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Research report

The effects of neonatal lesions in the amygdala or ventral hippocampus on social behaviour later in life

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Abstract

Disruption of normal social behaviour is seen in psychiatric neurodevelopmental disorders like schizophrenia or autism. In a rat model of neurodevelopmental disorders we investigated the social behavioural changes after damage of limbic brain areas, at two early stages of life. The effects of ibotenic acid lesions made on day 7 or 21 of life in the amygdala (AM) ((baso)lateral/medical) or ventral hippocampal area on social play behaviour, social behaviour unrelated to social play behaviour early in life, and social behaviour in adulthood were assessed. Lesions of the AM, but not lesions of the ventral hippocampal area, resulted in decreased social play behaviour, and no differences were found between lesions made on day 7 or 21 of life. Social behaviour unrelated to social play behaviour early in life and in adulthood was decreased in animals lesioned in the AM on day 7 but not in animals lesioned on day 21 of life. This effect was particularly present in animals with an additional lesion in the medial nuclei of the AM. Lesions in the ventral hippocampal area did not affect social behaviour. It is concluded that the AM is an important structure for social play behaviour. The effects on social behaviour that are dependent on the day of lesioning (day 7 vs. 21) are an indication of a neurodevelopmental deficit of structures connected to the (medial part) of the AM.

Keywords: Neurodevelopment; Social behaviour; Amygdala; Hippocampus; Schizophrenia; Autism

1. Introduction

Based on the hypothesis that psychiatric disorders like schizophrenia or autism are a result of a neurodevelopmental deficit (Schizophrenia: [8,11,36,57,58]; Autism: [12,31]) and on findings of abnormalities in specific brain structures in patients suffering from these disorders (Schizophrenia: [9,38,48,61]; Autism: [3,4]) a model for neurodevelopmental disorders was set up by lesioning the amygdala (AM) or ventral hippocampus (VH) on day 7 or 21 of life [59,60]. In postnatal life of rats brain development continues during the first 3 weeks [5]. On day 7 of life connections and topographical organizations of projections between different brain structures are not completed yet, whereas on day 21 of life brain structures are almost mature [10,25,28,56].

Lesioning the AM or VH on day 7 or 21 of life results in different patterns of locomotor activity later in life, while lesioning these structures on day 21 of life showed no changes in locomotor activity [13,59]. Behavioural changes seen in animals lesioned on day 7 of life and in animals lesioned on day 21 of life are most conceivably mediated by the lesioned structures, while behavioural changes seen in animals lesioned on day 7 of life but not in animals lesioned on day 21 of life are probably a result from malfunctioning of structures connected to the lesioned area. Thus, behavioural changes in animals lesioned on day 7 of life that are not present in animals lesioned on day 21 of life, are suggestive of a neurodevelopmental deficit.

Patients suffering from schizophrenia or autism have major impairments in social functioning. Social withdrawal and isolation are key-components of the negative symptoms of schizophrenia, and the core symptoms of

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autism include specific impairments of reciprocal social relationships [2]. Therefore, the present study focused on social behaviour.

Social play behaviour is the earliest form of non-mother-directed social behaviour in the rat. There are several indications that social play forms a separate, relevant category of behaviour [55] that contains behavioural patterns related to social, sexual, and agonistic behaviour [6]. Social play behaviour in rats can easily be recognized and is characterized by pinning behaviour, which is defined as one of the animals lying with its dorsal surface on the floor with the other animal standing over it [44]. In juvenile rats a distinction can be made between social behaviour related to social play behaviour (e.g. pinning) and social behaviour unrelated to social play behaviour (e.g. social exploration, contact behaviour). These forms of behaviour differ regarding their ontogenetic pattern. Social play behaviour mainly occurs between weaning and puberty [21,41,52], whereas social behaviour unrelated to social play behaviour occurs during the entire lifespan. Social behaviour related and social behaviour unrelated to social play behaviour may also differ regarding its function and underlying neurobiological mechanisms [55].

Interactive social play behaviour is an essential element in the development of affectional behaviour towards age-mates. Social play behaviour and social development are intimately tied. Impairment in social ability in adulthood is exhibited in monkeys [35] and rats [20] that were socially isolated early in life. This may be caused by a lack of opportunity during deprivation of social play behaviour to develop communicative skills that facilitate social interaction later in life. Social interaction tests in adulthood have widely been done in all kinds of mammals. Lesion studies revealed an important role for the AM in social behaviour [1,32,50].

The purpose of our study was to investigate the behavioural effects of moderately sized AM or VH lesions on day 7 or 21 of life on social play behaviour and social behaviour on two different time points. Ibotenic acid was used to produce lesions limited to the AM or VH with minimal damage to adjacent areas, fibres-of-passage, and the vasculature [18,22]. Social play behaviour and social behaviour unrelated to social play behaviour were investigated early in life, and social behaviour was investigated in adulthood. Our aim was to determine: (1) whether there are changes in social (play) behaviour following these lesions; (2) whether behavioural changes depend upon the day of life the lesions were made (day 7 or 21 of life); (3) whether the behavioural changes depend upon the lesioned structure (AM or VH); and (4) whether changes in social behaviour occur early in life or emerge during life.

In a former study we found that relatively large AM lesions, involving the lateral, basolateral, central, and medial nuclei of the AM, on day 7 or 21 of life changed social play behaviour in juveniles and social behaviour in adulthood [59]. On the basis of neuroanatomical connections the AM can be divided into basal, lateral, central and medial divisions. The afferent and efferent connections of the AM are suggestive of the various divisions being concerned with processing different categories of incoming and outcoming information [1]. McGregor and Herbert [33] reported a dissociation between the basolateral and corticomedial amygdaloid regions with respect to the contributions made to two social behaviours, i.e. sexual and agonistic behaviour. To determine the region(s) of the AM contributing to the changes in social behaviour in our former study, the lesions made in the present study were smaller, and the group of animals lesioned in the AM was divided into subgroups based upon the extent of the lesions.

2. Materials and methods

2.1. Subjects

Subjects were male offspring ($n = 160$) of Wistar rats (GDL, Utrecht, The Netherlands). Four (A–D) experiments were performed. In experiment A and C animals were operated in the AM on day 7 and 21 of life, respectively. In experiment B and D animals were operated in the VH on day 7 and 21 of life, respectively. The mothers, obtained at 18 days of gestation, were housed individually. One day after birth, litters were culled to nine puppies (Maximal nine males per litter. If a litter consisted of less than nine males, the litter was filled up to nine pups with females. Females were removed after weaning and did not participate in the behavioural tests). On the day of surgery the male offspring was randomized to sham and lesion status and was divided into AM lesioned, AM sham-operated animals or VH lesioned and VH sham-operated animals. Following surgery the animals operated on day 7 of life were returned to their mother within 30 min and were weaned and grouped 3–4 to a cage on day 19 of life. The animals operated on day 21 of life were weaned and grouped 3–4 to a cage within 3 h after surgery. After surgery all animals were housed in a dimly lit room (20–40 lux) under conditions as described above. The animals used for the behavioural experiments in the present study were also tested for locomotor behaviour in the large and small open field prepubertal (day 35 of life) or postpubertal (day 60 of life) (published separately [13]).

2.2. Surgery

Animals operated on day 7 of life were anaesthetized with fentanyl (0.3 mg/kg, sc) and immobilized in a David Kopf stereotaxic frame. To enable stable fixation

of the head a mould of condensation silicone was constructed in which rat puppies of a specific head size fit. A midline skin incision was made, the skull was perforated using a 1.0 mm dental drill, and ibotenic acid (3 μg/0.3 μl over 2 min) or vehicle (0.1 M phosphate-buffered saline, pH 7.4) was injected bilaterally by microinfusion pump (Harvard apparatus 22) through 0.33 mm stainless steel cannulae (coordinates: AP −1.0 mm, ML +3.8 mm, DV −6.0 mm (AM) (cannulae ∠ 4°) or AP −3.0 mm, ML +3.5 mm, DV −5.0 mm. (VH) (cannulae ∠ 0°) relative to bregma). The cannulae were withdrawn 4 min after the completion of the infusion. Subsequently, the skin was stitched and the animals were given naloxone (0.1 mg/kg, sc).

Animals operated on day 21 of life were anaesthetized with Hypnorm® (1 ml/kg, im) and immobilized in a David Kopf stereotaxic frame with the upper incisor bar at horizontal zero. An operation identical to that described above was performed (coordinates: AP −2.0 mm, ML +4.0 mm, DV −6.8 mm (AM) (cannulae ∠ 0°) or AP −4.0 mm, ML +4.4 mm, DV −5.8 mm (VH) (cannulae ∠ 0°) relative to bregma). Animals lesioned in the AM on day 21 of life received a higher concentration of ibotenic acid to produce a similarly sized lesion as in animals operated on day 7 of life (4 μg/0.3 μl over 2 min.).

2.3. Behavioural tests

2.3.1. Social (play) behaviour

2.3.1.1. Apparatus. The test arena consisted of an acrylic plastic cage measuring $35 \times 35 \times 50$ cm ($l \times w \times h$) with approximately 2 cm of wood shavings covering the floor. The test cage was illuminated by a 20 W red light bulb mounted 60 cm above the test cage. The behaviours of the animals were recorded on video tape (Sony VHS recorder).

2.3.1.2. Procedure. After surgery the animals were housed in a dimly lit room. Animals operated in the AM or VH on day 7 of life were tested for social play behaviour on day 21 of life. Animals operated in the AM or VH on day 21 of life were tested on day 28 of life. The test was performed under dim light/unfamiliar conditions [39,54], which means that the animals were tested under red light in a novel test cage. On the day of the experiment, the animals were socially isolated in macrolon cages measuring $22 \times 13 \times 20$ cm ($l \times w \times h$) for 3.5 h prior to the experiment. The test consisted of placing two animals from different litters into the test cage for 15 min. Every pair of tested animals consisted of two identically operated animals (lesion against lesion, sham against sham). Pairs were tested in a randomized order for lesion and the animals did not differ by more than 10 g in body weight. Testing took place between 11:30 and 15:30 h.

2.3.1.3. Behavioural analysis. Observations from the video tape recordings were performed afterwards without knowledge of lesion status. Behaviour was assessed per pair of animals, so behaviour of the individual animal was not analysed. Latency to pinning (one of the animals is lying with its dorsal surface on the floor of the test cage with the other animal standing over it) and total duration, frequency, and mean duration of pinning were measured. Latency to social behaviour unrelated to social play behaviour (following or approaching the test partner, mounting or crawling over the test partner, sniffing or grooming any part of the body of the test partner including the anogenital area) and total duration and frequency of social behaviour unrelated to social play behaviour were measured.

2.3.1.4. Statistical analysis. In general, data were analysed per 15 min, except where indicated. Group medians (for pinning) or group means ± SEM (other variables) were calculated. Since four separate experiments were performed (A–D), the lesioned animals in every experiment were compared to identically sham-operated animals. Pinning levels were not normally distributed, so data of latency, total duration and frequency related to pinning were analysed using Mann–Whitney non-parametric tests. Mean duration of pinning and social behaviours unrelated to social play behaviour were analysed by Student's t-tests to determine differences between lesioned animals and identically operated sham-operated animals. The statistical package SPSS was used.

2.3.2. Social behaviour in adulthood

2.3.2.1. Apparatus. The test arena consisted of an acrylic plastic cage measuring $70 \times 70 \times 50$ cm ($l \times w \times h$). The floor was divided into four equal squares by painted lines. The test cage was illuminated by two 60 W red light bulbs mounted 60 cm above the test cage. The behaviours of the animals were recorded on video tape (Sony VHS recorder).

2.3.2.2. Procedure. Three weeks before social behaviour was tested the animals were housed under reversed light conditions (lights off 07:00 h, lights on 19:00 h). Other conditions were as described above. One week prior to experimentation the lesioned (lesioned or sham-operated) animals were socially isolated. The stimulus (socially housed) animals were housed in groups of 3–4 animals per cage. All lesioned animals were tested between day 82 of life and day 92 of life. The test consisted of placing one lesioned animal and one stimulus animal into the test cage for 10 min. Animals

were tested in a randomized order for lesion, and the weight differences between test partners were kept as small as possible. Testing took place between 10:00 and 15:30 h.

2.3.2.3. Behavioural analysis. Observation from the video tape recordings was performed afterwards without knowledge of lesion status. Behaviour was assessed per individual animal (lesioned and stimulus). Latency to, and total duration, frequency, and mean duration of the following behaviours were measured: social exploration (sniffing or licking any part of the body of the conspecific except the anogenital region), anogenital investigation (sniffing or licking the anogenital area of the other rat), crawling/mounting (standing on hind legs and putting one or both forepaws on the back of the conspecific, or climbing over the conspecific), approaching/following (walking or running in the direction of the conspecific; the conspecific is staying where it is or moving away), and the non-social behaviours: ambulation (crossing one line), rearing, and self-grooming.

2.3.2.4. Statistical analysis. The behavioural data were analysed per total period of 10 min. Social behaviour was defined as a summation of social exploration, anogenital investigation, crawling and mounting behaviour. Group means ± SEM were calculated. Since four separate experiments were performed (A–D), the lesioned animals in every experiment were compared to identically treated sham-operated animals. Animals lesioned in the AM on day 7 or 21 of life were divided into animals lesioned only in the lateral and basolateral nuclei of the AM and animals with additional lesions in the medial nuclei of the AM. The data were analysed by analysis of variance (ANOVA) followed by Student–Newman–Keuls parametric tests. Data of animals lesioned in the VH on day 7 or 21 of life were analysed by Student's *t*-tests. The statistical package SPSS was used to determine differences between lesioned animals and identically treated sham-operated animals.

2.4. Histology

At the completion of behavioural testing, the animals were anaesthetized with Nembutal® (50 mg/kg, ip). The brains of all animals with a lesion and a representative sample of sham-operated animals were perfused with saline followed by a 4% formaldehyde solution. After removal the brains were kept in a 4% formaldehyde solution. At least 1 day before freezing in isopentane the brains were kept in a 15% sucrose solution. Frozen sections were cut in a cryostat at 20 μm and stained with Cresyl Violet (Nissl-stain). Subsequently, the sections were examined using light microscopy for the extent and locus of the lesions.

2.5. Materials

The following drugs were used: fentanyl, Janssen Pharmaceutics, Tilburg, The Netherlands; naloxone, Du Pont Pharmaceuticals, Wilmington Delaware; Hypnorm®, Janssen Pharmaceutics, Tilburg, The Netherlands; Nembutal®, Sanofi Sante BV., Maassluis, The Netherlands; ibotenic acid, Tocris Cookson, Langford, Bristol, UK; Dulbecco's PBS, ICN Biomedicals Inc., Aurora, OH. On the day of use ibotenic acid was dissolved in PBS and pH was set to 7.4 using NaOH.

3. Results

3.1. Histology

Nissl-stained sections through the brains of AM lesioned animals showed neuronal loss and microgliosis in part of the AM complex. The lesions made on day 7 of life (Fig. 1A1–2) were of the same extent and localization as lesions made on day 21 of life (Fig. 1C1–2) and affected the basolateral and lateral nuclei of the AM (Fig. 1A1 and C1) and in part of the animals also the medial nuclei of the AM (Fig. 1A2 and C2). Brain sections of the VH lesioned animals showed neuronal loss, microgliosis, and some cavitation in the ventral part of the hippocampal formation. Most of the dorsal hippocampal formation was spared. The lesions made on day 7 of life (Fig. 1B) were of the same extent and localization as lesions made on day 21 of life (Fig. 1D) and affected CA1–CA3 and portions of the dentate gyrus. No lesions were seen in the sham-operated animals. Only rats with bilateral AM or VH lesions were included for statistical analysis.

3.2. Behavioural results

3.2.1. Social (play) behaviour

3.2.1.1. Pinning behaviour. During the behavioural test on social (play) behaviour early in life couples of identically lesioned animals were tested (see Section 2). Social (play) behaviour early in life of all animals lesioned in the AM or VH on day 7 or 21 of life were compared to animals sham-operated in the same structure and on the same day of life.

When tested on day 21 of life animals lesioned in the AM on day 7 of life showed an increase in latency to pinning, and a decrease in total duration, frequency, and mean duration of pinning compared to sham-operated animals [latency (median: sham = 101.8 s vs. lesion = 172.0 s); $U = 23.0$, $P < 0.05$] [total duration; $U = 5.0$, $P < 0.001$, (Fig. 2A)] [frequency (median: sham = 21.0 vs. lesion = 4.0); $U = 16.0$, $P < 0.05$] [mean duration

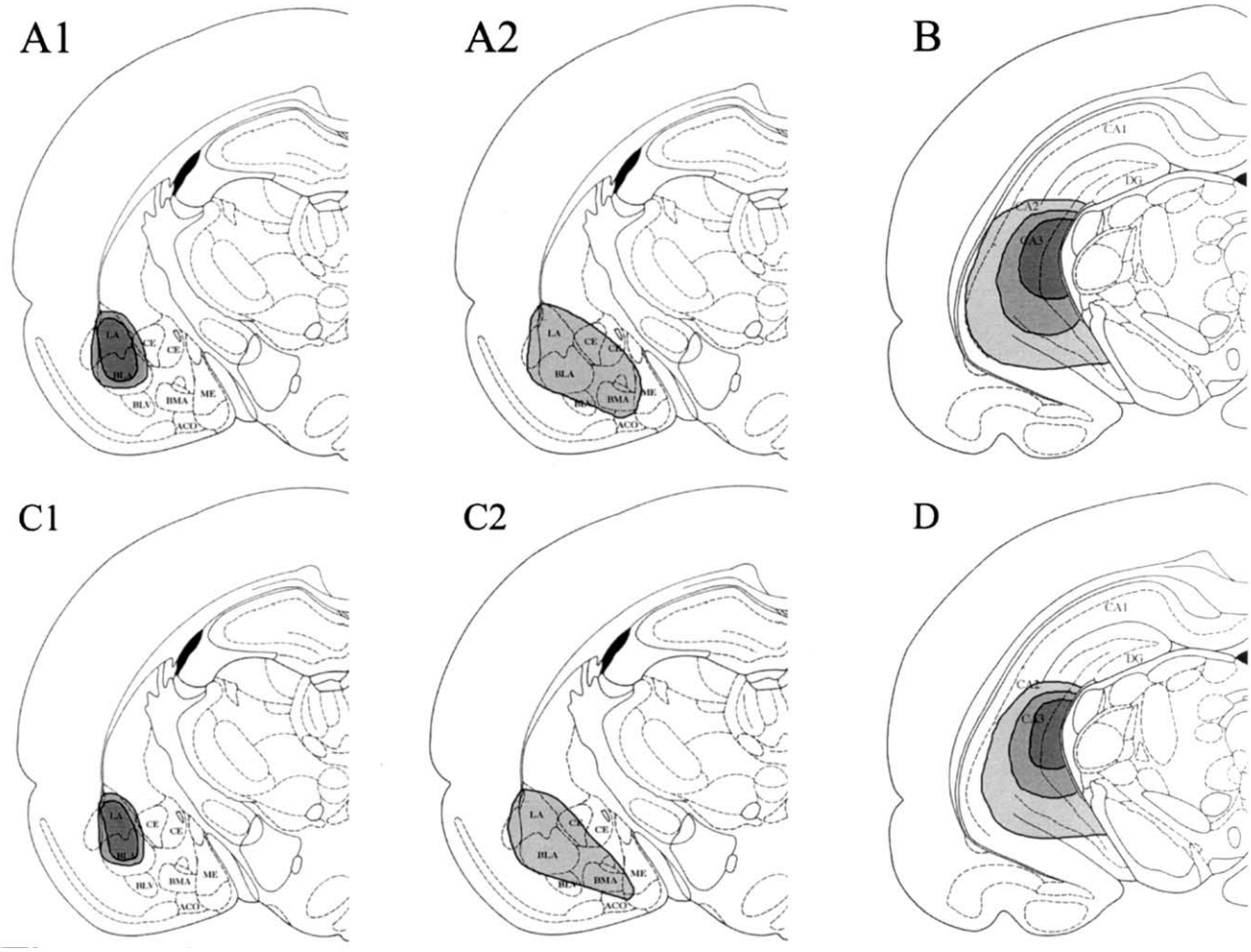

Fig. 1. (A, C) Lesion boundaries defined as the area of neuronal loss and microgliosis determined from Nissl-stained coronal sections from all rats with ibotenic acid lesions of the AM complex on day 7 (A) and 21 (C) of life limited to the basolateral and lateral nuclei (A1–C1) or with additional lesion to the medial nuclei (A2–C2). (B–D) Lesion boundaries defined as the area of neuronal loss and microgliosis determined from Nissl-stained coronal sections from all rats with ibotenic acid lesions of the ventral hippocampal area on day 7 (B) and day 21 (D) of life. The black area represents the area that was damaged in all animals, the dark-shaded colour represents the area that was damaged in about 80% of the animals, the light-shaded colour represents the area that was additionally damaged in part of the animals. Figures are derived from the stereotaxic atlas of Paxinos and Watson (bregma −2.30 mm (AM) and −4.80 mm (VH)) [42].

(mean: sham = 2.9 ± 0.2 s vs. lesion = 1.1 ± 0.2 s); $t(df = 17) = 1.97$, $P < 0.001$]. This decrease was present in each time block of 5 min. Animals lesioned in the VH on day 7 of life did not show any differences in pinning behaviour compared to sham-operated animals (Fig. 2B). Animals lesioned in the AM on day 21 of life showed a trend towards a decrease in latency to pinning, and a decrease in total duration and frequency of pinning behaviour [latency (median: sham = 174.4 s vs. lesion = 215.5 s); $U = 30.0$, $P = 0.057$] [total duration; $U = 14.0$, $P < 0.01$, (Fig. 2C)] [frequency (median: sham = 40.0 vs. lesion = 20.0); $U = 18.0$, $P < 0.01$] when tested on day 28 of life. The decrease in duration and frequency of pinning behaviour in animals lesioned in the AM on day 21 of life was present in each time block of 5 min. Mean duration of pinning behaviour was not affected in animals lesioned in the AM on day 21 of life (mean: sham = 4.1 ± 0.4 s vs. lesion = 4.0 ± 0.8 s). Animals lesioned in the VH on day 21 of life did not show any differences in pinning behaviour compared to sham-operated animals (Fig. 2D).

3.2.1.2. Social behaviour unrelated to social play behaviour early in life. Animals lesioned in the AM on day 7 of life showed a trend towards an increase in latency to social behaviour, and a decrease in total duration of social behaviour on day 21 of life compared to sham-operated animals [latency (mean: sham = 7.2 ± 0.9 vs. lesion = 14.5 ± 4.2); $t(df = 18) = 2.04$, $P = 0.056$; total duration; $t(df = 18) = 4.49$, $P < 0.001$, (Fig. 3A)]. This decrease in social behaviour during social play behaviour was not seen in the first time block of 5 min; the decline in social behaviour was present in the second [$t(df = 18) = 4.47$, $P < 0.001$] and third [$t(df = 18) =$

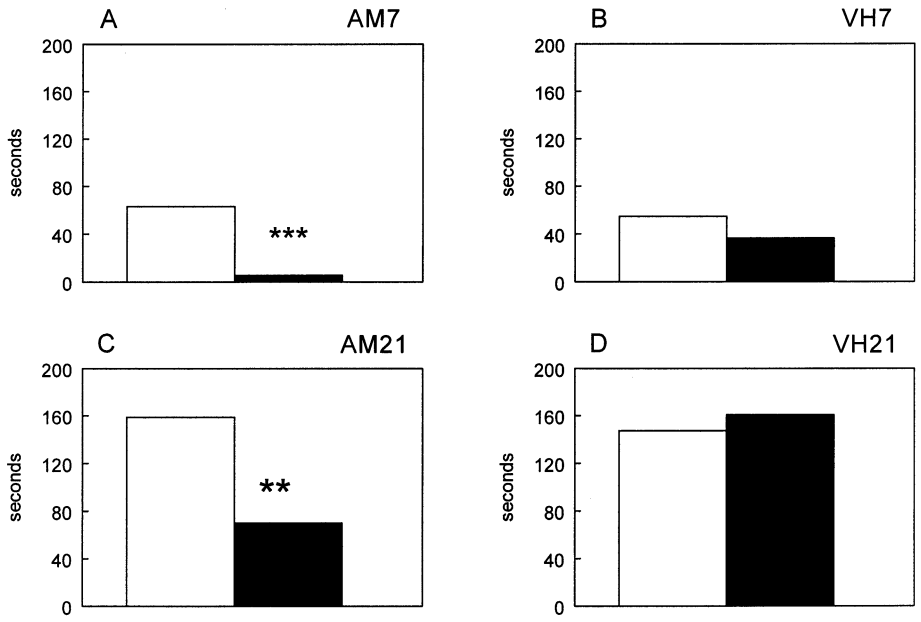

Fig. 2. Effects of AM and ventral hippocampal (VH) lesions on total duration of pinning behaviour (in seconds) in social play behaviour early in life during 15 min compared to sham-operated animals. Sham-operated and lesioned animals in the AM on day 7 of life (A), in the VH on day 7 of life (B), in the AM on day 21 of life (C), and in the VH on day 21 of life (D). Light bars represent sham-operated animals, dark bars represent lesioned animals. Data are expressed as median seconds ($n = 7$–9 lesioned couples/group; $n = 10$–13 sham-operated couples/group). $^{**}P < 0.01$, $^{***}P < 0.001$ (Mann–Whitney).

3.58, $P < 0.01$] time block [F(lesion × time blocks) (2, 17) = 5.25, $P = 0.017$]. Frequency of social behaviour was not affected in animals lesioned in the AM on day 7 of life (mean: sham = 145 ± 5 vs. lesion = 138 ± 10). No differences in total duration and frequency of social behaviour were seen in animals lesioned in the VH on day 7 of life or in the AM or VH on day 21 of life (duration; Fig. 3B–D).

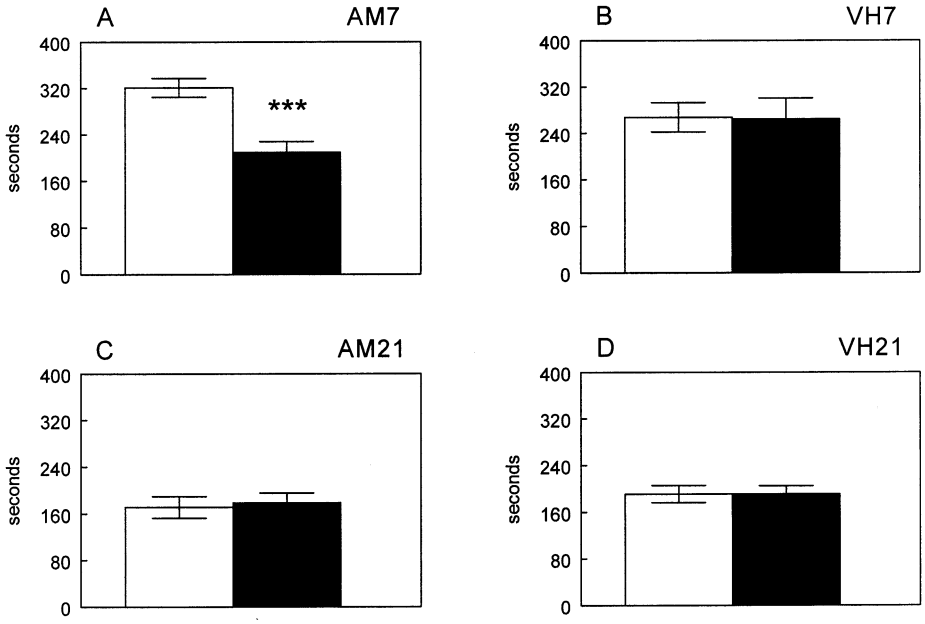

Fig. 3. Effects of AM and ventral hippocampal (VH) lesions on total duration of social behaviour unrelated to social play behaviour (in seconds) early in life during 15 min compared to sham-operated animals. Sham-operated and lesioned animals in the AM on day 7 of life (A), in the VH on day 7 of life (B), in the AM on day 21 of life (C), and in the VH on day 21 of life (D). Light bars represent sham-operated animals, dark bars represent lesioned animals. Data are expressed as mean seconds ($n = 7$–9 lesioned couples/group; $n = 10$–13 sham-operated couples/group). $^{***}P < 0.001$ (t-test).

3.2.2. Social behaviour in adulthood

In the social behaviour test in adulthood the isolated operated animals were tested against socially housed stimulus animals (see Section 2). Social behaviour in adulthood of animals lesioned in the AM on day 7 or 21 of life and sham-operated animals were compared with animals lesioned in the lateral and basolateral nuclei of the amygdala only (AM-L) and with animals with additional lesions in the medial nuclei of the amygdala (AM-M). Animals lesioned in the VH on day 7 or 21 of life were compared with animals sham-operated in the VH on the same day of life.

Social isolation increased social behaviour. In all groups of isolated sham-operated animals social behaviour was increased compared to socially housed stimulus animals [t-tests, AM7 $t(df = 44) = 6.24$, $P < 0.001$; AM21 $t(df = 44) = 9.93$, $P < 0.001$; VH7 $t(df = 40) = 4.63$, $P < 0.001$; VH21 $t(df = 40) = 7.23$, $P < 0.001$]. No differences in social behaviour were seen between rats with lesions on day 7 in the basolateral and lateral nuclei of the AM only and sham-operated animals (Fig. 4A). However, social behaviour was decreased in animals with additional lesions in the medial nuclei of the amygdala (AM-M) on day 7 of life compared to sham-operated animals and to animals lesioned only in the lateral and basolateral nuclei of the amygdala (AM-L) [total duration; $F(\text{lesion})(2, 35) = 4.85$, $P < 0.05$, frequency; $F(\text{lesion})(2, 35) = 5.21$, $P < 0.05$]. This effect was due in particular to a decrease in total duration (Fig. 4A) and frequency of social exploration [duration; $F(\text{lesion})(2, 35) = 4.84$, $P < 0.014$, frequency (mean; sham 39.0 ± 2.4 vs. AM-M 24.9 ± 5.0 vs. AM-L 48.0 ± 4.4); $F(\text{lesion})(2, 35) = 6.87$, $P < 0.003$]. In addition, latency to social exploration was prolonged [latency (mean; sham 18.9 ± 3.3 vs. AM-M 84.3 ± 31.9 vs. AM-L 28.4 ± 7.4); $F(\text{lesion})(2, 35) = 8.02$, $P < 0.001$]. Mean duration of social behaviour was not affected (mean; sham 2.0 ± 0.1 vs. AM-M 2.1 ± 0.3 vs. AM-L 1.7 ± 0.1). Although the differences did not reveal statistical significance, similar effects were observed for anogenital investigation and approaching/following. No differences were observed for crawling/mounting or the non-social behaviours (Table 1).

In animals lesioned in the AM on day 21 of life, no differences in social and in non-social behaviours were observed between the groups (sham-operated vs. AM-M vs. AM-L) (Fig. 4C). Social behaviours of animals lesioned in the VH on day 7 or 21 of life did not differ from social behaviours in sham-operated animals (Fig. 4B and D). In the animals lesioned in the VH on day 7 or 21 of life an increase in ambulation was observed compared to sham-operated animals [VH7; $t(df = 31) = 2.62$, $P < 0.05$, VH21; $t(df = 31) = 2.89$, $P < 0.05$] (Table 1).

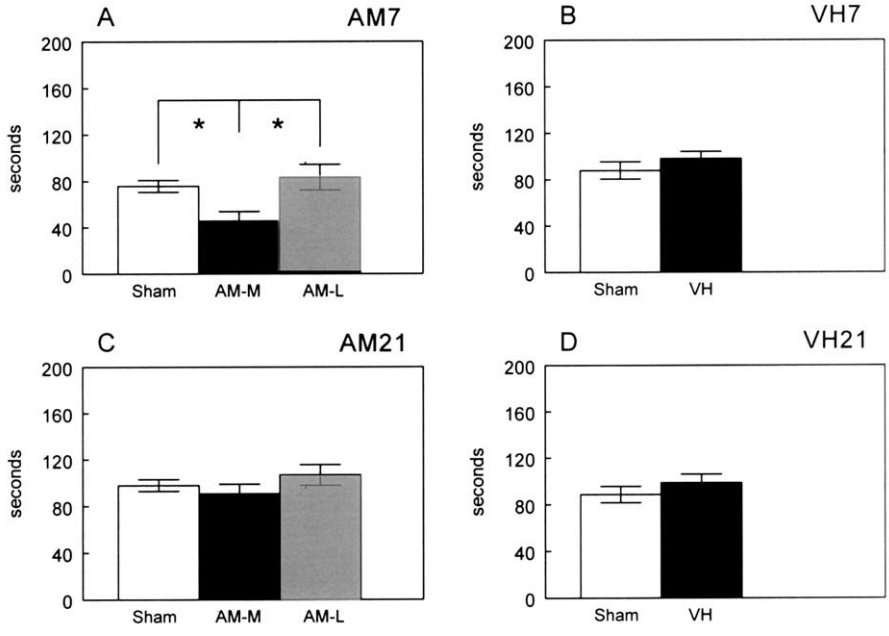

Fig. 4. (A, C) Effects of AM lesions with additional lesions in the medial nuclei of the amygdala on total duration of social exploration in adulthood (in seconds) during 10 min compared to sham-operated animals (sham) and animals lesioned only in the lateral and basolateral nuclei of the amygdala on day 7 (A) or 21 (C) of life. (B, D) Effects of ventral hippocampal lesions on total duration of social exploration in adulthood (in seconds) during 10 min compared to sham-operated animals on day 7 (B) or 21 (D) of life. Data are expressed as mean seconds ± SEM ($n = 7$–14 lesioned animals/group; $n = 20$–24 sham-operated animals/group). *Significant differences between groups according to Student-Newman-Keuls parametric tests following analysis of variances ($P < 0.05$).

Table 1
Social behaviour in adulthood

Behaviour		AMP7			VHP7	
		AM-Sh	AM-M	AM-L	VH-Sh	VH-Ls
Anog	frq	7.0±0.9	3.3±1.5	6.1±3.1	8.5±0.8	6.5±1.0
	lat	151.4±30.2	210.4±90.8	224.1±70.9	111.2±18.7	115.8±23.1
	dur	12.8±1.7	6.8±3.8	12.7±6.0	18.1±2.5	13.2±2.6
	mn	1.8±0.1	1.8±0.3	2.5±0.7	2.2±0.3	2.2±0.3
Appr/fllw	frq	47.3±2.3	37.0±7.5	51.0±5.4	40.7±4.1	46.3±4.6
	lat	9.8±2.4	31.3±8.3	21.7±15.2	24.7±7.5	15.6±5.2
	dur	42.8±3.3	37.7±8.1	43.0±6.8	49.6±2.6	49.6±4.0
	mn	0.9±0.1	1.0±0.1	0.8±0.1	1.4±0.1	1.2±0.1
Crwl/mnt	frq	4.2±1.0	5.1±1.5	6.3±1.1	7.5±1.6	6.6±1.6
	lat	260.6±32.3	164.1±37.0	182.8±25.6	169.6±17.9	188.8±35.6
	dur	2.5±0.9	2.7±1.0	2.8±0.5	4.1±0.8	3.1±0.8
	mn	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.05	0.4±0.04
Amb	frq	115.9±3.6	124.9±20.3	135.9±11.3	96.8±6.9	128.1±10.4*
	lat	5.0±0.9	12.2±7.4	3.2±1.0	5.0±1.0	4.6±0.9
Non.soc	frq	7.0±0.7	6.7±1.5	8.3±1.8	8.0±1.1	7.5±1.3
	dur	5.4±1.2	21.8±12.9	5.3±3.3	4.3±0.8	2.4±0.5
		AMP21			VHP21	
		AM-Sh	AM-M	AM-L	VH-Sh	VH-Ls
Anog	frq	10.0±1.0	9.6±1.6	8.6±1.7	7.8±0.9	8.1±1.4
	lat	119.6±25.8	145.5±37.1	117.5±47.0	78.2±20.0	129.0±31.9
	dur	20.6±2.3	18.0±3.1	16.4±3.8	15.6±2.1	16.7±3.0
	mn	2.1±0.2	1.9±0.2	1.8±0.2	2.2±0.3	1.9±0.2
Appr/fllw	frq	55.8±2.2	55.0±2.9	56.6±3.0	36.5±2.5	36.0±2.9
	lat	7.4±2.2	4.9±2.8	3.2±0.9	17.5±3.0	22.2±3.7
	dur	47.8±2.4	43.7±2.5	46.4±4.2	33.9±2.1	34.9±2.9
	mn	0.9±0.02	0.8±0.03	0.8±0.04	1.0±0.1	1.0±0.1
Crwl/mnt	frq	5.0±0.7	6.8±3.1	6.0±1.5	4.4±0.6	6.4±0.8
	lat	211.4±32.9	231.9±48.6	171.4±34.1	224.8±40.6	171.3±37.3
	dur	1.6±0.3	2.5±1.2	1.5±0.4	2.5±0.5	4.3±0.7
	mn	0.3±0.02	0.3±0.02	0.2±0.02	0.6±0.05	0.7±0.08
Amb	frq	124.6±3.5	134.6±9.8	125.3±7.2	121.2±3.5	142.2±7.34*
	lat	2.7±0.3	2.4±0.4	2.4±0.3	3.7±0.7	5.1±1.5
Non.soc	frq	9.1±0.8	10.9±2.1	7.6±1.1	12.2±1.6	10.7±1.5
	dur	4.0±0.6	3.3±0.9	1.8±0.3	5.5±1.4	3.3±0.6

Effects of AM lesions with additional lesions in the medial nuclei of the amygdala, animals lesioned only in the lateral and basolateral nuclei of the amygdala or ventral hippocampus (VH-Ls) on day 7 (AM7, VH7) or 21 (AM21, VH21) of life on latency to (lat), and frequency (frq), duration (dur) and mean duration (mn) of anogenital investigation (anog), approaching/following (appr/fllw), crawling/mounting (crwl/mnt) and the non-social behaviours during 10 min as compared to sham-operated animals (Sh). The non-social behaviours were split into latency to and frequency of ambulation counts (amb) and frequency and duration of rearing and self-grooming (non.soc). Data are expressed as mean counts (frq) or seconds (lat, dur, mn)±SEM.

* $P < 0.05$ as compared to sham-operated animals.

4. Discussion

4.1. Amygdala lesions

Pinning, the major characteristic behaviour of social play behaviour in rats, was markedly decreased in animals with moderately sized lesions in the AM on day 7 or 21 of life. This result confirmed earlier findings in animals with extensive lesions in the AM, involving the lateral, basolateral, central, and medial nuclei of the AM, on day 7 or 21 of life [59]. While a number of studies investigated changes in social behaviour in adulthood displayed by animals lesioned in the AM, only one study has investigated the effects of AM lesions on social play behaviour in juvenile rats. Male and female prepubertal rats differ in the frequency with which they engage in social play behaviour: males engage in more social play than females. This sexual

differentiation of play behaviour disappeared during observation between day 26 and 40 of life in males with electrolytic lesions in the AM made on day 21 or 22 of life [34]. All our experiments thus far have been done in male rats, so it is unknown whether our lesions would also decrease social play behaviour in females. Nevertheless, the AM seems to be of great importance for the appearance of pinning behaviour in social play behaviour in our male rats since lesions in the AM on both day 7 of life or day 21 of life show major deficits in pinning behaviour. Besides the AM other brain structures, among which the thalamus and brain stem [47] and the cortex [45] have been mentioned to influence play behaviour. Discrete electrolytic lesions of part of the thalamic area, lesions of the ventrolateral aspect of the brain stem and decortication in neonatal rats reduced pinning. The striatum seems to be important for maintaining sequential organization of social play behaviour [43].

Social behaviour unrelated to social play behaviour early in life (from now on referred to as social behaviour early in life) was decreased in animals lesioned in the AM on day 7 life and unaffected in animals lesioned in the AM on day 21 of life. The dissociation between behavioural effects on pinning and social behaviour early in life has been shown before. Several authors demonstrated that pinning behaviour does not correlate with social behaviour early in life [40,41,54]. Apparently, different mechanisms are involved in social behaviours expressed during the juvenile period. In contrast with the behavioural findings demonstrated in our former study [59], the results reported here show a decrease in social behaviour early in life only in animals lesioned on day 7 and not in animals lesioned on day 21 in the AM. It is not conceivable that in the present study the damaged AM accounts for the behavioural deficits, since animals lesioned on day 21 of life did not express the behavioural changes. This finding is suggestive of a neurodevelopmental deficit. The behavioural changes are most likely a result of malfunctioning of structures connected to the damaged AM.

Social play behaviour starts between day 15 and 16 of life, increases to a peak on day 30–36 of life, and declines gradually thereafter [51]. Animals lesioned in the AM on day 7 of life were tested on day 21 of life, whereas animals lesioned in the AM on day 21 of life were tested on day 28 of life. To exclude that the day of testing is responsible for the differences in the behavioural changes found between the two lesioned groups, a different cohort of animals was operated on day 7 of life and tested on day 28 of life (data not shown). These animals showed a similar decrease in pinning and social behaviour early in life as seen in animals operated on day 7 of life and tested on day 21 of life. Furthermore to exclude influences of body weight and body growth on the behavioural performances, body weight of animals lesioned or sham-operated in the AM on day 7 or 21 of life were measured during life (unpublished results). The lesioned animals consistently showed a lower weight compared to the corresponding sham-operated animals and a decrease (of less than 7%) was seen in animals lesioned on day 7 and in animals lesioned on day 21 of life. Decreases in weight in lesioned animals may indicate general impaired physical health of the animals, which may have influenced the performance on the behavioural experiments. However, this is most unlikely since the decreases in weight in animals lesiond in the AM on day 21 of life did result in only a few (present study) or no behavioural changes later in life [12].

Social behaviour in adulthood was analysed in subgroups of animals lesioned in the AM based on the extent of the lesions. Part of the animals lesioned in the AM proved to have bilateral lesions restricted to the basolateral and lateral AM, while another part of the animals did have additional lesions in the medial nuclei of the AM. Based on earlier findings of different behavioural effects depending upon the localization of the lesion in the AM [33], the AM lesioned animals were divided into two subgroups. Only animals lesioned in the basolateral AM with additional damage to the medial nuclei showed deficits in social behaviour in adulthood expressed by a decrease in social behaviour. This effect was due in particular to a decrease in social exploration, but it was also observed for anogenital investigation and approaching/following. Similar to the results on social behaviour early in life, behavioural changes on social behaviour in adulthood were only seen in (part of) the animals lesioned on day 7 of life, but not in animals lesioned on day 21 of life. According to our definition of the model of neurodevelopmental disorders, a neurodevelopmental deficit is likely.

Several studies have shown that lesions of the AM cause severe disruptions of social behaviour in monkeys [50], and in rats [23,53]. The deficits of animals lesioned in the AM seemed to consist of unresponsiveness to social stimuli [19]. All these studies showed similar extent and characteristics of the lesions. The lesions were large and localized in almost all nuclei of the AM. Electrolytic, aspiration and ablation techniques were used to produce the lesions in contrast with the more subtle technique of excitotoxic lesioning with axon-sparing ibotenic acid [22] as was performed in the present study. The results suggest that the lesioned area in the AM in our animals is not involved in social behaviour in adulthood, since no changes in social behaviour were observed in animals lesioned on day 21 of life in the AM. This is in agreement with the lack of effects on social interaction after small electrolytic lesions, particularly in the basolateral nuclei, of the AM [7].

In summary, on pinning behaviour an increase in latency and a decrease in total duration and frequency

was seen in animals lesioned in the AM on day 7 or 21 of life, and on social behaviour early in life as well as social behaviour in adulthood an increase in latency and a decrease in total duration and frequency was seen in animals lesioned in the AM on day 7 of life compared to sham-operated animals. In both paradigms, i.e. social (play) behaviour and social behaviour in adulthood, the animals were isolated, 3.5 h and 1 week, respectively, prior to testing. These isolation periods have previously been shown to induce a (half)-maximal increase in the amount of social (play) behaviour early in life [40] and a maximal increase in social behaviour in adulthood [39]. In the present experiments the increases in latency to social behaviour and decrease in duration of social (play) behaviour could be due to a deficit in this increase in social behaviour normally seen after isolation. A general effect on social behaviour resulting in social withdrawal and lack of interest or motivation for social (play) behaviour could also account for impaired social (play) behaviour in animals lesioned in the AM.

However, a neurodevelopmental deficit has to be present to explain the difference between the changes found on social behaviour early in life and in adulthood in animals lesioned in the AM on day 7 of life that were not present in animals lesioned on day 21 of life. It is very well possible that the underlying neurodevelopmental deficit responsible for impaired social behaviour early in life is identical to the underlying mechanism responsible for impaired social behaviour in adulthood in the subgroup of animals with additional lesions in the medial nuclei of the AM. Social behaviour early in life was assessed per pair of animals due to the reciprocal aspect of social (play) behaviour early in life [23]. If only one animal in a pair has additional lesions in the medial AM, this animal alone may account for a decrease in social behaviour early in life, as measured in a pair of lesioned animals.

Although further research has to be done to localize the neurodevelopmental disruption, one structure is worth considering here. In schizophrenia the medial prefrontal cortex (mPFC) has been mentioned to be involved in the social deficits shown in patients suffering from this disorder [8,14,49]. The AM massively projects to the mPFC and may play a role in the developmental plasticity reported for several functions of the mPFC, such as emotional and social behaviour [27]. Studied on the normal postnatal development of amygdaloid projections to the mPFC in rat showed that the transition from a diffuse fibre distribution to a characteristic bilaminar pattern occurred around day 12 of life [10,56]. Before this time innervation from the AM to the prefrontal cortex is scarce and still diffuse. Disruption of amygdaloid input to the mPFC on day 7 of life will most probably have different consequences for the functional development of the mPFC than damage to the AM on day 21 of life. The changes in social behaviour early and later in life in our animals lesioned in the AM on day 7 of life may reflect a neurodevelopmental deficit of the mPFC. This deficit may not be present in animals lesioned on day 21 of life because by that time projections from the AM to the mPFC are mature and the mPFC is almost completely developed.

Behavioural deficits in social behaviour in animals lesioned in the AM on day 7 of life occurred early in life, since it was already present before puberty (i.e. day 42–56 of life, [16,26] during social (play) behaviour. In autism social deficits are already present before the 3rd year of life or in early childhood [2]. Although the onset of the positive symptoms of schizophrenia is in early adulthood, there are indications that pre-schizophrenics already demonstrate difficulty in establishing normal relations before puberty [17,24].

4.2. Ventral hippocampus lesions

Animals lesioned in the VH on day 7 of life or on day 21 of life, showed no differences in social behaviour related or unrelated to social play behaviour early in life or in social behaviour in adulthood. Thus far only a few studies have investigated emotional or social behaviour in animals lesioned in the ventral hippocampal area. In monkeys separate contributions of the hippocampal formation and AM on memory functions and emotional behaviour were investigated by lesioning the hippocampus, the AM, or both structures [62]. Emotional behaviour was not disturbed in animals with damage in the hippocampal area only.

Sams Dodd et al. [46] studied the effects of neonatal ventral hippocampal lesions on social behaviour early (on day 35 of life) and later (on day 65 of life) in life. They described social interaction deficits on both day 35 and 65 of life. Active and passive social interaction was measured by automatical registration of the position of the two animals in one test-session of 10 min in an open arena ($150 \times 100 \times 40$ cm, $l \times w \times h$). Identically treated animals were tested against each other. The difference between the results of their study and the present study could be caused by the differences in test procedures, the differences in rat strains [30], or the differences in anxiety levels of the animals.

The enhanced locomotor activity during social behaviour in adulthood, as measured by an increase in ambulation counts, in both animals lesioned in the VH on day 7 or 21 of life differ from the increase in locomotor activity as was shown in these animals in the open field [13]. In the open field only animals lesioned in the VH on day 7 of life showed an increase in locomotor activity as measured by the distance moved. Apparently, changes in locomotor activity in animals lesioned in the VH depend upon the context in which this behaviour is measured. This may explain the conflicting reports about changes [15,29] or no changes [37] in locomotor

activity after ventral hippocampal lesions. In the present study, the enhanced locomotor activity during social behaviour is probably mediated by the VH, since it is both present in animals lesioned in the VH on day 7 and 21 of life.

5. Conclusions

1) Changes in social (play) behaviour early in life were present in animals lesioned in the AM on day 7 or 21 of life. On social behaviour early in life and in adulthood, deficits were only seen in animals lesioned in the AM on day 7 of life.
2) A neurodevelopmental disturbance on social behaviour early in life (before puberty) and in adulthood (after puberty) was seen in animals lesioned in the basolateral and medial AM on day 7 of life, since rats with similar lesions induced on day 21 of life did not show deficits in social behaviour.
3) Lesions in the ventral hippocampal area did not affect social (play) behaviour early in life or social behaviour in adulthood.
4) In summary, impairment in social behaviour is one of the major symptoms of psychiatric disorders like schizophrenia or autism. In the context of these disorders, the disturbances in social (play) behaviour induced by lesions in the AM on day 7 of life contribute to the validity of this model of neurodevelopmental disorders.

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References

[1] Aggleton JP. The contribution of the amygdala to normal and abnormal emotional states. Trends Neurosci 1993;16:328–33.
[2] American Psychiatric Association. In: Diagnostic and statistical manual of mental disorders (DSM-IV). Washington DC: APA, 1994.
[3] Bachevalier J, Merjenian PM. The contribution of medial temporal lobe structures in infantile autism: a neurobehavioral study in primates. In: Bauman ML, Kemper TL, editors. The neurobiology of autism. London: The Johns Hopskins University Press, 1994:146–69.
[4] Bauman M, Kemper TL. Histoanatomic observations of the brain in early infantile autism. Neurology 1985;35:866–74.
[5] Bayer SA, Altman J, Russo RJ, Zhang X. Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. Neurotoxicology 1993;14:83–144.
[6] Bekoff M. Social play and play-soliciting in infant canids. Am Zool 1974;14:323–40.
[7] Birbaumer N, Grodd W, Diedrich O, Klose U, Erb M, Lotze M, Schneider F, Weiss U, Flor H. fMRI reveals amygdala activation to human faces in social phobics. Neuroreport 1998;9:1223–6.
[8] Bloom FE. Advancing a neurodevelopmental origin for schizophrenia. Arch Gen Psychiatry 1993;50:224–7.
[9] Bogerts B, Lieberman JA, Ashtari M, Bilder RM, Degreef G, Lerner G, Johns C, Masiar S. Hippocampus-amygdala volumes and psychopathology in chronic schizophrenia. Biol Psychiatry 1993;33:236–46.
[10] Bouwmeester H, Wolterink G, Van Ree JM. Neonatal development of projections from the basolateral amygdala to prefrontal, striatal, and thalamic structures in the rat. J Comp Neurol 2002;442:239–49.
[11] Chua SE, Murray RM. The neurodevelopmental theory of schizophrenia: evidence concerning structure and neuropsychology. Ann Med 1996;28:547–55.
[12] Courchesne E. Brainstem, cerebellar and limbic neuroanatomical abnormalities in autism. Curr Opin Neurobiol 1997;7:269–78.
[13] Daenen EW, Wolterink G, Gerrits MA, Van Ree JM. Amygdala or ventral hippocampal lesions at two early stages of life differentially affect open field behaviour later in life; an animal model of neurodevelopmental psychopathological disorders. Behav Brain Res 2002;131:67–78.
[14] Deutch AY. The regulation of subcortical dopamine systems by the prefrontal cortex: interactions of central dopamine systems and the pathogenesis of schizophrenia. J Neural Transm Suppl 1992;36:61–89.
[15] Dhume RA, Gogate MG. Significance of spatial and temporal dysfunction exhibited by enhanced running activity in hippocampally-lesioned rats. Indian J Physiol Pharmacol 1983;27:209–16.
[16] Dohler KD, Wuttke W. Changes with age in levels of serum gonadotropins, prolactin and gonadal steroids in prepubertal male and female rats. Endocrinology 1975;97:898–907.
[17] Done DJ, Crow TJ, Johnstone EC, Sacker A. Childhood antecedents of schizophrenia and affective illness: social adjustment at ages 7 and 11. Br Med J 1994;309:699–703.
[18] Dunn LT, Everitt BJ. Double dissociations of the effects of amygdala and insular cortex lesions on conditioned taste aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid. Behav Neurosci 1988;102:3–23.
[19] Gloor P. Inputs and outputs of the amygdala: what the amygdala is trying to tell the rest of the brain. In: Livingston KE, Hornykiewicz O, editors. Limbic mechanisms, the continuing evolution of the limbic system concept. New York: Plenum Press, 1978:189–209.
[20] Hol T, Van den Berg CL, Van Ree JM, Spruijt BM. Isolation during the play period in infancy decreases adult social interactions in rats. Behav Brain Res 1999;100:91–7.
[21] Hole G. Temporal features of social play in the laboratory rat. Ethology 1988;78:1–20.
[22] Jarrard LE. On the use of ibotenic acid to lesion selectively different components of the hippocampal formation. J Neurosci Methods 1989;29:251–9.
[23] Jonason KR, Enloe LJ. Alterations in social behavior following septal and amygdaloid lesions in the rat. J Comp Physiol Psychol 1971;75:286–301.
[24] Jones P, Rodgers B, Murray R, Marmot M. Child development risk factors for adult schizophrenia in the British 1946 birth cohort. Lancet 1994;344:1398–402.
[25] Kalsbeek A, Voorn P, Buijs RM, Pool CW, Uylings HB. Development of the dopaminergic innervation in the prefrontal cortex of the rat. J Comp Neurol 1988;269:58–72.

[26] Knorr DW, Vanha-perttula T, Lipsett MB. Structure and function of rat testis through pubescence. Endocrinology 1970;86:1298–304.
[27] Kolb B. Functions of the frontal cortex of the rat: a comparative review. Brain Res 1984;320:65–98.
[28] Kolb B, Whishaw IQ. Plasticity in the neocortex: mechanisms underlying recovery from early brain damage. Prog Neurobiol 1989;32:235–76.
[29] Lanier LP, Isaacson RL. Activity changes related to the location of lesion in the hippocampus. Behav Biol 1975;13:59–69.
[30] Lipska BK, Weinberger DR. Genetic variation in vulnerability to the behavioral effects of neonatal hippocampal damage in rats. Proc Natl Acad Sci USA 1995;92:8906–10.
[31] Lotspeich LJ, Ciaranello RD. The neurobiology and genetics of infantile autism. Int Rev Neurobiol 1993;35:87–129.
[32] Maaswinkel H, Baars AM, Gispen WH, Spruijt BM. Roles of the basolateral amygdala and hippocampus in social recognition in rats. Physiol Behav 1996;60:55–63.
[33] McGregor A, Herbert J. Differential effects of excitotoxic basolateral and corticomedial lesions of the amygdala on the behavioural and endocrine responses to either sexual or aggression-promoting stimuli in the male rat. Brain Res 1992;574:9–20.
[34] Meaney MJ, Dodge AM, Beatty WW. Sex-dependent effects of amygdaloid lesions on the social play of prepubertal rats. Physiol Behav 1981;26:467–72.
[35] Mickley GA, Ferguson JL. Enhanced acoustic startle responding in rats with radiation-induced hippocampal granule cell hypoplasia. Exp Brain Res 1989;75:28–34.
[36] Murray RM. Neurodevelopmental schizophrenia: the rediscovery of dementia praecox. Br J Psychiatry Suppl 1994:6–12.
[37] Myhrer T. Locomotor, avoidance,and maze behavior in rats with selective disruption of hippocampal output. J Comp Physiol Psychol 1975;89:759–77.
[38] Nelson MD, Saykin AJ, Flashman LA, Riordan HJ. Hippocampal volume reduction in schizophrenia as assessed by magnetic resonance imaging: a meta-analytic study. Arch Gen Psychiatry 1998;55:433–40.
[39] Niesink RJ, Van Ree JM. Short-term isolation increases social interactions of male rats: a parametric analysis. Physiol Behav 1982;29:819–25.
[40] Niesink RJ, Van Ree JM. Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. Neuropharmacology 1989;28:411–8.
[41] Panksepp J. The ontogeny of play in rats. Dev Psychobiol 1981;14:327–32.
[42] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. San Diego, CA: Academic Press, 1986.
[43] Pellis SM, Castaneda E, McKenna MM, Tran Nguyen LT, Whishaw IQ. The role of the striatum in organizing sequences of play fighting in neonatally dopamine-depleted rats. Neurosci Lett 1993;158:13–5.
[44] Pellis SM, Pellis VC. Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat Rattus norvegicus. Aggresive Behav 1987;13:227–42.
[45] Pellis SM, Pellis VC, Whishaw IQ. The role of the cortex in play fighting by rats: developmental and evolutionary implications. Brain Behav Evol 1992;39:270–84.
[46] Sams Dodd F, Lipska BK, Weinberger DR. Neonatal lesions of the rat ventral hippocampus result in hyperlocomotion and deficits in social behaviour in adulthood. Psychopharmacology 1997;132:303–10.
[47] Siviy SM, Panksepp J. Juvenile play in the rat: thalamic and brain stem involvement. Physiol Behav 1987;41:103–14.
[48] Syvalahti EK. Biological factors in schizophrenia. Structural and functional aspects. Br J Psychiatry Suppl 1994:9–14.
[49] Tamminga CA, Thaker GK, Buchanan R, Kirkpatrick B, Alphs LD, Chase TN, Carpenter WT. Limbic system abnormalities identified in schizophrenia using positron emission tomography with fluorodeoxyglucose and neocortical alterations with deficit syndrome. Arch Gen Psychiatry 1992;49:522–30.
[50] Thompson CI, Bergland RM, Towfighi JT. Social and nonsocial behaviors of adult rhesus monkeys after amygdalectomy in infancy or adulthood. J Comp Physiol Psychol 1977;91:533–48.
[51] Thor DH, Holloway WR. Developmental analyses of social play behavior in juvenile rats. Bull Psychon Soc 1984;22:587–90.
[52] Thor DH, Holloway WR, Jr.. Social play in juvenile rats: a decade of methodological and experimental research. Neurosci Biobehav Rev 1984;8:455–64.
[53] Van den Berg CL, Hol T, Van Ree JM, Spruijt BM, Everts H, Koolhaas JM. Play is indispensable for an adequate development of coping with social challenges in the rat. Dev Psychobiol 1999;34:129–38.
[54] Vanderschuren LJ, Niesink RJ, Spruijt BM, Van Ree JM. Influence of environmental factors on social play behavior of juvenile rats. Physiol Behav 1995;58:119–23.
[55] Vanderschuren LJ, Niesink RJ, Van Ree JM. The neurobiology of social play behavior in rats. Neurosci Biobehav Rev 1997;21:309–26.
[56] Verwer RW, Van Vulpen EH, Van Uum JF. Postnatal development of amygdaloid projections to the prefrontal cortex in the rat studied with retrograde and anterograde tracers. J Comp Neurol 1996;376:75–96.
[57] Waddington JL. Schizophrenia: developmental neuroscience and pathobiology. Lancet 1993;341:531–6.
[58] Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry 1987;44:660–9.
[59] Wolterink G, Daenen EWPM, Dubbeldam S, Gerrits MAFM, Van Rijn R, Van Ree JM. Early amygdala damage in the rat as a model for neurodevelopmental psychopathological disorders. Eur Neuropsychopharmacol 2001;11:51–9.
[60] Wolterink G, Daenen EWPM, Van Ree JM. Animal models for schizophrenia. Neurosci Res Comm 2000;27:143–54.
[61] Yeo RA, Hodde Vargas J, Hendren RL, Vargas LA, Brooks WM, Ford CC, Gangestad SW, Hart BL. Brain abnormalities in schizophrenia-spectrum children: implications for a neurodevelopmental perspective. Psychiatry Res 1997;76:1–13.
[62] Zola Morgan S, Squire LR, Alvarez Royo P, Clower RP. Independence of memory functions and emotional behavior: separate contributions of the hippocampal formation and the amygdala. Hippocampus 1991;1:207–20.