Pineal Cross-Transplantation (Old-to-Young and Vice Versa) as Evidence for an Endogenous "Aging Clock"

VLADIMIR A. LESNIKOV[a] AND WALTER PIERPAOLI[b,c]

Institute of Experimental Medicine
Russian Academy of Medical Science
St. Petersburg, Russia

and

[b]*Novera H. Spector Neuroimmunomodulation Laboratory*
Biancalana-Masera Foundation for the Aged
Via Birarelli, 8
60121 Ancona, Italy

We have shown that both exogenous, chronic circadian (night) administration of the pineal neurohormone melatonin (N-acetyl-5-methoxytryptamine) to old mice or grafting of the entire pineal gland from young donors into the thymus of aging mice delays aging and prolongs a state of juvenile, disease-free life.[1-14] However, in both models used, some aspects were as yet amenable to criticism, which required clarification. In fact, in those time-consuming experiments one could not completely discriminate the positive effects of chronic melatonin administration or pineal grafting from changes related to different food intake, drinking habits, chronic stimulation of the thymus, etc., which would not allow drawing conclusions about a real life-prolonging effect of the treatment when compared with the control groups.

We devised experiments which would permit evaluating beyond any possible doubt whether the life-prolonging or aging-delaying effects observed were due to the intrinsic properties of the pineal and not to nonspecific chronic factors, in that the mice used were themselves controls, donors and at the same time recipients of an "old" or of a "young" pineal gland.

A few years ago we initiated a new series of long-term experiments with a genetically pure, inbred strain of mice, namely the BALB/cJ, in which the entire pineal gland adhering to the bone fragment of the skull was removed simultaneously from the skull of young-adult (3–4-month-old) or old (18-month-old) mice of the same inbred strain and sex, and in which the size- and shape-matched skull fragment with the "young" or the "old" pineals were adapted to the skull of the young or old recipient. This operation is described in FIGURE 1.

[a] Present address: R/D Haemoderivitaves, SCLAVO SpA, Via Fiorentina, 1, 53100 Siena, Italy.

[c] To whom requests for reprints should be addressed.

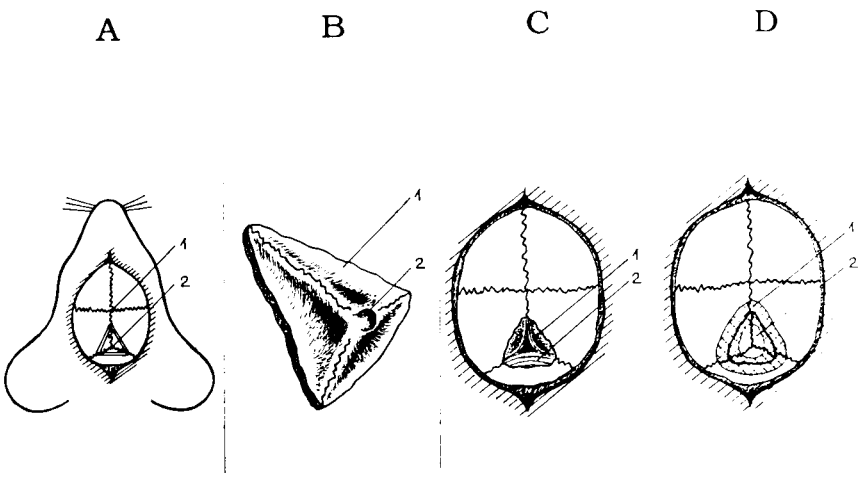

FIGURE 1. Pineal cross-transplantation: sequential steps of the operation. **(A)** Operation field on the mouse head. The bone-pineal graft (*triangle*) is outlined by a *double line*. 1: bregma; 2: lambda. **(B)** Bone-pineal graft. 1: skull fragment inside the head bone; 2: pineal gland. **(C)** Cranial window in the recipient mouse. 1: cortex; 2: cerebellum. **(D)** Donor bone-pineal graft fitted to the recipient mouse. 1: cement; 2: bone-pineal graft fitted to the recipient mouse.

Pineal Cross-Transplantation

Groups of female, young-adult (3–4-month-old) and old aging (18-month-old) BALB/cJ mice maintained in our animal rooms were used. They were kept at 22°C and had free access to food pellets and tap water. Lighting of the rooms was 7 a.m. light-on and 7 p.m. light-off. The animals were anesthesized with the barbiturate hexenal (200 mg/Kg). The upper part of the skull was accurately shaven and the operation field was treated and disinfected with 70 percent ethanol, trying to avoid an irritation to the eyes. The head skin was cut sagittally along the midline for 10–12 mm, and the aponeurosis and other soft tissues were removed from this part of the skull (see FIG. 1A). The surface of the bone was rinsed with 5 percent H_2O_2 and dried with 70 percent ethanol, in order to visualize the fissures of the calvarium. In order to fasten the mouse and to turn its head in a proper operational position, and original stereotaxical instrument developed at the Institute of Experimental Medicine, St. Petersburg, Russia, was used.[5,6]

A triangle-shaped skull fragment located at the intersection of the sagittal and occipital fissures (FIG. 1A) was cut with the help of a dental drilling machine, trying not to produce any lesion to the underlying cortex and cerebellum. The next steps of the operation were carried out with the help of a dissection microscope. After turning the animal's head around its midline horizontal axis, the skull fragment, including its adherent pineal gland in its membranes and ligaments was removed. Bleeding from the brain venous sinuses was stopped by repeated rinsing with saline and absorption with tissue pads until spontaneous cessation. The pineal ligaments connected with dura mater and forming the brain sinuses were cut with

TABLE 1. Pineal Gland, Young-to-Old and Old-to-Young Cross-Transplantation in BALB/cJ Female Mice Results in Prolongation or Abbreviation of Their Life Span under Conventional Laboratory Conditions[a]

Group	No.	Treatment	Survival (Days ± SE)*
A	30	sham-operated	719 ± 32
B	10	"young" pineal into old mice	1021 ± 56***
C	10	"old" pineal into young mice	510 ± 36**

[a] Young (4-month-old) and old (18-month-old) female inbred BALB/c mice were used simultaneously both as donors and as recipients of an intact pineal gland. The mice were left undisturbed after ear-marking as long as they lived. Their body weight was taken monthly. Results are of two identical experiments in the course of three years.

* Standard error; ** C vs A, $p < 0.01$; *** B vs A, $p < 0.002$. For statistical analysis the Student t test was used.

fine scissors and the skull fragment together with the adherent pineal gland was removed and kept in ice-cooled medium TC 199 (no serum added). It is also possible to use the same procedure to obtian skull fragments with epiphyses in their original position from mice which have been sacrificed as donors of an intact pineal gland. In all cases the bone-pineal graft must be examined microscopically to ensure that the pineal gland is in its original position and undamaged (FIG. 1B).

The pineal gland in its original position from the donor-recipient mouse is then positioned in the corresponding and size-matched, appropriate recipient-donor cranial window and the adapted skull fragment is glued with cement (polymer BF-6, FIG. 1C and D). After drying, the scalp skin is sutured with silk stitches and the sealed operation field is sprayed with antibiotic powder. The duration of one intervention of cross-transplantation between an old and a young mouse is between one and a half and two hours. Sham-operated mice undergo the same procedure, but the mouse is grafted again with its own skull fragment and pineal gland.

In the evaluation of the effects produced by pineal cross-transplantation between young and old mice, a noninvasive approach was chosen in order to assess the existence of a pineal "aging clock." We examined only body weights, physical conditions and the life span of the pineal, cross-grafted mice. A first series of mice (5 in each group) was cross-grafted in April 1990 and a second series in November 1991. TABLE 1 shows the results of both experiments. A remarkable acceleration of aging and death was seen in the young mice grafted with an "old" pineal, while a very significant delay of aging and death was observed in the old mice grafted with a "young" pineal gland. At one year after the pineal cross-grafting, no evidence of age difference consequent to physical attrition and decay could be observed between the younger and the older mice (FIG. 2). A progressive increase of body weight was measured in the older mice grafted with a "young" pineal while the body weight of the younger mice grafted with an "old" pineal became progressively similar or identical to those of the older mice at about one year after pineal cross-grafting. In both cases death was preceded by a rapid loss of weight. This weight decrease was more marked in the younger mice grafted with an "old" pineal gland. Without exception, young-to-old or old-to-young

pineal grafting respectively delayed or accelerated aging of a good one-third (6 months), which is about one-fourth the life duration of BALB/cJ mice in our animal rooms.[4] These results largely surpass the life prolongation and/or aging-delaying effects obtained with melatonin administration and/or pineal grafting into the thymus.[1-4] They clearly point to a distinctive and central role of the pineal gland in the initiation and progression of aging. This model deserves repetition and extension aimed at elucidating the mechanism by which the "aging clock" in the pineal scans the time of life and death.[7]

SUMMARY

Circadian (night), chronic administration of melatonin and young-to-old pineal grafting into the thymus have provided evidence for the existence of an endogenous, primary and central "aging clock" in the pineal gland. The new model described here serves to definitely demonstrate that the replacement of the pineal gland of an old mouse with the pineal from a young, syngeneic donor mouse remarkably prolongs its life and, conversely, the "old" pineal transplanted into a younger mouse will considerably shorten its life span. Pineal cross-transplanta-

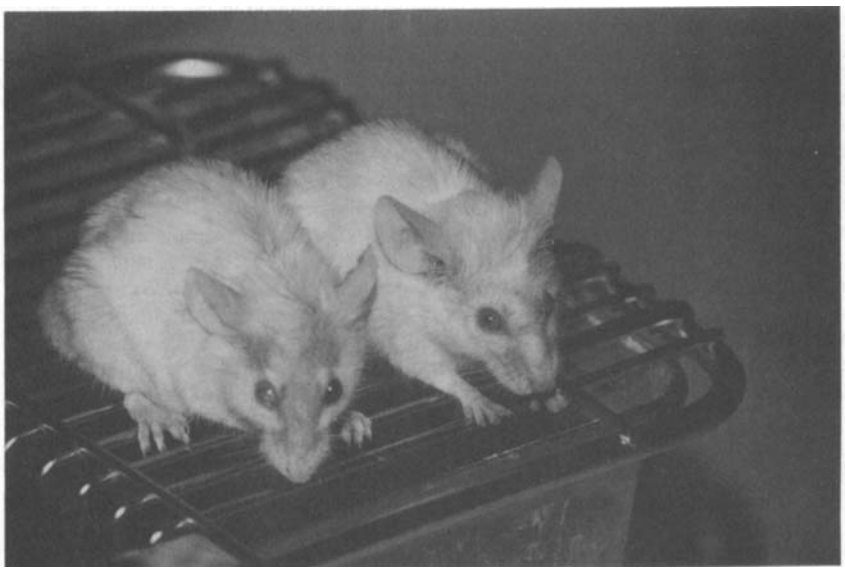

FIGURE 2. Pineal cross-transplantation: effects on somatic conditions. The illustration shows two female inbred BALB/c mice at one year after pineal cross-grafting. The mouse on the *left* side was grafted at 540 days of age with the pineal gland from a 110-day-old donor. This mouse lived 1061 days. The mouse on the *right* side was grafted at 110 days of age with the pineal gland from the 540-day-old donor-recipient (on the *left* side of the picture). This younger mouse lived only 476 days. At one year after pineal cross-grafting no difference of somatic conditions (fur, posture, skin, weight) can be observed between the "old" and the "young" mouse.

tion thus provides clear-cut evidence for the central role of the pineal gland in the initiation and progression of senescence. It offers a novel basis for interventions in the aging process.

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