ELSEVIER

Physiology & Behavior, Vol. 60, No. 1, 55–63, 1996

0031-9384/96 $15.00 + .00

SSDI 0031-9384(95)02233-3

Roles of the Basolateral Amygdala and Hippocampus in Social Recognition in Rats

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Received 30 August 1995

MAASWINKEL, H., A.-M. BAARS, W.-H. GISPEN AND B. M. SPRUIJT. *Roles of the basolateral amygdala and hippocampus in social recognition in rats.* PHYSIOL BEHAV **60**(1) 55–63, 1996.—Lesions of the amygdala or hippocampus have a large impact on social behavior of rats. In this study we investigated whether a social recognition test was also affected by those lesions. An NMDA-induced lesion of the basolateral amygdala did not impair the ability to distinguish a familiar from an unfamiliar juvenile rat. It was argued that the cortico-medial amygdala may be more important for social recognition than the basolateral amygdala. Fimbria-transected rats could no longer distinguish a familiar from an unfamiliar juvenile. Moreover, during all encounters they spent less time investigating the juvenile. The precise nature of this deficit, especially the reason for the overall reduced social investigation time, could not be specified with the classical procedure of the social recognition test.

Basolateral amygdala Hippocampus Object recognition Social recognition
Social performance Stimulus–reward association

THOR and Holloway (58) introduced a procedure to test social recognition in rats. In this test, juvenile rats are placed twice in the home cage of a single adult male rat for 5 min, with a variable intertrial interval. If the same juvenile is presented again after a short interval, the adult rat spends less time on social investigation of the juvenile than during the first encounter. If the interval between the encounters increases, the difference between the duration of the investigations decreases. If, however, during the second encounter an unfamiliar juvenile is presented, even after a short interval, the investigation will not differ in duration.

Ploeger et al. (45) suggested that the nucleus accumbens plays a role in social recognition. The basolateral amygdala and hippocampus are among the main structures that project to this nucleus (55), and thus the question is, whether one of these brain structures is involved in social recognition.

Originally it was thought that a lesion of either the amygdala or the hippocampus results in an impairment in object recognition as measured by the delayed (no-)matching-to-sample task (36,39). Now we know that in many of these studies parts of the parahippocampal region (i.e., entorhinal, perirhinal, parahippocampal/postrhinal cortices) were affected as well. In many studies limiting the lesion to the amygdala or the hippocampus, without affecting the adjacent cortical areas, a deficit in recognition memory was not found, whereas it was usually found after lesion of the perirhinal or entorhinal cortices (17,37,38,42,65). However, in some cases of hippocampal lesions a deficit in the delayed (non-)matching-to-sample was seen, the most frequently used recognition test. They are critically discussed by Shaw and Aggleton (52): in some cases the task made use of spatial orientation [e.g., Jagielo (25)], in others the "objects" were surface textures [e.g., Raffaelle (49)], in contrast to "three dimensional 'junk' stimuli." The impression is then that the hippocampus is only indirectly involved in recognition, namely only if the procedure involves some other kinds of processing such as spatial orientation. The recognition cue in the social recognition test seems to be of olfactory nature (12,47). Otto et al. (42) have shown that in odor recognition tests, the hippocampus is not involved, but the rhinal cortex is. Thus, because the social recognition test does not depend on spatial information processing, there is no reason to expect that after a lesion confined to the amygdala or to the hippocampus rats should be impaired in recognizing juveniles, if we suppose that this is a form of object recognition.

However, both the amygdala (27,32,50,57) and hippocampus (16,30,31,33,35) are involved in aspects of social behavior and could therefore have a role in general or recognition-dependent

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social performance in the "social recognition task." It is furthermore possible that individual recognition depends, in contrast to object recognition, on aspects of social evaluation (see below).

A bilateral lesion of the amygdala disrupts social and nonsocial behavior in monkeys and in rats: fall in social hierarchy, social withdrawal, "tameness," reduced fear in novel situations, etc. (27,28,30,50,57). It was suggested by Weiskrantz (61) that the amygdala is primarily involved in identifying reinforcing stimuli. This has led to the current view that the amygdala is the basis for secondary reinforcement (8,19,29) in that it plays a role in associating a biological or affective value to stimuli (41). Familiarization of rats with mice can suppress mouse-killing. This effect is abolished by amygdala lesions (59). In monkeys, cells responding to faces and complex social stimuli were found in the amygdala (7,34). In humans with a bilateral damage to the amygdala, the recognition of emotions in facial expressions is disrupted (2,64). An impairment in learning new faces was also found (64), perhaps indicating a role of emotional evaluation in the acquisition of new faces. Seeck et al. (51) found, mainly in the amygdala, variations in the frequency of evoked potentials due to familiarity of faces: according to the authors this could be due to the emotional and motivational significance of familiar faces, which seems to be mediated by the amygdala. The conclusion of all these findings is that the amygdala is involved in recognition, affective evaluation of, and/or responses to social signals (32,34). The amygdala may have a role in social recognition by way of affective evaluation. Alternatively, the amygdala may be responsible for the social response upon recognition.

Social behavior is also disrupted after lesions of the hippocampus (16,30,31,33,35). Hippocampal-lesioned mice have been shown to be less aggressive (16). In rats with hippocampus lesion nest-building (30) and maternal behavior (31) are disorganized, and sexual behavior is more rigid (35). Although recognition itself should not be disrupted directly by the lesion, because this would be a function of the parahippocampal region (42), we expect that social interest is changed. This could also indirectly influence recognition per se.

In this study the lesion of the amygdala is restricted to the basolateral amygdala, because we wanted to study areas projecting to the nucleus accumbens. We limited ourselves to the difference between familiarity and unfamiliarity, and therefore compared social investigation behavior of lesioned vs. sham-operated rats in their ability to distinguish a familiar from an unfamiliar juvenile after a short interval (5 min). Longer intervals

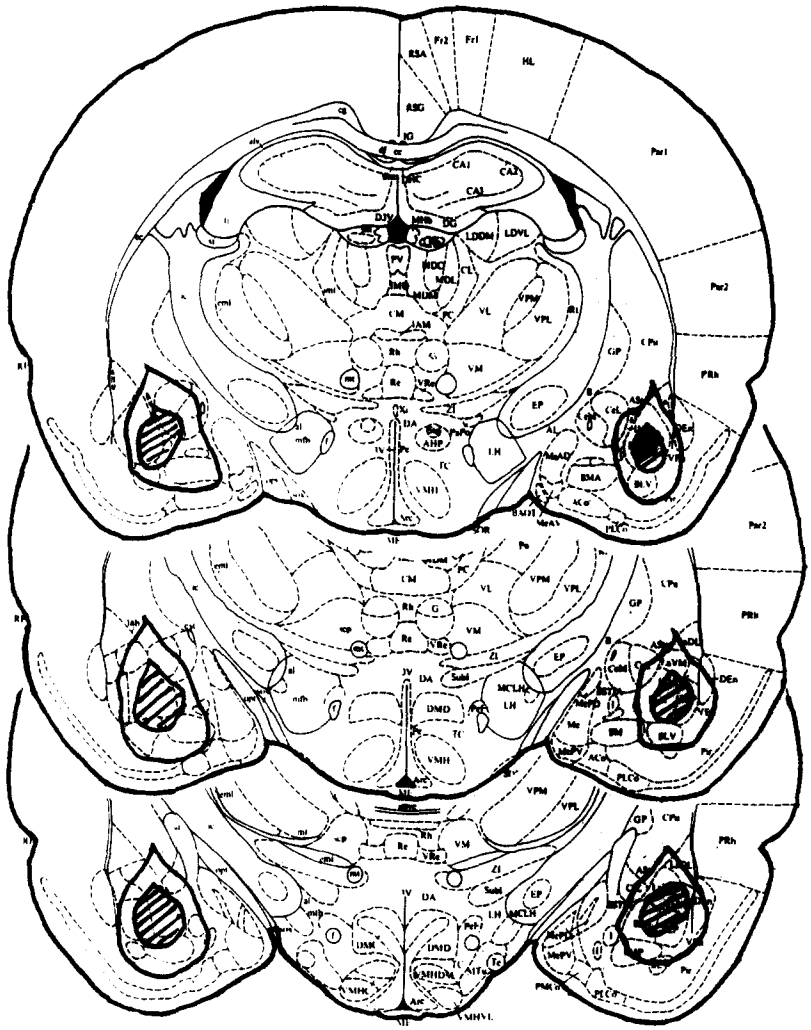

FIG. 1. NMDA lesion of the basolateral amygdala. The striped area represents the smallest lesion, the blank area the largest lesion (combination of all rats). Adapted from Paxinos and Watson (43).

were not used because we were not interested in long-term recognition. Because both brain areas are directly involved in social behavior, we also compared the absolute value of social investigation as a measure for social interest. Bilateral lesions of the basolateral amygdala were made by using *N*-methyl-D-aspartate (NMDA), and bilateral lesions of the hippocampus were made by transecting the fimbria mechanically.

METHOD

Animals

Adult male Wistar rats (from our own stock), weighing 265–340 g at the time of the lesion, were housed individually in Macrolon cages with chow and water available ad lib and under a reversed day–night rhythm (dark period: 0800–2000 h). The juveniles were approximately 18 days of age on the first day of the experiment and were housed in groups of five animals in Macrolon cages under the same conditions as the adults.

Surgery

In the first experiment 15 rats with basolateral NMDA lesions and 14 rats with sham lesions were used. Stereotaxic surgery was performed under anesthesia with 0.05 ml/100 g IM Hypnorm (Duphar) and 0.1 ml/100 g SC Dormicum (Roche). In accordance with the study of Everitt et al. [(10), but using another stereotactic atlas], the lesion animals received four injections of 0.3 μl of a 120 m*M* NMDA phosphate buffer solution (adjusted to pH 7.4) at the following sites [according to the stereotactic atlas of Paxinos and Watson (43)]: posterior to bregma 2.3 and 3.3 mm, lateral right and left 5.0 mm, and ventral 8.5 mm. The injection, via a Hamilton syringe, was carried out over approximately 15 s and was performed manually. The needle was left in place for 2 min. In the sham-operated rats the needle was inserted less deep, 7.5 mm, and no injection was performed. After 2 min the needle was removed. The purpose of the NMDA lesion was to damage the cell bodies without affecting the passing fibers (55), as it now is generally accepted that lesion of these fibers and destruction of the cell bodies in situ has additional effects to those resulting from mere damage to the cell bodies (15). The sham animals received no injection at all, because the issue was not to study the effects of NMDA but to study neuronal damage vs. no damage (9). The animals were allowed to recover for at least 2 weeks before the experiment was started.

In the second experiment 12 rats with fimbria lesions and 12 rats with sham lesions were used. The anesthesia was the same as for the basolateral amygdala operation. The fimbria transection was made by lowering a knife at 1.9 mm posterior to bregma, $\pm$1.9 mm lateral, and 5.5 mm ventral from the top of the skull and moving it along a lateral groove to $\pm$3.0 mm lateral. The sham-operated animals underwent the operation but were not given a lesion. The animals were allowed 2 weeks to recover before the experiment was started.

Histology

After the experiments the rats were killed with an overdose of sodium pentobarbital. The brains were removed and kept in 4% formaldehyde. Slices of 35 μm were made and stained with thionine. The extent of the lesions was assessed microscopically. The basolateral amygdala was largely destroyed; there were lesions of varying severity in the surrounding areas (central

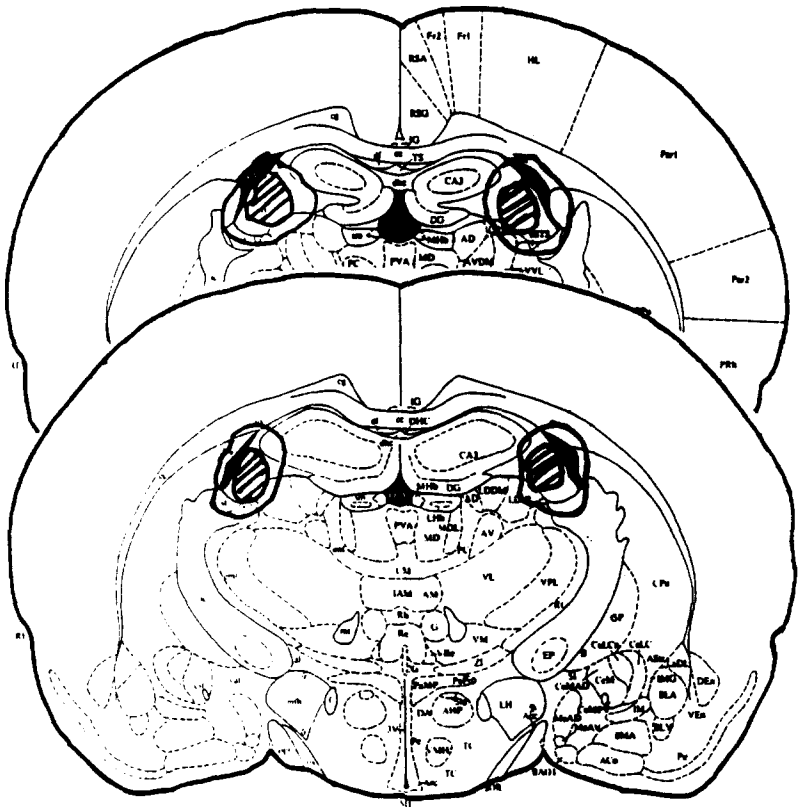

FIG. 2. The fimbria transection. The striped area represents the smallest lesion, the blank area the largest lesion (combination of all rats). Adapted from Paxinos and Watson (43).

amygdala, basomedial and endopiriform nuclei, ventral part of the caudatus putamen) (Fig. 1). The transection of the fimbria was always complete and the lesions of surrounding areas (hippocampus proper, thalamus, caudatus putamen) were variable (Fig. 2).

Experimental Procedure

The experiments were carried out in the home cage of the adult rats during the dark period. The cages were placed at least 2 h in advance on the observation table in the same room in which the rats were housed. The experiment for each lesion group lasted 3 days. New juveniles were used each day.

During the first day the adult rats were habituated to the procedure. A juvenile was introduced into the cage (first encounter) and removed after 5 min. Five minutes later it was reintroduced again for another 5 min (second encounter). The first experimental trial (second day) was identical but the behavior of the adults was recorded. In the second experimental trial (third day), the juvenile rat used in the second encounter was not the same as the one used in the first encounter. This trial was also recorded.

Data Analysis

For the first and second encounters of the first and second experimental trials, we calculated the total time of social investigation (SIT), which we define as the combination of all forms of affiliative social activities the adult rat showed towards the juvenile (anogenital grooming, social grooming, social sniffing, and following). We were interested in two measurements: the total recognition-independent and the recognition-dependent social interest.

To obtain a parameter for the recognition-independent social interest we compared the SITs of the first encounters, because the lesions could already have had an effect on these parameters. An ANOVA was used for statistical analysis.

To assess the recognition-dependent social interest, usually the RID (ratio of investigative duration) is used, which is calculated by dividing the SIT of the second encounter by the SIT of the first encounter. This parameter was not suited for our purpose, because we wanted to compare the RIDs of a trial using the same juvenile during the second encounter with a trial using a novel one. Because we used the same adult rats in both trials, we had to exclude habituation effects. This can be done by introducing new parameter, the novelty index (NI):

$$\mathrm{NI} = \mathrm{RID(second\ trial)} - \mathrm{RID(first\ trial)} = \frac{\mathrm{SIT\ [2nd\ encounter\ (second\ trial)]}}{\mathrm{SIT\ [1st\ encounter\ (second\ trial)]}} - \frac{\mathrm{SIT[2nd\ encounter\ (first\ trial)]}}{\mathrm{SIT[1st\ encounter\ (first\ trial)]}}$$

The NI has some resemblance to the recognition index (46). However, the recognition index compares different drug treatments. The NI is a measure for the recognition of a familiar from an unfamiliar juvenile (the terms "familiar" and "unfamiliar" are in this context defined by the procedure). An ANOVA was used to assess whether there was a difference in discrimination between familiar and unfamiliar juveniles.

The NI only reveals whether there is any effect of the treatment. A lower NI means impaired recognition, which does not mean that recognition is totally absent. To check this we also tested whether NI is different from zero, that is, whether RID (second trial) is significant different from RID (first trial). For this we used a paired t-test. This analysis was carried out per group.

Behavioral Assessment of the Effectiveness of the NMDA-Induced Lesion

The size of the lesion was assessed histologically, and the behavioral effectiveness of the lesion was assessed in the passive avoidance test 1 week after the social investigation test. Dunn and Everitt (15) have shown that an excitotoxic lesion (ibotenic acid) of the basolateral amygdala reduces the step-down latency. In earlier experiments (unpublished results) with an NMDA-induced lesion, we obtained a similar result with the step-through passive avoidance test: the step-through latency was markedly reduced.

The apparatus consisted of a dark compartment (40 × 40 cm) with an electrifiable grid floor. On one side, there was an entrance with a sliding door. In front of it on the outside was a small platform, illuminated with a 40-W bulb. The procedure (1) was as follows. On the first day the rats were placed individually for 2 min in the dark compartment with the door closed. Immediately afterwards they were placed on the platform with their heads away from the door. After they had entered the dark compartment, the door was shut and the rats were left in it for about 10 s. On the second day they received a second habituation trial, but this time they were immediately placed on the platform. Three hours later they received the acquisition trial, which was identical with the previous habituation trial, except that they received an electric foot shock (0.4 mA for 2 s) upon entering the dark compartment. The retention trial was given 24 h later. The rats were again placed on the platform with their head away from the sliding door. The time until entering the dark compartment (step-through latency) was measured, with a maximum of 300 s.

RESULTS

Behavioral Assessment of the Effectiveness of the NMDA-Induced Amygdala Lesion

The step-through latencies of the acquisition and the retention trial are presented in Fig. 3. For the acquisition trial, there was no significant difference between groups, $F(1, 27) = 3.32$, $p > 0.1$.

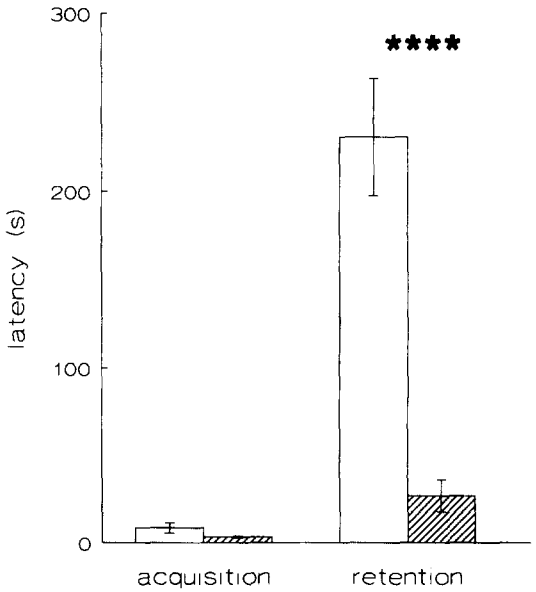

FIG. 3. Step-through latency (mean ± SEM) for the acquisition and retention trials. Open bars: sham-operated rats, hatched bars: amygdala-lesioned rats.

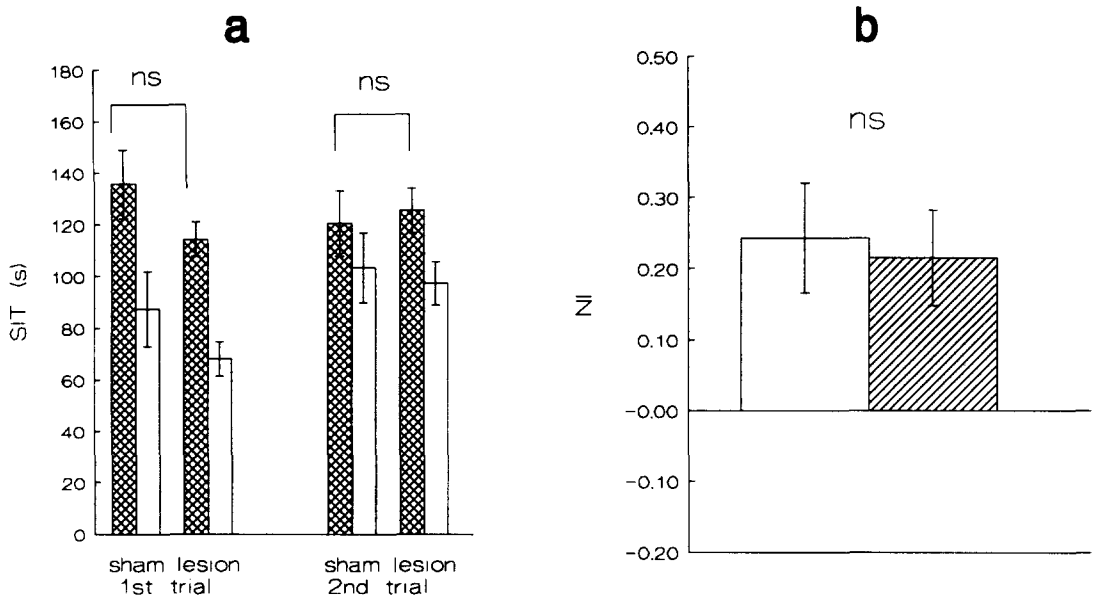

FIG. 4. (a) Mean ± SEM social investigation time for all encounters of the basolateral amygdala-lesioned group and of the sham-operated group. Hatched bars: first encounters, open bars: second encounters. (b) Mean ± SEM novelty index (NI). Open bar: sham-operated rats, hatched bar: basolateral amygdala-lesioned group, ns: nonsignificant.

During the retention trial, the lesioned rats had a significantly shorter latency, $F(1, 27) = 41.35$, $p < 0.001$. All the lesioned animals used in the social recognition test displayed an impaired avoidance behavior.

Behavioral Testing—Amygdala Lesion

The SITs of all encounters of both trials are presented in Fig. 4a. For the first and the second trials the SIT of the first encounter of the lesioned group was not statistically different from the SIT of the first encounter of sham-operated group [$F(1, 27) = 2.13$, $p > 0.15$ and $F(1, 27) = 0.11$, $p > 0.70$, respectively]. Thus, during the first encounters the amygdala-lesioned rats spent the same amount of time on social investigation as the sham-operated rats.

The NI is presented in Fig. 4b. There was no statistical difference between the amygdala-lesioned and the sham-lesioned group, $F(1,27) = 0.10$, $p > 0.75$.

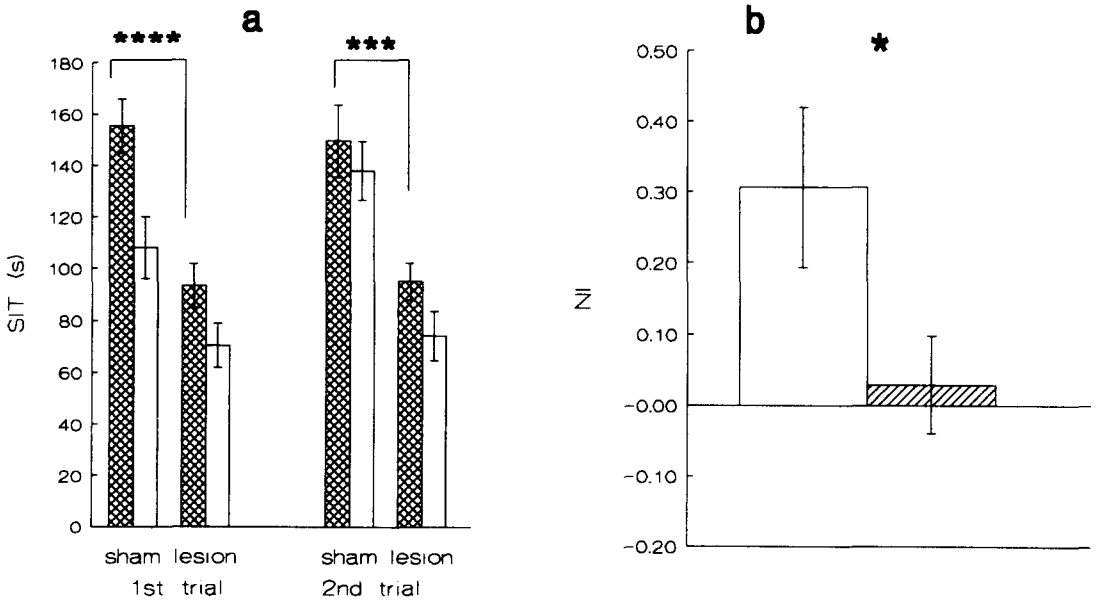

FIG. 5. (a) Mean ± SEM social investigation time (SIT) for all encounters of the fimbria-transected rats and their sham-operated counterparts. Hatched bars: first encounters, open bars: second encounters. (b) Mean ± SEM novelty index (NI). Open bar: sham-operated rats, hatched bar: fimbria-transected rats.

Both the amygdala-lesioned rats and the sham-operated rats could distinguish between the familiar and the unfamiliar juvenile [$t(14) = 3.16$, $p < 0.01$. and $t(13) = 3.13$, $p < 0.01$, respectively].

Behavioral Testing—Hippocampal Lesion

The SITs of all encounters of both trials are presented in Fig. 5a. For the first trial the SIT of the first encounter of the lesioned group was significantly lower than the SIT of the first encounter of the associated sham group, $F(1, 22) = 20.815$, $p < 0.001$. This was also true for the second trial, $F(1, 22) = 11.95$, $p < 0.005$. Thus, the lesioned rats spent less time on social investigation than the sham-operated rats.

The NI is presented in Fig. 5b. The NI was significantly lower for the hippocampus-lesioned rats than for the sham-operated rats, $F(1, 22) = 4.36$, $p < 0.5$. The lesioned rats had more difficulty discriminating between a familiar and an unfamiliar juvenile. In fact, a paired t-test revealed that the lesioned rat could not make this distinction at all, $t(11) = 0.42$, $p > 0.65$, whereas the sham-operated rats could, $t(11) = 2.65$, $p < 0.05$.

DISCUSSION

The basolateral amygdala-lesioned rats did not differ in any parameter from their controls, whereas the fimbria-transected rats had a lower SIT than the sham-operated rats for the first encounters of both trials. Their NI score was significantly lower than that of their controls. Fimbria-transected rats could not distinguish between familiar and unfamiliar juvenile rats.

The absence of any effect of the basolateral lesion cannot be attributed to the lack of an unsuccessful lesion as the step-through latency during the retention trial of the passive avoidance test was significantly shorter in the lesioned rats than in the control rats. Our initial assumption was that the amygdala could have a role in social recognition, because of its supposed role in emotional evaluation of social significant stimuli or because of its role in responding to such stimuli. The lack of any effect of the lesion on social recognition is not consistent with our assumption. We can think of two possible explanations for this result. Firstly, the cue by which the juvenile is recognized is not processed by the basolateral amygdala, but by other parts of the amygdala. Secondly, this part of the amygdala does not mediate the association of socially significant stimuli to the conditioned stimulus (recognition cue) or to the response. There is support for both arguments.

Popik et al. (47) investigated recognition cues in social memory. They found that anosmic rats were impaired in recognition. They found further that the preputial gland in juveniles was the source of the scents. Preputialectomized juveniles could not be recognized. Both findings imply that the recognition cue is olfactory in nature. In contrast to the medial and the cortical amygdaloid nuclei, the nuclei of the basolateral amygdala do not (or only sparsely) receive direct projections from the olfactory bulb (48). We may ask whether the basolateral amygdala mediates emotional evaluation of or responses to simple olfactory cues. This can be investigated using the secondary reinforcement paradigm. Hatfield et al. (22) have shown that basolateral lesions do not influence odor discrimination in a place preference task. However, taste-potentiated odor aversion learning is impaired by the lesion. They hypothesized that the basolateral amygdala uses multimodal sensory cues as a basis for associations. This may be true for olfactory and gustatory stimuli [conditioned taste aversion is not mediated by the basolateral amygdala (15)], but not for visual and auditory ones (14). We know that the insular cortex receives olfactory input from the primary olfactory cortex and projects to the basolateral amygdala (48). The role of the insular cortex in odor-based secondary reinforcement and odor–taste association is not yet clear.

A second possible reason for the absence of any effect of the lesion of the basolateral amygdala on social recognition is that this part of the amygdala is not involved in the association of socially significant stimuli to the recognition cue (odor) or to the response. There are some indications that the cortico-medial amygdala is more important in this function. We know, for instance, that this region of the amygdala is involved in social learning (60) and in the initiation of social behavior, such as sexual behavior (21) and aggression (28,53) in rats [but compare (50) for involvement of the basolateral amygdala in aggression in the rhesus monkey]. In primates, it is mainly the cortico-medial amygdala and medial parts of the accessory basal nucleus that are involved in face recognition (34) and recognition of other social relevant stimuli (7). It may well be that social investigation of the juvenile is an innate social action that is mediated by the cortico-medial amygdala. There are some indications that the basolateral amygdala is more important than the cortico-medial amygdala for the acquisition of new responses in an unpredictable context. The projections of the basolateral amygdala to the nucleus accumbens and the neostriatum (48) point in this direction. Both structures are involved, in different ways, in a noninnate selection of motor programs (6,11).

In summary, our prediction that an amygdala lesion disrupts social recognition could not be proved. In this study the lesion was restricted to the basolateral amygdala. Future research has to show whether other parts of the amygdala are involved in social recognition. One complication is that the medial amygdala plays, among others, a role in the vomeronasal system. Bluthé and colleagues (4) have shown that the high activity of this system in male rats elevates social interest, but reduces recognition. This problem could be discarded by using female rats.

The reduction of the NI in the fimbria-transected rats compared to their controls indicates that the lesioned animals were less successful in distinguishing a familiar from an unfamiliar juvenile. It is not clear whether the reason is a deficit in social memory, because the initial SIT was also affected. These animals seemed to have an overall lack of social interest. It is known that male rats that are castrated (5) or that have undergone a lesion of the vomeronasal organ (4) show, after a recovery period of approx. 2 weeks, a reduced SIT, like untreated females. However, their recognition is improved. In fact, like female rats (3), they are able to recognize the juvenile for more than 2 h, in contrast to the normal 40 min for untreated male rats. Unlike those male rats, our fimbria-transected male rats showed a combination of reduced SIT with total lack of social recognition, even after 5 min. The relation between SIT and recognition is complex. Logically one would expect that less investigation would lead to worse recognition. However, in normal male rats the elevated social interest (high SIT) compared to female rats seems to have just the opposite effect. It would therefore be advisable to use female rats in this test if, as in our case, the study is of neuropsychological nature.

Terranova et al. (56) found after cerebral ischemia absence of recognition after 30 min. Whether this lesion had any influence on the SIT was not reported. They suggest that the reason for impaired recognition is the destruction of neurons of the CA1 layer of the hippocampus after ischemia. They argued that the hippocampus is involved in working memory (in this interpretation, object recognition is obviously taken as an instance of working memory). However, we know now, especially since the research of Jarrard and colleagues (13,26), that the hippocampus proper is primarily involved in spatial and contextual information

processing (18,24), but probably not in recognition or configural association. The rhinal cortex, not the hippocampus, is involved in recognition memory, as already pointed out in the Introduction. Furthermore, it may be doubtful whether the effects of cerebral ischemia are indeed limited to the hippocampus. Wood et al. (62) found impairment of object recognition memory after an "ischemia-induced damage to the hippocampus." However, the same authors reported that object recognition was only impaired by lesion of the rhinal cortex (37), but only very slightly by a lesion of the hippocampus (38). They call these results "paradoxical." We know now (40) that silver impregnation reveals neuronal damage following ischemia in other areas of the brain, such as the cingulate area. The main conclusion of all this is that it is not clear whether our fimbria transection is comparable to the lesion in the study by Terranova et al. (56), though the effect on recognition is comparable. Another issue is that the explanation they give for their results, namely impaired working memory, is hardly able to explain our results: why should impaired object recognition (if this was indeed an acceptable explanation) reduce SIT? In castrated males and untreated females a reduced SIT even accompanies a prolonged recognition.

Some researchers have suggested that rats with a hippocampal lesion are more susceptible to distraction (54). Indeed, unlike a classical recognition test, such as a delayed matching-to-sample test, the social recognition test is not centered around a discrete reward (like food), which could bind the attention of the hippocampus-lesioned rat. But, although we cannot exclude the distraction explanation, it is not very likely. Firstly, the experiment was carried out in the home cage of the adult rat. The only "distractor" was the juvenile, which should then be responsible for an elevation of the SIT, which was not found. In fact, the SIT was heavily reduced. Secondly, though the nonsocial behaviors of the adults were unfortunately not recorded, these rats were clearly more passive than the sham-operated rats. The elevated immobility of hippocampus-lesioned rats in some situations has been seen previously [e.g., Woodruff et al. (63)].

The social deviations after hippocampal lesions could be the result of a disruption of sequential behavior, that is, an instance of conditional association. This was already postulated by Hirsch (23). Michal (35) has shown that rats with a hippocampal lesion were more rigid in their sexual behavior. Cannon et al. (10) demonstrated that sequences of self-grooming were eliminated. We (Maaswinkel et al., submitted) have analyzed dyadic social interactions between male rats after bilateral fimbria transection. The total time spent on social interaction was not different between groups; the behavior of the lesioned rats was much less dependent on the behavior of the social partner, as revealed by information statistics and sequential analysis. In this study, we did not score the behavior of the juveniles, nor the interaction between adults and juveniles. One difference between the social recognition test used here and the above-mentioned dyadic social interaction test is that in this last one both social partners were lesioned. Another difference is that in the social recognition test the lesioned adult rats spent less time on social behavior than the sham-lesioned rats, which is more comparable to the changes in maternal behavior after hippocampus lesion (31).

In summary, our prediction about the disruptive effect of the fimbria transection on the social recognition was verified, in that the lesioned rats were impaired in social recognition. However, the procedure of the social recognition task does not allow to explore the precise nature of this impairment. One possible way to meet this problem would be to study the interaction between adult and juvenile rats. In fimbria-transected rats, social interactions are heavily reduced (Maaswinkel et al., submitted). We also need to take into account another possible reason for the behavioral deviation of the fimbria-transected rats: it is known that, under certain circumstances, habituation is retarded in hippocampus-lesioned rats (20). This could be studied by applying more trials. Furthermore, the nonsocial behaviors should be scored in more detail. It would also be a good idea to use female rats for this kind of study to avoid the complex social interest/social recognition interaction of male rats, which has been revealed in the studies by Bluthé and colleagues (3–5).

As a final remark, we have to be aware that the "social recognition test" does not dissociate individual recognition and social performance. To measure exclusively recognition, the reinforcer of such a test should be nonsocial, such as is usually the case in the delayed (non-)matching-to-sample test. Thus, "social recognition" may concern: individual recognition, general social performance, or recognition-dependent social performance.

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