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Neonatal Amygdala Lesions and Juvenile Isolation in the Rat: Differential Effects on Locomotor and Social Behavior Later in Life

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Pervasive developmental disorders such as autism are characterized by deficits in social interaction and communication. Disturbed development of limbic structures such as the amygdala might underlie these deficits. The authors examined the effects of amygdala lesions on Postnatal Day 7 and juvenile isolation (2 weeks of individual housing during Weeks 4 and 5 of life) on rat locomotor and social activity later in life. Before puberty, but more pronounced after puberty, lesioned rats displayed enhanced locomotor activity. Adult social behavior was selectively disturbed by the lesion and the isolation procedure. In particular, the combination of neonatal lesions and juvenile isolation severely disrupted social interaction. These results suggest that a combination of neonatal amygdala damage and juvenile isolation may serve as an animal model of certain psychopathological neurodevelopmental disorders, such as autism.

Autism has been described as a neurodevelopmental disorder by many authors (Bachevalier, 1994; Lotspeich & Ciaranello, 1993; Rapin & Katzman, 1998). The pathogenesis of autism has not been identified yet, but results from neuroanatomical studies indicate that medial temporal lobe structures may be implicated. Several postmortem studies have demonstrated amygdala abnormalities in autistic subjects, such as small neuronal size and increased cell-packing density (Kemper & Bauman, 1998). In addition, it has been reported that children with severe temporal lobe damage caused by encephalitis, tumors, or other factors develop autistic-like symptoms (for a review, see Sweeten, Posey, Shekhar, & McDougle, 2002). Further evidence for abnormal development of the amygdala in autism is presented by lesion studies in monkeys. Lesions to the medial temporal lobe in infant rhesus monkeys have been shown to result in long-term deficits in social behavior, an effect that was absent in monkeys receiving similar lesions in adulthood (Bachevalier, Malkova, & Mishkin, 2001).

Neonatal ibotenic acid lesion of the amygdala in the rat has been proposed as an animal model of neurodevelopmental disorders such as autism (Daenen, Van der Heyden, Kruse, Wolterink, & Van Ree, 2001; Wolterink et al., 2001). Excitotoxic lesions of the amygdala in the neonatal rat (i.e., on Postnatal Day [PD] 7) produce multiple behavioral abnormalities persisting into adulthood, such as disturbed locomotor behavior and a significant reduction in adult social activity. Effects on locomotor behavior are absent after later (i.e., on PD 21) amygdala damage, which is an indication of neurodevelopmental deficits of structures connected to the amygdala. Deficits in social interactions were also observed in PD 21 lesioned rats but were less pronounced, suggesting that the effects may depend on the day of life the lesion was induced. Together, these findings indicate that lesions of the amygdala on PD 7 mimic certain aspects of autism, a neurodevelopmental disorder characterized by (among other symptoms) such deficits in social behavior as a failure to develop peer relationships and isolation from social contact. This becomes prominent in early childhood: Autistic children engage much less in social activities compared with children of the same age that develop normally (American Psychiatric Association, 1994).

Social skills depend not only on the integrity of the amygdala, but also on early social experiences, which are very important for normal social development. Long-term effects of early social deprivation have been established for most mammals, including humans and nonhuman primates (Harlow, Dodsworth, & Harlow, 1965; Yarrow, 1961). In addition, several studies have shown that when rats are deprived of social experiences in early life, they exhibit decreased levels of social behavior at adult age (for a review, see Hall, 1998). For example, 2 weeks of juvenile isolation (isolation housing during Weeks 4 and 5 of life) results in reduced

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levels of social activity persisting into adulthood. This effect cannot be reversed by rehousing with either identically reared rats or socially reared rats (Hol, Van den Berg, Van Ree, & Spruijt, 1999). This indicates the importance of this particular period, during which rats display high levels of social play behavior (Thor & Holloway, 1984; Vanderschuren, Niesink, & Van Ree, 1997).

In order to increase face validity of the abovementioned animal lesion model for autism, we investigated the effects of neonatal amygdala damage in the rat in combination with juvenile isolation on social and nonsocial responsiveness. For this purpose, rats received bilateral ibotenic acid infusions in the amygdala and were housed in isolation during Weeks 4 and 5 of life. In Week 6 (before puberty) and Week 11 (after puberty), effects on general locomotor activity were measured in an open field test, and in Week 14, the rats were subjected to a social interaction test to determine possible effects on social responsivity. The purpose of this study was to investigate (a) whether a combination of neonatal amygdala lesions with juvenile isolation induces social deficits that are more severe than those produced by each individual manipulation and (b) whether these effects are restricted to the social domain or, in addition, affect general locomotor activity.

Method

The experimental procedures were approved by the Ethical Committee for Animal Experiments of Utrecht University.

Subjects and Housing

Subjects were male offspring of Wistar rats (U:WU; GDL Utrecht, the Netherlands). The mothers were obtained at Day 17 of gestation and were housed individually in Macrolon cages measuring 40 cm long × 26 cm wide × 20 cm high, with tap water and standard rat chow freely available. The rats were housed in a temperature- and humidity-controlled room with lights on between 7 a.m. and 7 p.m. One day after birth, litters were culled to 9 pups. (If a litter consisted of fewer than 9 males, the litter was filled up with females. Females were removed after weaning.) Surgery was administered to male offspring on PD 7. On PD 21, the males were weaned and were housed either in groups of 2–3 ($n = 33$; juvenile social housing [SOC]) or in social isolation ($n = 36$; juvenile isolation [ISO]). Throughout the whole experiment, the rats were housed in a single room, so that juvenile isolation deprived the rats of physical contact but not auditory, olfactory, and visual cues. After 2 weeks of isolation, the rats were socially rehoused in groups of 2–3 per cage with identically reared rats. Lesioned (AMX) and sham-operated (SHAM) rats were housed together.

Surgery

On the day of operation, rats were randomly assigned to lesion or sham-operated groups and subsequently received injections of either neurotoxin or vehicle in the amygdala. The rats were anesthetized subcutaneously with 0.3 mg/kg fentanyl (0.05 mg/ml, Janssen Pharmaceuticals, Tilburg, the Netherlands) and immobilized in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). A specially constructed silicone head mold provided stable fixation of the head. A midline skin incision was made, and the skull was perforated with the aid of a 1.0-mm dental drill. Excitotoxic lesions were induced by 2-min bilateral infusion of ibotenic acid (10 μg/μl in 0.1M pH 7.4 phosphate-buffered saline [PBS]; Tocris Cookson, St. Louis, MO), made with a microinfusion pump aimed at the basolateral nucleus of the amygdala, at a rate of 0.15 μl/min. Sham-operated rats received bilateral vehicle (PBS) injections. The coordinates for the positioning of the needles were 3.8 mm lateral to the midline, 1.0 mm posterior to bregma, and 6.0 mm below the surface of the skull at an angle of 4°. Four minutes after completion of the infusion, the cannulas were withdrawn and the skin was sutured. Finally, the rats were subcutaneously injected with 0.1 mg/kg naloxone (5 μg/100 ml 0.9% NaCl; Du Pont Pharmaceuticals, Wilmington, DE) to antagonize the effects of fentanyl and were returned to their mothers.

Behavioral Procedures

Testing was performed between 9 a.m. and 4 p.m. The rats were handled before each experiment. Experiments and analysis were performed by researchers blind to the rats' lesion status. Because of the rather large amount of rats, each experiment was performed in 2 days. Locomotor activity was tested during the light phase, both before and after puberty, on PD 38–39 and PD 71–72, respectively. Social activity was measured on PD 92–93, during the dark phase.

Open field test. Locomotor behavior of the rats was assessed by means of an open field test. The experiment was performed in a sound-attenuating, red-lit room. The arena consisted of a large circular black open field (i.d. 130 cm, height 36 cm) which was novel to the rats. An object (∅ 9 cm, height 17 cm) was placed in the center of the open field to provoke the rats to explore the middle of the open field. The open field was divided into two zones, an inner and an outer zone. On the day of testing, the rats were transported to the experimentation room, at least 1 hr prior to testing. The rat was placed in the outer zone of the open field, and locomotor behavior was registered for a 15-min period by a fully automated observation system (EthoVision; Noldus Information Technology B.V., Wageningen, the Netherlands). The quantified parameters were total distance traveled (both zones together), distance traveled in each zone, time spent in each zone, latency to enter the inner zone, and frequency of visits to both zones.

Social interaction test. Three weeks prior to experimentation, the day–night cycle was reversed (lights on between 7 p.m. and 7 a.m.). One week before the test, the experimental rats were housed individually in order to induce a maximal increase in social interaction (Niesink & Van Ree, 1982). Naive stimulus rats were housed in groups of 3 in similar Macrolon cages. Testing was performed under red-light conditions in a sound-attenuating room. The testing arena—which was unfamiliar to the rats—consisted of an acrylic plastic cage (measuring 70 cm long × 70 cm wide × 50 cm high), with a black floor demarcated in a 2 × 2 square grid. The testing arena was illuminated by two 60-W red light bulbs.

An experimental rat and a weight-matched stimulus rat (weight difference < 10 g) were placed in the arena for 10 min. Social interactions were videotaped and analyzed afterward with the aid of an observation system (The Observer; Noldus Information Technology B.V.). Frequency, latency, duration, and mean duration (duration divided by frequency) of the following behavioral elements were measured: social sniffing (exploration of the partner's body by sniffing), anogenital sniffing (exploration of the partner's anogenital region and tail by sniffing), social grooming (mouthing and licking the fur of the other rat), crawling over/under (climbing over or crawling under the other rat), and approaching/following (moving in the direction of or pursuing the other rat). Total social activity was determined by calculating the total duration and frequency of the behavioral elements mentioned above. Locomotor activity was determined by measuring the total number of square crossings.

Histological Verification

At the end of the experiments, the rats were decapitated and their brains were collected. The brains were frozen and stored at −80 °C until assay. Frozen brains were sectioned at 20 μm and stained with hematoxylin acid/eosin for assessment of lesions. Lesions were evaluated by accurately defining the area of neuronal loss and microgliosis. Only rats with substantial bilateral amygdala damage and without damage to adjacent struc-

tures were regarded as having correct lesions and were included for statistical analysis.

Data Analysis

Data from the open field test were analyzed by a three-way analysis of variance (ANOVA) including repeated measures (pre- and postpubertal testing), with lesion status (sham or lesion) and rearing condition (juvenile isolation or juvenile social housing) as independent factors. In case of significant Test × Lesion interaction effects, data from the prepubertal and postpubertal test were analyzed separately by two-way ANOVA (independent factors: lesion status and rearing condition) to detect any possible differences between the two tests with respect to lesion status.

For the social interaction test, a two-way ANOVA was used, with lesion status and rearing condition as independent factors. In case of statistically significant interaction effects, further comparisons were made by means of Tukey's post hoc tests.

The SPSS (Version 9.0; SPSS, Chicago, IL) statistical package was used. Differences were significant at $p < .05$.

Results

Histology

Histological analysis revealed that ibotenic acid infusion induced bilateral damage to the amygdala in 18 rats (10 of which were isolation reared: Group AMX–ISO; and 8 of which were reared socially: Group AMX–SOC). In most rats, these lesions affected the basolateral, lateral, central, and medial nuclei of the amygdala. Lesions from the Groups AMX–ISO and AMX–SOC were comparable with respect to localization and size of the lesioned area. Figure 1 illustrates the largest and smallest areas of lesion damage observed in neonatally lesioned rats. All sham-operated rats (19 reared in isolation: Group SHAM–ISO, and 17 socially reared: Group SHAM–SOC) were included in the analysis.

Locomotor Activity

Data from the open field test are displayed in Table 1 and Figure 2. The experiment included 2 consecutive days of testing. Because no significant differences were revealed between testing days, data were analyzed per experiment. For none of the parameters were significant Test × Rearing, Test × Lesion × Rearing Condition or main effects of rearing established, therefore only Test × Lesion and main effects of lesion and test are discussed.

Distance traveled. Overall analyses, using the repeated measures paradigm, revealed a significant Test × Lesion interaction effect for total distance traveled in the open field, $F(1, 50) = 16.16$, $p < .01$. Sham-operated rats showed a decrease in total distance traveled, whereas this measure was not changed in lesioned rats when comparing the first test (before puberty) with the second test (after puberty). Further analysis by two-way ANOVA revealed that before puberty, the four experimental groups traveled equivalent total distances in the large open field: Lesion Status × Rearing Condition interaction effect, $F(1, 50) = .09$, *ns* (see Figure 2A). After puberty however, total distance traveled in the open field was higher in AMX rats than in SHAM rats, $F(1, 50) = 23.12$, $p < .01$ (Figure 2B).

Distance traveled in the inner zone was increased in all groups in comparing the first with the second test, $F(1, 50) = 5.14$, $p < .05$. In addition, a significant lesion effect was found, $F(1, 50) = 8.87$, $p < .01$, with AMX rats traveling a shorter distance in the inner zone than SHAM rats tested both before and after puberty. Further analysis showed that before puberty, AMX rats traveled a smaller distance in the inner zone than SHAM rats, $F(1, 50) = 9.80$, $p < .01$. After puberty, no effects were seen for distance traveled in inner zone: lesion effect, $F(1, 50) = 2.95$, *ns.* Distance traveled in the outer zone was decreased in SHAM rats, whereas no change was observed in AMX rats, resulting in a significant Test × Lesion interaction effect, $F(1, 50) = 6.70$, $p < .05$. In addition, a significant main effect of lesion was observed for distance traveled in the outer zone, $F(1, 50) = 15.63$, $p < .01$, meaning that during both tests, AMX rats traveled a longer distance in the outer zone than SHAM rats. Before puberty, AMX rats traveled a longer distance in the outer zone than SHAM rats, $F(1, 50) = 4.55$, $p < .05$. This effect was even larger after puberty, $F(1, 50) = 19.24$, $p < .01$.

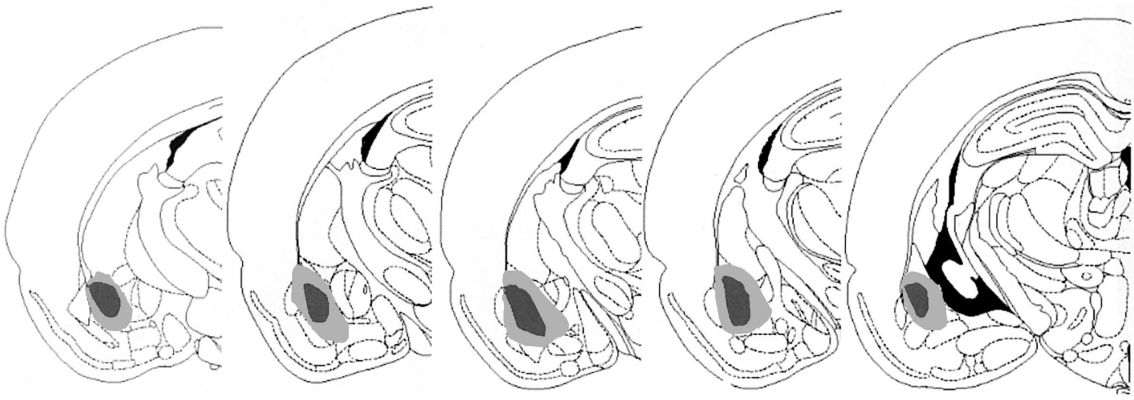

Figure 1. Diagrams of coronal sections (−1.88, −2.30, −2.80, −3.30, −3.80 mm posterior to bregma, from left to right) on which the extent of cell loss and gliosis observed after bilateral ibotenic acid infusions aimed at the basolateral amygdala is depicted. Largest regions of damage are depicted in light gray, smallest regions in a darker gray. Reprinted from *The Rat Brain in Stereotaxic Coordinates,* 4th ed., G. Paxinos and C. Watson, Figures −1.88, −2.30, −2.80, −3.30, and −3.80, Copyright 1998, with permission from Elsevier.

Table 1
Effects of Neonatal Amygdala Lesion and Juvenile Isolation on Open Field Performance in the Rat, Before and After Puberty

Performance measure, open field zone, and rearing condition	Prepubertal		Postpubertal	
	SHAM	AMX	SHAM	AMX
Distance traveled (m)				
Inner				
SOC	17 ± 2	9 ± 2††	17 ± 1	14 ± 2††
ISO	15 ± 1	11 ± 2††	17 ± 1	14 ± 3††
Outer				
SOC	66 ± 3	74 ± 7*	49 ± 2	74 ± 10**
ISO	67 ± 2	75 ± 4*	50 ± 1	63 ± 7**
Frequency of visits				
Inner				
SOC	24 ± 3	17 ± 2	25 ± 2	26 ± 2
ISO	21 ± 1	20 ± 2	23 ± 2	24 ± 3
Outer				
SOC	27 ± 3	19 ± 3*	26 ± 2	29 ± 2
ISO	24 ± 1	22 ± 2*	24 ± 2	27 ± 3
Duration of visits (s)				
Inner				
SOC	199 ± 21	115 ± 29†††	273 ± 20	206 ± 32†††
ISO	198 ± 13	125 ± 26†††	258 ± 17	173 ± 33†††
Outer				
SOC	699 ± 21	782 ± 29†††	626 ± 20	693 ± 32†††
ISO	698 ± 13	774 ± 27†††	642 ± 17	724 ± 32†††
Latency to inner zone (s)				
SOC	65 ± 11	33 ± 9*	43 ± 9	33 ± 13
ISO	54 ± 10	29 ± 7*	36 ± 9	54 ± 10

Note. Data are expressed as means (± *SEM*). SHAM = sham-operated rats; AMX = amygdala-lesioned rats; SOC = socially reared; ISO = reared in isolation.
* $p < .05$, ** $p < .01$ (two-way analysis of variance [ANOVA] of each experiment).
†† $p < .01$, ††† $p < .001$ (main effect of lesion, three-way ANOVA).

Frequency of visits. Frequency of visits to the inner zone was increased in all experimental groups during the second test, $F(1, 50) = 7.22$, $p < .01$. No differences between AMX and SHAM rats were established: Test × Lesion interaction effect, $F(1, 50) = 3.54$, *ns.* A significant Test × Lesion interaction effect was determined for frequency of visits to the outer zone, $F(1, 50) = 7.44$, $p < .01$; AMX rats showed an increase in frequency of visits to the outer zone during the second test, an effect that was absent in SHAM rats. Before puberty, AMX rats displayed a lower frequency of visits to the outer zone than SHAM rats, $F(1, 50) = 4.21$, $p < .05$, an effect that was absent after puberty, $F(1, 50) = 2.27$, *ns.*

Duration of visits. Duration of visits to the inner zone was increased in all experimental groups: test effect during the second test, $F(1, 50) = 35.33$, $p < .01$, with SHAM rats staying longer in the inner zone than AMX rats both before and after puberty: lesion effect, $F(1, 50) = 14.44$, $p < .01$. This effect was accompanied by a decrease in duration of visits to the outer zone during the second test in all experimental groups, $F(1, 50) = 35.06$, $p < .01$. In addition, a significant lesion effect was found for duration of visits to the outer zone, $F(1, 50) = 14.34$, $p < .01$, with AMX rats displaying higher values than SHAM rats measured before and after puberty.

Latency. Finally, latency to enter the inner zone was found to be reduced in SHAM, but not AMX, rats during the second test, Test × Lesion effect, $F(1, 50) = 4.76$, $p < .05$. An additional

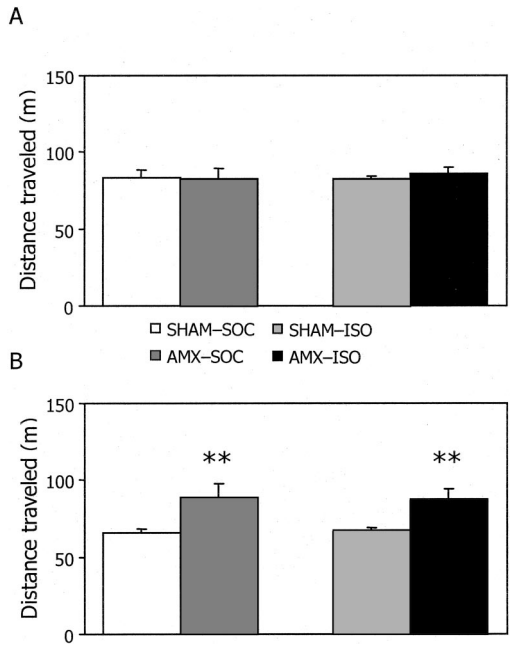

Figure 2. Effects of neonatal amygdala lesions (AMX) and sham lesions (SHAM) in combination with juvenile isolation (ISO) or social rearing (SOC) on total distance traveled in the open field during 15 min of testing, before (A) and after (B) puberty. Data are expressed as means (± *SEM*). ** $p < .01$ (analysis of variance).

two-way ANOVA revealed that, before puberty, latency to enter the inner zone was significantly lower in AMX rats than in SHAM rats, $F(1, 50) = 6.56$, $p < .05$. However, after puberty, latency to enter the inner zone was not different between the four experimental groups, $F(1, 50) = 0.16$, *ns.*

Social Activity

The social interaction test included 2 consecutive days of testing. No significant differences were revealed between testing days, therefore data were analyzed per experiment. Data from the social interaction test are displayed in Table 2 (data per behavioral element) and Figure 3 (total frequency of social behavior and total duration of social behavior). Total frequency of social activity was found to vary dependent on lesion status and rearing condition, $F(1, 50) = 7.23$, $p < .01$ (Figure 3A). Post hoc Tukey's analyses showed that total frequency of social activity was lower in Group AMX–ISO compared with Groups AMX–SOC ($p < .05$), SHAM–ISO ($p < .01$), and SHAM–SOC ($p < .05$). A similar interaction effect was found for total duration of social activity, $F(1, 50) = 6.70$, $p < .05$, as depicted in Figure 3B. Group AMX–ISO rats spent less time interacting socially than rats in Groups AMX–SOC ($p < .05$), SHAM–ISO, ($p < .01$), and SHAM–SOC ($p < .01$).

Qualitative aspects of social behavior were also affected by lesion status and/or rearing condition (Table 2). Significant main effects of lesion were found for the following parameters: duration of anogenital sniffing, $F(1, 50) = 7.72$, $p < .01$; mean duration of anogenital sniffing; $F(1, 50) = 7.92$, $p < .01$, and mean duration of following/approaching, $F(1, 50) = 24.77$, $p < .01$, with AMX rats having lower scores than SHAM rats.

Main effects of rearing condition were also found. ISO rats spent less time on social sniffing than SOC rats, $F(1, 50) = 4.78$, $p < .05$. A similar effect was found for mean duration of social sniffing, $F(1, 50) = 5.40$, $p < .05$, and duration of anogenital sniffing, $F(1, 50) = 6.39$, $p < .05$.

Frequency of anogenital sniffing tended to vary depending on lesion status and rearing condition, $F(1, 50) = 3.20$, $p = .08$. Post hoc analyses revealed that AMX–ISO rats displayed lower frequencies than SHAM–ISO rats ($p < .05$) and SHAM–SOC rats ($p < .05$). In addition, latency and mean duration of crawling over/under varied depending on lesion status and rearing condition: latency, $F(1, 50) = 5.11$, $p < .05$; mean duration, $F(1, 50) = 7.48$, $p < .01$. Post hoc analysis showed that mean duration of crawling over/under tended to be lower in AMX–ISO rats compared with AMX–SOC rats ($p = .07$). Tukey's post hoc analyses revealed no further differences for crawling over/under latency. Following and approaching behavior also varied depending on lesion status and rearing condition. Significant Lesion × Rearing interaction effects were determined for frequency, $F(1, 50) = 9.17$, $p < .01$; duration, $F(1, 50) = 6.95$, $p < .05$; and latency, $F(1, 50) = 4.09$, $p < .05$, of this behavioral element. Frequency of following/approaching was lower in AMX–ISO rats as compared

Table 2
Effects of Neonatal Amygdala Lesion and Juvenile Isolation on Adult Social Behavior in the Rat

Behavioral element and treatment group	Frequency	Duration (s)	Latency (s)	Mean duration (s)
Social sniff				
SHAM–SOC	21.0 ± 3.0	21.0 ± 4.0	17 ± 3	0.9 ± 0.1
AMX–SOC	30.0 ± 4.0	28.0 ± 5.0	17 ± 5	0.9 ± 0.0
SHAM–ISO	20.0 ± 3.0	17.0 ± 2.0$^{\$}$	29 ± 9	0.8 ± 0.0$^{\$}$
AMX–ISO	21.0 ± 2.0	16.0 ± 3.0$^{\$}$	29 ± 10	0.8 ± 0.1$^{\$}$
Anogenital sniff				
SHAM–SOC	36.0 ± 3.0	58.0 ± 6.0	22 ± 4	1.5 ± 0.1
AMX–SOC	35.0 ± 6.0	49.0 ± 9.0††	24 ± 10	1.3 ± 0.1††
SHAM–ISO	35.0 ± 2.0	51.0 ± 3.0$^{\$}$	21 ± 3	1.5 ± 0.1
AMX–ISO	21.0 ± 4.0$^{\#}$	26.0 ± 6.0††$^{\$}$	25 ± 6	1.1 ± 0.1††
Social groom				
SHAM–SOC	2.1 ± 0.6	4.1 ± 1.3	429 ± 43	1.6 ± 0.4
AMX–SOC	1.4 ± 0.8	5.9 ± 4.3	447 ± 63	1.6 ± 0.8
SHAM–ISO	2.0 ± 0.5	4.5 ± 1.1	410 ± 39	2.3 ± 0.9
AMX–ISO	0.8 ± 0.5	1.6 ± 1.0	526 ± 47	0.5 ± 0.3
Crawl over/under				
SHAM–SOC	4.5 ± 1.1	3.4 ± 0.8	284 ± 48	0.5 ± 0.1
AMX–SOC	5.4 ± 1.1	4.7 ± 1.3	192 ± 42	0.8 ± 0.1
SHAM–ISO	5.1 ± 0.8	3.5 ± 0.5	199 ± 37	0.7 ± 0.1
AMX–ISO	4.8 ± 0.6	3.4 ± 1.4	355 ± 82*	0.4 ± 0.1**
Follow/approach				
SHAM–SOC	29.0 ± 3.0	43.0 ± 6.0	37 ± 9	1.4 ± 0.1
AMX–SOC	25.0 ± 3.0	30.0 ± 6.0	25 ± 10	1.2 ± 0.1†††
SHAM–ISO	38.0 ± 2.0	62.0 ± 5.0	28 ± 5	1.6 ± 0.1
AMX–ISO	14.0 ± 2.0**	17.0 ± 3.0*	60 ± 20*	1.2 ± 0.0†††

Note. Adult social behavior in sham-operated and socially reared rats (SHAM-SOC), amygala-lesioned and socially reared rats (AMX-SOC), sham-operated rats that underwent juvenile isolation (SHAM-ISO), and lesioned rats that underwent juvenile isolation (AMX-ISO). Data are expressed as means (± *SEM*).
Lesion effects: †† $p < .01$, ††† $p < .001$. Rearing effects: $^{\$}$ $p < .05$. Lesion × Rearing interaction effects: $^{\#}$ $p = .08$, * $p < .05$, ** $p < .01$, two-way analysis of variance.

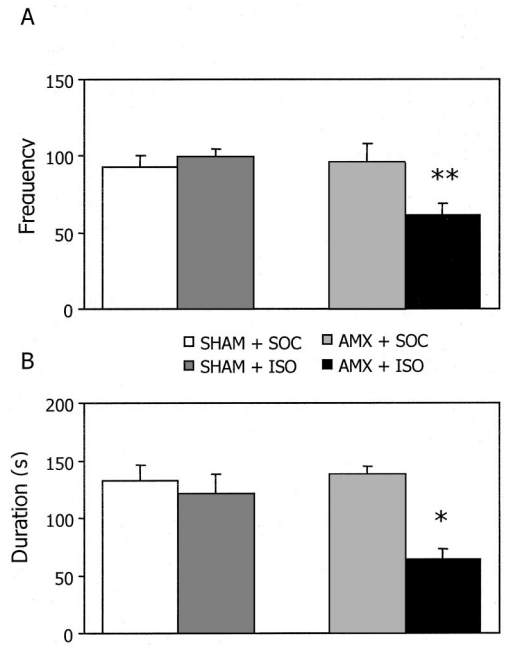

Figure 3. Effects of amygdala lesions (AMX) and sham lesions (SHAM) on Postnatal Day 7 in combination with juvenile isolation (ISO) or social rearing (SOC) on total frequency (A) and duration (B) of social activity during 10 min of testing with a weight-matched, unfamiliar stimulus rat. Data are expressed as means (± *SEM*). * $p < .05$, ** $p < .01$ (two-way analysis of variance).

with SHAM–ISO and SHAM–SOC rats ($p < .001$ and $p < .01$, respectively). In addition, AMX–SOC rats displayed a lower frequency of following/approaching than SHAM–ISO rats, $p < .05$. Duration of following/approaching was lower in AMX–ISO rats compared with SHAM–ISO and SHAM–SOC rats ($p < .01$ and $p < .05$, respectively). AMX–SOC rats spent less time on following/approaching than SHAM–ISO rats ($p < .01$). Post hoc analyses revealed no further differences for following/approaching latency.

No group differences were observed for social grooming behavior or locomotor activity, $F(1, 50) = 0.60$, *ns.* Mean number of square crossings were SHAM–SOC rats = 78 ± 5, AMX–SOC rats = 72 ± 10, SHAM–ISO rats = 82 ± 4, and AMX–ISO rats = 80 ± 6.

Discussion

The present data demonstrate that excitotoxic lesions of the amygdala in neonatal rats produce deficits in locomotor and social behavior. The locomotor effects emerged before puberty but were more pronounced after puberty. Social isolation during Weeks 4 and 5 of life did not further affect locomotor activity. Adult social behavior was also disturbed in rats with neonatal amygdala damage but was most seriously affected in rats that were also deprived from social stimuli during the juvenile period. The differential effects of neonatal amygdala lesions and subsequent juvenile isolation on social and nonsocial responsiveness is discussed below.

Locomotor Activity

Locomotor activity was studied in a large open field before and after puberty. Rats with neonatal lesions in the amygdala displayed altered locomotor responses, an effect that was independent of rearing condition. A prominent behavioral disturbance in these rats included increased locomotor activity in the open field. This effect was apparent only after puberty and was mainly due to increased activity in the outer zone of the open field. Locomotor hyperactivity after puberty and a tendency to stay in the outer zone of the open field have been reported previously in rats with neonatally induced lesions to the basolateral, medial, and central nucleus of the amygdala (Daenen et al., 2001; Wolterink, Daenen, Dubbeldam, Gerrits, van Rijn, Kruse, van der Heijden, & Van Ree, 2001). A putative explanation for the preference to stay in the outer zone of the open field may be that lesioned animals are more anxious. However, because latency to enter the inner zone was lower (before puberty) or not different from that of sham-operated rats (after puberty) and the number of entries to the inner zone was unchanged, a lesion-induced difference in basal anxiety levels probably does not account for the observed behavioral changes. A more likely explanation is locomotor stereotypy displayed by rats with neonatal amygdala lesions. Such non-goal-oriented behavior has been reported previously by Daenen, Wolterink, Gerrits, and Van Ree (2002a), showing that neonatally amygdala-lesioned rats continuously walked along the edges of the open field. Although walking patterns were not analyzed in the present study, the increased distance traveled in the outer zone might reflect a similar preference for the edge of the open field. These effects were not found after lesioning the amygdala later in life (on PD 21), suggesting that integrity of the amygdala is not crucial for this effect (Daenen et al., 2002a). Instead, it was hypothesized that early loss of the amygdala interferes with normal development of other brain structures. The (basolateral) amygdala has strong reciprocal connections with many other brain structures, such as the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAC; Bouwmeester, Smits & Van Ree, 2002; Bouwmeester, Wolterink & Van Ree, 2002; Groenewegen, Berendse, Wolters, & Lohman, 1990). Both the mPFC and the NAC have been shown to play a key role in locomotor responses to novelty. For example, local infusions of dopaminergic agents in either the mPFC or the NAC have been shown to affect horizontal activity in an unfamiliar environment (Broersen, Feldon, & Weiner, 1999; Hooks & Kalivas, 1995; Staton & Solomon, 1984). Therefore, the observed locomotor changes may be a consequence of anatomical miswiring and, thus, altered development of the mPFC and the NAC. In support of this hypothesis, Bouwmeester et al. (2002) have demonstrated altered tissue levels of noradrenaline, as well as dopamine and its metabolites, in the mPFC and NAC in rats with neonatal basolateral amygdala damage.

Social Activity

In addition to changed locomotor activity, disturbances in adult social behavior were demonstrated after neonatal amygdala damage, that is, decreases in follow and approach behavior and anogenital sniffing behavior. These effects are comparable to those reported by Wolterink et al. (2001) who showed similar decreases in follow and approach activity and social exploration behavior (i.e., social sniffing plus social grooming behavior) in rats with neonatally induced damage to the amygdala. A reduction of social behavior has also been reported in monkeys with neonatal aspiration lesions of the amygdala (Bachevalier, 1994). Monkeys with

excitotoxic amygdala lesions, however, displayed increased social anxiety during dyadic social interactions, but social behavior itself was unaffected by the lesion (Prather et al., 2001).

One can argue that the observed effects of amygdala damage on social behavior are not age dependent, as decreased social behavior has also been demonstrated for adult rats with amygdala lesions (Jonason & Enloe, 1971). Nevertheless, a previous report indicates that those reductions in social behavior are more pronounced in neonatally amygdala-lesioned rats compared with rats with amygdala damage induced later in life, when the brain is almost mature (Wolterink et al., 2001). The latter is probably due to the more subtle technique of lesioning with axon-sparing ibotenic acid (Jarrard, 1989). In addition, in another study, PD 21 amygdala-lesioned rats did not differ from sham-operated rats with respect to social behavior (Daenen et al., 2002b). Together, these data suggest that the observed effects on social behavior depend at least partially on the day of life the lesion was induced and that a neurodevelopmental deficit may at least partly underlie the disturbed behavior. The amygdala is reciprocally connected to the orbitofrontal cortex, an area which—together with the amygdala—is considered to be part of the so-called "social brain." This particular connection has not fully matured by PD 7 (Bouwmeester, Wolterink, & Van Ree, 2002). Neonatal amygdala damage may negatively affect this connectivity, and consequently result in aberrant social activity.

Isolation housing during Weeks 4 and 5 of life also affected the quality of social activity in adulthood. Rearing in isolation during this particular period resulted in decreased duration and mean duration of social sniffing. These findings are in line with previous experiments (Van den Berg, Hol, et al., 1999) and may be an indication of reduced social motivation. In support of this hypothesis, it has been demonstrated that rats reared in isolation during Weeks 4 and 5 of life fail to show conditioned place preference for social contact (Van den Berg, Pijlman, et al., 1999).

However, the most disturbing effects were observed after the combination of neonatal amygdala damage and juvenile isolation. The most striking effect was that neonatal amygdala lesions in combination with juvenile isolation reduced the total amount of time the rats interacted socially. In addition, a marked reduction in total frequency of social activity, a reduction in frequency and duration of following and approaching behavior, and increased latency of following and approaching were reported after neonatal amygdala damage and juvenile isolation. Together, these data suggest that neonatal amygdala damage in combination with juvenile isolation has deleterious effects on adult social functioning.

Behavioral (social) stimuli in the first weeks of life have shown to be critically involved in the functional maturation of several neuronal structures. For example, it has been shown that dopamine and serotonin levels in the amygdala, striatum, and certain cortical regions are altered after isolation rearing in the rat (Hall, 1998). In addition, an in vitro autoradiography study in isolation-reared rats reported upregulation of mu- and kappa-opioid receptors in the basolateral amygdala and several cortical areas (Van den Berg, Van Ree, Spruijt, & Kitchen, 1999). Therefore, it may be hypothesized that the absence of essential, social stimuli interacts with the lesion-induced disturbed maturation of (orbitofrontal) cortical regions, and hence produce the selective impairment in social behavior.

Taken together, the present results show that neonatally amygdala-lesioned rats show certain reductions in social behavior; however, these deficits are limited when rats have the opportunity to interact with conspecifics during Weeks 4 and 5 of life. When neonatally amygdala-lesioned rats are deprived of such vital social stimuli during this particular period of life, they persistently fail to show normal social behavior.

Conclusion

The present data demonstrate that excitotoxic lesions of the amygdala in neonatal rats and juvenile isolation have differential long-term effects on responsiveness to social and nonsocial stimuli. Social behavior was found to be sensitive to both manipulations separately but was most seriously disturbed in rats that were neonatally lesioned in the amygdala and subsequently deprived from social stimuli during Weeks 4 and 5 of life. Nonetheless, locomotor hyperactivity induced by neonatal lesioning was not further affected by the juvenile isolation procedure. This indicates that the combined effects are specific to social behavior and thus cannot be a consequence of a general disturbance of all behaviors. These characteristics resemble symptoms seen in neurodevelopmental psychiatric disorders such as autism, namely, altered responses to novel environments and severe impairments in social interaction (American Psychiatric Association, 1994). Accordingly, the results of the present study indicate that a combination of neonatal excitotoxic lesions of the amygdala and social isolation rearing may be a useful tool for studying functional and neurobiological disturbances involved in autism and autism-related disorders. Furthermore, it may be speculated that the social isolation early in life may have a deteriorating impact on symptomatology of autistic patients.

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Correction to Rhodes et al. (2003)

The article "Exercise Increases Hippocampal Neurogenesis to High Levels but Does Not Improve Spatial Learning in Mice Bred for Increased Voluntary Wheel Running," by Justin S. Rhodes, Henriette van Praag, Susan Jeffrey, Isabelle Girard, Gordon S. Mitchell, Theodore Garland Jr., and Fred H. Gage (*Behavioral Neuroscience,* 2003, Vol. 117, No. 5, pp. 1006–1016), contained an error.

In Figure 3, the symbols representing the "Control runners" and "Selected no wheels" groups were reversed in Panel B. They should match the legend in Panel A: Open circles should appear as solid squares, and solid squares as open circles.