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Neonatal pinealectomy in rats — a simple micro-suction technique

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Abstract: To determine the role of the pineal gland and its secretory product melatonin on various aspects of the functioning of the organism, the gland can be easily surgically removed in rats within 18 hours after birth. We performed pinealectomy in rats in a state of deep hypothermia under an operating microscope, using a micro-suction device of our own construction. The rats were induced into a state of suspended animation by placing them in the freezing compartment at minus 20 Celsius degrees. The cessation of respiration and heart beat lasted for about 15 minutes. During that time the pinealectomy was performed. In some cases there was minor hemorrhage that was easily controlled. There were no major side effects or mortality following surgery. All rats recovered within 15 minutes after the end of the procedure. The pinealectomy procedure described in this study is simple, rapid, effective and safe, and can be easily performed with instruments commonly available in most laboratories.

Key words: melatonin, neonate, pinealectomy, pineal gland, rat.

Introduction

Since the 1930s, pinealectomy (PINX) in rats has been an excellent model for studying the impact of the major neurohormone secreted by the pineal gland, melatonin (N-acetyl-5-methoxytryptamine), on the physiology and pathology of mammals. Melatonin has been reported to possess numerous biological functions including sleep initiation, vasomotor control, anti-excitatory actions, and regulation of mitochondrial functions [1, 2]. Melatonin and its metabolites were also found to have important immunomodulatory and antioxidant properties, owning to their direct and indirect antioxidant actions by scavenging free radicals and by upregulating antioxidant pathways [1, 3–7].

It is well established that melatonin plays a role in the regulation and reset of circadian rhythms, with involvement in the measurement of day length, an environmental variable used for seasonal timing of reproduction, metabolism, and behavior in animal species [1, 8–10]. Furthermore, melatonin has been demonstrated to have the capability to regulate leukocyte function, and contributes to the control of inflammation in tissues, acting as both an activator and inhibitor of the inflammatory and immune responses [11–14]. Melatonin administration increases the proliferative response of rat lymphocytes, increases the number of NK cells, stimulates the release of pro-inflammatory cytokines interleukin 1 (IL-1), and tumor necrosis factor (TNF-alpha), enhances phagocytes, and modulates apoptosis [2]. The various regulatory roles of melatonin in the inflammatory process have made it a potential candidate drug for the treatment of a variety of chronic inflammatory diseases such as liver fibrosis [15] and non-alcoholic fatty liver disease [16].

Additionally, melatonin can act on energy metabolism, by stimulating mitochondrial biogenesis and increasing the efficacy of the electron transport chain mitochondria, thereby limiting electron leakage and free radical generation [17, 18]. Melatonin can also increase mitochondrial glutathione levels, leading to protection against free oxygen species [19].

Neonatal PINX in rats is an important tool for further studying the role and influence of melatonin on many immunological, physiological, and pathophysiological processes in the human body. Neonatal PINX removes a major source of melatonin in the very early stage of growth of the organism, and allows researchers to assess the effects of a lack of this hormone on various biological properties. Previously described rat PINX procedures have reported a variety of difficulties and perioperative complications, including neurologic damage, intracranial hemorrhage and death [20]. The aim of this manuscript is to present a safe, effective, and easy to perform PINX procedure for neonatal rats.

Methods

The PINX procedures were carried out on 62 newborn Sprague-Dawley rats (36 females, 26 males) within 18 hours after birth. All procedures were performed at the Department of Immunobiology, Institute of Pharmacology, Polish Academy of Sciences in Krakow, Poland, and were conducted in accordance with the principles of the Animal Husbandry Institute of the Polish Academy of Sciences in Krakow, Poland.

The surgical procedure was performed with the rats in a state of deep hypothermia. To induce hypothermia, each of the newborns was placed in an open cardboard box and placed in the freezer compartment of a commercial refrigerator at minus 20 degrees Celsius for a period of 8–10 minutes [21]. This resulted in cessation of respiration and heartbeat. The animals remained in a state of suspended animation for the duration of the PINX procedure, a period of about 15 minutes.

The operation was performed under an operating microscope (OPMI Pico, Zeiss) at twenty-fold magnification. The hypothermic animal was placed on a precooled surface. A midline longitudinal incision was performed, and the scalp and its muscles were retracted aside, which exposed the occipital and parietal bones of the skull. Next, the periosteum was retracted and a U-shaped skull flap was made by incising the bone with a small lancet around the junction of the sagittal and transverse sinuses (confluence of sinuses) [21]. The flap was then lifted up and bent posteriorly to expose the pineal gland. Using a 0.5 mm injection needle, a slit was made into the dura, exposing access to the pineal gland, which was subsequently removed by using a surgical micro-suction device of our own construction.

The PINX suction device was made by elongating a normal glass suction pipe over a gas burner to achieve a minimum internal diameter (Fig. 1.1–2). Once the

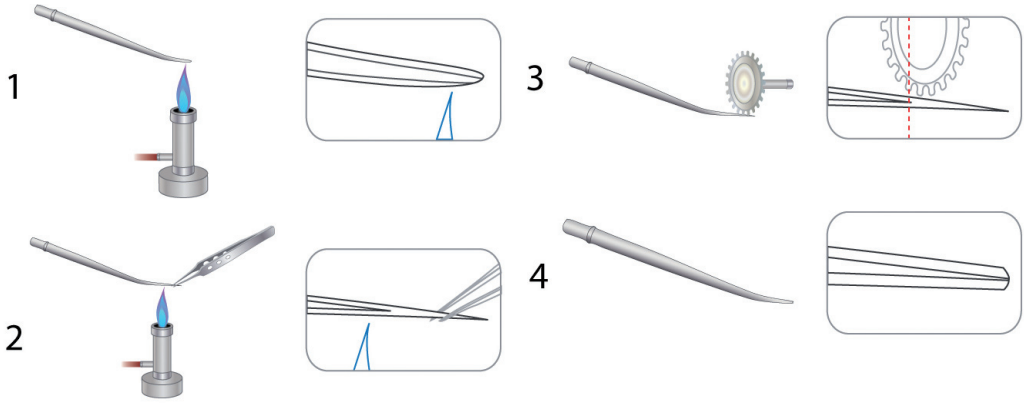

Fig. 1. Making of the Micro-Suction Device.
The glass micro-suction tube is placed over a flame (1), and elongated to achieve a minimum internal diameter (2). Once the tube lumen is no longer visible under an operating microscope, it was removed from the flame, and using a diamond cutter, the suction was cut right above its thinnest point (3), thus obtaining a micro-suction with a tiny lumen seen only under an operating microscope (4).

tube reached the point where there was no longer a visible lumen in the tube, it was removed from the flame. Then, using a diamond cutter, the suction was cut right above its thinnest point, thus obtaining a micro-suction with a tiny lumen seen only under an operating microscope (Fig. 1.3–4).

During the PINX, in 11 cases, there was minimal hemorrhage, which was easily controlled by light compression with a gauze swab. The bone flap was replaced and the scalp was sutured using Mersilk 5-0 (Ethicon). The wound was then sealed with Cutanol spray (Pfizer Inc.).

After the procedure, the animals were then placed in an open cardboard box with a thermometer, and with a heat lamp (75 W) above the box in order to achieve an optimum temperature of 28 degrees Celsius.

Results

All of the animals recovered from the deep hypothermic state, regaining respiratory and cardiac activity within 15 minutes. When the neonates were mobile, they were restored to the maternal cage. There were no deaths or no major adverse effects. In all rats post-PINX, normal life functions and no abnormal behavior was observed. Furthermore, no macroscopic or microscopic cortical or epithalamic damage was observed as a consequence of the PINX (Fig. 2, 3). Completeness of the removal of the pineal gland was verified in all case by histology (Fig. 3).

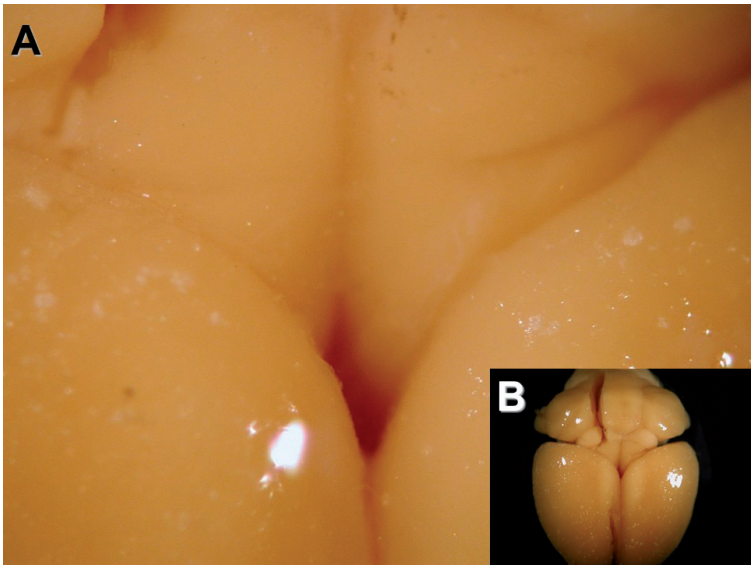

Fig. 2. Macroscopic view of a Neonatal Rat Brain Post-Pinealectomy.
A — Close-up gross view of the site of pineal gland excision; B — Whole brain.

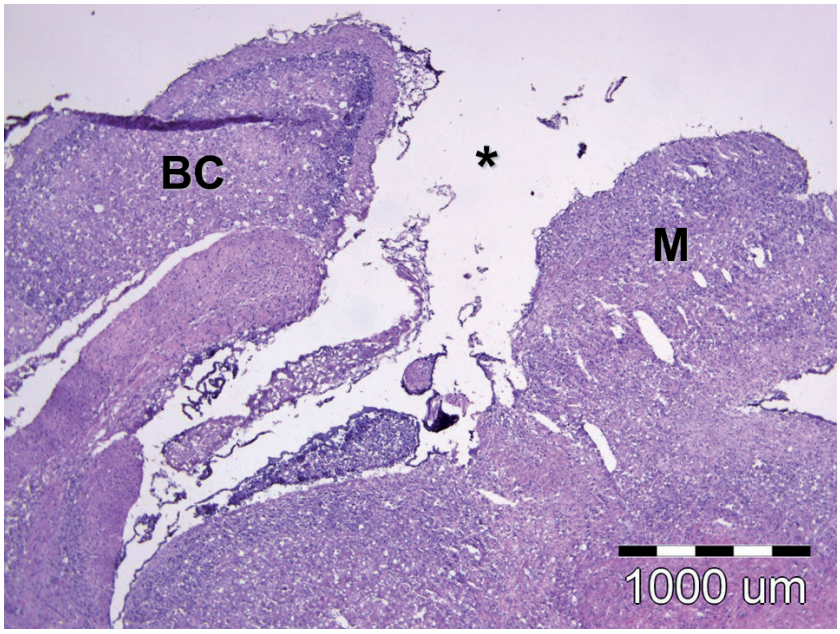

Fig. 3. Microscopic view of a Neonatal Rat Brain Post-Pinealectomy.
Hematoxylin & Eosin stained slide showing the site of pineal gland removal. BC — brain cortex; M — mesencephalon; * — site of pinealectomy.
Bar = 1000 um

Discussion

The pineal gland in rats is a tiny, round, rather translucent organ, situated on top of the brain at the confluence of the superior sagittal and transverse sinuses, located between cerebral hemispheres and the cerebellum [22, 23]. The pineal gland represents a complex, rather than a single organ in the rat brain. Within the complex, one can distinguish 3 main parts: the deep pineal tissue in the intercommissural region, a superficial pineal which represents the major part of the pineal complex, and a parenchymal stalk of variable length [24].

Pinealectomies are known to remove the normal nocturnal elevation of serum melatonin level and decrease overall melatonin concentrations. It has been previously suggested that reduction of serum levels of melatonin after pinealectomy increases oxidative stress in numerous tissues [5]. Furthermore, lower serum levels of melatonin after PINX has been demonstrated to influence immunity and aging [12–14]. As such, PINX in rats remains an important model for further studying the effects of melatonin in organisms.

The technique of neonatal PINX described in this study is simple, rapid, effective, and safe. The PINX may be easily performed with instruments commonly available

in laboratories, with slight modifications as described in the methods section of this paper.

In contrast to other common techniques, where troubles such as neurological deficits, intracranial hemorrhage, and death have been reported, we observed no serious side effects or mortality as a result of the procedure in any of the 62 newborn rats [20, 25, 26]. In a technique described by Maganhin *et al.* [20], which used a modified direct visual approach and tweezers to remove the pineal gland in rats, a 6.3% mortality rate was observed. The authors primarily attributed the perioperative mortality to anesthesic-induced central nervous system depression [20]. They further reported that the duration of the combined xylazine and ketamine induced anesthesia was long due to slow metabolism of the drugs, lasting in some cases for over 40 minutes. Thus, we used and recommend an induced state of deep hypothermia as opposed to traditional drug-induced anesthesia. Deep hypothermia is quick and easy to perform, and induces a period of anesthesia of only 15 minutes, allowing rapid recovery and reduced risk of perioperative death and complications.

A high level of precision in neonatal pinealectomy is very important, and for this reason the operation should be performed under an operating microscope. The anatomy of important structures such as the transverse sinus, superior sagittal sinus, and the confluence of sinuses should be taken into consideration during the procedure to avoid intracranial hemorrhage. Care should also be taken to avoid any iatrogenic injury to the encephalon. Lastly, completeness of PINX should always be confirmed by histological examination.

Author contributions

Bohdan Pawlicki — study design, conduction of experiments, analysis of histological samples, analysis and interpretation of data, critical revision of the manuscript.

Brandon Michael Henry — study design, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript.

Krzysztof A. Tomaszewski — study design, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript.

Mariusz Gajda — preparation of histological samples, analysis of histological samples, critical revision of the manuscript.

Iwona Brzozowska — conduction of experiments, critical revision of the manuscript.

Jerzy A. Walocha — study design, conduction of experiments, meritorical oversight, critical revision of the manuscript.

Anna Skowron-Cendrzak — study design, meritorical oversight, obtaining funding for the study, critical revision of the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

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