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Long-term impairment of social behavior, vocalizations and motor activity induced by bilateral lesions of the fastigial nucleus in juvenile rats

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Abstract

The cerebellum is increasingly recognized to be involved in limbic and cognitive-associative functioning. Cerebellar cognitive affective syndromes may result from various types of injuries. Cerebellar mutism may occur in children after resection of midline tumors in the posterior fossa, which has been thought to be related to damage to the cerebellar vermis. Here, we investigated whether bilateral lesions of the fastigial nucleus, which is located within the upper vermis, would affect social behavior in a rat model. Juvenile male Sprague–Dawley rats, aged 23 days, underwent bilateral thermocoagulation of the fastigial nucleus via stereotaxically implanted electrodes under general anesthesia. Electrodes were inserted without application of electric current in a sham-lesion group and naïve rats served as additional controls. All groups underwent standardized examination before surgery and on specific time points up to 49 days after surgery to investigate locomotor activity, motor coordination, social behavior, and ultrasound vocalizations during social interaction. Finally, lesions were verified histologically. Playing behavior and vocalizations were reduced up to 4 weeks after surgery in rats of the lesion group compared to rats with sham-lesions and controls. After surgery in rats of the lesion group, locomotor activity was disturbed for 3 days as compared to sham-lesion rats, but for 4 weeks as compared to controls. Motor coordination measured by the rotarod and balance beam test was compromised until adulthood. Bilateral lesions of the fastigial nucleus in juvenile rats cause a severe and long-lasting reduction of social interaction and motor coordination in juvenile rats, which has some similarities to cerebellar cognitive affective syndromes in the human context. This indicates a modulating role of the fastigial nucleus with regard to neural circuitries relevant for social behavior, such as the limbic system and the prefrontal cortex.

Keywords Fastigial nucleus · Juvenile rats · Mutism · Motor activity · Social behavior · Ultrasound vocalizations

Introduction

There is increasing evidence that the cerebellum is not only involved in motor control, but that it is also relevant in limbic and cognitive-associative functioning (Ramnani 2006; Strick et al. 2009; Peloquin et al. 2012; Strata 2015; Zhang et al. 2016). Cerebellar injury or dysfunction may result in a wide variety of deficits affecting cognition, speech and emotion. The term cerebellar cognitive affective syndrome has been coined to describe neuropsychological deficits in adults associated with various cerebellar disorders, comprising personality changes, impairment of executive functions, and language deficits including agrammatism and dysprosodia (Schmahmann and Sherman 1998). The terms cerebellar mutism and posterior fossa syndrome have been used to describe a mostly transient postoperative state after resection of midline cerebellar or brainstem tumors in childhood, which is characterized by diminished speech progressing to mutism, emotional lability, and reduced motor activity (Van Calenbergh et al. 1995; Riva and Giorgi 2000; Gelabert-González and Fernández-Villa 2001; Kossorotoff et al. 2010).

Damage to midline structures of the cerebellum has been suggested to be the anatomical substrate for the occurrence of cerebellar mutism after surgery in children (Van Calenbergh et al. 1995; Catsman-Berrevoets et al. 1999; Robertson

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et al. 2006; Pitsika and Tsitouras 2013), and injury of the vermis has been described in more than 90% of patients with postoperative mutism syndrome after removal of midline tumors (Dailey et al. 1995). However, although splitting of the vermis is thought to constitute a relevant risk factor, cases of cerebellar mutism have also been described when using vermis-saving approaches (Zaheer and Wood 2010), suggesting that other structures may be involved, as well.

The fastigial nucleus is the phylogenetically oldest cerebellar nucleus which is highly conserved throughout mammalian evolution (Ito 2006). As opposed to the dentate and the interpositus nuclei, the fastigial nucleus is located within the cerebellar vermis and some of its fibers also decussate within the vermis (Haroian et al. 1981; Diedrichsen et al. 2011). It is also the main relay for fibers arising from the vermian cortex and has monosynaptic and polysynaptic connections to widespread areas in the brainstem, the thalamus, the limbic system and the neocortex (Zhang et al. 2016). Therefore, direct injury of the fastigial nucleus during surgery may play an important role in the development of motor and non-motor cerebellar deficits, including cerebellar mutism after resection of midline cerebellar tumors.

Here, we aimed to investigate the possible role of the fastigial nucleus in mediating social interactions and motor function, and whether lesions restricted to the fastigial nucleus would result in transient or permanent deficits. Rats show a distinct playing behavior, typically after weaning at the age of 21 days until young adulthood (Meaney and Stewart 1981; Brunelli et al. 2006). Since playing rats communicate by ultrasound at frequencies of about 50 kHz (Brunelli et al. 2006), and since vermian splitting in juvenile rats produces transient disturbances of social behavior and vocalizations (Al-Afif et al. 2013), we analyzed social playing and vocalization behavior also in the present study.

Materials and methods

Juvenile male Sprague–Dawley rats (Charles River Laboratories), weight between 48 and 73 g, were separated from their mothers at postnatal day (PND) 21 and housed in cages (Makrolon Type IV) under a 14/10 h light/dark cycle with water and food ad libitum. All tests were performed during the light phase. The rats were kept in a scantainer (Scanbur) with humidity of 55% ± 10% and at a temperature of 22 °C ± 2 °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.

Experimental design

Overall, 50 animals were obtained from 11 litters. At PND 23, the rats were randomly divided into three groups: (1) control group ($n = 16$), (2) sham-lesion group ($n = 16$), and (3) lesion group ($n = 18$). Rats were kept in groups of four to six animals per cage, mixed from at least two different groups (e.g., lesions and sham-lesions). In the lesion group, two electrodes were stereotaxically inserted bilaterally into the fastigial nucleus and lesions were made by electrical thermocoagulation. In the sham-lesion group, the electrodes were inserted just above the fastigial nucleus on both sides without applying electrical current. One group of naïve rats served as controls. In addition, 18 age-matched naïve rats served as partners for assessment of social behavior of the three experimental groups.

All rats were assessed for motor activity, motor coordination, social behavior and ultrasound vocalizations before surgery (d0) and on days 1, 2, 3, 4, 7, 14, 21, 28, and 49 after surgery. All behavioral tests were done between 9 a.m. and 4 p.m.

Stereotaxic surgery for bilateral lesioning of the fastigial nuclei

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, the head was fixed in a stereotaxic frame. A skin incision was made with microscissors in the midline from the coronal suture to the level of foramen magnum. The suboccipital muscles were retracted laterally until the skull was exposed from the bregma to approximately 1 cm caudal to the lambdoid sutures and a skin retractor was positioned. The sagittal suture, the coronal suture and both lambdoid sutures were completely visualized and bregma and lambda positioned in the same transversal plane. The sites for the burr holes in relation to lambda were marked on the skull and two small holes were made using a small diamond drill. Then the dura was opened carefully and an electrode was inserted with two trajectories on each side into the fastigial nucleus with the following coordinates (in mm relative to lambda): two trajectories in the anterior–posterior direction with − 2.0 and − 2.5, both trajectories lateral ± 1.0 and ventral 6.4. In the lesion group, electrical current (300 µA for 60 s) was applied via the monopolar electrodes. The coordinates and the lesion settings had been determined in pilot experiments earlier to achieve a specific lesion to the fastigial nucleus. In sham-operated controls, the electrode was inserted just above the fastigial nucleus without application of electrical current. Finally,

the electrodes were removed and the skin was closed using single stitches. All operated rats as well as rats from the control groups were housed individually in single cages overnight and then relocated to their original groups.

Behavioral testing

Motor activity

Locomotor activity was assessed in an open field (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. The total distance was analyzed by the computer program TopScan (TopView Analyzing System 2.0; Clever Sys Inc.).

Social behavior

Rats were kept individually in cages (Makrolon Type III) for 1 h before testing to have a standardized condition (Niesink and van Ree 1982). For testing, an experimental rat and a naïve partner (weight difference < 10 g) were placed in the open field for 5 min. Social interactions were videotaped with a video camera above the box and analyzed off-line. Frequency and duration of social interactions were evaluated: following behavior (moving in the direction of or approaching the partner), playing (biting the tail and the body or raiding of the partner (Panksepp et al. 1984; Schneider and Koch 2005) and sniffing behavior.

Ultrasound vocalizations

For recording of ultrasound vocalizations, two rats from the same group (control, sham-lesion and lesion) 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 a monitor. The occurrence of ultrasound vocalizations in the 50 kHz range was manually counted for 5 min. Additionally, social interactions were videotaped and analyzed off-line as described above.

Motor coordination

Rotarod test A rotarod chamber with the dimensions of $10.5 \times 43 \times 43$ cm (rotarod, series 8, IITC Life Science) was used to assess motor coordination and balance. For testing, the rat was placed on a rod that rotated with accelerating speed starting with five rotations per minute (rpm) and 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 values of these trials were used for statistical analysis.

Balance beam test Since previous studies showed that rats climb down the rotarod once they reach a certain size (Al-Afif et al. 2013), their motor coordination was additionally tested on day 49 after surgery with the balance beam test. For this test, a specific wooden beam was used (18 mm wide, 150 cm long), which was placed 85 cm above the ground. The platforms at both ends of the beam were filled with pellets to encourage the animals to run to the ends. The rat was placed in the center of the beam and the time was recorded until the animal fell from the beam. In case the rat would not fall down after 60 s or when it reached one of the platforms on either side, a maximum time of 60 s was used for analysis.

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 1 day, horizontal brain sections of 50 μm thickness were obtained using a cryostat. The extent of the lesion in the fastigial nucleus on both sides was qualitatively determined in Nissl-stained sections (thionine-staining) with a Zeiss light microscope. The absence of lesions was verified in sham-lesion rats.

Statistical analysis

For statistical analysis, a two-way repeated measure analysis of variance (ANOVA, Systat Software Inc., 2006) was used with group as between factor and day as within factor. Since analysis showed that the data were not normally distributed and the equal variance failed in about half of the data, in case of significance we used non-parametric rank tests for post hoc analysis; the Kruskal–Wallis one-way analysis was used to compare between groups on each postoperative day, and Friedman's analysis to compare differences within groups to day 0, followed by post hoc Tukey test in case of significance ($p < 0.05$). Since it is known that ultrasound vocalizations are related to social behavior, we additionally tested whether ultrasound vocalizations would correlate with playing, following and sniffing behavior (Pearson correlation test). Further it is known that motor abilities are necessary for social behavior, we also tested whether the distance moved in the open field would correlate with playing, following and sniffing behavior with the naïve social partner.

Fig. 1 Lesion sizes in the fastigial nucleus after bilateral thermocoagulation. The extent of the smallest lesion (marked in black) and the largest lesion (marked in gray) within the boundaries of the fastigial nucleus (marked by a point-shaped line) is depicted on a schematic drawing of a coronar section (**a**) from the atlas of Paxinos and Watson (1998) and on coronal Nissl-stained sections of the cerebellum obtained 49 days after sham operation (**b**), and after bilateral lesions of the fastigial nucleus (**c**, **d**). Scale bar corresponds to 1 mm
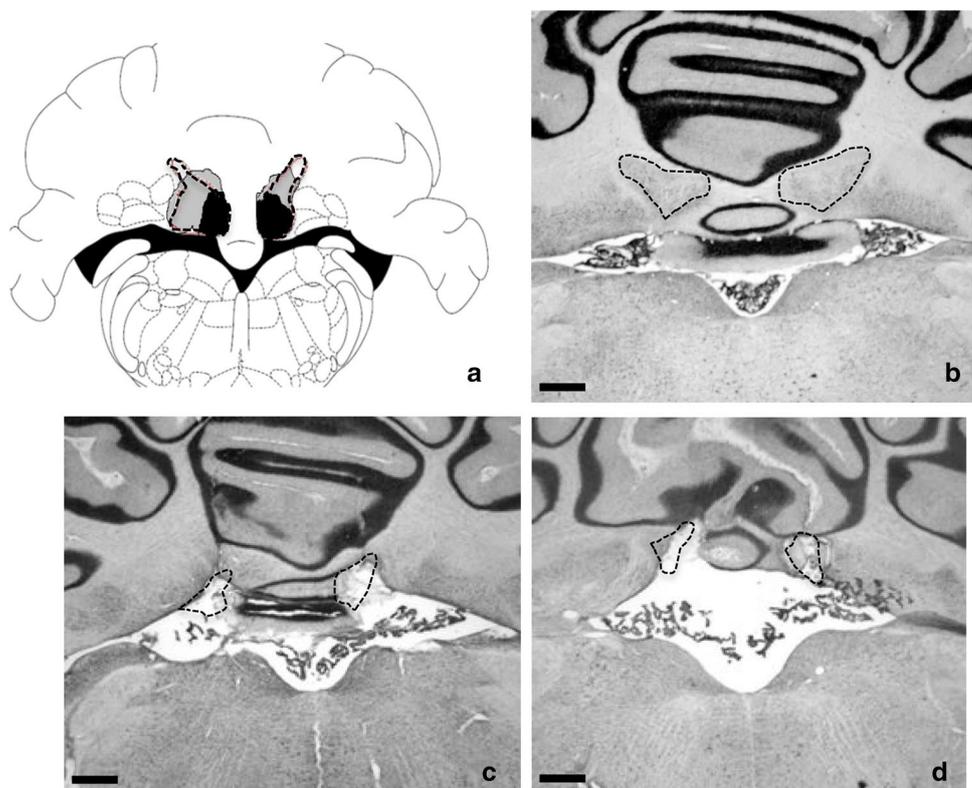
All tests were performed two-sided and $p < 0.05$ was considered to be significant. Since the balance beam test was used only on day 49 after surgery, a one-way ANOVA was used to compare between groups. All graphs were plotted with SigmaPlot 10 showing mean $\pm$ SEM (Systat Software Inc., 2006).

Results

Overall clinical outcome

All operated rats survived surgery. Direct observation in the cage revealed that the lesion group showed postoperative postural instability, gait ataxia and some difficulties in locomotion. The food pellets were therefore placed inside the cages for the first three postoperative days. Nevertheless, all operated rats postoperatively gained weight. Seven days after surgery, only mild impairment of motor coordination was directly visible in rats of the lesion group. From the 14th day after surgery, animals in the lesion group did not display obvious motor or behavioral disturbances upon direct observation without using the specific tests. One rat in the sham-lesion group died on the seventh postoperative day without any obvious reason. All other rats in the sham-lesion group did not show behavioral disturbances during the entire observation period.

Histology

Only rats with evident lesions of the fastigial nucleus on both sides and with less than 10% damage of neighboring regions (e.g., interposed and lateral cerebellar nuclei) were used for analysis. Two rats from the lesion group were excluded from further analysis because of lack of discernible lesions, leaving $n = 16$ rats for this group. The extent of the smallest and largest lesion of the fastigial nucleus is depicted on schematic drawings (Fig. 1a). Sham-lesion rats showed no damage of the fastigial nucleus (Fig. 1b), while in rats of the lesioned group the fastigial nuclei were lesion without affecting adjacent nuclei (Fig. 1c, d). Overall, for statistical analysis, 16 lesion rats, 15 sham-lesion rats, and 16 control rats were used.

Social behavior and interaction with the naïve social partner

Interaction playing with the naïve social partner

While playing behavior of naïve and sham-lesion rats increased after surgery, it was reduced in rats of the lesion group as compared to preoperative measures. Also, rats of the lesion group played less with their naïve social partner than control and sham-lesion rats after surgery for about 1 and 2 weeks, respectively. Statistical analysis with

ANOVA showed a significant effect for the factors group ($F_{2,401} = 14.474$; $p < 0.001$), day ($F_{9,401} = 17.802$; $p < 0.001$) and interaction between factors ($F_{18,401} = 5.935$; $p < 0.001$). Post hoc testing with Kruskal–Wallis revealed no differences between groups on the day before surgery ($p = 0.159$), while except for day 7 after surgery rats of the lesion group played significantly less than controls until day 14, and significantly less than sham-lesion rats until day 7 (all Kruskal–Wallis and post hoc tests: $p < 0.05$). In comparison to the preoperative measures, the time of playing significantly increased after surgery for days 1, 3, 14, and 21 in sham-lesion rats, and for day 21 in controls (Friedman's and post hoc tests: $p < 0.05$), while in rats of the lesion group the playing times were significantly reduced for the first postoperative day (Friedman's and post hoc tests: $p < 0.05$; Fig. 2a; Table 1).

Interaction playing in the same group

Differences of the playing time of the rats within groups were even stronger and longer lasting. Pairs of rats of the lesion group played less than control and sham-lesion rats for more than 4 weeks after surgery. Statistical analysis with ANOVA showed a significant effect for the factor group ($F_{2,180} = 29.445$; $p < 0.001$), the factor day ($F_{9,180} = 6.136$; $p < 0.001$) and the interaction between factors ($F_{18,180} = 2.961$; $p < 0.001$). Post hoc testing revealed no differences between groups on the day before surgery (Kruskal–Wallis test: $p = 0.091$), while except for day 4 after surgery rats of the lesion group played significantly less than controls until day 49, and significantly less than sham-lesion rats until day 28 (Kruskal–Wallis and post hoc tests: $p < 0.05$). In comparison to the preoperative measures, the time of playing significantly increased after surgery for day 21 in sham-lesion rats, and for days 1, 2, and 21 in controls (Friedman's and post hoc tests: $p < 0.05$), while in rats of the lesion group the playing times were reduced postoperatively, which, however, did not reach the level of significance (Fig. 2b; Table 1).

Following behavior with naïve social partners

The following behavior time with the playing partner did not change after surgery in controls and sham-lesion rats, but was reduced in rats of the lesion group early after surgery. Also, lesion rats followed their social partners less than controls and sham-lesion rats for about 4 days after surgery. Statistical analysis with ANOVA showed a significant effect for the factor day ($F_{9,401} = 34.6$; $p < 0.001$), and for the interactions between day and group ($F_{18,401} = 18.7$; $p < 0.001$), but not for the factor group ($F_{2,401} = 0.15$; $p = 0.865$). Post hoc testing revealed no differences between groups on the day before surgery (Kruskal–Wallis test: $p = 0.589$), while after surgery rats of the lesion group followed the naïve

partner significantly less than controls until day 4, and significantly less than sham-lesion rats from days 1 to 3 and day 21 (Kruskal–Wallis and post hoc tests: $p < 0.05$). In comparison to the preoperative measures, following the naïve partner significantly decreased after surgery for days 1–3 in lesion rats (Friedman's and post hoc tests: $p < 0.05$), while no differences were found for sham-lesion and control rats (Fig. 2c; Table 1).

## Following behavior within experimental groups

Changes in the following behavior of pairs of rats within the same experimental groups paralleled the observations made with naïve social partners. Statistical analysis with ANOVA showed a significant effect for the factor group ($F_{2,180} = 11.414$; $p < 0.001$), the factor day ($F_{9,180} = 7.332$; $p < 0.001$) and the interaction between factors ($F_{18,180} = 1.849$; $p = 0.023$). Post hoc testing revealed no differences between groups on the day before surgery (Kruskal–Wallis test: $p = 0.572$), while after surgery rats of the lesion group followed their partner significantly less than controls until day 4, and significantly less than sham-lesion rats until day 3 (Kruskal–Wallis and post hoc tests: $p < 0.05$). In comparison to the preoperative measures, rats of the lesion group followed each other significantly less for the first two postoperative days (Friedman's and post hoc test: $p < 0.05$), while no differences were found for sham-lesion and control rats (Fig. 2d; Table 1).

Sniffing behavior with naïve social partners

The sniffing behavior with the naïve playing partner was only reduced in rats with lesions on the first postoperative day. Statistical analysis with ANOVA showed a significant effect for the factor day ($F_{9,401} = 37.541$; $p < 0.001$), but not for the factor group ($F_{2,401} = 0.573$; $p = 0.568$) and for the interactions between day and group ($F_{18,401} = 1.341$; $p = 0.158$). Post hoc testing revealed only a significant reduction of sniffing behavior in lesion rats as compared to control rats on the first postoperative day (Kruskal–Wallis and post hoc tests: $p < 0.05$), while on all other days no differences between groups were found. In comparison to the preoperative measures, sniffing behavior was not altered in either group (Fig. 2e; Table 1).

Sniffing behavior within experimental groups

Changes in the sniffing behavior of pairs of rats within the same experimental groups paralleled the observations made with naïve social partners. Statistical analysis with ANOVA showed a significant effect for the factor group ($F_{2,180} = 7.051$; $p = 0.005$), for the factor day ($F_{9,180} = 9.839$; $p < 0.001$) and for the interactions between day and group
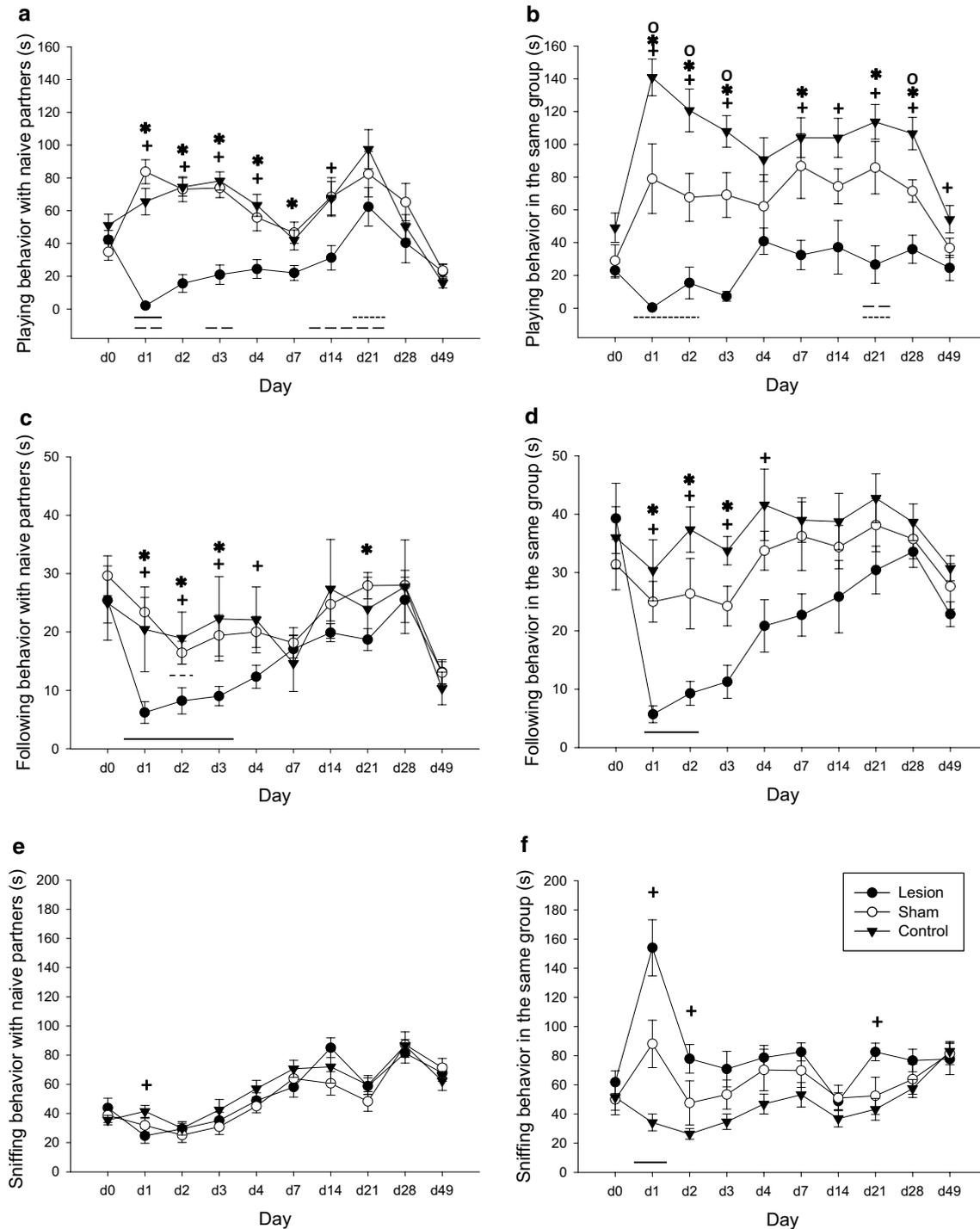
Fig. 2 Social interaction of rats with lesions and sham-lesions of the fastigial nucleus and naïve controls. Graphs show mean ± S.E.M of playing (**a**, **b**), following (**c**, **d**) and sniffing behavior (**e**, **f**) in seconds (s) with naïve social partners (**a**, **c**, **e**) and in the same group (**b**, **d**, **f**). Differences between lesion and naïve control rats are shown as pluses, between lesion and sham-lesion rats as asterisks, and between sham-lesion rats and naïve control rats as circles; differences to day 0 (d0) within lesion rats are depicted by solid line within sham-lesion rats by dashed lines and within naïve controls by dotted lines (ANOVA with post hoc Tukey test; $p < 0.05$)

($F_{18,180} = 4.748$; $p = 0.001$). Post hoc testing revealed a significant difference of sniffing behavior in lesion rats as compared to control rats on postoperative days 1, 2, and 21

(Kruskal–Wallis and post hoc tests: $p < 0.05$), while on all other days no differences between groups were found. In comparison to the preoperative measures, sniffing behavior

Table 1 The days of statistically significant reduced playing and following behavior, and enhanced sniffing behavior in lesion as compared to control and sham-lesion groups, and sham-lesion as compared to control group

	Partner of the same group			Naïve partner		
	Playing	Following	Sniffing	Playing	Following	Sniffing
Lesion vs. control	1–49 (excl. 4)	1–4	1, 2, 21	1–14 (excl. 7)	1–4	1
Lesion vs. sham	1–28 (excl. 4)	1–3	–	1–7	1–3, 21	–
Sham vs. control	1–3, 28	–	–	–	–	–

Note that the number of days with reduced playing behavior in the lesion group clearly outlasts the reduced following behavior, which indicates that the reduced playing behavior cannot only be explained by impaired motor activity. Please also note that the lesion group shows more playing behavior when tested with naïve partners, indicating that when motivated by naïve rats they are physically able to play
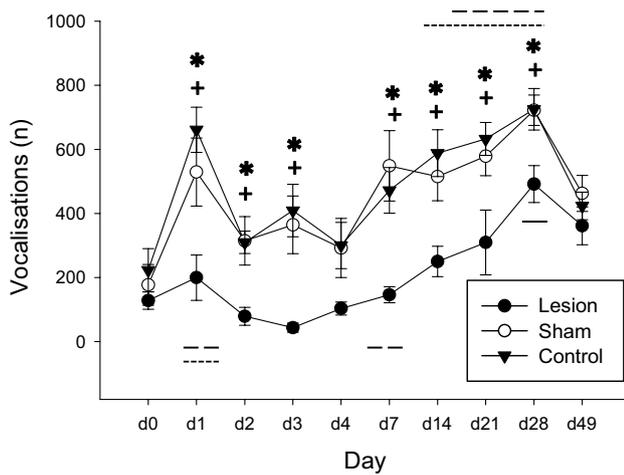
Fig. 3 Ultrasound vocalizations of rats with lesions and sham-lesions of the fastigial nucleus and naïve controls. Graphs show the mean ± SEM of the number of vocalizations. Differences between lesion and naïve control rats are shown as pluses, and between lesion and sham-lesion rats as asterisks; differences to day 0 (d0) within lesion rats are depicted by solid line, within sham-lesion rats by dashed lines and within naïve controls by dotted lines (ANOVA with post hoc Tukey test; $p < 0.05$)

was only reduced in lesion rats on the first postoperative day (Friedman's and post hoc tests: $p < 0.05$; Fig. 2f; Table 1).

Ultrasound vocalizations

Ultrasound vocalizations were recorded from pairs of rats, i.e., the lesion, the sham-lesion, and the control group in the open field. Rats of the lesion group vocalized less than control and sham-lesion rats for about 4 weeks after surgery (Fig. 3). Statistical analysis with ANOVA showed a significant effect for the factor group ($F_{2,180} = 10.021$; $p < 0.001$), for the factor day ($F_{9,180} = 22.475$; $p < 0.001$) and for the interactions between day and group ($F_{18,180} = 1.669$; $p = 0.048$). Post hoc testing did not reveal differences between groups on the day before surgery (Kruskal–Wallis test: $p = 0.530$), while after surgery rats of the lesion group

vocalized significantly less than controls and sham-lesion groups until day 28, except day 4 (Kruskal–Wallis and post hoc tests: $p < 0.05$). In comparison to the preoperative measures vocalizations were not reduced in either group, but enhanced on the first postoperative day (Friedman's test and post hoc test: $p < 0.05$).

Correlation analysis showed that playing behavior positively correlated ultrasound vocalizations for almost all days, while following behavior only correlated with about half of the days, and sniffing behavior negatively correlated with for most postoperative days (see Table 2 for exact values).

## Motor activity and motor coordination

Open field

Rats of the lesion group covered less distance in the open field for about 4 weeks after surgery as compared to control rats, while this was the case for only 3 days when compared to sham-lesion rats. This difference was mainly due to reduced locomotion of rats of the lesion group after surgery, while locomotion of sham-lesion rats and controls did not change during testing (Fig. 4a). Statistical analysis with ANOVA showed significant effects for the factor group ($F_{2,401} = 10.658$; $p < 0.001$), for the factor day ($F_{9,401} = 7.161$; $p < 0.001$) and for the interactions between day and group ($F_{18,401} = 3.114$; $p < 0.001$). Post hoc testing revealed no differences between groups on the day before surgery (Kruskal–Wallis test: $p = 0.838$), while rats of the lesion group covered less distance than controls until postoperative day 28, and compared to sham-lesion rats until postoperative day 3 and on day 28 (Kruskal–Wallis and post hoc tests: $p < 0.05$). In comparison to the preoperative measures, the distance of lesion rats was significantly decreased as compared to the preoperative day on days 1–3 and day 49 (Friedman's and post hoc tests: $p < 0.05$), while no differences were found in control and rats of the sham-lesion group.

Table 2 Correlations between ultrasound vocalizations with playing, following and sniffing behavior within the same group of all rats with *p* values and correlation coefficients for all days

| | Correlation of ultrasound vocalizations with | | | | | |
| | Playing | | Following | | Sniffing | |
	p	Corr coeff	*p*	Corr coeff	*p*	Corr coeff
d0	**< 0.001**	**0.753**	**< 0.001**	**0.607**	0.059	− 0.398
d1	**< 0.001**	**0.766**	**0.003**	**0.586**	**< 0.001**	**− 0.7**
d2	**< 0.001**	**0.647**	0.086	0.366	**< 0.001**	**− 0.672**
d3	**< 0.001**	**0.795**	**0.011**	**0.524**	0.151	− 0.309
d4	**< 0.001**	**0.753**	0.263	0.243	**< 0.001**	**− 0.624**
d7	**< 0.001**	**0.818**	0.074	0.379	**< 0.001**	**− 0.726**
d14	**< 0.001**	**0.793**	**0.008**	**0.538**	**0.005**	**− 0.57**
d21	**< 0.001**	**0.85**	**0.008**	**0.537**	**< 0.001**	**− 0.644**
d28	**< 0.001**	**0.608**	0.912	0.0243	0.055	− 0.406
d49	0.051	0.412	0.097	0.354	0.155	0.307

Significant correlations as analyzed with Pearson correlation test are highlighted as bold

Fig. 4 Motor behavior of rats with lesions and sham-lesions of the fastigial nucleus and naïve controls. Graphs show data as mean ± SEM of locomotor activity in the open field measured as distance covered (**a**), time until falling down the rotarod (**b**), and motor coordination on the balance beam test on day 49 after surgery (**c**). Differences between lesion rats and controls are shown as pluses and between lesion rats and sham-lesion rats as asterisks; differences to day 0 (d0) within lesion rats are shown by solid lines (ANOVA with post hoc Tukey test; *p* < 0.05)
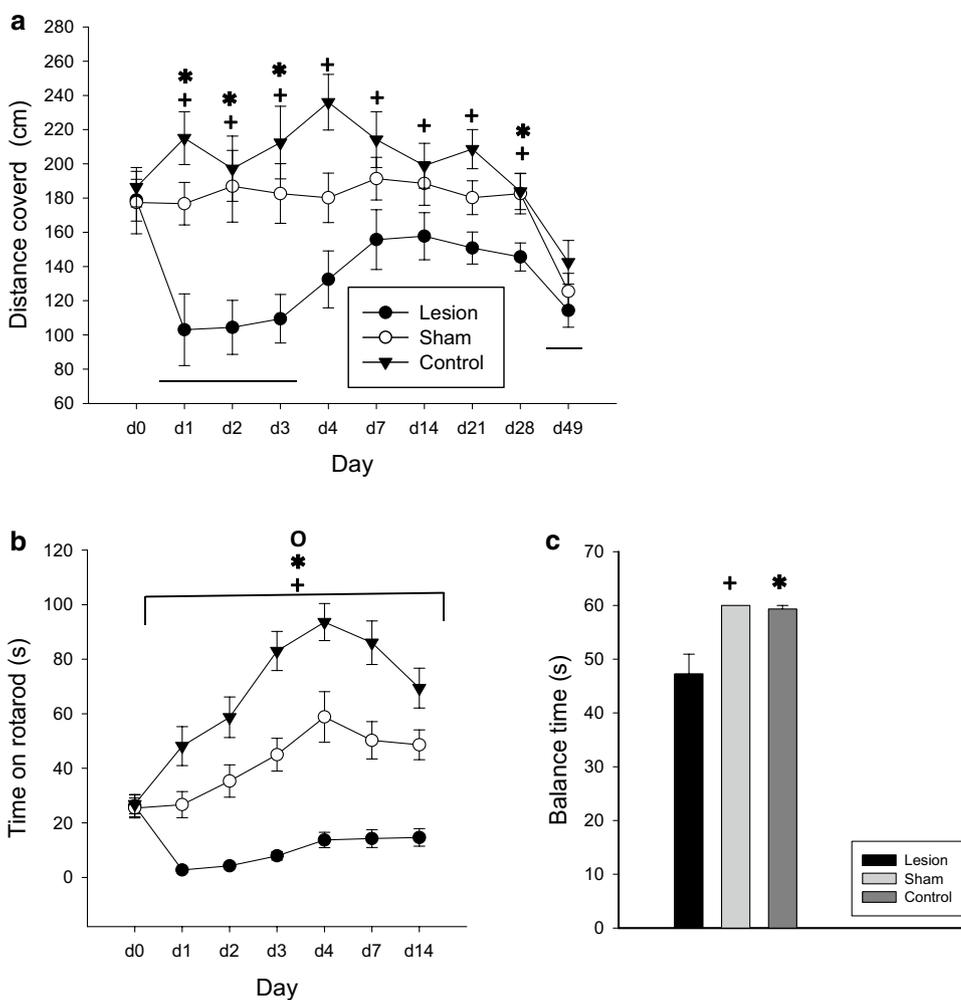
Correlation analysis showed that playing behavior positively correlated to the distance in open field on most postoperative days, but only for days 2, 4, and 14 for the following behavior, while almost no correlation was found between sniffing behavior and distance (see Table 3 for exact values).

Table 3 The correlation between distance covered in the open field with playing, following and sniffing behavior within the naïve partner of all rats with *p* values and correlation coefficients for all days

	Correlation of distance with					
	Playing		Following		Sniffing	
	p	Corr coeff	*p*	Corr coeff	*p*	Corr coeff
d0	0.444	0.113	0.626	0.072	0.099	− 0.241
d1	**< 0.001**	**0.524**	0.053	0.281	0.435	0.115
d2	**< 0.001**	**0.577**	**< 0.001**	**0.562**	0.107	− 0.235
d3	**< 0.001**	**0.493**	0.212	0.184	0.964	− 0.007
d4	**< 0.001**	**0.418**	**0.012**	**0.359**	0.344	− 0.14
d7	0.051	0.284	0.791	0.039	0.39	− 0.127
d14	**< 0.001**	**0.578**	**< 0.001**	**0.479**	**< 0.001**	**− 0.648**
d21	**< 0.001**	**0.457**	0.792	0.039	0.098	− 0.245
d28	**< 0.001**	**0.536**	0.567	0.086	0.424	− 0.119
d49	0.1	0.243	0.853	0.028	0.687	0.06

Significant correlations as analyzed with Pearson correlation test are highlighted as bold

Rotarod

The latency to drop off from the rotarod could only be tested for the first 2 weeks after surgery, because thereafter nearly all rats had learned to climb down once they were placed on the rod. During the first 2 weeks, rats of the lesion group dropped off from the rotarod faster than control and sham-lesion rats, but the sham-lesion rats also dropped off faster than controls (Fig. 4b). Statistical analysis showed significant differences for the factor group ($F_{2,345} = 56.609$; $p < 0.001$), the factor day ($F_{6,345} = 24.088$; $p < 0.001$) and the interaction between factors ($F_{12,345} = 11.06$; $p < 0.001$). After surgery, in the control group, the time spent on the rotarod was significantly increased as compared to the presurgical measurements on all days (Friedman's and post hoc tests: $p < 0.05$), while in the sham-lesion group it was increased from day 3 on (Friedman's and post hoc tests: $p < 0.05$). Rats of the lesion group spent less time on the rotarod than before surgery for the first 3 days after surgery (Friedman's and post hoc tests: $p < 0.05$), thereafter their behavior did not differ from the preoperative measure. Rats of the lesion group spent less time on the rod than sham-lesion rats or controls on all days, and sham-lesion rats spent less time on the rod than controls for all days (Kruskal–Wallis and post hoc tests: $p < 0.05$).

Analysis of performance on the balance beam on day 49 after surgery showed that rats of the lesion group stayed shorter on the beam than rats of the control and sham-lesion groups ($p = 0.002$ after significant ANOVA with F = 9.376; $p < 0.001$; Fig. 3c).

Discussion

Thus far it has not been fully clarified which cerebellar structures are involved in the occurrence of the cerebellar cognitive affective syndrome and in other disease states such as autism spectrum disorders. Here, we provide further evidence that the fastigial nucleus may play a pivotal role in mediating the quality and intensity of social behaviors. More recent works strongly also suggest that some specific cerebellar regions are responsible for non-motor functions. Carta et al. showed direct connections between the deep cerebellar nuclei and the ventral tegmentum and stressed the role of the cerebellum in the control of motivated and social behavior. Altered cerebellar connectivity might also play a role in autism spectrum disorders (Stoodley et al. 2017; Carta et al. 2019).

The present study shows that bilateral lesions of the fastigial nucleus in juvenile rats lead to impairment of social interaction and ultrasound vocalizations without recovery until adulthood which outlasted motor deficits at least to some extent. In particular, playing interaction was markedly disturbed for at least 4 weeks as compared to both sham-lesion and naïve controls. This effect was even enhanced when pairs of rats of the lesion group were tested together. It has been reported that during development playing behavior is most intense between the age of 21 and 30 days, and thereafter decreases until young adulthood (Meaney and Stewart 1981; Brunelli et al. 2006). This course of playing interaction was found in both control groups (sham-lesion and controls), whereas after lesioning of the fastigial nuclei playing behavior never developed. Since adult rats normally do not show playing behavior any longer, it remains open, whether other aspects of social behavior would be disturbed in adult rats.

The reduction of ultrasound vocalizations in the 50-kHz domain in the lesion group also indicates reduced motivation for social interaction and communication. Ultrasound vocalizations at 50 kHz are present in appetitive situations and they have been suggested to reflect a positive affective state equivalent to happiness (Wöhr and Schwarting 2013). High levels of 50 kHz vocalizations are also found during short periods of isolation, indicating a high motivation for social interaction stressing its communicative value (Panksepp and Burgdorf 2000; Wöhr and Schwarting 2013). However, more detailed analysis of ultrasound vocalizations revealed that rats mainly vocalized during playing and following interaction, indicating that this measure just accompanies decreased social interaction. Our results are in line with other studies in which ultrasound vocalizations at 50 kHz are positively correlated with social interaction in juvenile rats (Wöhr and Schwarting 2012). In our previous study, we showed that splitting of the inferior part of the vermis produces a similar reduction of social interaction and of ultrasound vocalizations in juvenile rats, however, only for a few days after surgery (Al-Afif et al. 2013). Instead, bilateral fastigial lesions cause long lasting and deteriorating effects on social behavior.

Locomotor activity was impaired for 4 weeks after surgery compared to controls, but only for 3 days as compared to sham-lesion rats. Playing behavior correlated to some extent with motor function, and it is, therefore, difficult to unequivocally relate disturbed function of either behavioral domain to the fastigial nucleus, which is a limitation of this study. Nevertheless, at least part of the deficits in the playing behavior in the rats with fastigial lesions may not simply be a consequence of reduced motor activity for the following reasons. (1) When rats with lesions were tested with naïve playing partners, the playing behavior normalized after 1 week (as compared to sham-lesion rats), and after 2 weeks (as compared to the naïve control group). However, when rats were tested with partners of the same group (e.g., two lesion rats), playing behavior was disturbed within groups of lesion rats until reaching adulthood (4 weeks as compared to sham-lesion, and 7 weeks as compared to the control group). One possible explanation is that the motivation to initiate or to maintain playing behavior in the lesion group is reduced until adulthood, whereas the ability to perform playing behavior (including motor performance) recovers earlier when lesion rats are tested with naïve a playing partner, which takes on the role to initiate and motivate the lesion rat to play. (2) Analysis of "following behavior", which strongly depends on motor abilities, showed that this behavior normalizes in rats with lesions after 4 days, both when tested with a naïve partner and when tested with a lesion partner, although actual playing behavior was longer disturbed. This observation also indicates that the reduction of the playing behavior in the lesion group could not simply be explained by reduced motor abilities. Notably, both following and sniffing behavior did not correlate with motor activity.

Another limitation of this study is that sham-lesion rats exposed some deficits in motor and social functions, which were less pronounced than those deficits observed in the lesion rats. These effects may be attributed to a small lesion of the vermis caused by the transient insertion of the electrodes, which provides most afferent fibers to the fastigial nuclei (Al-Afif et al. 2013).

In social interaction, following and sniffing in rats represent explorative behavior (Ku et al. 2016), whereas playing is necessary to establish a stable social relationships (Panksepp 1981). A similar observation that playing behavior is more prominently reduced than social exploration was already observed in juvenile rats with neonatal lesions of the medial prefrontal cortex and was interpreted as reduced motivation for playing (Schneider and Koch 2005).

It has to be mentioned that gait ataxia measured on the rotarod and balance beam persisted in the lesion group until adulthood. This corroborates previous studies which investigated motor behavior after lesions of the fastigial nucleus in rats (Wankhar and Rathinasamy 2015), monkeys (Noda et al. 1990) and human patients (Ilg et al. 2008; Chambers and Sprague 1955; Bastian 2006; Thach and Bastian 2004).

The fastigial nucleus is a small but highly connected structure in the midline of the cerebellum (Ito 2006). Its critical role for axial motor function has been well recognized. Additionally, there is growing evidence for its involvement in non-motor functions, which is supported by its widespread connectivity to many other brain regions. Besides various afferents from different cerebellar structures, the fastigial nucleus receives input from the brainstem and the hypothalamus. Efferent pathways are organized via descending and ascending systems. Descending output contains vestibulospinal and reticulospinal tracts, while the ascending output projects to the thalamus, various brainstem nuclei, the hypothalamus, the periventricular gray, the hippocampus, the amygdala, and other structures. These pathways are involved in motor control, oculomotor control, feeding behavior, cardiovascular and respiratory function, visceral control, and last not least emotional and higher cognitive functioning (Ramnani 2006; Zhang et al. 2016).

Anatomical studies in rats showed that fibers arising from the fastigial nucleus decussate within the vermian white matter, course through the rostral third of the contralateral fastigial nucleus and continue laterally to form the uncinate fasciculus, which "hooks around the superior cerebellar peduncle" (Haroian et al. 1981). An ascending part of this fasciculus projects via the superior cerebellar peduncle to the dorsal midbrain tegmentum (Rasmussen 1933). Then, the fastigiothalamic fibers separate from the superior cerebellar peduncle and continue rostrally in the dorsal tegmentum to enter the thalamus via the thalamic fasciculus and the

internal medullary lamina. In rats, this pathway terminates at the medial dorsal nucleus and the medial part of the ventral medial and ventral lateral nuclei in the thalamus (Haroian et al. 1981). The thalamic dorsal medial nucleus has widespread connections to the prefrontal cortex, which resulted in the assumption that the fastigial nucleus is part of a diffusely activating system of the prefrontal cortex (Steriade 1995). Supporting this theory, electrical stimulation of the fastigial nucleus in rats evoked distinct local field oscillatory responses in the medial prefrontal cortex (Watson et al. 2009, 2014). Also in cats, stimulation of the fastigial nucleus induced alterations of cortical EEG over widespread cortical areas (Steriade 1995). Another study in macaque monkeys postulated that there is a complete neural circuitry between vermis, fastigial nucleus and cerebral cortex (Coffman et al. 2011). Nevertheless, although fastigial connections to the thalamus are highly conserved in mammalian and primates like cats and monkeys (Batton et al. 1977; Angaut and Bowsher 1970; Jansen and Jansen 1955; Carpenter 1959), they have not been well studied in humans.

With regard to the connectivity of the fastigial nucleus with the hypothalamus, the septal nuclei, the hippocampus, the nucleus accumbens, and the amygdala via the ventral tegmental areal (Harper and Heath 1972), a cerebellar limbic system has been postulated with the fastigial nucleus and the vermis as an extension of the Papez circuitry (Schutter and van Honk 2005; Strata 2015). Furthermore, electrophysiological studies showed that stimulation of the fastigial nucleus and associated regions of the vermal cortex evoked responses in the cingulate cortex, the amygdala and other regions of the limbic system (Anand et al. 1959; Snider and Maiti 1976). In addition, direct stimulation of the fastigial nucleus may alter behaviors like attack, grooming, and eating in cats (Reis et al. 1973). A more recent study showed that electrical stimulation of the fastigial nucleus can alleviate depressive-like behaviors in a post-stroke depression rat model (Zhang et al. 2017). Bilateral lesions of the fastigial nuclei will, therefore, interrupt the vermian neural circuits to various regions, which may produce both limbic and cognitive functional disturbances.

Functional MRI studies suggest a functional topography within the cerebellum in humans with regard to non-motor function involving the vermian cortex (Stoodley et al. 2010). The projections via the mesocortical system and the ventral tegmental area to the prefrontal cortex have been linked to neuropsychiatric disorders such as schizophrenia (Parker et al. 2014) Interestingly, also congenital malformations affecting the cerebellar vermis have been linked to affective and social disturbances (Tavano et al. 2007). Hypoplasia of vermal lobules VI and VII, which have extensive connections to the fastigial nuclei, is one of the most reported abnormalities in autism spectrum disorders (Gould and Graybiel 1976; Stoodley et al. 2017).

The incidence of cerebellar mutism after resection of medulloblastomas is higher than after resection of other cerebellar midline tumors, such as ependymomas (Gelabert-González and Fernández-Villa 2001). This may be attributed to the fact that medulloblastomas mainly arise from the roof of the 4th ventricle, in the so called "fastigium", whereas cerebellar ependymomas arise from the floor of the 4th ventricle. Due to its location near to the midline in the superior vermis immediately above the roof of the 4th ventricle, the fastigial nucleus may be especially vulnerable to bilateral damage during surgery for medulloblastomas (Dimitrova et al. 2002; Ozimek et al. 2004; Diedrichsen et al. 2011). Since nowadays cure is possible even in malignant tumors like medulloblastomas using advanced microsurgical techniques and improved adjuvant therapy options, quality of life in these patients deserves more and more attention. Various vermian-saving approaches for resection of such tumors have been described in the literature and are now commonly used (Mussi and Rhoton 2000; El-Bahy 2005; Hermann et al. 2008; Abla and Lawton 2014; Tomasello et al. 2015). Additionally, avoiding damage to the fastigial nucleus located in the upper vermis during resection of tumors involving the 4th ventricle may also be crucial to prevent long-term motor and non-motor deficits.

In conclusion, we demonstrate in this study that bilateral injury of the fastigial nuclei in juvenile rats results in long-lasting non-motor deficits in social behavior, which parallels observations in patients with cerebellar cognitive affecting syndromes caused by different cerebellar pathologies. Further studies are needed to specify the role of the fastigial nuclei and the involvement of their afferent and efferent connectivity in this context.

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