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Research report

Splitting of the cerebellar vermis in juvenile rats—Effects on social behavior, vocalization and motor activity

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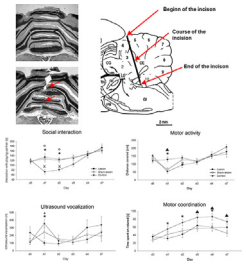
HIGHLIGHTS

- Testing the effect of vermian splitting in juvenile rats on social behavior, vocalization and motor activity.
- Deficient social behavior and vocalization after surgery are related to vermian splitting.
- These results indicate that vermian splitting can reduce communicative drive in the early postsurgical phase.

GRAPHICAL ABSTRACT



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ABSTRACT

Radical resection of malignant midline tumors of the posterior fossa in childhood followed by adjuvant therapies like chemotherapy or radiotherapy often leads to longterm survival and even healing of such patients. Therefore, quality of life becomes particular important. Postoperative neurological deficits, such as cerebellar mutism and ataxia have been attributed to splitting of the cerebellar vermis to remove these tumors.

Here, we tested the effect of vermian splitting in juvenile rats on social behavior, vocalization and motor activity. Juvenile male Sprague Dawley rats, aged 23 days, underwent vermian splitting under general anesthesia after medial suboccipital craniotomy (lesioned group, $n = 16$). In sham-lesioned rats, only craniotomy was performed and the dura was opened with release of cerebrospinal fluid ($n = 16$). Naïve rats served as controls ($n = 14$). All groups were tested on day 0 (before surgery), and on days 1–4 and 7 after surgery for locomotor activity, motor coordination, social behavior, and ultrasound vocalization during social interaction. Finally, splitting of the vermis was histologically verified.

Social interaction was reduced for two days after surgery in lesioned rats compared to sham-lesioned rats and controls. Vocalization was decreased for one day compared to controls. Locomotor activity was disturbed for several days after surgery in both lesioned and sham-lesioned rats as compared to controls.

Deficient social behavior and vocalization after surgery are related to vermian splitting in juvenile rats. These results indicate that similar to the human context vermian splitting can reduce communicative drive in the early postsurgical phase.

1. Introduction

Brain tumors constitute the second most common neoplastic entity in childhood just after leukemia. Two thirds of brain tumors in pediatric patients occur in the posterior fossa during the first decade of life. These tumors are often located in the midline involving or displacing the cerebellar vermis. The treatment of these tumors includes neurosurgical resection followed by

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adjuvant radio- and chemotherapy. With increasing survival rates and cure, even in highly malignant tumors like medulloblastomas, postoperative quality of life becomes increasingly important [1,2].

The aim of surgery is complete tumor resection whenever possible while preserving or improving neurological function. One common surgical approach to resect midline tumors of the posterior fossa is the transvermian approach, which usually consists of splitting the inferior part of the cerebellar vermis [3,4]. Recent data show that this fast and straightforward technique may be associated with neurological and neuropsychiatric problems, which are independent of postoperative radiotherapy [5–7,2]. Several studies reported that lesions of the cerebellar vermis may result in ataxia and staggering of gait [8,9]. Additionally, cerebellar autism is a very impressive and still unexplained neurological and neuropsychological syndrome after tumor resection of the posterior fossa in childhood [10–12]. This phenomenon includes a temporary inability to speak along with unimpaired consciousness, and impairment of motor activity, motor coordination and social behavior. Some of these symptoms recover completely after weeks to months but others, such as speech and ataxia may recover only incompletely [1]. The precise cause and the neuroanatomical substrates for the "cerebellar mutism" syndrome after posterior fossa tumor resection are still unknown. Involvement of the vermis has been described in more than 90% of patients [13], but some investigators argue against direct operative vermis injury or resection being the sole mechanism for cerebellar mutism [14,15]. So far, only few experimental studies have investigated this issue [16–18].

Rats show a distinct playing behavior, especially between the age of 21 and 30 days [19,20]. Playing rats communicate by ultrasound at frequencies of about 50 kHz [21,19]. Combined reduction of playing behavior and 50 kHz vocalization may serve as an endophenotype for the cerebellar mutism seen in children after damage to the vermis. With that reasoning, we here tested the effect of vermian splitting on social behavior and ultrasonic vocalization in juvenile rats. Further, these rats were tested for locomotor activity in the open field and for motor coordination on the rotarod.

2. Materials and methods

Juvenile male Sprague-Dawley rats (Charles River Laboratories) weighing between 48 g and 73 g, which were separated from their mothers at postnatal day (PND) 21, were used for this study. The animals were housed in cages (Makrolon Type IV) under a 14/10 h light/dark cycle with water and food ad libitum. The cages were kept in a scantainer (Scanbur) with a humidity of 55% and at a temperature of 22 °C. All efforts were undertaken to minimize the number of animals used and their suffering. Experiments were done in accordance with the German Animal Welfare Law and with the European Community Council Directive 86/609/EEC for the protection of animals used for experimental purposes. All experiments were approved by the Local Institutional Animal Care and Research Advisory Committee and authorized by the local government (AZ #10/0055).

2.1. Experimental design

Overall, 52 animals were obtained from 11 litters. These rats were randomly divided into three groups: (1) control group (n = 18 rats), (2) sham-lesioned group (n = 16 rats) which underwent only suboccipital craniotomy and opening of the dura for the release of cerebrospinal fluid, and (3) lesioned group (n = 18 rats) with additional splitting of the inferior part of the cerebellar vermis. Additionally, 18 rats served as playing partners (stimulus rats) for assessment of social behavior of the three experimental groups.

All rats were assessed for motor activity, motor coordination, social behavior and ultrasound vocalization before surgery (d0) and on the days 1–4 and 7 after surgery. All behavioral tests were conducted on each day and performed between 9 am and 4 pm.

2.2. Surgery

For surgery animals were anesthetized by intraperitoneal injection of chloral hydrate (360 mg/kg). After shaving their head from the coronal suture to the neck, they were fixed in a stereotaxic frame with the head in an anteflexed position. A skin incision was made with microscissors in the midline from the coronal suture to the level of the spinal process of the second cervical vertebra. Then, the suboccipital muscles were reflected laterally until the suboccipital part of the skull, the protuberantia occipitalis externa, the lamina of the atlas and the atlanto-occipital membrane were completely visualized. A skin retractor was positioned and the atlanto-occipital membrane was incised in the midline and removed. The dura mater, which was very thin, was visualized and separated carefully from the inner part of the suboccipital skull by using a microdissector. After that, an osteoclastic suboccipital craniotomy was performed with a small rongeur opening the foramen magnum up to below to the confluens sinuum. The confluens sinuum and both transverse sinuses were transparent through the thin skull of the juvenile rats allowing their protection during craniotomy. Then, the dura mater and the arachnoid were opened in the midline by microscissors to release cerebrospinal fluid and to visualize the cerebellar vermis just below from the confluens sinuum down to the obex. For the sham-lesioned group, surgery was stopped at this point and the wound was closed by cutaneous sutures.

For the lesion group, a blunt microdissector was inserted carefully through the foramen of Magendie into the fourth ventricle and was positioned on the floor of the fourth ventricle using a surgical microscope. Thereafter, the vermis was slowly lifted with the dissector to protect the brainstem while splitting the cerebellar vermis. Using a microblade, the inferior part of the cerebellar vermis was cut longitudinally in the midline. Bleeding was controlled by gently putting a small piece of surgical cotton on the bleeding parts of the vermis. After bleeding was controlled the wound was closed. After operation all rats as well as rats from the control groups were housed individually in a single cage for 12 h.

2.3. Performance of tests

2.3.1. Motor activity

Locomotor activity was assessed in an open field, i.e., a 62 cm long and 62 cm wide arena with a 30 cm wall (made from black polyamide). The rats were accustomed to this box on PND 22, initially alone for 5 min and thereafter with a playing partner for further 5 min.

For experimental testing the rat was placed in the open field and its locomotor behavior was recorded by a video camera located above the box for 5 min. Thereafter the total distance was analyzed by the computer program TopScan (TopView Analyzing System 2.0; Clever Sys Inc.).

2.3.2. Social behavior

Rats were kept individually in cages (Makrolon Type III) for approximately 1 h before testing social behavior in order to have a standardized condition to elicit social interaction [22]. For experimental testing an experimental rat and a weight-matched stimulus rat (weight difference <10 g) were placed in the open field for 5 min after testing for locomotor activity. Social interactions were videotaped with a video camera above the box and analyzed off-line. Frequency and duration of the following behavioral elements were measured: sniffing (exploration of the stimulus rat's body by sniffing), crawling over/under, approaching/following (moving in the direction of or pursuing the stimulus rat) and fighting (biting the tail and the body of the stimulus rat). Total social activity was determined by calculating the total duration of behavioral elements mentioned above.

2.3.3. Ultrasound vocalization

For recording of ultrasound vocalization (USV), two rats from the same group (control, sham-lesioned and lesioned) with the same age were placed in the activity box. A microphone above the box recorded vocalizations in the ultrasound range, which were visualized by the program Avisoft SAS Lab Pro (Avisoft Bioacustics) on the monitor. The amount of USV in the range of 50–75 kHz was manually counted for 5 min. Additionally, videos were analyzed for social interaction to calculate the amount of vocalizations per second of interaction.

2.3.4. Motor coordination

A rotarod chamber with the dimensions of 10.5 cm × 43 cm × 43 cm (Rota-rod, series 8, IITC Life Science) was used to assess motor coordination and balance. For testing, the rat was placed on a rod, which rotated with accelerating speed (starting with 5 rotations per minute (rpm) accelerating up to 15 rpm within 60 s). Thereafter, the speed remained constant for further 60 s. For analysis, the latency to drop off was compared for the different groups. Rats were tested three times on the rotarod and the mean value of these trials used for statistical analysis.

2.4. Histology

After behavioral testing was completed the animals were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde fixation-solution. After immersion in 30% sucrose for at least one day, horizontal brain sections of 50 μm thickness were obtained using a cryostat. Every second section was mounted on a gelatine-coated slide, Nissl-stained with thionine and evaluated under a light microscope to determine location, length and extent of vermian splitting according to the cytoarchitectonic structure of the lobules of the vermis and the adjacent nuclei of the cerebellum and brainstem. The atlas of Paxinos and Watson (1998) [23] was used as reference. The extent and the depth of the vermian incision was reconstructed through the connection of the adjacent nucleus of the brainstem and the