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Research report

Social attachment in juvenile monkeys with neonatal lesion of the hippocampus, amygdala and orbital frontal cortex

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Abstract

Non-human primates, like humans, develop and maintain social relationships and attachments throughout their life. The first and most crucial relationship in a primate life is that with its mother. Yet, in absence of their biological mother, infant primates form attachment to surrogate mothers. Although, this early attachment is critical for the development of normal species-typical social and emotional skills, the neural substrates underlying the formation of social relationships in primates are still unclear. The present study assessed, in infant rhesus monkeys (*Macaca mulatta*) reared by human caregivers and social interactions with peers, the effects of bilateral neonatal (1–2 weeks of age) ibotenic acid lesions of the amygdala and hippocampus ($N=6$ in each group), aspiration lesions of the orbital frontal cortex ($N=6$) or sham lesions ($N=5$) on the development of a social attachment with the principal human caregiver. A specific preference for the later was assessed at 11 months of age, in a two-choice discrimination task, opposing the principal human caregiver to another familiar human, in a familiar environment. None of the lesions impaired the expression of preferential responses toward the principal human caregiver. Nevertheless, lesions of the orbital frontal cortex led to a weaker preference, suggesting that this structure may play a role in the quality and/or strength of the infant/mother relationships. The present non-human primate findings are discussed in terms of their relevance for autism.

Keywords: Non-human primates; Social attachment; Preference; Human caregiver; Autism; Neural structures

1. Introduction

1.1. Filial attachment and autism

Autism and, more specifically autism spectrum disorders (ASD), is a pervasive developmental disorder characterized by disruption of socio-emotional behavior and communication. Early clinical diagnostic features or theories have indicated that failure to develop social attachment and, particularly attachment with the mother in early infancy, may be at the source of this neurodevelopmental disorder [32,62,102]. Recent studies, however, indicate that individuals with autism do indeed form attachment with their mother [99,107]. Thus, during separation/reunion situations in unfamiliar laboratory settings [3], children with autism, like children without autism matched for mental age, showed higher preferential responses (increased orientation, approach, physical contact, proximity seeking, contact maintenance, language or affective display) toward their caregiver than toward a stranger, especially upon reunion episodes [38,39,92,93,106,108]. In addition, they can also display extreme distressed behaviors (i.e. crying, calling and holding) when separated from their mothers [40]. This indicates that children with autism have the capacity to discriminate and preferentially select their caregiver. Furthermore, children with autism, like normally developing children and children with Down syndrome, use their caregiver as a secure and comforting base for exploring the environment, especially when the environment becomes challenging or alarming [38]. Finally, the proportion of children with autism securely or insecurely attached to their caregiver is similar to that observed among those with developmental language disorder or mental retardation [38,39,93,106,123], although children with autism,

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especially those with lower mental development, show more signs of insecurity than their matched comparison groups and are less responsive to contacts with their caregivers [99]. Yet, there are some concerns that the classification commonly used to assess the quality of the attachment relationship with the caregiver (strange situation procedure [3]) is not adapted for children with cognitive disabilities, such as Down syndrome [89,117] and the same restriction might applied for autistic children with severe mental retardation [99]. Nevertheless, children with autism appear to possess the separation/reunion equilibrium that has been described as one of the main characteristic of the mother–infant attachment [4].

What remains unknown, however, is whether the development and maintenance of filial attachment in autistic individuals is based on behavioral and cognitive processes (e.g. early pattern of reciprocity, synchronization and reciprocal modulation within the mother–infant dyad, as well as security patterns) normally seen in typically developing children in the first 2 years of life. Thus, based on the relation between quality of attachment (i.e. patterns of behavior and security scores) and cognitive and social abilities found in children with autism [26,39,92,93,123], some researchers have suggested that early development of filial attachment in children with autism might either be delayed [93,107] or induced via different cognitive compensatory strategies, especially in higher-functioning autistic children [99,107]. Yet, if such compensatory strategies exist and allow the early formation of filial attachment, they may not be sufficient for the later development of more complex reciprocal social interactions or relationships. For instance, impairments in "theory of mind" tasks, which required internal working model of self, others and their interdependence, in children with autism have been interpreted as reflecting a lack of normal progression from a basic psychobiological level of filial attachment toward a more advanced psychological stage requiring an internal working model of the relationship with the caregiver [26,40,92,93,99]. The presence of a deficient working model of social relationships in autistic children in the first years of life may in turn lead to protracted deficits in forming and maintaining reciprocal social interactions later in life. Thus, a better understanding of the early development of filial attachment as well as of its neurobiological substrate appears critical to shed lights on the origin of the socio-emotional dysfunction in autism.

1.2. Filial attachment in primates

Primates, humans and non-humans, live in societies characterized by complex and dynamic social structures that are maintained through multiple and specific social relationships among the individuals in the society. Attachment to the biological mother is the first social relationship that primates develop in early infancy. In addition to provide basic survival needs, such as energy via food and warmth, the mother serves as a source of comfort and reassurance when infants are faced with dangers or challenges during exploration of their environment [3,22,77,121]. The presence of the mother, thus, facilitates the development of emotional stability in the infant [5]. This early social attachment, or filial attachment, is characterized by proximity-seeking and proximity-maintaining behaviors, and its disruption results in intense distress biobehavioral symptoms, such as changes in heart rate, body temperature, immunological variables, CSF metabolites levels, plasma cortisol concentrations, sleep patterns, locomotion and vocalizations (see [91] for review). These stress responses rapidly disappear following reunion with the mother [51,78]. In the complete absence of the mother, non-human infant primates will develop attachment to other conspecific peers [20], to heterospecific individuals, such as dogs [75], and even to biologically improbable objects, such as cloth-covered cylinder [54], presumably in an attempt to reach emotional stability. Research in humans have shown that attachment to a specific being, or object, represents a long lasting emotional bond as well as an integrative perceptual/cognitive achievement that requires several cognitive processes linking sensory inputs to motor outputs and including motivation, attention, memory and social recognition [22]. These processes develop progressively and are influenced by many variables that include maturity of the subject, individual and species differences, characteristics of the attachment object or being, and early rearing environment conditions [49,77,96]. The significance of mother–infant attachment for the normal development of emotional bonding later in life has mainly been demonstrated in studies following social skills in children or infant monkeys that have encountered abnormal filial attachment in early infancy.

1.3. Filial attachment and social competence

Infant monkeys reared with inanimate surrogate mothers during the first few months of life exhibit persistent social deficits together with inadequate development of affiliative behaviors [53,115]. Rearing with peers only partially compensates for many of the socio-emotional deficits seen in surrogate-reared monkeys. Thus, peer-reared, as compared to mother-reared, infant monkeys display higher distress responses when placed in a novel setting even in presence of their favorite peer [55], suggesting that they have developed insecure social relationships [5]. Peer-reared infant monkeys have also difficulties in assessing social dominance status (indicator of social success in rhesus monkeys) not only as juveniles, but even more so as adults [11]. Finally, the quality of the relationship with the mother also influences the social and emotional development of the young primate. Thus, infant monkeys reared in semi-naturalistic social groups by naturally abusive mothers, or by experimentally induced highly stressed mothers (i.e. by imposing environment with unpredictable foraging demands), developed insecure filial attachment and, although they appeared to develop normally, they became less socially competent, more fearful, and hyperresponsive to stressful stimuli as they mature [73,97,104]. Similarly, human children developing less secure attachment to their mother are often less socially competent than children having a secure filial attachment [88]. For instance, children raised by abusive caregivers develop disordered/disorganized attachment to their caregivers, and show difficulties in peer relationships, cognitive delay, alterations in hormonal response to stress and higher frequency for depressive symptoms, conduct disorders and drug abuse tendencies [31]. Children raised

with no particular figure of attachment, such as those placed in institutional care from an early age, display severe and persistent (even after adoption in a caring family) social, cognitive and emotional difficulties, such as motor stereotypies (abnormal repetitive behaviors), abnormal aggressivity, language delays, as well as unusual, often disordered attachment patterns, associated with lack of selectivity in social relationships and interactions, i.e. indiscriminate friendliness [29,98,100,101,110,127–129].

All of these findings indicate that both the socio-emotional stimulation provided by the mother early in life and the quality of the mother–infant relationship play a critical role in the development of normal and adaptive social and emotional competences in both human and non-human primates. Indeed, Nelson and Panksepp [82] have argued that filial attachment constitutes the roots of affiliative behavior expressed throughout life and that the neural mechanisms underlying its formation are organized into a "socially directed motivational system". This motivational system modulates the development and expression of concurrent and future affiliative behaviors, including the formation of later attachments, such as adult heterosexual and parental attachment [21,22]. Based on an evolutionary perspective, it seems reasonable to suppose that the neural mechanisms underpinning filial attachment early in development are also those underlying social bonds, more generally [67].

1.4. Neural basis of social attachment

Despite the importance of mother–infant attachment for social competence later in life, the neural systems underlying the formation and maintenance of filial attachment and social relationships in primates are still poorly understood. Because non-human primates, like humans, develop and maintain social relationships [28,35] through their life and particularly early in infancy with their biological mother [113], or, in absence of their mother, with other individuals [20,75], they provide an excellent animal model to study the neural basis of social attachment.

A growing number of neurobiological primate studies provide evidence for a complex neural network, including the amygdala and the ventral frontal lobe (e.g. orbital frontal cortex), underlying the regulation of socio-emotional/affective cognition [1,2,17,19,41,71]. The amygdala is involved in the perception and recognition of socially salient stimuli, such as facial expression of emotion, eye-gaze directions, body postures and movements, as well as in the identification of social significance, initial social judgment of other individuals and social interpretation of ambiguous situations [1,23,24,86,87,126]. The amygdala also plays a role in stimulus-reward association, i.e. in the process of associating discrete stimuli with their intrinsic reward value, or biological significance [15]. By contrast, the orbital fontal cortex is less important for perceiving and identifying the significance of stimuli, but rather, anticipates, monitors and uses this information to evaluate, guide and adjust goal-directed behaviors upon changing contexts [7,16,18,34,36,37,56,57,68,94,95,116]. Finally, both brain structures have been involved in the formation of social attachments in non-primates species, such as sheep [63–66], voles [124,125], and rats [81]. Thus, both structures are likely to play a critical role in the formation and/or maintenance of early social attachments in primates as well. There is only one study in non-human primates that has directly investigated the role of the amygdala in the development of filial attachment. Bauman et al. [12] observed that animals with neonatal damage to the amygdala displayed apparently normal interactions with their mother and showed recognition behaviors. However, unlike sham-operated controls and those with neonatal hippocampus lesions, they showed no preference for their mother immediately after weaning at 6 months of age. The authors attributed this lack of preference to an inability to perceive potential danger (e.g. the unfamiliar environment or unfamiliar stimulus monkey), rather than a disruption of the filial attachment formation per se. Thus, this finding suggests that the quality of the attachment to the mother, rather than the attachment itself, may have been affected by the early amygdala lesion. Similarly, as reviewed above, autistic children display attachment behaviors toward their caregiver, but the quality of the filial relationship may be different to that of normally developing children. Thus, it is possible that similar neural systems, including the amygdala and the orbital frontal cortex, may underlie the development of filial attachment in autism as well. This possibility is strengthened by growing evidence from neuroimaging studies in autistic individuals demonstrating dysfunction of a neural network that includes the amygdala and orbital frontal cortex [8]. To further assess the respective contribution of the amygdala and orbital frontal cortex in filial attachment, the present study investigated the effects of bilateral neonatal lesions of the amygdala, orbital frontal cortex or hippocampus, on the expression of a preference for a principal human caregiver as compared to a familiar human, in juvenile rhesus monkeys. Preliminary results from this study have been presented elsewhere [48].

2. Method

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Texas at Houston, and were conformed to the NIH Guide for the Care and Use of Laboratory Animals (HHS publication 85-23, 1985). Prior to this experiment, all animals had received behavioral and cognitive testing to assess maturation of visual orientation, motor coordination, irritability, and discrimination of facial social cues (1–4 weeks of age), object and spatial recognition memory (2, 6 and 8 months of age), defensive reactions to a human intruder (2 and 5 months of age) and dyadic social interactions with peers (3 and 6 months of age).

2.1. Subjects

Twenty-four full-term infant rhesus monkeys (*Macaca mulatta*), born from multiparous mothers, served as subjects in the present study. They arrived in three cohorts of eight animals of both males and females born approximately a year apart. Between 1 and 4 days of age they were brought from the breeding facility of the University of Texas, M.D. Anderson Cancer Center Science Park (Bastrop, TX) to the primate-nursery of the M.D. Anderson Cancer Center (Houston, TX) and were raised by human caregivers and with age-matched peers. Each cohort included two infants from each of the following experimental groups: group Neo-A_{ibo} received bilateral neurotoxic lesion of the amygdala, group Neo-H_{ibo} received bilateral neurotoxic lesion of the hippocampus, group Neo-O_{asp} received bilateral aspiration lesion of the orbital frontal cortex, and group Neo-C received bilateral sham operations. Each group included six animals, three males and three females, except group Neo-H_{ibo} which included four males and two females, and all surgical procedures occurred between 10 and 15 days of age.

At their arrival in the laboratory, all infant monkeys were first hand-fed a diet of infant Similac formula (SMA with iron). At around 3–4 weeks of age, when self-feeding had been acquired, their diet was supplemented with banana pellets (190 mg, P.J. Noyes, Cleveland, OH). At 3 months of age, the diet of all infant monkeys was supplemented with Purina monkey chow (PMI Feeds), and pieces of fresh fruits were given every day. From 8 months to 1 year of age, fresh fruits served as a reward after behavioral or cognitive testing. Water was always available. To follow normal ponderal growth, infants' weight was monitored daily until 3 months of age and weekly thereafter.

2.2. Rearing conditions

Upon their arrival in the primate nursery, the 1–4-day-old infant monkeys were placed individually in open plastic box within larger wire cages (40 cm × 30 cm × 40 cm) and under open radiant incubators. The incubator accommodated two wire cages allowing somato-sensory contacts between two infants in adjacent wire cages. In addition, the wire cages allowed visual, auditory and olfactory contacts with all other infants in the nursery. Contact comfort was provided by a synthetic plush surrogate (approximately 30 cm in length) and a cotton towel hung from the top of the cage as well as several towels placed on the floor and the sides of the plastic box. At 1 month of age, the plastic boxes were removed allowing the animals to freely move within the wire cages. At 3 months of age, animals were transferred to larger wire cages and housed individually, although physical contacts were possible between pairs of infants through the large central mesh separating two adjacent cages.

Each cohort was raised by a principal human caregiver who fed and handled them several times a day from the day they arrived in the primate nursery until the end of the present experiment. The principal human caregiver spent approximately 6 h daily, 5 days a week in the primate nursery with the infants. On week-end, familiar human caregivers fed, handled and played with the infants 2–3 times a day for a total of 2–4 h both days. In addition, at 3 months of age until approximately 9 months of age, all infants received daily social interactions (3–4 h, 5 days/week) with three other age- and sex-matched peers of the same cohort and in the presence of the principal human caregiver and 1–3 of the familiar human caregivers. Socialization took place in a play pen/cage located in the primate nursery and containing toys and towels. Finally, when the infants reached approximately 7 months of age, one adult multiparous female rhesus monkey was introduced in the nursery room and placed in an individual cage allowing visual, auditory and olfactory, but not physical, contacts with the infants.

2.3. Surgical procedures

2.3.1. Magnetic resonance imaging (MRI) procedures

Because of the inter-individual variability between monkey brains, MR images of the brain of each subject were acquired immediately prior to surgery (pre-surgical MRI scans). This procedure allowed selection and calculation of the injection site coordinates (bilaterally) within the amygdala or the hippocampus (see [74] for details) and localization of the lateral and medial orbital frontal sulci, which were used as lateral and medial borders for the orbital frontal cortex lesions. On the morning of surgery, the infant monkey was anesthetized by isofluorane inhalation (1–2% to effect), intubated with an endo-tracheal canula to maintain adequate levels of anesthesia through the MRI scans and surgical procedures. The monkey's head was then secured into a non-ferromagnetic stereotaxic apparatus (CRIST instruments Co., Inc., Damascus, MD). Measurements of the positions of ear bars, mouth plate, and mouth piece were recorded to allow precise re-positionning of the animal in the stereotaxic apparatus for ulterior MRI scanning (post-surgical MRI scans, see below). The stereotaxic apparatus was then aligned in the scanner and the monkey's brain was imaged.

The MR images were acquired with a GE Signa 1.5 T Echo Speed scanner (GE Medical Systems, Milwaukee, WI) using a 7.5 cm diameter surface coil. The earbars and midline sinus were always used as reference. For all animals of the four experimental groups, including those receiving sham-operations, a first structural series of images (T1-weighted spin-echo sequence, echo time (TE) = 11 ms, repetition time (TR) = 450 ms, contiguous 4 mm sections, 12 cm field of view (FOV), 256 × 256 matrix) was acquired in the sagittal plane and used to align the next series of images. The second series (3D T1-weighted fast spoiled gradient (FSPGR)-echo sequence, TE = 2.6 ms, TR = 10.2 ms, 25° flip angle, contiguous 1 mm sections, 12 cm FOV, 256 × 256 matrix) was acquired in the coronal plane. In addition, animals in groups Neo-A_{ibo} and Neo-H_{ibo} received three series of images (Fluid Attenuated Inversion Recovery (FLAIR) sequence, TE = 140 ms, TR = 10,000 ms, inversion time (TI) = 2200 ms, 90° flip angle, contiguous 3 mm sections, 14 cm FOV, 256 × 256 matrix) acquired in the coronal plane and offset by 1 mm in the anterior/posterior axis. The FLAIR sequence reveals tissue T2 prolongation with cerebrospinal fluid suppression. An entire MRI session lasted from 45 to 60 min, after which the animal was kept anesthetized in the stereotaxic apparatus, placed in an incubator, and immediately brought to the surgical suite.

Approximately one week following surgery, animals in groups Neo-A_{ibo} and Neo-H_{ibo} received a second MRI session (post-surgical MRI scans) using the same procedures and sequences as those used during the pre-surgical MRI session. This second MRI procedure was used to evaluate the location and extent of the lesion [74,83] by comparing pre- and post-surgical FLAIR images (see below) to identify hypersignals (optimal for less than 2 weeks after injection of ibotenic acid) resulting from edema caused by neurotoxin-induced cell death. Animals of group Neo-O_{asp} also received a second structural post-surgical MRI scan (3D T1-FSPGR), which was compared to the pre-surgical T1 scan to evaluate the extent of the orbital frontal lesions. This post-surgical scan was done 1 month after surgery to provide sufficient time for brain tissue around the lesion to ill and reduce artifacts on the MR images. The sham-operated animals also received a second MRI scan, approximately a month after their sham-surgery.

2.3.2. Surgical procedure

The single-stage surgeries followed immediately the MRI scanning session and were performed aseptically. Throughout surgery the animals were maintained under gas anesthesia (isoflurane/O_2, 1–2% to effect) and vital signs (heart and respiration rates, blood pressure, body temperature and expired CO_2) were monitored. An intra-venous drip of 5% dextrose and 0.5% sodium chloride maintained normal hydration for the duration of the surgery. The animal's head was shaved, the skin was disinfected with Novalsan solution, and a long-acting local anesthetic (Marcaine 25%, 1.5 ml) was injected subcutaneously along the midline skin incision. After the midline incision, the connective tissue was gently displaced laterally to expose the skull.

For the ibotenic acid injections in the hippocampus or amygdala, two craniotomies were made bilaterally, in front of bregma and above the respective neural structure and the dura was incised to expose the brain. The injections of ibotenic acid (Biosearch Technologies, Novato, CA, 10 mg/ml in PBS, pH 7.4) were made simultaneously in the two hemispheres using 30 gauge needles attached to 10 μl Hamilton syringes held in Kopf manipulators (David Kopf Instrument, Tujunga, CA). The needles were slowly lowered at each injection site, and 0.4–0.6 μl (hippocampus lesion) or 0.2–0.4 μl (amygdala lesion) of ibotenic acid was manually injected at a rate of 0.2 μl/min. After each injection, the needles were left in place for an additional 3 min period to allow diffusion of the drug at the tip of the needle and minimize its spread in the needle track during retraction of the needles. The needles were then slowly raised out of the brain and their tips were swabbed to remove any residual neurotoxin or blood before being lowered at the next injection site. A total of 2.8–4.2 μl of ibotenic acid was injected into 7–8 sites along the hippocampus and was intended to target the uncus and the hippocampus along its entire length (Fig. 1, left column). A total of 0.8–1.6 μl of ibotenic acid was injected into four sites centered within the amygdala and was intended to target all amygdala nuclei (Fig. 2, left column). To control for potential brain swelling, before the last neurotoxin injection, the animal was given 30 ml of Mannitol (20%, i.v.) at a rate of 1 ml/min.

The bilateral orbital frontal lesions were made by subpial aspiration lesions [79]. Following a craniotomy (approximately 2.5 cm × 1.5 cm) above each orbit, the bone of the supra-orbital ridge was slightly eroded with a drill. Bilateral incisions were made in the dura, and the frontal lobe was gently elevated to access its ventral aspect. Microscope-guided aspiration of the orbital frontal cortex was made through small 21 and 23 gauge aspirating probes. The lesion of the orbital frontal cortex was intended to include cytoarchitectonic areas 11 and 13 [9,27]. Borders for the intended orbital frontal lesions (Fig. 3, left column) included a line joining the anterior tips of the lateral and medial orbital sulci anteriorly, the medial lip of the lateral orbital sulcus laterally, the olfactory stria medially, and ended posteriorly at a level where the olfactory striae turn laterally. Using these landmarks, the orbital frontal lesions was largely restricted to areas 11 and 13 anteriorly, but extended slightly into area I_a (anterior insular area) more posteriorly.

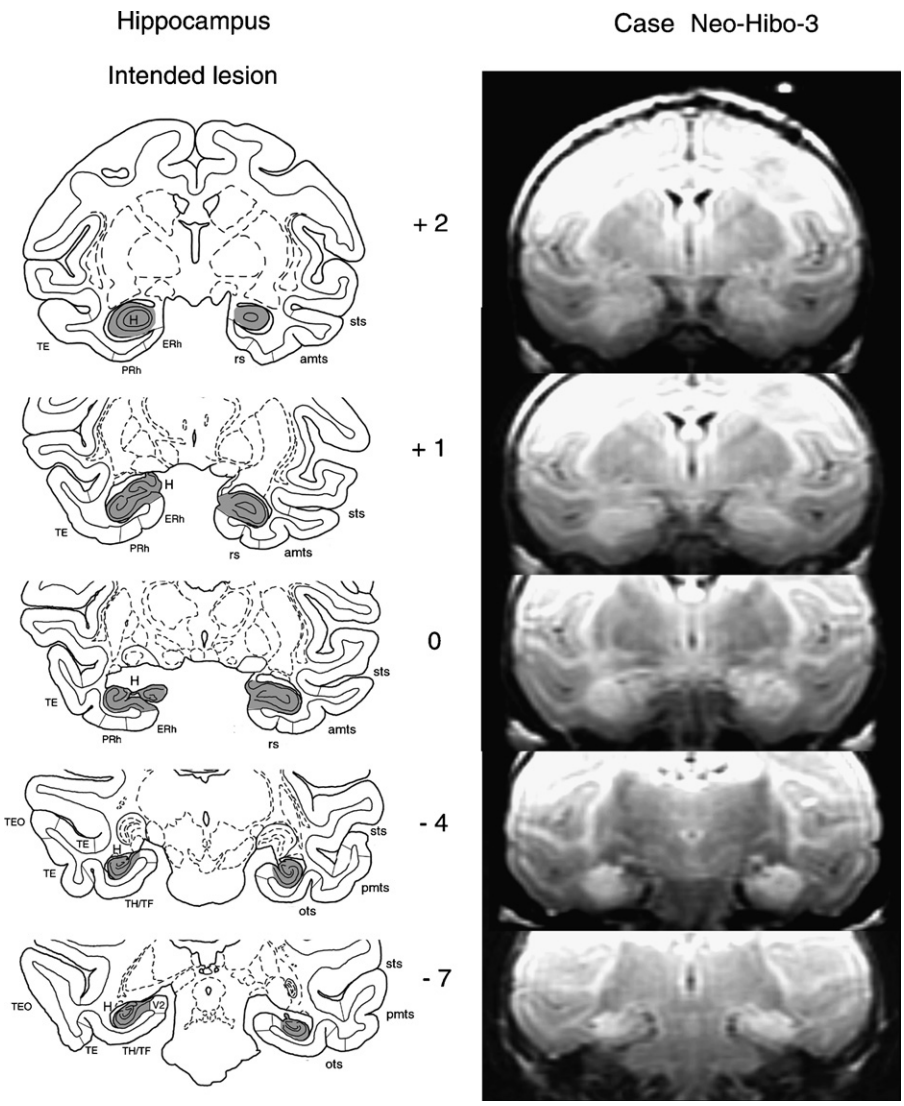

Fig. 1. Drawings of coronal sections through five levels of the hippocampus of a normal 1-week-old infant monkey brain depicting the intended damage in gray (left column) and coronal fluid attenuated inversion recovery (FLAIR) magnetic resonance (MR) images at the corresponding levels for case Neo-H_{ibo}-3 (right column). On the FLAIR MR images, areas of hypersignals (white areas) indicate edema induced by injections of the neurotoxin (ibotenic acid). For each section, the numbers indicate the distance in millimeters from the interaural plane. Abbreviations: amts, anterior medial temporal sulcus; H, hippocampus; ERh, entorhinal cortex; ots, occipito-temporal sulcus; pmts, posterior medial temporal sulcus; PRh, perirhinal cortex; rs, rhinal sulcus; sts, superior temporal sulcus; TE, TEO and TH/TF, ventral cortical areas of the temporal lobe; V2, extrastriate visual area V2.

Sham-operations included bilateral opening of the skull and dura at approximately the same location as for the amygdala and hippocampal lesions, but no needle penetration and no injection were performed.

At the completion of all surgical procedures, the wound was closed in anatomical layers with vicryl sutures (Ethicon Inc., Johnson & Johnson, NJ), after which the infant monkey was removed from the stereotaxic apparatus and placed in an incubator ventilated with oxygen until it regained complete consciousness.

2.3.3. *Pre- and post-surgical treatments*

All infants including the sham-operated monkeys received the following pharmacological treatments. As a prophylactic measure against infection, cephalexin (25 mg/kg, p.o.) was administered the night prior surgery and was continued once a day up to 7 days after the surgery. As a precaution against brain edema, dexamethasone sodium phosphate (0.4 mg/kg, s.c.) was given the night before and the day of surgery, and was continued for 4 days after surgery with progressive withdrawal of the drug. For analgesia, acetaminophen (Tylenol, 10 mg/kg, p.o.) was given twice after completion of surgery and was continued for 5 days after surgery. In addition, to prevent infection of the wound, all infants were diapered the night following surgery, the wound was cleaned daily, and antibacterial ointment (bacitracin–neomycin–polymyxin) was applied to the skin sutures twice a day for as long as necessary. No major complications were encountered either during or following all surgical procedures.

2.4. *Lesion verification*

For each animal of groups Neo-H_{ibo} and Neo-A_{ibo}, each coronal FLAIR image was matched to a series of drawings of coronal sections of a 1-week-old normal rhesus monkey brain (J. Bachevalier, unpublished data), acquired at 1 mm interval. The extent of hypersignals on each MR image was visually identified and plotted onto the corresponding images of the brain of the normal infant monkey. These drawings were then imported into ImageJ® to measure the surface area (in pixels) of damage to intended target area (hippocampus and amygdala), as well as to unintended adjacent areas (entorhinal and perirhinal cortex, parahippocampal areas TH/TF, areas TE, TEO and V2). For each intended and unintended structures in each hemisphere, the measured surface area on each

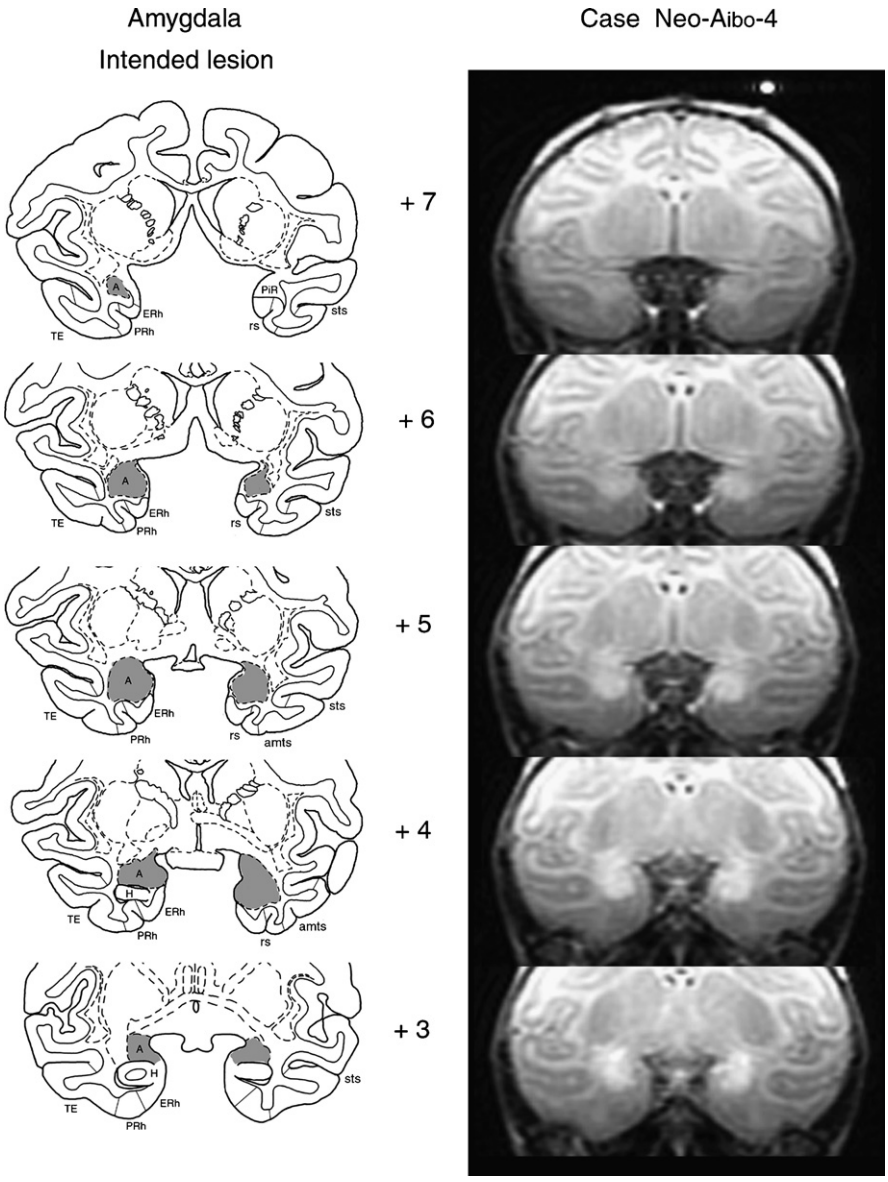

Fig. 2. Drawings of coronal sections through five levels of the amygdala of a normal 1-week-old infant monkey brain depicting the intended damage in gray (left column) and coronal fluid attenuated inversion recovery (FLAIR) magnetic resonance (MR) images at the corresponding levels for case Neo-A_{ibo}-4 (right column). A, amygdala; PiR, piriform cortex; for other abbreviations and details on the FLAIR scans see legend of Fig. 1.

section was summed and then multiplied by image thickness (1 mm) to calculate a total volume of damage [50]. The volume of damage to each structure (intended or unintended) was then expressed as a percentage of the normal volume (previously estimated from the normal infant rhesus monkey brain, using similar method) for that structure (see [83] for details).

For each animal of group Neo-O_{asp}, the extent of cortical removal was visually evaluated on each coronal MR images, using the pre- and post-surgical FSPGR scans. Using the same procedures as those described for groups Neo-H_{ibo} and Neo-A_{ibo}, the extent of cortical damage was first plotted onto matched coronal images of the brain of the normal infant monkey, and percentage of intended damage to cortical areas 11 and 13, and to unintended damage to adjacent cortical fields (10, 14, 12, and I_a) were calculated.

2.5. Behavioral procedure

2.5.1. Apparatus

The two-choice discrimination task was performed into a large rectangular enclosure (3.2 m × 1.8 m × 2.1 m, Fig. 4), which was made of wire-mesh (back and front walls, ceiling, and floor) and clear Plexiglas (opposite side walls) to allow video-recording during testing. This enclosure was divided into four three-dimensional zones (front left, front right, back left and back right) of 1.6 m × 0.9 m × 2.1 m each, relative to the position of the stimuli. A neutral zone included the release cage, positioned securely in the center of the back zone, as well as the central pole with perches, positioned in the very center of the enclosure. In addition, the left and right front zones were further divided to include a proximity zone (0.7 m × 0.3 m × 0.9 m) just in front of each of the stimuli located outside of the enclosure. To optimize visual exploration between the infant monkey and the stimuli, two transparent Plexiglas windows (30 cm × 50 cm) were positioned in the wall separating the stimuli (outside the enclosure) from the monkey (inside the enclosure), in the middle of each proximity zone (see Fig. 4B). When the infant monkey was in the proximity zones, the wire mesh around both plastic windows allowed physical contacts with the stimuli. Finally, a door in the back zone allowed introduction of the animal, within the release cage, inside the enclosure. A pulley system permitted the experimenter (hidden behind a curtain) to open the release cage door from a distance (see Fig. 4B).

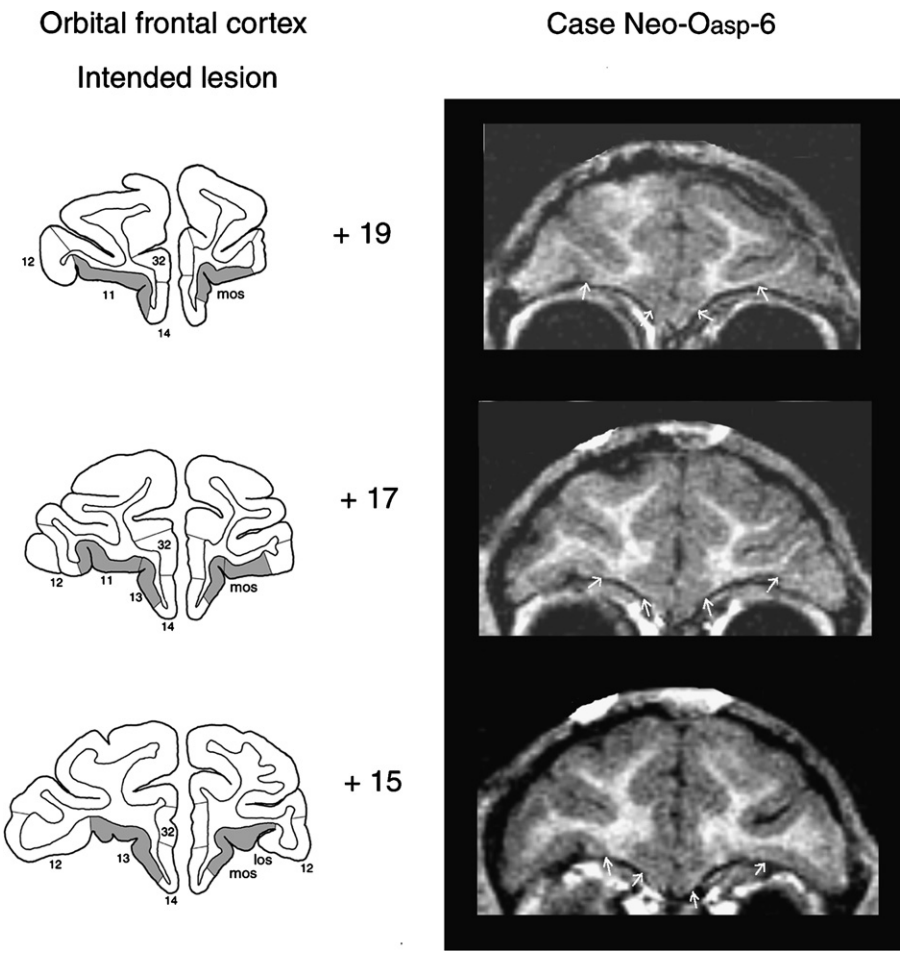

Fig. 3. Drawings of coronal sections through three levels of the orbital frontal cortex of a normal 1-week-old infant monkey brain depicting the intended damage in gray (left column) and coronal post-surgical T1-weighted fast spoiled gradient (FSPGR, see text for details) MR images at the corresponding levels for case Neo-O_{asp}-6 (right column). Areas 11, 12, 13, 14, 25 and 32 are cytoachitectonic fields of the ventral frontal cortex as defined by Carmichael and Price (1994). Abbreviations: mos, medial orbital sulcus; los, lateral orbital sulcus. The white arrows (right column) indicate the borders of cortical damage.

2.5.2. Stimuli

The principal human caregiver and another familiar human caregiver served as stimuli. Though the principal human caregiver differed between the three cohorts, the other familiar human caregiver remained the same for all subjects.

2.5.3. Habituation period

At 9 months of age, infant monkeys of the same cohort were socialized daily (1 h, 5 days a week, for approximately 2 months) in the large enclosure to familiarize them with the handling procedures and testing environment. This training was performed by one of the experimenter (APG) to ensure that none of the human stimuli to be used in the discrimination task could be seen in the testing room by the infant monkeys.

2.5.4. Discrimination task

At 11 months of age, each infant monkey received a 10 min discrimination task in the large enclosure, during which it could choose between the principal human caregiver and another familiar human caregiver (Fig. 4). Before the onset of the task, the human stimuli were notified to accept and reciprocate physical contact initiated by the infant monkey but to never initiate it. The two human stimuli then sat on the floor behind each Plexiglas window of the proximity zone. The position (left or right) of the principal human caregiver was randomized across animals. The animals were tested in a pre-determined order independently of their experimental group. At the beginning of the task, after the two stimuli were positioned, the infant monkey was brought into the release cage, and after 10 s, the door of the release cage was open from a distance by the hidden experimenter and the monkey was allowed to freely explore the large enclosure for the 10 min session. The entire session was recorded with two digital video-camera recorders (Sony, model DCR-TRV 250) positioned in front of each of the two Plexiglas walls.

2.5.5. Behavioral measures

The Observer Video-Pro Software (Noldus, Netherlands) (Noldus, 1991) was used to collect behavioral data on each animal. Several parameters were recorded for each zone (proximity, back, front and neutral): frequency of visits and time spent in each zone, position relative to the stimuli (principal human caregiver side or familiar human side) within each zone, latency to reach each proximity zone as well as frequency of each type of vocalizations (coo, scream, grunt, bark and girn, see Table 1). In addition, in order to specify the type of behavior displayed by the animal while in proximity with the stimuli, three contact zones were also defined: human-contact, mesh/Plexiglas-contact and no-contact (Table 1). Latencies, frequencies of visits and time spent in each of these contact zones were recorded for each animal. Finally, to directly assess the preference for the principal human caregiver, an index of preference (IP, defined by Goursaud and Nowak [46]) was calculated as follows: IP = (duration of proximity with caregiver − duration of proximity with familiar human)/total duration of proximity with both stimuli. The IP maximum value is +1 (all the time in proximity with the principal human caregiver) and its minimum value is −1 (all the time in proximity with the other stimulus). Thus, a positive IP value signifies that the animal displays a preference for the principal human caregiver, whereas a negative IP value indicates that the animal preferred the other stimulus (familiar human), and a null IP value signifies that the animal expressed no preference for either stimuli.

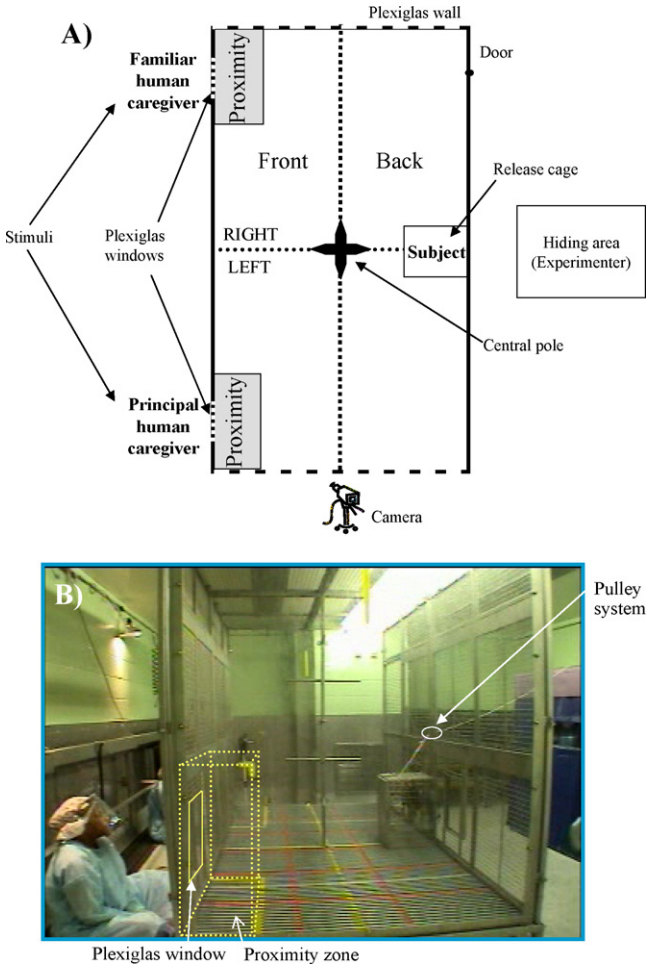

Fig. 4. (A) Schematic representation of a top view of the enclosure used for the two-choice discrimination task (A). Details of the experimental apparatus and behavioral testing procedure are given in the text. (B) Photograph of the side view of the testing enclosure. The dotted rectangular 3D box is a schematic representation of the left proximity zone and of the position of the Plexiglas window (see text for details).

2.6. Statistical analysis

The data (first 2 min and total 10 min of the task) were analyzed using one-, two- and three-ways parametric multivariate analyses of variance. Group was always the between-subjects factor. The Huynh–Feldt correction for degree of freedom was used for the repeated measures. Pairwise comparisons used univariate repeated analysis of variance and/or Bonferoni corrected *t*-tests, as appropriate. In addition, the index of preference (IP) was compared to chance level (i.e. IP value = 0) using one-sample paired *t*-tests. For data not normally distributed, non-parametric Kruskal–Wallis, Mann–Whitney *U*-test or Wilcoxon signed ranks test were used as appropriate. Pearson correlation test was used to assess relationship between percent tissue damaged and IP values. For all analyses, significance was set at $p \leq 0.05$ (two-tailed). Yet, given the small sample size in each group, values of p between 0.05 and 0.08 were also reported and considered as showing a tendency toward significance. Since the analysis of the first 2 min of the discrimination task gave results similar to that of the total 10 min, only the results for the total 10 min sessions are presented below.

Finally, a first set of analysis led us to exclude one animal from group Neo-C from all further analysis. This infant monkey was born prematurely, had a slower maturational rate than the other monkeys of the group, and displayed no preferential attachment responses in the discrimination task.

Table 1
Definitions of proximity zones and vocalizations

Parameters	Description
Proximity zones (states)	
Human-contact	In physical contact with the stimulus
Mesh/Plexiglas-contact	In physical contact with wire mesh of the enclosure or Plexiglas of the window but not in contact with the stimulus
No-contact	In proximity but not in contact with either the stimulus, wire mesh of the enclosure, or Plexiglas of the window
Vocalizations (events)	
Coo	Long duration call with initial rise then fall in frequency; lips rounded and pursed (affiliative vocalization to facilitate maternal retrieval)
Scream	High-frequency and high intensity sound; lips pulled back showing the teeth (fear/distress call)
Grunt	Soft and guttural, low frequency sound; mouth closed (affiliative vocalization)
Bark	Short, sharp and guttural, low frequency sound; mouth wide open (aggressive vocalization)
Girn	Low frequency, soft, nasal whine. Lips are closed or slightly open. Often given in bouts (infant affiliative or contact vocalization, often emitted when reunion with conspecifics, especially mother)

3. Results

3.1. Assessment of lesion extent

Table 2 displays for each monkey the volume of damage estimated from the FLAIR MR-images in groups Neo-H_{ibo} and Neo-A_{ibo} and from the structural T1-weighed images for group Neo-O_{asp}.

3.1.1. Group Neo-H_{ibo}

The extent of hippocampal damage across the two hemispheres ranged from 3.9% to 87.4% (average: 48.1%). Cases Neo-H_{ibo}-2 and Neo-H_{ibo}-3 had extensive (mean *X*: 77.5%) and symmetrical (mean *W*: 59.8%) lesions. Fig. 1 (right column) displays coronal FLAIR images through the hippocampus lesion in case Neo-H_{ibo}-3. Three cases (Neo-H_{ibo}-1, Neo-H_{ibo}-4 and Neo-H_{ibo}-5) had a moderate (mean *X*: 43.2%) and asymmetrical (mean *W*: 11%) lesions, with extensive damage on one side (mean *X*: 71.8%) but less on the other side (mean *X*: 14.6%). The sparing included the hippocampus almost entirely on the right for case Neo-H_{ibo}-1, and the medial portion of the body and/or the uncus on the left for cases Neo-H_{ibo}-4 and Neo-H_{ibo}-5. Finally, case Neo-H_{ibo}-6 had no damage to the hippocampus bilaterally, except for minor damage (<8%) to the most anterior portion of the left hippocampus. Damage to the adjacent cortical areas or the amygdala was negligible in all cases (see Table 2).

3.1.2. Group Neo-A_{ibo}

The extent of bilateral amygdala damage in all cases ranged from 47.1% to 76.0% (average: 62.5%). Fig. 2 (right column)

Table 2
Intended and unintended damage after neurotoxic lesions of the hippocampus (Neo-H_{ibo}) and amygdala (Neo-A_{ibo}) and aspiration lesions of the orbital frontal cortex (Neo-O_{asp})

Group		Intended damage				Unintended damage																			
Subjects	Symbols[a]	Hippocampus				Amygdala				TH/TF				TE				ERh				PRh			
		L%	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*
Neo-H_{ibo}																									
Neo-H_{ibo}-1	♦	63.6	2.9	33.2	1.8	14.0	0.0	7.0	0.0	3.1	0.5	1.8	0.0	0.0	0.0	0.0	0.0	2.6	0.0	1.3	0.0	0.0	0.0	0.0	0.0
Neo-H_{ibo}-2	□	54.4	80.9	67.6	44.0	0.0	0.0	0.0	0.0	21.4	2.7	12.1	0.6	0.6	0.0	0.3	0.0	0.0	0.0	0.0	0.0	5.4	0.5	2.9	0.0
Neo-H_{ibo}-3	▲	78.5	96.3	87.4	75.6	1.7	0.0	0.8	0.0	6.1	5.5	5.8	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-H_{ibo}-4	×	20.3	67.3	43.8	13.6	0.0	4.7	2.4	0.0	15.3	0.0	7.6	0.0	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-H_{ibo}-5	■	20.7	84.4	52.6	17.5	0.0	4.9	2.4	0.0	6.1	4.0	5.1	0.2	0.0	0.0	0.0	0.0	0.0	1.5	0.7	0.0	0.0	0.5	0.3	0.0
Neo-H_{ibo}-6	+	7.9	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean		40.9	66.4	48.1	25.4	2.6	1.6	2.1	0.0	8.7	2.1	5.4	0.2	0.3	0.0	0.1	0.0	0.4	0.2	0.3	0.0	0.9	0.2	0.5	0.0

Group		Intended damage				Unintended damage																			
Subjects	Symbols[a]	Amygdala				Hippocampus				ERh				PRh				TE				TG			
		L%	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*
Neo-A_{ibo}																									
Neo-A_{ibo}-1	♦	89.0	59.8	74.4	53.2	5.1	3.1	4.1	0.2	0.1	4.7	2.4	0.0	2.0	10.1	6.0	0.2	0.0	1.8	0.9	0.0	6.5	35.0	20.7	2.3
Neo-A_{ibo}-2	□	42.0	77.6	59.8	32.6	0.0	0.8	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-A_{ibo}-3	▲	33.0	61.1	47.1	20.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-A_{ibo}-4	×	62.1	90.0	76.0	55.9	1.9	3.0	2.4	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-A_{ibo}-5	■	41.2	66.6	53.9	27.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-A_{ibo}-6	+	52.1	75.6	63.8	39.3	5.6	10.3	8.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean		53.2	71.8	62.5	38.1	2.1	2.9	2.5	0.1	0.0	0.8	0.4	0.0	0.3	1.7	1.0	0.0	0.0	0.3	0.2	0.0	1.1	5.8	3.5	0.4

Group		Intended damage								Unintended damage											
Subjects	Symbols[a]	Area 11				Area 13				Area 12				Area 14				Ia			
		L%	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*	*L%*	*R%*	*X%*	*W%*
Neo-O_{asp}																					
Neo-O_{asp}-1	♦	80.5	92.7	86.6	74.7	93.0	73.5	83.3	68.4	40.2	11.0	25.6	4.4	8.0	10.2	9.1	0.8	11.6	3.4	7.5	0.4
Neo-O_{asp}-2	□	62.6	95.6	79.1	59.9	99.3	100	99.6	99.3	9.3	1.4	5.4	0.1	31.9	6.8	19.4	2.2	78.5	57.7	68.1	45.3
Neo-O_{asp}-3	▲	98.7	100	99.4	98.7	94.0	82.4	88.2	77.4	22.3	21.6	22.0	4.8	18.7	11.6	15.1	2.2	16.5	13.8	15.1	2.3
Neo-O_{asp}-4	×	84.1	93.9	89.0	79.0	87.3	95.6	91.4	83.4	2.8	4.0	3.4	0.1	9.7	12.6	11.2	1.2	82.5	64.6	73.6	53.3
Neo-O_{asp}-5	■	84.0	98.9	91.5	83.1	96.8	97.2	97.0	94.1	18.5	22.8	20.6	4.2	6.5	11.0	8.8	0.7	87.0	67.8	77.4	59.0
Neo-O_{asp}-6	+	58.1	61.2	59.6	35.5	84.0	77.0	80.5	64.7	11.2	6.0	8.6	0.7	13.7	6.8	10.2	0.9	99.9	65.8	82.9	65.8
Mean		78.0	90.4	84.2	71.8	92.4	87.6	90.0	81.2	17.4	11.2	14.3	2.4	14.7	9.8	12.3	1.3	62.7	45.5	54.1	37.7

Mean: average damage per group; *L%*: percent damage in the left hemisphere; *R%*: percent damage in the right hemisphere; *X%*: average damage to both hemispheres; W%: weighted average damage to both hemispheres ($W\% = (L\% \times R\%)/100$). ERh: entorhinal cortex; Ia: insular area; PRh: perirhinal cortex; TE, TH/TF and TG: ventral cortical areas of the temporal lobe. Closed and open symbols represent females and males, respectively.

[a] These symbols are used in Fig. 7 to represent each individual.

shows the extent of amygdala damage in a representative case (Neo-A_{ibo}-4). For three cases (Neo-A_{ibo}-1, Neo-A_{ibo}-4 and Neo-A_{ibo}-6), the damage was substantial and symmetrical (mean: 75.1% and 67.7% in the right and left, respectively). The sparing in these three cases included the medial aspect of the right amygdala in case Neo-A_{ibo}-1, the dorsal region anteriorly and the ventral region posteriorly of the left amygdala in case Neo-A_{ibo}-4 (see Fig. 2, right column), and the anterior portion as well as the lateral region more posteriorly of the left amygdala in case Neo-A_{ibo}-6. The remaining three cases (Neo-A_{ibo}-2, Neo-A_{ibo}-3 and Neo-A_{ibo}-5) had moderate and asymmetrical amygdala damage, ranging from 33.0% to 42.0% in the left side and 61.1–77.6% in the right side. Finally, extent of unintended damage to the adjacent cortical areas and the anterior portion of the hippocampus was negligible for all cases, except for case Neo-A_{ibo}-1 for which unintended damage also included the ventral portion of the right claustrum and bilaterally the tail of the putamen and of the caudate nucleus just above the anterior portion of the hippocampus.

3.1.3. Group Neo-O_{asp}

All six cases with orbital frontal lesions had complete and symmetrical damage of areas 11 and 13 (average: 84.2% and 90.0%, respectively). Fig. 3 (right column) displays coronal T1 images through the orbital frontal cortex lesion in case Neo-O_{asp}-6. Unintended damage to adjacent cortical areas or to the white matter beneath the cortex was negligible, except for area 12 on the left side (40.2%) in case Neo-O_{asp}-1 and area I_a bilaterally in cases Neo-O_{asp}-2, Neo-O_{asp}-4, Neo-O_{asp}-5 and Neo-O_{asp}-6 (mean: 75.5%).

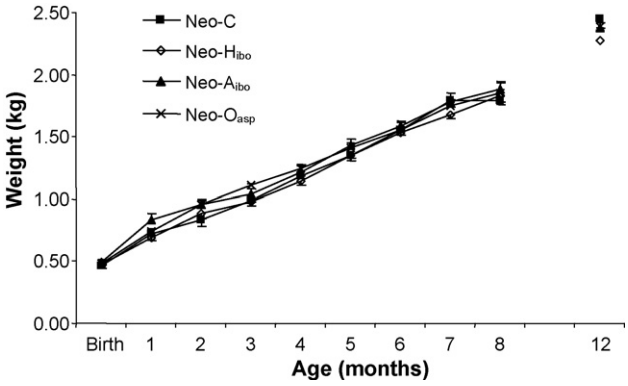

Fig. 5. Ponderal growth (kg ± S.E.M.) over the first 12 months of life for group Neo-C (sham-operated controls), group Neo-H_{ibo} (animals with neonatal bilateral neurotoxic lesion of the hippocampus), group Neo-A_{ibo} (animals with neonatal bilateral neurotoxic lesion of the amygdala) and group Neo-O_{asp} (animals with neonatal bilateral aspiration lesion of the orbital frontal cortex).

3.2. Ponderal growth, general activity, and vocalizations

All animals, independently of the lesion, had a similar growth over their first year of life (group effect: $F_{(3,6)} = 2.001$; $p = 0.215$, age effect: $F_{(\text{Huynh–Feldt: } 9,10.5)} = 47.743$; $p < 0.001$; see Fig. 5). In addition, none of the lesions significantly affected exploration of each zone (proximity, front, back, and neutral) or motor and vocal activities ($p > 0.05$ in all cases; see Table 3).

Table 3
General activity and vocalizations

	Neo-C ($N = 5$)	Neo-H_{ibo} ($N = 6$)	Neo-A_{ibo} ($N = 6$)	Neo-O_{asp} ($N = 6$)
Durations (s)				
Total proximity	161.84 ± 56.36	132.82 ± 19.33	130.53 ± 49.24	153.36 ± 72.02
Total front	100.59 ± 23.26	150.99 ± 66.99	119.81 ± 24.72	118.91 ± 20.15
Total back	208.52 ± 49.14	153.14 ± 33.35	211.41 ± 33.46	191.55 ± 46.61
Total neutral	129.05 ± 26.68	163.04 ± 39.22	138.24 ± 26.08	136.18 ± 32.72
Frequencies				
Total proximity	29.20 ± 9.87	20.83 ± 6.71	26.83 ± 8.98	31.33 ± 7.19
Total front	67.20 ± 15.78	36.67 ± 10.98	60.00 ± 15.85	77.50 ± 19.77
Total back	54.20 ± 12.61	26.00 ± 6.38	48.83 ± 7.17	54.17 ± 16.61
Total neutral	34.60 ± 7.59	24.50 ± 5.67	33.00 ± 5.68	26.00 ± 6.22
Total zones crossed	185.20 ± 28.01	108.00 ± 26.17	168.67 ± 34.22	189.00 ± 43.24
Length of bouts (s)				
Total proximity	5.24 ± 1.98	8.83 ± 2.52	6.28 ± 1.73	13.20 ± 8.59
Total front	3.13 ± 1.17	17.43 ± 12.39	5.23 ± 1.67	4.09 ± 1.28
Total back	7.81 ± 1.94	12.48 ± 2.47	10.58 ± 2.07	9.55 ± 3.32
Total neutral	4.27 ± 1.33	6.44 ± 0.61	4.71 ± 1.13	5.50 ± 1.66
Vocalization frequencies				
Total vocalizations	223.80 ± 41.57	296.33 ± 94.34	207.17 ± 62.86	147.67 ± 40.13
Coo	127.00 ± 18.19	174.83 ± 48.59	91.00 ± 40.10	78.17 ± 31.13
Scream	60.20 ± 26.47	67.00 ± 37.14	26.00 ± 19.32	13.50 ± 12.12
Grunt	1.80 ± 1.36	0.33 ± 0.21	0.33 ± 0.33	0.33 ± 0.33
Bark	11.60 ± 4.84	7.67 ± 4.93	17.33 ± 8.08	6.17 ± 2.33
Girn	23.20 ± 6.30	46.50 ± 22.23	72.50 ± 34.61	49.50 ± 23.62

Notes: The values represent the means ± S.E.M.

Table 4
Discrimination scores

	Neo-C ($N=5$)	Neo-H_{ibo} ($N=6$)	Neo-A_{ibo} ($N=6$)	Neo-O_{asp} ($N=6$)
Latencies (s)				
Proximity caregiver	88.38 ± 57.84	182.70 ± 40.38	140.28 ± 73.29	34.85 ± 10.90
Proximity familiar	288.59 ± 108.94	600.00 ± 151.92	355.15 ± 82.61	140.52 ± 75.15
Durations (s)				
Proximity caregiver	149.31 ± 50.61	125.40 ± 19.08	125.39 ± 48.85	134.36 ± 73.93
Proximity familiar	12.53 ± 9.65	7.42 ± 3.42	5.14 ± 1.54	19.00 ± 3.62
Front caregiver	82.29 ± 21.47	117.61 ± 72.07	84.40 ± 14.15	72.20 ± 13.64
Front familiar	18.30 ± 6.27	33.39 ± 11.94	35.41 ± 12.45	46.70 ± 9.33
Back caregiver	168.35 ± 38.68	65.57 ± 35.06	130.99 ± 29.10	120.14 ± 27.11
Back familiar	40.17 ± 12.59	87.57 ± 27.15	80.43 ± 7.45	71.41 ± 24.94
Frequencies				
Proximity caregiver	23.20 ± 6.92	17.33 ± 5.34	24.00 ± 8.57	20.17 ± 5.30
Proximity familiar	6.00 ± 3.41	3.50 ± 1.50	2.83 ± 0.79	11.17 ± 3.05
Front caregiver	48.40 ± 10.72	24.33 ± 6.63	45.00 ± 12.58	45.50 ± 11.77
Front familiar	18.80 ± 5.62	12.33 ± 4.88	15.00 ± 3.70	32.00 ± 8.88
Back caregiver	38.00 ± 7.94	12.33 ± 3.84	33.50 ± 5.05	31.33 ± 9.30
Back familiar	16.20 ± 5.00	13.67 ± 5.20	15.33 ± 3.05	22.83 ± 8.19
Length of bouts (s)				
Proximity caregiver	5.39 ± 1.30	10.45 ± 2.75	5.09 ± 0.65	11.16 ± 8.58
Proximity familiar	1.00 ± 0.52	1.68 ± 0.68	2.00 ± 0.82	2.04 ± 0.45
Front caregiver	2.14 ± 1.03	14.31 ± 12.77	2.74 ± 0.93	2.33 ± 0.96
Front familiar	0.99 ± 0.19	3.11 ± 1.20	2.49 ± 0.82	1.76 ± 0.36
Back caregiver	5.05 ± 1.50	4.45 ± 2.40	4.69 ± 1.21	5.96 ± 2.87
Back familiar	2.77 ± 0.47	8.03 ± 1.69	5.88 ± 0.89	3.58 ± 0.59

Notes: The values represent the means ± S.E.M. caregiver = principal human caregiver; familiar = familiar human caregiver.

3.3. Discrimination scores and behaviors in the proximity zone

To investigate whether the animals discriminated the two stimuli and had a preference for the principal human caregiver, we compared for each zone the duration, frequency and length of bout on the side of the principal human caregiver to that on the side of the familiar human, and calculated the latency to reach each proximity zone (Table 4 and Fig. 6). All animals reached the proximity zone on the side of the principal human caregiver faster than that of the familiar human (stimulus effect: $F_{(1,19)}=12.943$, $p=0.002$) and remained in proximity with the principal human caregiver for similar amount of time (group effect: $F_{(3,19)}=1.294$; $p=0.305$). Animals in all groups also entered more often (location × stimulus interactions: $F_{(1,22)}=13.155$, $p=0.001$; $F_{(1,22)}=31.196$, $p<0.001$; $F_{(1,22)}=28.510$, $p<0.001$, for the frequencies in the back, front and proximity zones, respectively) and spent more time (location × stimulus interactions: $F_{(1,22)}=5.810$, $p=0.025$; $F_{(1,22)}=7.273$, $p=0.013$; $F_{(1,22)}=25.160$, $p<0.001$, for the durations in the back, front and proximity zones, respectively, see Fig. 6) on the side of the principal human caregiver than on the side of the familiar human. Finally, all animals displayed more frequent contacts (no-contact, mesh/Plexiglas-contact and human-contact) in the proximity zone on the side of the principal human caregiver than on that of the familiar human (Table 5). This was confirmed by significant interactions between location and stimulus or the three contact zones ($F_{(1,22)}=27.130$, $p<0.001$; $F_{(1,22)}=28.291$, $p<0.001$ and $F_{(1,22)}=11.533$, $p=0.003$, for no-contact, mesh/Plexiglas-contact and human-contact zones, respectively). In addition, all animals reached the contact zones on the side of the principal human caregiver significantly faster than those on the side of the familiar human (group Neo-H_{ibo}: $p=0.028$, for both mesh/Plexiglas-contact and human-contact zones; group Neo-A_{ibo}: $p=0.028$, for both mesh/Plexiglas-contact and no-contact zones; and group Neo-O_{asp}: $p=0.068$, for human-contact zone only). Interestingly, although five of the six subjects in group Neo-O_{asp} reached the mesh/Plexiglas-contact zone on the side of the familiar human, only one subject in group Neo-C and three in both groups Neo-A_{ibo} and Neo-H_{ibo} did reach this zone (Table 5).

3.4. Index of preference

The index of preference (Fig. 7) clearly confirmed that all groups were not only able to discriminate their principal human caregiver from a familiar human, but also displayed a preference for the former (IP mean ± S.E.M.: 0.84 ± 0.08 for group Neo-C; 0.88 ± 0.05 for group Neo-H_{ibo}; 0.87 ± 0.05 for group Neo-A_{ibo}, and 0.40 ± 0.20 for group Neo-O_{asp}). However, this preference was weaker for group Neo-O_{asp} (group

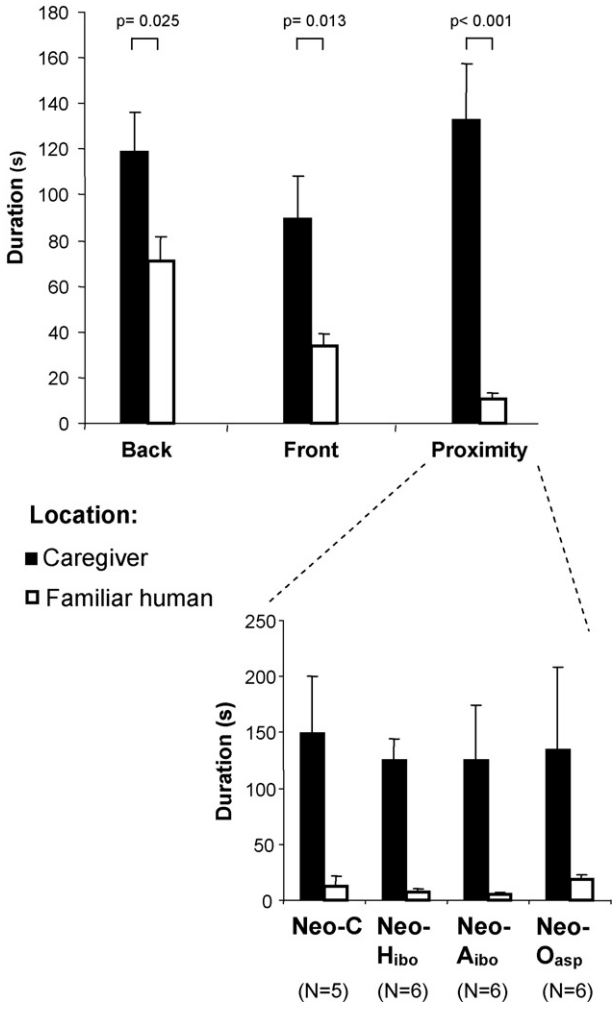

Fig. 6. Duration (mean ± S.E.M.) in seconds (s) in the back, front and proximity zones on the side of the principal human caregiver (black bars) and the familiar human (white bars) across all four groups, and in the proximity zone for each separate group. Abbreviations as in Fig. 5.

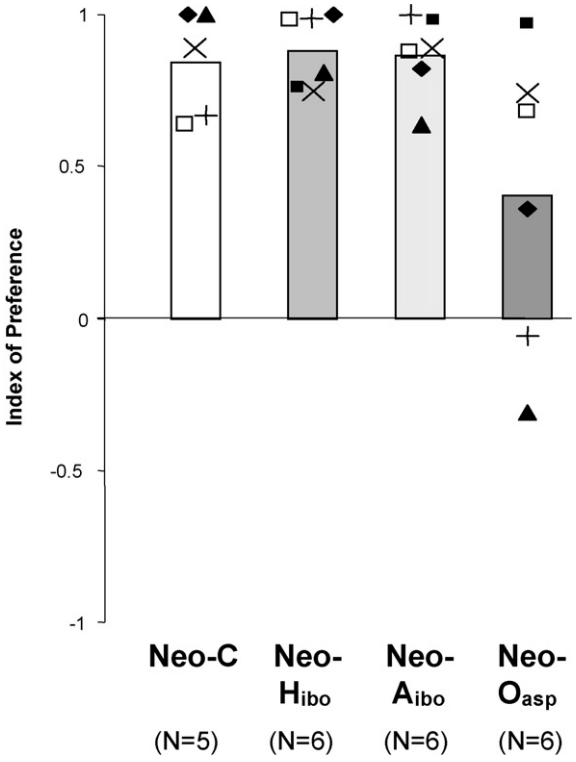

Fig. 7. Bars represent the average index of preference for each group and symbols (see Table 2 for details) depict individual index of preference values. Abbreviations as in Fig. 5.

effect: $F_{(3,19)} = 4.078$; $p = 0.022$). Thus, group Neo-O_{asp} had a significantly lower mean IP than groups Neo-A_{ibo} and Neo-H_{ibo} ($p = 0.056$ and $p = 0.047$, respectively). In addition, the mean IP values were significantly greater than chance for groups Neo-C, Neo-H_{ibo} and Neo-A_{ibo} ($t_{(4)} = 10.729$, $p < 0.001$; $t_{(5)} = 17.912$, $p < 0.001$ and $t_{(5)} = 16.324$, $p < 0.001$, respectively), but not for group Neo-O_{asp} ($t_{(5)} = 1.954$, $p = 0.108$). It is also interesting to note that the only group for which the individual IP values varied greatly was group Neo-O_{asp} (Fig. 7). Thus, although cases Neo-O_{asp}-2, Neo-O_{asp}-4, and Neo-O_{asp}-5 displayed a significant preference for the principal human caregiver (mean IP = 0.80; $t_{(2)} = 9.014$, $p = 0.006$), case Neo-O_{asp}-1 displayed a weaker preference for the principal human caregiver (IP = 0.36; $t_{(2)} = 4.941$, $p = 0.019$), case Neo-O_{asp}-6 showed no preference for either stimulus (IP = −0.06), whereas case Neo-O_{asp}-3 showed a weak preference for the familiar human (IP = −0.31). Finally, when cases Neo-O_{asp}-3 and Neo-O_{asp}-6 were removed from the analysis, the mean IP of group Neo-O_{asp} became positive and significantly greater than chance (IP = 0.69 ± 0.13, $t_{(3)} = 5.465$, $p = 0.006$). Furthermore, the group effect disappeared ($F_{(3,17)} = 1.284$, $p = 0.312$). For all groups, there were no significant correlation between the index of preference and extent of lesion, as well as no effect of sex.

4. Discussion

The present experiment revealed several important findings. First, sham-operated infant monkeys separated from their biological mother in the first few days of life and reared by human caregivers together with high level of socialization with age-matched peers and regular cognitive testing and human handling, are able to develop a clear preference for their principal human caregiver as compared to another familiar human. Second, neonatal bilateral lesion of the amygdala or hippocampus in monkeys separated from their biological mother in the first few days of life and reared by human caregivers and age- and sex-matched peers did not affect the expression of social discriminative capacities and preference for the principal human caregiver. Third, neonatal bilateral lesion of the orbital frontal cortex did not preclude preference for the human caregiver although it affected the strength and/or quality of the relationship with the principal human caregiver. These findings as well as their relevance for autism are discussed below.

4.1. Social preference for the principal human caregiver in nursery-reared sham-operated infant monkeys

The rearing conditions used in this study, although species-atypical, were adequate and sufficient for the development of a

Table 5
Contact behavior in the proximity zone

	Neo-C ($N=5$)	Neo-H_{ibo} ($N=6$)	Neo-A_{ibo} ($N=6$)	Neo-O_{asp} ($N=6$)
Latencies (s)				
No-contact caregiver	88.31 ± 57.85	40.41 ± 28.49	130.10 ± 69.56	34.84 ± 10.88
No-contact familiar	288.53 ± 108.96	151.85 ± 90.06	315.52 ± 96.68	140.41 ± 75.17
Mesh/Plexiglas caregiver	155.51 ± 111.54	101.57 ± 52.20	166.41 ± 87.68	72.22 ± 29.39
Mesh/Plexiglas familiar	492.11 ± 107.89	405.62 ± 88.87	513.35 ± 46.63	270.90 ± 93.75
Contact caregiver	326.65 ± 113.17	255.92 ± 52.92	370.78 ± 114.26	298.44 ± 110.38
Contact familiar	520.43 ± 79.57	564.95 ± 35.05	600.00 ± 0.00	600.00 ± 0.00
Durations (s)				
No-contact caregiver	51.71 ± 17.07	47.75 ± 6.01	51.90 ± 14.09	34.13 ± 10.57
No-contact familiar	5.68 ± 3.28	5.73 ± 3.03	4.30 ± 1.72	16.05 ± 3.15
Mesh/Plexiglas caregiver	67.40 ± 27.24	63.50 ± 18.38	56.50 ± 25.42	41.67 ± 19.57
Mesh/Plexiglas familiar	5.17 ± 5.17	2.36 ± 1.27	1.93 ± 1.06	5.97 ± 2.69
Contact caregiver	34.60 ± 20.58	16.50 ± 4.54	19.67 ± 12.29	62.00 ± 55.69
Contact familiar	2.72 ± 2.72	0.16 ± 0.16	0.00 ± 0.00	0.00 ± 0.00
Frequencies				
No-contact caregiver	33.60 ± 11.40	21.83 ± 4.18	31.67 ± 11.41	24.83 ± 6.51
No-contact familiar	6.80 ± 4.06	4.67 ± 2.01	3.17 ± 0.95	13.00 ± 3.08
Mesh/Plexiglas caregiver	22.00 ± 8.54	15.33 ± 2.76	18.67 ± 7.49	12.33 ± 4.16
Mesh/Plexiglas familiar	3.40 ± 3.40	1.17 ± 0.54	1.00 ± 0.52	2.67 ± 0.80
Contact caregiver	4.40 ± 2.04	2.67 ± 0.56	5.33 ± 3.65	3.00 ± 1.59
Contact familiar	0.60 ± 0.60	0.17 ± 0.17	0.00 ± 0.00	0.00 ± 0.00
Lenght of bouts (s)				
No-contact caregiver	5.63 ± 3.32	2.82 ± 1.37	4.93 ± 2.11	1.51 ± 0.39
No-contact familiar	3.45 ± 3.22	0.69 ± 0.19	1.32 ± 0.78	1.67 ± 0.50
Mesh/Plexiglas caregiver	1.32 ± 0.47	2.83 ± 0.25	6.59 ± 3.65	1.98 ± 0.31
Mesh/Plexiglas familiar	0.10 ± 0.10	0.86 ± 0.52	1.72 ± 1.23	2.11 ± 1.03
Contact caregiver	1.52 ± 0.87	5.80 ± 2.08	1.03 ± 0.57	3.44 ± 1.87
Contact familiar	0.31 ± 0.31	0.09 ± 0.09	0.00 ± 0.00	0.00 ± 0.00

Notes: The values represent the mean ± S.E.M. caregiver = principal human caregiver; familiar = familiar human caregiver; no-contact = no-contact zone (in proximity but not in contact with either wire mesh of the enclosure, Plexiglas of the window or stimulus); mesh/Plexiglas = mesh/Plexiglas-contact zone (in proximity and in physical contact with wire mesh of the enclosure or Plexiglas of the window but not in contact with one of the human stimuli); contact = human-contact zone (in proximity and in physical contact with one of the human stimuli).

filial attachment with a being from a different primate species, as indicated by the clear preference for their principal human caregiver over the familiar human, during the discrimination task. The newborn primate has the propensity to assimilate the first agent it encounters into a "maternal schema" with whom it will progressively develop a filial attachment [75,76]. Although this agent is generally the biological mother, in its absence, infant monkeys will develop substitute attachment to objects or other conspecifics [20,54] or heterospecific individuals, such as dogs [75] or, as in the present study, a principal human caregiver. For nursery-reared infant monkeys, using a human being as the attachment figure has several advantages. First, a human caregiver can provide response-contingence stimulations to the infant monkeys in probably the closest fashion from that provided by the biological mother [103]. For instance, a human caregiver can reward species-typical behaviors and discipline unsuitable behaviors more appropriately than other beings, such as age-matched peers or dogs. Second, human caregivers trained to provide care to infant monkeys can be used for all animals involved in a single study, reducing the individual variability occurring from different mothering styles in biological mothers [6,43,44,72,109]. Maternal style has been shown to influence the behavioral and cognitive development of the offspring [10,43,105]. Thus, reduction in inter-individual variability is critical specifically in developmental research in non-human primates that generally involves small sample sizes.

When normally reared by their biological mother, infant monkeys spent the first two postnatal months in ventro-ventral contact, during which time they develop a secure attachment with their mother and progressively acquire independence and species-typical socio-emotional competences over the next 4–6 months (see [47,113] for reviews). The present rearing conditions differ from such species-typical rearing conditions, especially in terms of the regular separations (nights and week-ends) from the principal caregiver. Earlier studies have shown that repetitive separations from the mother in mother-reared infants between the 3rd and 9th months of life induced an avoidance of the mother during preference tests at 1.5 year of age [114], suggesting that the multiple separations impaired the development of an attachment to the biological mother and even

induced an aversion for it. By contrast, in the present study, despite the separations from the human caregiver during the nights and week ends, the sham-operated infants displayed a clear preference for the principal human caregiver as compared to another familiar human caregiver, suggesting that the frequent separations did not significantly affect the development of the early social attachment. Thus, the high level of daily social interactions with familiar humans and peers, cognitive testing and human handling counterbalanced the potentially negative effect of the routine separations from the primary caregiver. The findings obtained in the sham-operated monkeys are clearly important for primate developmental and behavioral research in which infant monkeys need to be reared independently from their mothers. Yet, although these control animals appeared to have developed an attachment to their principal human caregiver, their pattern of attachment may not be entirely typical and similar to that of mother-reared infant monkeys. Thus, similar to the findings on institutionalized children that show that, although these children are able to form specific attachment, they express it in disordered/disorganized fashion, leading to later socio-emotional problems and eventually psychopathologies [29,119,127,129], the sham-operated monkeys of the present study might display greater socio-emotional problems later in life as compared to mother-reared infants. Ongoing studies on these same sham-operated animals are evaluating the effect of the particular rearing conditions described here on the development of socio-emotional abilities and emotional reactivity to threatening stimuli in adolescence and adulthood.

4.2. *Intact social preference after neonatal lesion of the hippocampus or amygdala*

Like sham-operated monkeys, those with neonatal hippocampal or amygdala lesions discriminated the two stimuli and displayed a clear preference for the principal human caregiver over a familiar human caregiver. The sparing of social discrimination after neonatal hippocampal lesions confirmed that observed previously [12] in 6-month-old infant monkeys with neonatal lesion of the hippocampus in discrimination tasks opposing the biological mother to a familiar monkey. By contrast, the sparing of social discrimination after neonatal amygdala lesions differs from the weaker preference for the mother obtained by Bauman et al. [12]. Although in this later study amygdala-operated infant monkeys approached their mother first and more frequently than the other stimulus, they spent less time in proximity with their biological mother, maintained a greater distance away from her but a closer distance from the other monkey stimulus and displayed less distress vocalizations than the control and hippocampus-operated animals. Several possible factors could explain the different results in the two studies. The first factor relates to the age of the infants at testing, i.e. 6-month-old in Bauman et al. [12] and 11-month-old in the present study. Normally, by 3 months of age, infant monkeys are the primary responsible for maintaining proximity and physical contact with their mother, suggesting that by this age they have established a preferential and exclusive relationship with their mother (see [112] for review). Because social attachment in early infancy requires several cognitive processes linking sensory inputs to motor outputs, including motivation, attention, memory and social recognition [22], it is possible that the development of one or more of these cognitive processes could have been delayed by neonatal amygdala lesion, and resulted in a protracted formation of this early social bond. However, such explanation seems unlikely. Indeed, although the amygdala-operated monkeys in Bauman et al. [12] did not maintain proximity with their mother during the discriminations tasks, they spent more time in contact with their mother than their controls in all other familiar and social situations, between 4 and 6 months old, suggesting that by this age, they had developed all cognitive skills to maintain the bond with the mother.

The second more likely factor relates to the familiarity of the infant monkeys with the experimental environment. In potentially threatening novel situations, infant monkeys seek reassurance and maintain contact with their attachment figure [54,77]. However, selective amygdala lesions in infancy or adulthood have been shown to increase exploratory behaviors to novel stimuli [59,61,80,90,111]. Thus, as discussed by Bauman et al. [12], an inability to detect potential danger in a novel environment and with an unfamiliar monkey stimulus could have heightened exploration and reduced proximity to the mother in the amygdala-operated infant monkeys, especially since in other, more familiar, experimental settings the same neonatally amygdala-operated monkeys did recognize their mother and displayed normal mother–infant relationship. By contrast, in the present study, the amygdala-operated infant monkeys were placed in a familiar environment and in the presence of familiar stimuli, thus reducing their propensity to explore the novel environment and stimuli and enhancing the expression of proximity-maintaining behaviors with and preference for the principal human caregiver.

The final possible factor is the presence or absence of physical contact allowed with the stimuli, and especially the mother, or principal human caregiver, during the discrimination tasks. Infant monkeys in the Bauman et al. study [12] could not enter in physical contacts with the stimuli, while in the present study they could seek physical contacts through the wire mesh of the cage. The absence of possible physical contacts with the biological mother could have increased frustration in the amygdala-operated infant monkeys, and, in a certain extent, resulted in avoidance of the mother. Supporting this claim, physical contacts seem to be a particularly important aspect of the mother–infant relationship in 6-month-old infant monkeys with neonatal amygdala lesion, since increased duration of contact with the mother, was the only measure that differentiated these animals from sham-operated controls, in all familiar social situations [12]. In addition, during dyadic interactions with other age-matched infants, amygdala-operated infants displayed higher frustration/irritation behaviors, such as temper tantrums, when their partners were trying to break physical contacts [90]. Whatever the reasons explaining these differences, the findings in both studies demonstrate that early damage to the amygdala or hippocampus does not drastically alter filial attachment either in nursery-reared or mother-reared monkeys. These data also suggest that the atypical rearing conditions used

in the present study provide a promising model to assess the neural basis of filial attachment in primates.

4.3. Weaker social preference after neonatal lesion of the orbital frontal cortex

Infant monkeys with neonatal orbital lesions clearly discriminated both stimuli and displayed a preference for the principal human caregiver as opposed to a familiar human. However, the preference index was weaker in this group than in all other experimental groups, suggesting that perhaps the strength and/or quality of the relationship with the principal human caregiver may have been affected by the neonatal lesion. Yet, the weaker preference for the human caregiver in group Neo-O_{asp} was only due to three out of the six subjects. Several explanations may account for the absence of preference for the principal human caregiver in these three subjects. The first possibility relates to the extent of damage to the orbital frontal cortex and/or inadvertent damage to adjacent cortical areas. However, like in the other experimental groups, no relations between extent of lesion and expression of a preference for the principal human caregiver were found. Specifically, case Neo-O_{asp}-3 and Neo-O_{asp}-6, which showed no preference for the principal human caregiver, had as extensive damage into areas 11 and 13 than case-O_{asp}-5, which displayed a strong preference for the principal human caregiver. Nevertheless, because the orbital frontal lesions were more complete than the neurotoxic amygdala and hippocampal lesions, it remains possible that the lack of effects following the amygdala and hippocampal lesions could have resulted from incomplete lesions. This is also unlikely since the two cases with the most complete amygdala (Neo-A_{ibo}-1 and Neo-A_{ibo}-4) and hippocampal (Neo-H_{ibo}-2 and Neo-H_{ibo}-3) lesions displayed as strong preference for the principal caregiver as those with the least extended lesions (see Fig. 7). Another explanation relates to individual temperament and/or preference for the human caregiver. The three operated animals that did not show a preference for the principal human caregiver might in fact have developed a stronger attachment to other caregivers regularly present in the primate nursery, including the familiar human stimulus participating in the experiment described here. Indeed, Case Neo-O_{asp}-3 (Fig. 7) showed a stronger preference for the familiar human stimulus than for the principal human caregiver. Nonetheless, it is surprising that only some animals in group Neo-O_{asp}, but none in the other experimental groups, would have shown such individual preference.

A final and more likely explanation is related to the involvement of the orbital frontal cortex in self-regulation of social and emotional behaviors, including impulsiveness or behavioral disinhibition [8], especially in situations that could induce negative consequences [19]. Interestingly, five of the six animals in group Neo-O_{asp} (83.3%) reached the mesh/Plexiglas proximity zone on the side of the familiar human, whereas only one monkey in group Neo-C (16.6%), and three in both groups Neo-A_{ibo} and Neo-H_{ibo} (50.0%) did reach this zone. This pattern of results indicates that, although infant monkeys with neonatal orbital frontal lesions appeared to have developed a social attachment with the principal human caregiver, they were more likely to explore the proximity zone of the familiar human stimulus than all other infant monkeys, suggesting perhaps greater disinhibition or impulsivity. Interestingly, such social disinhibition have been observed in institutionally-reared human infants as evidenced by their indiscriminate friendliness (even with strangers), that persisted even after adoption in families and even after they had developed a discriminative attachment with their new foster/adoptive parents [29,84,110,128,129]. In addition, when assessed at 8.8 years of age, these post-institutionalized adopted children showed significantly decreased metabolic activity in several brain regions, including the orbital frontal cortex bilaterally, as compared to normal adults and age-matched epileptic children [30]. Hence, an involvement of the orbital frontal cortex in socio-emotional self-regulation processes, but not in the direct development of filial attachment, is an attractive explanation of the present data, especially when considering the functional development of the orbital frontal cortex. Thus, the orbital frontal cortex in humans has a protracted functional maturation, with some functions appearing between 1 and 2 years of life and some others continuing to mature until adulthood [52,85]. In addition, cognitive deficits (object discrimination reversal and delayed-response) after early orbital frontal cortex lesions (8 weeks) in monkeys emerged around 15 months of age in few animals but affected all animals only at 18 months of age [45]. These data suggest not only that the behavioral effects of early damage to the orbital frontal cortex may remain silent until a time when it normally becomes functional [8,42,60,70,71], but also that the age at which orbital frontal cortex becomes functional varies between individuals. Thus, it is possible that, in the present study in which filial attachment was assessed at an age (11 months of age) when the orbital frontal cortex begin to attain functional maturity, only few animals showed a weaker preference for the principal human caregiver, suggestive of an emergence of impulsivity and disinhibition. Such an interpretation of our results corresponds well with the protracted and increasingly more severe social self-regulation deficits (impulse control) seen in children with early damage to the prefrontal cortex, including the orbital frontal cortex [42]. Thus, the orbital frontal cortex, via its critical involvement in self-regulatory processes, may play a role in the maintenance of social relationships. Further investigations of the animals of the current study on their abilities to form and maintain social bonds in adolescence and adulthood will provide additional insights on the specific role of the orbital frontal cortex in the development of filial attachment and social behavior.

Yet, a combined effect of the atypical rearing conditions with that of the lesion cannot be entirely excluded. Further studies assessing the effects of orbital frontal cortex lesion in mother-reared infant monkeys could help to dissociate the influence of the two factors on the development of social preferences and characterize the role of the orbital frontal cortex in filial attachment.

4.4. Comparison of present findings with the non-primates data

The present findings revealed that, as previously suggested by others [12], the hippocampus and amygdala do not seems to play

a critical role in the development and maintenance of early social bond. A similar conclusion can be drawn for the orbital frontal cortex, although it may play an indirect role in the strength, quality and/or maintenance of such early relationships, via its critical role in self-regulatory processes. These results differ from those that have shown that both the amygdala and orbital frontal cortex are involved in the formation of social attachment in sheep, voles and rats [63–66,124,125]. Although one explanation for such differences could be related to species differences in the brain circuits mediating the formation and maintenance of social bond, other possible factors are likely. First, the type of social attachment differs between the primate and non-primate studies. Filial attachment to a caregiver was studied in primates whereas adult relationships (maternal attachment and pair-bonding) were studied in sheep and voles, respectively. Thus, the neural structures, including the amygdala and the orbital frontal cortex, involved in the formation of social attachments in adults may not play a similar role in the neonates [81]. Although this possibility cannot be excluded at this time, it does not fit with current views proposing that filial attachment in early infancy constitutes the roots of social bonding later in life [21,22,82], and that it is mediated by neural mechanisms similar to those described for social bonding in adults [67]. A final possible explanation relates to differences in methodology and/or timing of brain manipulation. In the primate studies, permanent neural damage was performed in early infancy, whereas in the non-primates investigations, reversible neural inactivation techniques along with functional activation were used in adulthood. Permanent damage to a neural structure, especially at an early stage of development, may induce important cellular and chemical brain reorganization offering the potential for compensatory mechanisms to support the functions normally performed by the damaged structure [25,58,120,122]. If this last possibility proves to be correct, the results in primates do not imply that the amygdala and orbital frontal cortex are unnecessary for filial attachment, but rather that, in the absence of one of these two structures early in development, filial attachment could be mediated by one of the remaining structures or by other compensatory mechanisms.

4.5. Relevance of the present findings for autism

The pattern of results that we have described above in monkeys with damage to selective neural structures may provide some insights into the understanding of neural dysfunction and social deficits observed in autism. Generally, children with autism show clear signs of early attachment to their primary caregiver, usually the mother, although they often display less contact seeking and contact maintaining behaviors with their mother than do children without autism (see introduction). Similarly to some of the monkeys in group Neo-O_{asp} that displayed impulsive/disinhibited interest toward the familiar human, many children with autism demonstrate attentional deficits and greater impulsivity, than children with attention deficit hyperactivity disorder or typically developing children (e.g. see [33]). In addition, some parents of autistic children report that their children frequently approach strangers inappropriately or that they run off in public places if not supervised closely (Loveland, 2006, personal communication). Nonetheless, these behaviors do not signify that they do not understand that different persons (or different context) have different meaning. Indeed, several studies have found that children with autism are able to distinguish and respond differentially to different persons, and in different contexts [69,108]. It is worth noting here that all studies that assessed filial attachment in children with autism have used some version of the well-defined strange situation procedure [3] that compares the child's behavior during reunion/separation episodes between the caregiver and a stranger. To our knowledge, no studies compared empirically the behavior of children with autism toward familiar individual other than the mother (or primary caregiver). Yet, we could speculate that if they are disinhibited/impulsive, children with autism would be more likely to approach less familiar individuals and thus could appear to possess a weaker preference for their mother (or primary caregiver), similarly to what we observed during the discrimination task in a subset of monkeys that received neonatal orbital cortex lesion. Such speculation will obviously need to be assessed empirically.

Thus, as for the monkeys with orbital frontal lesions, it is possible that in infancy the neural systems that are dysfunctional in autism may not affect the formation of the bond with the mother, but may be inadequate to support the normal progression toward more complex cognitive stages of filial attachment, such as the development of internal working models of social relationships [26,40,92,93]. The absence of such psychological progression could later lead to more severe and pervasive impairments in social interactions and reciprocal relationships (see [118] for a recent review). Interestingly, there exists growing evidence to support the existence of dysfunction in a complex neural network, including the amygdala and the orbital frontal cortex, in autism (see [8] for review).

This hypothesis will be empirically tested in the monkeys of the present study as they will reach adolescence and adulthood. Some evidence for it has already been provided in recent studies. Monkeys with neonatal amygdala lesions that displayed attachment to their mother in early infancy [12], did show changes in social interactions with conspecifics as juveniles [13] and reduced social dominance as adolescents [14]. These data suggest that, although damage to the amygdala and perhaps the orbital frontal cortex might not alter the development of early filial attachment, it may affect the development of higher order cognitive processes necessary for the maintenance of social relationships and the development of new ones, later in life.

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