after a hemiface allograft transplant in rats under tacrolimus immunosuppression monotherapy.

Material and methods

Experimental design

All animal housing, care, and surgeries were in accordance with the European Union Guidelines for Research Animals (24 November 1986), and the procedure was approved by the Ethics Committee for Animal Research at our institution. All procedures were carried out under sterile conditions. During interventions, the animals were kept warm with an electric blanket at 38 °C. Anaesthesia was induced using a mixture of ketamine 25 mg, diazepam 20 mg, and atropine 1 mg, giving a subcutaneous dose of 1 mL per 300 g weight. Hair of the head and neck was shaved and the skin was prepared with clorhexidine 0.05%. Subcutaneous fluid resuscitation consisting of

3 mL of lactated Ringer's solution was given after surgery, and doses of 3 mL were repeated every 8 h for the first day. The eyes were protected with standard eye ointment. After surgery, animals were housed independently in flat-bottom cages, fed standard rat chow, and given water *ad libitum*. Postoperative medication was given subcutaneously, at doses of 2 mg per kg per 8 h of metamizol and 4 mg per kg per12 h of ciprofloxacine for 48 h.

A total of 48 animals were used in three experimental groups. In group I (anatomic study group, $n = 12$), Wistar–Lewis rats were used for trialling in hemifacial flap-harvesting technique, and gelatin–barium angiographies were performed. In group II (allograft control group, $n = 12$), non-vascularised hemifacial allografts were transplanted in order to check if the hemifacial flap could be taken as a graft. In group III, 24 vascularised hemifacial allografts were transplanted from Brown–Norway (RT^n) rats to Wistar–Lewis (RT^l) rats under tapered tacrolimus immunosuppression monotherapy. In subgroup III_a (non-functional allograft, $n = 12$), innervation of the hemiface was prevented, creating a 10 mm nerve gap and coagulating the nerve stump. In subgroup III_b (functional allograft, $n = 12$), re-innervation of the flap was sought by repairing the branches of the facial and trigeminal nerves (Table 1).

Surgical procedure

Preparation of the donor. The hemifaces were harvested according to previous reports,[16] and modifications were done as follows. Eyelids, nose, and lips were respected. The flap incisions were traced on the middle nasofrontal line, then perinasally and then following the labial margin. The nasofrontal incision was deepened to the periosteal frontal plane, and the ZyO branch of the facial nerve running on the upper border of the orbicularis oculii muscle was harvested along with the levator labii superioris (LLS) muscle, bipolating the angular branch of the facial artery. Dissection proceeded under the LLS muscle and just over the oral mucosa to include the facial artery, the anterior facial vein, the IoN, and the mystacial pad along with the whiskers in the flap. The IoN was transected at its origin in the infraorbital fissure. In the lateral region of the neck, the dissection was continued superiorly over the sternocleidomastoid muscle to the angle of the mandible and the masseter muscle, where the uMM and BL branches were isolated and included in the flap. The external jugular vein and its main branches (anterior and posterior facial veins) were preserved and included in the flap. At the middle line of the neck, the submandibular gland was excised after ligation of its vessels. Incision was deepened in the medial side of the sternocleidomastoid muscle to find the common carotid artery. The posterior belly of the digastric muscle was freed and the greater horn of the hyoid bone transected. The hypoglossal nerve crossing the external carotid artery was transected. The internal carotid artery, superior thyroid artery, ascending pharyngeal artery, lingual artery, ascending palatine artery, and internal maxillary artery were ligated. Careful dissection was carried out on below the external ear canal, where the superficial temporal artery and the internal maxillary artery and pterygoid venous plexus can be easily damaged. The ear was included in the flap along with the posterior auricular artery and vein, but the large retromandibular veins were ligated. The flap was vascularised by the facial and temporal superficial arteries and drained through the external jugular vein. The flap was then perfused with heparine-saline solution.

Preparation of the recipient. The hemiface skin of the recipient was elevated in full thickness from 1 cm over the shoulder to the nose, including the ear and respecting a 2 mm margin in the periocular and perioral areas. The mystacial pad was resected, including the LLS muscle along with the vibrissal follicles, but respecting the oral mucosa. The uMM, BL, ZyO, and IoN nerve branches were prepared for repair. The flap was dropped to cover the defect. Epineural neurorraphies were performed followed by definite insetting and suturing except for the neck. The common carotid artery of the flap was repaired end-to-side to the common carotid artery of the recipient, and the external jugular vein was repaired end-to-end using the standard interrupted suture technique. Then the flap was finally sutured, and rapid resuscitation proceeded using 3 mL of lactated Ringer's solution.

Immunosuppressive protocol

Group III was maintained under daily monotherapy immunosuppression using tacrolimus (Astellas Pharma

Table 1 Experimental design and allograft survival

Group	Vascularisation	Purpose	Procedure	Sample size	Outcome	Survival (days)
I		Anatomical study group	Harvesting trial run and angiographical study	$n = 12$	The facial artery and mystacial vascular network were traced	
II	Non-vascularised allotransplants	Allograft control group	Non-vascularised allograft	$n = 12$	100% necrosis	All survived indefinitely
III	Vascularised allotransplants	Non-functional alloflap subgroup III_a	Vascularised alloflap without nerve repairs	$n = 12$	No clinical, histological, or neurophysiological recovery was observed	56,56,0,56,56,21, 56,0,56,56,40,56
		Functional alloflap subgroup III_b	Vascularised alloflap with nerve repairs	$n = 12$	Clinical, histological and neurophysiological signs of recovery were noted	56,56,56,0,56,56, 56,56,56,32,56,56

GmbH, Munich, Germany), tapering doses from 8 mg per kg per day to 2 mg per kg per day for 4 weeks. The animals were inspected daily for rejection, infection or weight loss.

Clinical, neurophysiological, and histopathological evaluation

Evaluation included appearance of the face, macroscopic appearance of the allotransplants, and clinical, electrophysiological, and histological examination. After 6 weeks, recipients were examined by a neurophysiologist unaware of the nerve-repair procedure. First, sensitivity was assessed by pulling the whiskers, observing whether there were evasive behaviour and defence reactions or no response at all. Afterwards, the animals were sedated using ketamine (10 mg per kg ip), and electroneurograms (ENG) of the facial nerve and electromyograms (EMG) of the mystacial muscles were registered in the normal hemiface and the transplanted hemiface. The presence of denervation activity or electrical silence in the resting EMG was registered. In the voluntary EMG, the records were classified into normal voluntary activity, moderate activity, or absence of activity according to a visual scale. Regarding ENG, the amplitude and duration of the conduction potentials were registered, should they be present. Zero value was assigned where no potential was observed. After 8 weeks, the animals were electively put down and biopsies of the mystacial region were taken. Haematoxylin–eosin preparations were processed to observe the presence of the neurokeratin artefact, which traduces the presence of successively and spirally rolled Schwann cells plasmatic membrane around the axons that provide the myelin to the nerve fibres.[42] In the absence of axons, Schwann cells do not roll over them and the neurokeratin artefact is not observed. The neurokeratin presence was noted for each sample as positive or negative.

Statistical analysis

Quantitative variables are presented as mean ± standard deviation and qualitative variables as percentages. Pair wise comparisons of the duration and amplitude of ENG in the group III_b based in the explored side were performed using the Wilcoxon test. The Mann–Whitney U-test was used for the comparison of these variables in the transplanted hemiface between the groups III_a and III_b. The differences in resting EMG, voluntary EMG, sensitivity test, and histology in the transplanted side based on these groups were performed using Fisher's exact test. A p-value of less than 0.05 was considered as statistically significant. Statistical analysis was performed using the SPSS Statistical Package, version 13 for Windows.

Results

Follow-up and survival

In group I, 12 hemifacial flaps were elevated, and the vascular network of the flaps was confirmed by gelatin–barium angiographies in two animals (Figure 1). The superficial temporal artery and the facial artery were traced, and the mystacial pad showed a rich vascularisation.

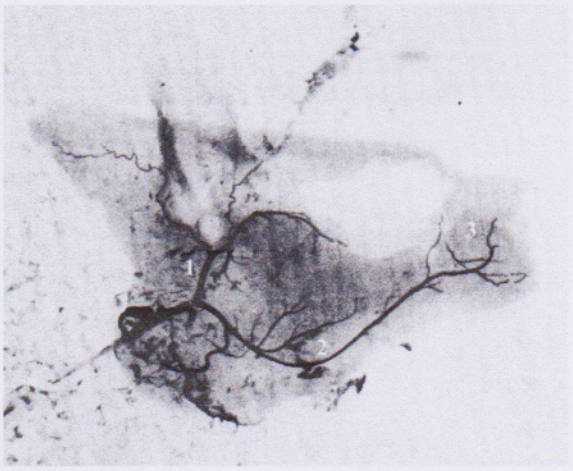

Figure 1 Gelatin–barium angiographical studies showed hemifacial flap vascularisation and mystacial vascular network. 1 – superficial temporal artery; 2 – facial artery; 3 – mystacial vascular network.

In group II, all non-vascularised allografts necrosed within a week.

In group III, 24 hemifacial vascularised allografts were transplanted. The surgical procedure required an average of 7 h (range 5–11 h), and the average time of ischaemia was 2 h. Eighteen animals (75%) from group III survived the experiment. Animals were put down on day 56. Survival for each individual is specified in Table 1. The causes of death were intraoperative (three cases, 12.5%), including haemorrhage and aspiration. Postoperative death was seen in three cases (12.5%), due to poor general condition and anorexia. No necropsies were done; no episodes of rejection were seen; and no difficulty of breathing or feeding was observed. After 3 days the animals returned to their usual routine of drinking and eating. Perioral scarring did not affect lips. No bruising was observed under the flaps, although blood clots were seen in the external ear canal for several days. Oedema was observed for a week approximately. Preauricular desquamation, probably due to scratching, was seen, but spontaneously resolved within 2 weeks. The hair grew again after 30 days (Figure 2), although whiskers never recovered their normal spatial orientation.

Clinical, neurophysiological, and histopathological evaluation

Clinical explorations of subgroup III_a showed no response to pulling the whiskers in any case. In contrast, in subgroup III_b, defence reactions and evasive behaviour were seen in all animals when whiskers were pulled (Table 2).

In ENG examinations of subgroup III_a, no conduction potentials were observed. In the EMG of the III_a subgroup, positive sharp waves and fibrillation potentials were observed, both compatible with denervation activity. In subgroup III_b, conduction potentials were seen in the ENG; these had less amplitude and were longer as compared to

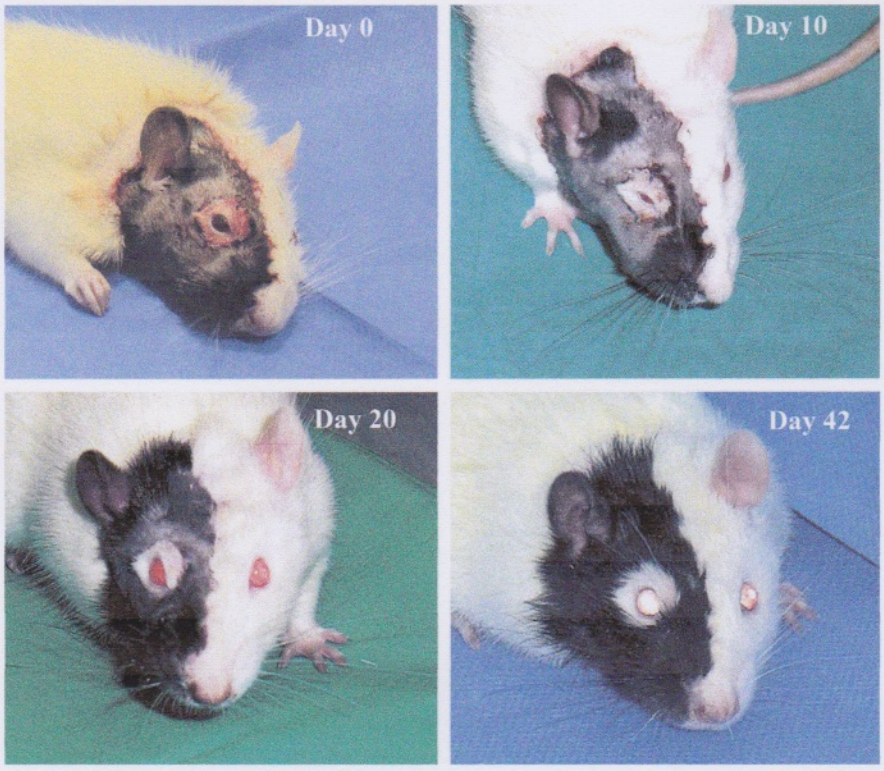

Figure 2 Appearance of the transplant recipients in the postoperative period days 0, 10, 20, and 42.

the normal contralateral hemifaces. In the EMGs of the III_b subgroup, no denervation activity was seen, but electrical silence while resting and a moderate motor voluntary activity while awake were observed (Figures 3 and 4). The results measured in each animal are shown in Table 2.

The haematoxylin–eosin biopsies revealed the entrance of the nerve fascicles into the vibrissal follicles. Nerve sheaths in subgroup III_a did not show the neurokeratin artefact in any case, traducing that fibres had not been remyelinated. All biopsies in nerve sheaths of subgroup III_b showed the neurokeratin artefact, traducing the presence of myelin (Figure 5).

Table 3 lists the analysed variables descriptively. Comparisons are made, in group III_b, between the normal hemiface and the transplanted hemifaces, and the statistical significance is now denoted as p^*. Comparisons between transplanted hemifaces of III_a and III_b subgroups are made, and the statistical significance is denoted as $p\dagger$. This table shows that there are statistically significant differences between face allograft recipients depending upon whether the facial and trigeminal nerve branches were repaired or not. In subgroup III_b, comparison between transplanted hemifaces and the normal hemifaces showed that the amplitude and conduction potentials diminished, and this difference was also statistically significant.

Discussion

Severe panfacial injuries require staged reconstruction procedures that rarely achieve aesthetic and functional results. Theoretically, a face transplant would provide the best tissue to reconstruct 'like' with 'like'. This reconstruction would not only be a vascularised facial skin transplant, but also a multi-functional, facial sensorimotor subunit transplant. Return of motion and sensitivity to these facial subunits would be paramount for the success of a face transplant in humans.

Evidence of recovery of facial function after a face transplant in animal models would support at some point an expected functional outcome in humans but, to the best of our knowledge, there is no report as yet on this matter. Animal models in face transplants are difficult to manage because critical features like breathing, feeding, or blinking cannot be jeopardised, and therefore the nose, the lips, and the eyelids, respectively, 'must' be preserved. The mystacial pad is a paradigm of sensitivity and movement in a rat face because it is a specialised subunit that contains the whiskers to explore the environment. Its main advantage is that it can be transplanted and monitored without sacrificing vital functions of the face, thus decreasing the morbidity and avoiding ethical issues.

In our study, recipients in which nerve branches to the mystacial pad were repaired (subgroup III_b) showed restoration of sensitivity, facial nerve conduction potentials, moderate voluntary motor activity, and histological signs of remyelination. No evidence of sensory or motor recovery was found in the non-nerve repair III_a subgroup, and this was accompanied by biopsies in which no myelin could be observed around axons. As we expected, comparison between the non-nerve repair (III_a) and nerve repair (III_b)

Table 2 Records of each receptor after 6 weeks

Explored side	ENG				EMG		Sensitivity test	Histology
	L1	L2	Duration	Amplitude	Resting EMG	Voluntary EMG		
25 – Normal hemiface	1.36	2.7	1.34	5.2	Electrical silence	Normal	Defence	Neurokeratin +
25 – Transplanted hemiface	0	0	0	0	Denervation activity	Absent	No response	Neurokeratin –
26 – Normal hemiface	1.29	2.73	1.44	5.4	Electrical silence	Normal	Defence	Neurokeratin +
26 – Transplanted hemiface	0	0	0	0	Denervation activity	Absent	No response	Neurokeratin –
27 – Intraoperative death								
28 – Normal hemiface	1.38	2.77	1.39	4.9	Electrical silence	Normal	Defence	Neurokeratin +
28 – Transplanted hemiface	0	0	0	0	Denervation activity	Absent	No response	Neurokeratin –
29 – Normal hemiface	1.34	2.64	1.3	4.3	Electrical silence	Normal	Defence	Neurokeratin +
29 – Transplanted hemiface	0	0	0	0	Denervation activity	Absent	No response	Neurokeratin –
30 – Died in postoperative day 21								
31 – Normal hemiface	1.1	2.5	1.4	4.4	Electrical silence	Normal	Defence	Neurokeratin +
31 – Transplanted hemiface	0	0	0	0	Denervation activity	Absent	No response	Neurokeratin –
32 – Intraoperative death								
33 – Normal hemiface	1.56	2.87	1.31	4.6	Electrical silence	Normal	Defence	Neurokeratin +
33 – Transplanted hemiface	0	0	0	0	Denervation activity	Absent	No response	Neurokeratin –
34 – Normal hemiface	1.35	2.71	1.36	4.1	Electrical silence	Normal	Defence	Neurokeratin +
34 – Transplanted hemiface	0	0	0	0	Denervation activity	Absent	No response	Neurokeratin –
35 – Died in postoperative day 40								
36 – Normal hemiface	1.28	2.61	1.33	3.9	Electrical silence	Normal	Defence	Neurokeratin +
36 – Transplanted hemiface	0	0	0	0	Denervation activity	Absent	No response	Neurokeratin –
37 – Normal hemiface	1.46	2.8	1.34	3.8	Electrical silence	Normal	Defence	Neurokeratin +
37 – Transplanted hemiface	0.98	3	2.02	1.2	Electrical silence	Moderate	Defence	Neurokeratin +
38 – Normal hemiface	1.13	2.45	1.32	4.8	Electrical silence	Normal	Defence	Neurokeratin +
38 – Transplanted hemiface	1	2.78	1.78	1.5	Electrical silence	Moderate	Defence	Neurokeratin +
39 – Normal hemiface	0.8	2.13	1.33	3.9	Electrical silence	Normal	Defence	Neurokeratin +
39 – Transplanted hemiface	1.13	3.34	2.21	1.8	Electrical silence	Moderate	Defence	Neurokeratin +
40 – Intraoperative death								
41 – Normal hemiface	1.06	2.33	1.27	4.6	Electrical silence	Normal	Defence	Neurokeratin +
41 – Transplanted hemiface	0.82	2.79	1.97	1.5	Electrical silence	Moderate	Defence	Neurokeratin +
42 – Normal hemiface	1.09	2.36	1.27	4.4	Electrical silence	Normal	Defence	Neurokeratin +
42 – Transplanted hemiface	1.12	2.9	1.78	1.4	Electrical silence	Moderate	Defence	Neurokeratin +
43 – Normal hemiface	0.75	2.1	1.35	3.7	Electrical silence	Normal	Defence	Neurokeratin +
43 – Transplanted hemiface	0.69	3.56	2.87	1.5	Electrical silence	Moderate	Defence	Neurokeratin +
44 – Normal hemiface	1.23	2.52	1.29	4.3	Electrical silence	Normal	Defence	Neurokeratin +
44 – Transplanted hemiface	0.88	2.66	1.78	1.7	Electrical silence	Moderate	Defence	Neurokeratin +
45 – Normal hemiface	0.9	2.21	1.31	4.7	Electrical silence	Normal	Defence	Neurokeratin +
45 – Transplanted hemiface	0.91	3.78	2.87	1.5	Electrical silence	Moderate	Defence	Neurokeratin +
46 – Died in postoperative day 32								
47 – Normal hemiface	1.07	2.41	1.34	5.2	Electrical silence	Normal	Defence	Neurokeratin +
47 – Transplanted hemiface	1.23	3.76	2.53	1.3	Electrical silence	Moderate	Defence	Neurokeratin +
48 – Normal hemiface	0.9	2.22	1.32	4.8	Electrical silence	Normal	Defence	Neurokeratin +
48 – Transplanted hemiface	0.57	2.68	2.11	1.5	Electrical silence	Moderate	Defence	Neurokeratin +

ENG = electroneurogram; EMG = electromyogram; L1 = peak ; L2 = off-peak.
All measures are in milliseconds.

subgroups revealed that nerve repair is essential for face allograft re-innervation. In the III_b subgroup, the EMG and ENG revealed a partial recovery with the presence of a moderate voluntary activity and conduction potentials that were longer and had less amplitude, and these observations were statistically significant. This partial functional recovery shows that motion and sensitivity can return to facial allograft if short time is allowed to pass.

This is the first report on face transplant under tacrolimus immunosuppression. Previous studies were performed using cyclosporine A (CsA), but there are reasons that support the use of tacrolimus in face-transplant research. Compared to CsA, tacrolimus has demonstrated effectiveness at lower doses and earlier nerve regeneration after peripheral nerve transplantation (both in the research model and in humans), accelerated return of function after nerve transection with immediate neurorrhaphy, and superior nerve regeneration histologically.[34–39,41] The optimum dose of tacrolimus for maximum acceleration of nerve regeneration in rats was 5 mg per kg per day in

Figure 3 Neurophysiological tests were performed after 6 weeks. Stimulus with two-needle over facial nerve exit, monopolar measure on vibrissae. Reference on the neck.

a dose-dependent study.[43] Tapered doses of 8 mg per kg per day to 2 mg per kg per day after 4 weeks were used in our study. We observed anorexia as a major adverse effect, probably due to drug overdose, but none of the recipients showed signs of rejection. Regarding surgical complications, 12.5% of the animals (three cases) died because of bleeding or aspiration in the last hour of the procedure. As many as 75% (18 cases) of the recipients survived up to 8 weeks. Our procedure lasted an average of 7 h (2 h longer than previous reports), and, like other groups,[15,16,19] we had high mortality rates.[15,16,19] This was probably due to the complexity of the procedure. Those animals that survived surgery showed few complications and a good tolerance to the daily injection of immunosuppression.

This work has some limitations, which we now discuss:

1. There is no analogue subunit of the mystacial pad in humans. The human neonates employ their upper lip and gingiva to explore small objects, as homologues of the mystacial pad. The vibrissae in the human nasal vestibule are quite sensitive, but obviously neither move nor differentiate. Therefore, the use of the mystacial pad in the rat does not parallel that of the human face in any case.
2. Spontaneous innervation from a healthy bed would aid to innervate the flap if the recipients lived longer, and we do not overlook this fact.
3. Ideally the motor re-innervation should be expressed in terms of vibrissal movement measured by high-speed cinematography in unrestrained rats. This was impossible in this experiment because some vibrissae were lost during the insensitivity period, and the new ones did not orientate like the original ones due to scarring.
4. This experiment had aimed for a qualitative demonstration of re-innervation, not for an immunological or survival study. A period of time that would allow axons to grow into the mystacial pad (about 15 mm) was selected. Extending the observation period would probably have improved the quality and quantity of re-innervation.
5. Pulling the whiskers is not a validated test for sensory recovery, although it gives qualitative evidence of re-innervation, and similar sensitivity tests after ear allotransplant have been published.[19]

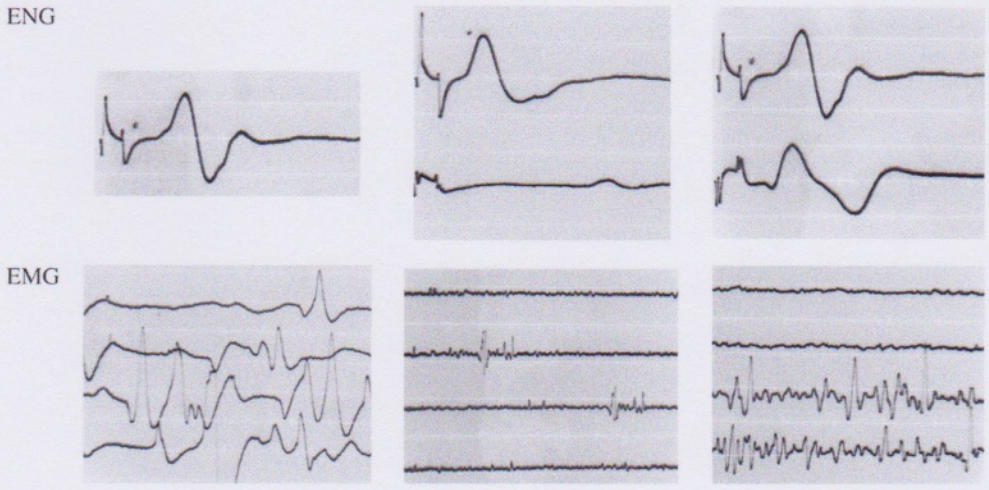

Figure 4 Electrophysiological study of the mystacial region. ENG, electroneurogram; EMG, electromyogram. (Above, left) Facial nerve conduction potentials in the normal hemifaces. (Above, centre) Morphology of the facial nerve conduction potential in normal hemifaces as compared with the transplant III_a subgroup, in which no waves were observed. (Above, right) The morphology of the conduction potentials in the transplanted hemifaces of the subgroup III_b was similar to those of the normal hemifaces. (Below, left) Voluntary motor activity of the mystacial muscles in normal hemifaces. (Below, centre) Denervation activity and isolated polyphasic motor potentials observed in subgroup III_a. (Below, right) Moderate voluntary motor activity was observed when nerves were repaired (subgroup III_b).

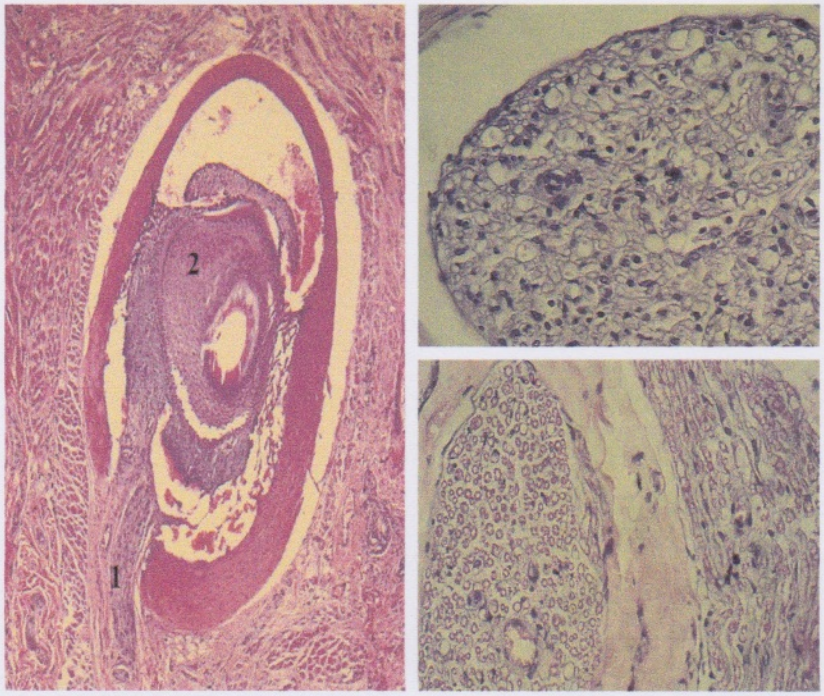

Figure 5 Mystacial biopsies. (Left) A normal infraorbital fascicle (1) enters a vibrissal follicle (2). (Above) A transverse section of a nerve from the subgroup III_a is displayed, showing that nerve fascicles were empty of neurokeratin, traducing the absence of Schwann cells wrapping the axons and lack of remyelination. (Below) Cross section and longitudinal section of a nerve fascicle in the subgroup III_b. The neurokeratin artefact observed as a result of the presence of Schwann cells supporting the axons growing in the allograft.

Table 3 Summary Neurophysiological, Clinical and Histological Evaluation

	III_a ($n = 8$)		III_b ($n = 10$)		p^*	$p^\dagger$
	Normal hemiface	Transplanted hemiface	Normal hemiface	Transplanted hemiface		
ENG						
Duration	1.35 ± 0.05	0	1.31 ± 0.03	2.19 ± 0.42	0.005	<0.001
Amplitude	4.6 ± 0.53	0	4.42 ± 0.49	1.49 ± 0.17	0.005	<0.001
Resting EMG (%)						
Electrical silence	100	0	100	100	–	<0.001
Denervation activity	0	100	0	0		
Voluntary EMG (%)						
Normal	100	0	100	0		
Moderate	0	0	0	100	–	<0.001
Absence	0	100	0	0		
Sensitivity test (%)						
Defence	100	0	100	100	–	<0.001
No response	0	100	0	0		
Histology (%)						
Neurokeratin +	100	0	100	100	–	<0.001
Neurokeratin –	0	100	0	0		

Data are *n*, means ± SD, or %.
* Normal hemiface vs. Transplanted hemiface in the group III_b.
† III_a vs. III_b Transplanted hemiface.

6. The histological study indicates re-innervation through indirect signs, like the neurokeratin artefact. The neurokeratin artefact was chosen because it is an easy recognizable sign of myelin presence traducing that Schwann cells support the axons growing in the allograft.[42]
7. Neither a re-plantation model nor an isograft model is reported in this paper. Previous research in our laboratory confirmed that face re-plantation and facial isograft transplants could recover both sensitivity and movement, and this was reported elsewhere.[44,45] In addition, previous works in rats have largely demonstrated that lower-extremity allografts can re-innervate as well as isografts.[46–48]

The above-listed reasons make further investigations in larger animal models essential before a wider application of facial transplantations in humans.

As stated by the American Society of Reconstructive Microsurgery, "the technical issues surrounding facial transplantation are generally touted as being solved although very little data exist to support this conclusion."[49] Few publications regarding the technical aspects of face transplants have been published,[14,23–28] and some details are still under discussion.[29,30] Most previous papers have focused on the advisability and ethics of a face transplant,[50–54] but few have insisted on the functionality of the reconstruction.[30] The reported cases of full-face reconstruction with autologous flaps in humans have not included functional reconstruction of areas such as eyelids or lips.[30,55,56] Results in these cases were poorly functional in spite of a big surgical effort. A patient that does not need to have a functional reconstruction of the face would be a good candidate for such reconstructions. On the other hand, a patient needing a functional reconstruction, especially for the eyelids and lips with or without other facial subunits, would be a candidate for face transplant. The partial face transplant reported in France[1] included the nose and the lips. The orbicularis orii could only neurotise on one side, and the oral sphincter was insufficient for some months. However, follow-up at 8 months has confirmed excellent lower-lip tone recovery.[57] Nevertheless, there was no previous evidence in research models to support this. In our study, return of function to the hemifacial allograft transplant in rats was observed when motor and sensory nerves were repaired. This is the first confirmation of functional recovery after a face transplant in the experimental model, and we hope our data will aid in further research in functional face transplant.

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