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Letter to Neuroscience

INCREASED SOCIAL FEAR AND DECREASED FEAR OF OBJECTS IN MONKEYS WITH NEONATAL AMYGDALA LESIONS

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The amygdala has been implicated in the mediation of emotional and species-specific social behavior (Kling et al., 1970; Kling and Brothers, 1992; Kluver and Bucy, 1939; Rosvold et al., 1954). Humans with bilateral amygdala damage are impaired in judging negative emotion in facial expressions and making accurate judgements of trustworthiness (Adolphs et al., 1998, 1994). Amygdala dysfunction has also been implicated in human disorders ranging from social anxiety (Birbaumer et al., 1998) to depression (Drevets, 2000) to autism (Bachevalier, 1994; Baron-Cohen et al., 2000; Bauman and Kemper, 1993). We produced selective amygdala lesions in 2-week-old macaque monkeys who were returned to their mothers for rearing. At 6–8 months of age, the lesioned animals demonstrated less fear of novel objects such as rubber snakes than age-matched controls. However, they displayed substantially more fear behavior than controls during dyadic social interactions. These results suggest that neonatal amygdala lesions dissociate a system that mediates social fear from one that mediates fear of inanimate objects. Furthermore, much of the age-appropriate repertoire of social behavior was present in amygdala-lesioned infants indicating that these lesions do not produce autistic-like behavior in monkeys. Finally, amygdala lesions early in development have different effects on social behavior than lesions produced in adulthood.

Bilateral amygdala lesions in mature macaque monkeys produce decreased fear of inanimate objects and increased affiliative social behavior (Emery et al., 2001; Zola-Morgan et al., 1991). We concluded from these behavioral changes that the amygdala normally functions as a protection device. It acts as a brake on both social and non-social behavior prior to the evaluation of biologically important stimuli. The amygdala is also involved in determining whether an object or organism is potentially dangerous. If danger is detected, it coordinates a variety of other brain regions to produce a species-typical response to avoid the danger. The amygdala appears to be a highly plastic brain region that contributes to learning what is dangerous in the environment (LeDoux, 2000). If the amygdala is essential for learning contingencies of danger, it is reasonable to predict that early lesions would produce a lack of fear responses. Bachevalier (1994) has proposed that early amygdala lesions might also produce autistic-like symptomatology in monkeys, particularly lack of social interaction. This might be expected if the amygdala is essential for learning the nuances of appropriate interpretation and/or production of social signals, such as facial expressions, and for initiating social interaction.

We have directly evaluated the consequences of early neonatal amygdala lesions on social and non-social behavior in three macaque monkeys. All lesion surgeries were carried out at 2 weeks after birth, the earliest age at which accurate stereotaxic procedures could be performed. The animal was anesthetized and placed in an MRI-compatible stereotaxic apparatus. T1-weighted images, 1 mm thick, were taken to determine the location of the amygdala in stereotaxic coordinates. Lesions were made in one stage and entailed bilateral injections of ibotenic acid in the amygdala. Following surgery, animals were placed in an incubator, provided with intravenous fluids and monitored continuously until they regained normal activity. They were returned to their

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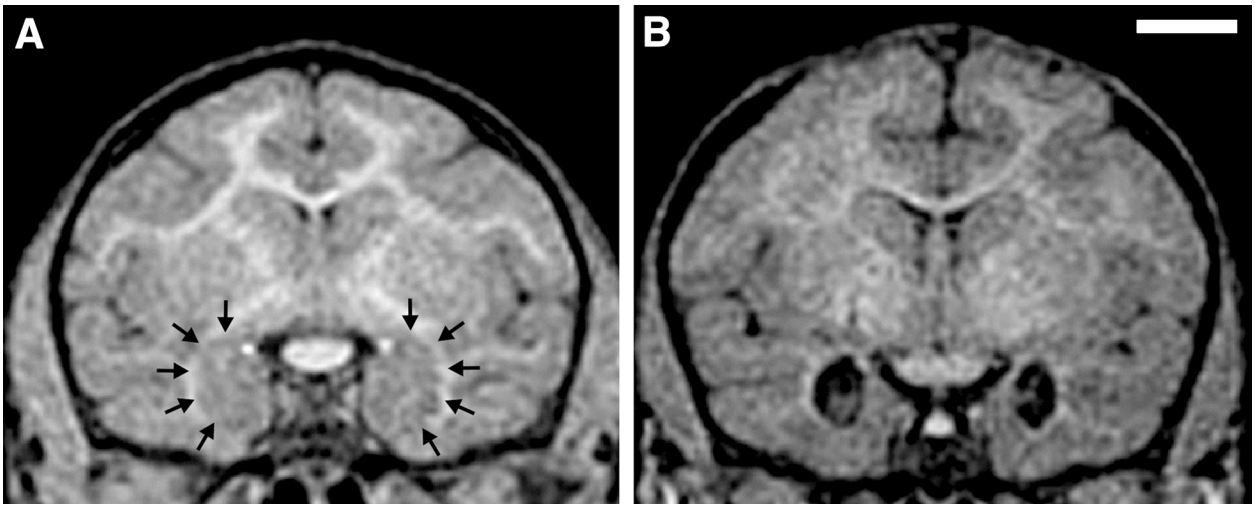

Fig. 1. MRI analysis of the amygdala lesions at 8.5 months. T1-weighted images at a mid-rostrocaudal level of the amygdala. (A) Non-operated control animal; the arrows indicate the extent of the amygdala. (B) Amygdala-lesioned animal. Dark oval structures in the region of the amygdala are expanded ventricles. Scale bar = 10 mm.

mothers within 24 h and the pair was monitored continuously to insure that the mother would nurture the infant. Three age-matched controls were also used for behavioral observations. Behavioral observations were carried out for 8.5 months until the animals were killed for histological evaluation of the lesions. All three lesioned animals sustained complete or very substantial bilateral amygdala damage (Figs. 1 and 2). In two lesioned animals, there was no detectable residual amygdala; in the third animal there were patches of cells in the amygdalohippocampal area and the posterior cortical nucleus. In one animal, collateral damage was minimal and consisted of focal damage in the sulcus of the superior temporal gyrus, area 35, and the dorsomedial portion of the entorhinal cortex. In the other two animals, there was more extensive damage of the entorhinal and perirhinal cortex. In all animals, there was some damage of the most rostral levels of the hippocampal formation.

Social and non-social behavior was recorded using a catalog of 36 categories of behavior commonly used for this species (Capitanio, 1984; Capitanio et al., 1998), modified to include mother–infant-specific behaviors (Hansen, 1966; Hinde and Spencer-Booth, 1967). Behavioral observations of mother–infant interactions were done in their home cage. There were no differences in mother–infant interactions between amygdala-lesioned and control infants. For example, the time spent in ventral–ventral contact or breast contact was similar for lesioned and control infants. The growth curves of the amygdala-lesioned animals were delayed initially following surgery, but recovered during the remainder of the testing period. These results confirm earlier studies showing that neonatal amygdala lesions have little, if any, effect on general development and mother–infant interactions (Kling and Green, 1967).

The main behavioral findings were obtained after weaning. We examined the infants' responses to novel inanimate objects placed inside their home cage. Objects were of two types: neutral objects such as luggage tags, and fearful objects such as rubber snakes. We recorded the frequency and duration of contact with the neutral objects, and the latency to retrieve food placed adjacent to a fearful object. The amygdala-lesioned animals engaged in greater manual and oral exploration of the neutral objects than the controls did (Fig. 3, $z = 1.964$, $P = 0.0463$). Their latency to retrieve food next to a fearful stimulus was also shorter (Fig. 3, $z = 1.964$, $P = 0.0495$). In fact, the latency to retrieve food by the lesioned animals was not affected by the presence of these objects (Fig. 3, $t(2) = 0.159$, $P = 0.88$), whereas the fear stimuli delayed food retrieval by the controls (Fig. 3, $t(2) = 8.547$, $P = 0.0134$). This change in behavior is similar to the decreased fear of objects demonstrated by mature animals who received amygdala lesions as adults (Zola-Morgan et al., 1991). This is a robust finding that we have also replicated in mature animals with ibotenic acid lesions (Amaral et al., 1997).

The most surprising behavioral observation came from the dyadic social interactions of amygdala-lesioned infants with other infants. Each infant was paired with every other infant on four separate occasions. Focal observations were conducted as described by Altmann (1974). Amygdala-lesioned animals demonstrated less oral and manual exploration of the cage or objects in the cage during social interactions (Fig. 4, $z = 1.964$, $P = 0.0495$). Moreover, the lesioned animals produced more fear grimaces and screams than the controls during social interactions (Fig. 4, fear grimaces: $z = 1.964$, $P = 0.0495$; screams: $z = 1.964$, $P = 0.0495$). Although the lesioned animals appeared to be somewhat calmer when the control animals maintained physical contact with them, the lesioned animals would often generate a tantrum when the controls withdrew. This complex pattern of behavior was consistent with a generally heightened state of fear during the dyadic social interactions for the amygdala-lesioned animals.

The first conclusion from these preliminary studies is that neonatal amygdala lesions do not eliminate fear responses, but actually lead to increased fear during social interactions. Given that the amygdala is generally

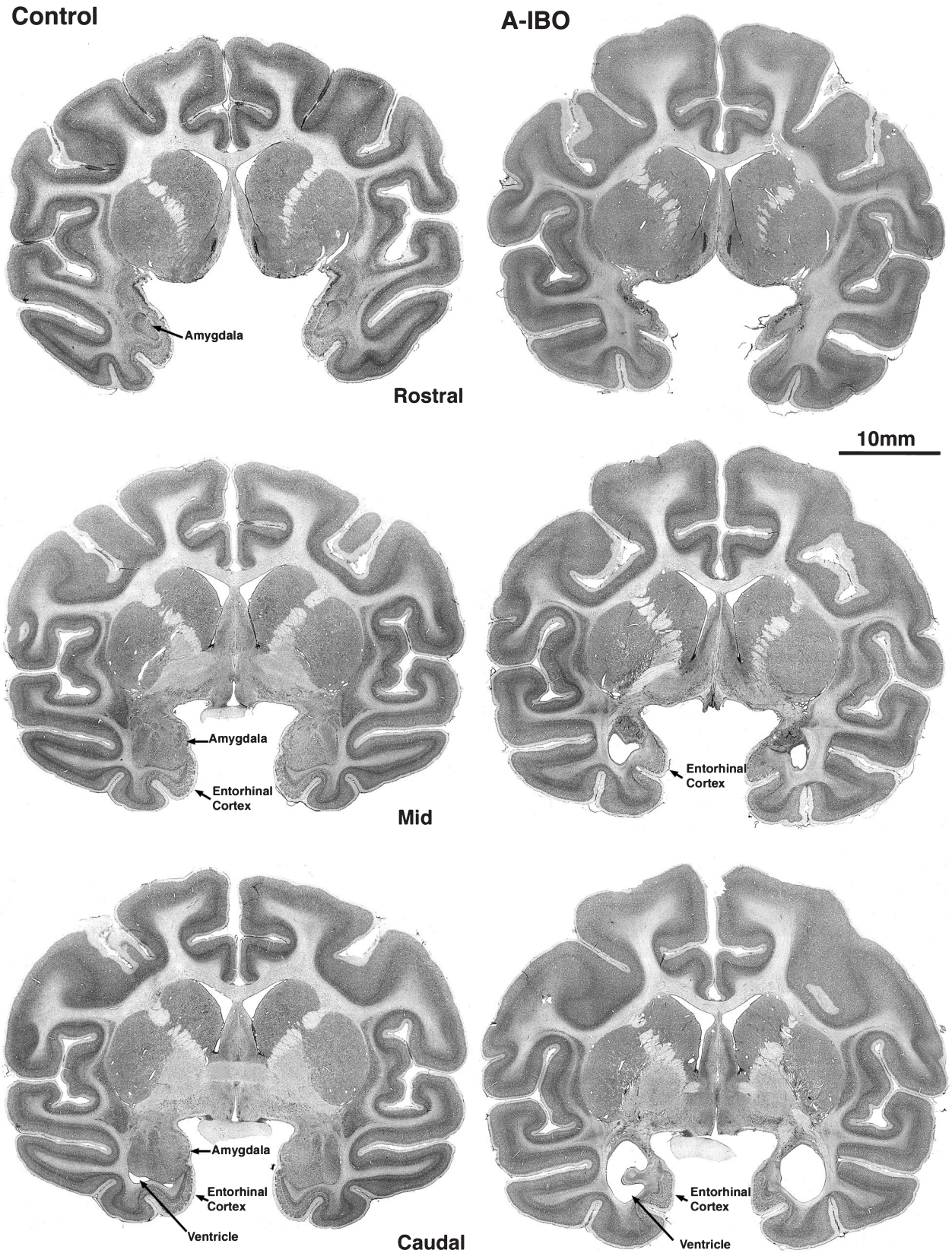

Fig. 2. Coronal sections through three levels (rostral, mid and caudal) of a 3-month-old animal used in another study (left panels) and one of the amygdala, ibotenic acid-lesioned animals (A-IBO; right panels). The amygdala in this animal was completely eliminated while adjacent structures such as the entorhinal cortex were largely intact. Note that the ventricle has greatly expanded and occupied space vacated by the shrunken amygdala. 30-μm-thick sections.

Fig. 3. Responsiveness testing. (A) Average number (per session) of manual and oral explorations of the novel object during trial 1 (neutral object only). (B) Latency to take the food during 'food only' trials and during trials where food was placed adjacent to a fear stimulus on day 4 of responsiveness testing. Comparisons of performance between groups were conducted with non-parametric Mann–Whitney tests. Comparisons of performance between conditions were conducted with two-tailed paired comparison *t*-tests on the log-transformed values of the data.

considered to generate such species-typical fear responses in mature monkeys (Emery and Amaral, 2000), the first question that arises is what brain region mediates the heightened fear in the neonatal amygdala-lesioned animals in social situations. The amygdala has generally been considered a central component of the brain circuitry involved in the regulation of fear behavior and in some descriptions, it is portrayed as the primary generator of fear behavior (Davis, 1992; LeDoux, 2000). Currently, we are not able to speculate as to which brain region might be supporting this amygdala-independent fear behavior that we observed in the neonatally lesioned animals.

Second, amygdala lesions early in development have different effects on social behavior than lesions produced in adulthood. Mature monkeys with bilateral amygdala lesions engage in greater amounts of affiliative social interactions than control monkeys (Emery et al., 2001). We believe this is because the lesioned monkeys do not engage in the appropriate period of cautious familiarization that normally occurs when unfamiliar animals are introduced. In contrast, the neonatal amygdala-lesioned animals did not show any indication of increased affiliative behavior and actually demonstrated heightened fear behavior during dyadic social interactions. These differential findings are not necessarily contradictory. If the amygdala is essential for learning the nuances of appropriate interpretation and/or production of social signals, but this information is not stored in the amygdala, then the adult lesioned animals would have had the opportunity to gain social knowledge (and would have access to it to initiate social interactions) but the neonates would not have. The increased fear demonstrated by the neonates may be due to their approaching a complex social situation without the benefit of knowledge concerning appropriate social behavior. Obviously, more information is needed on the short- and long-term consequences of amygdala lesions on the development of species-typical social behavior.

Third, selective neonatal amygdala lesions do not produce autistic-like behavior in macaque monkeys. The neonatal lesioned animals were clearly attentive to the control animals and produced a variety of social signals. These results contrast with reports indicating that early

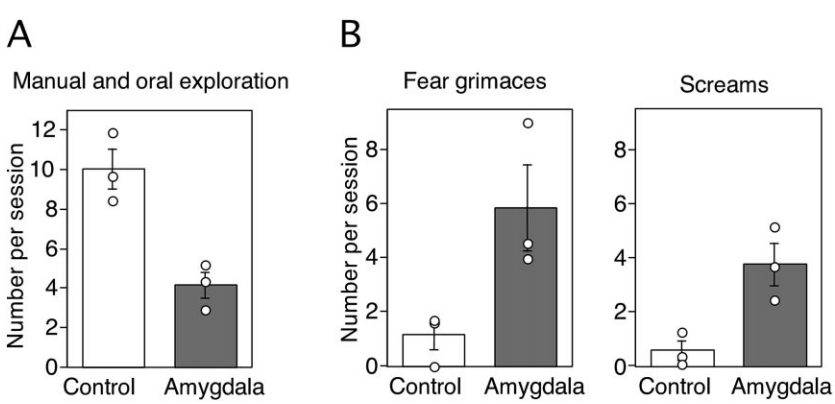

Fig. 4. Dyadic social interaction. (A) Average number (per session) of manual and oral explorations of the cage and objects placed in the cage during dyadic social interactions. (B) Average number (per session) of fear grimaces and screams produced during dyadic social interactions. Comparisons of performance between groups were conducted with non-parametric Mann–Whitney tests.

lesions of the medial temporal lobe induce social withdrawal, passivity and loss of affect (Bachevalier, 1994). The discrepancies between Bachevalier's studies and ours could be explained by the lesion techniques (aspiration versus selective ibotenic acid lesions), the rearing conditions (peer-reared versus mother-reared) or the ethograms used to assess behavior (nine versus 36 categories of behavior). Our findings, however, substantiate an earlier report showing that infant monkeys reared in near isolation who received two-stage aspiration lesions of the amygdala at 2 months of age demonstrated increased fear in dyadic social interactions (Thompson et al., 1969). Our findings, using selective lesion techniques in 2-week-old infants raised by their mother, further indicate that much of the age-appropriate repertoire of social behavior is present in amygdala-lesioned infants.

In conclusion, this study revealed three unexpected and original findings. First, selective neonatal amygdala lesions dissociate a system that mediates social fear from one that mediates fear of inanimate objects. These data support Kagan's (1998) suggestion that "the concept of a single fear state should be replaced with a family of terms for related but distinct states, each with its own class of incentives, neurophysiological profiles and behavioral reactions". Second, neonatal amygdala lesions have different effects on social behavior than lesions produced in adulthood. Third, these lesions do not produce autistic-like behavior in monkeys. A network of brain areas in the temporal lobe, the orbitofrontal cortex and the anterior cingulate cortex have been implicated in social cognition (Adolphs et al., 1998; Damasio, 1995). A better definition of the development and contributions to social cognition of these brain regions may provide insight into which areas are impaired by limited or pathological social interactions during human development (Rutter et al., 1999). This knowledge will be a first step to developing and implementing strategies for affective rehabilitation of socially deprived children.

EXPERIMENTAL PROCEDURES

Subjects and living conditions

Six infant rhesus monkeys (*Macaca mulatta*) were used in this study. Three infants (two males, one female) received bilateral amygdala lesions at 14 days (±2 days) after birth, while the other three infants (two males, one female) served as controls. Infants were housed with their mothers in standard home cages (61 cm W×66 cm D×81 cm H) until they were on average 5.5 months old, when the mothers were removed. Following weaning, the infants remained in their home cage and were allowed access to an age-matched peer for 3 h per day. Each pair consisted of one amygdala-lesioned and one control infant.

Surgical procedures

Infants were anesthetized with ketamine hydrochloride (15 mg/kg i.m.) and metatomadine (0.3 mg/kg), then placed in an MRI-compatible stereotaxic apparatus (Crist Instruments Co., Damascus, MD, USA). Their brain was imaged using a General Electric 1.5 T Gyroscan magnet; 1.0-mm-thick sections were taken using a T1-weighted Inversion Recovery Pulse sequence (TR = 21, TE = 7.9, NEX 3, FOV = 8 cm, matrix, 256×256). The MRI images were evaluated for the location of the amygdala and a stereotaxic atlas for the subject animal was prepared to calculate the coordinates for injection of ibotenic acid. Electrophysiological analyses were performed to confirm the dorsoventral extent of the amygdala. A tungsten microelectrode was lowered through the amygdala and extracellular multi- and single-unit responses were recorded along its trajectory. Bilateral ibotenic acid injections were made at multiple sites within the amygdala using two 10-μl Hamilton syringes (26-gauge beveled needles). One microliter of ibotenic acid (Biosearch Technologies, 10 mg/ml in 0.1 M phosphate-buffered saline) was injected at each site at a rate of 200 nl/min. The total amount of ibotenic acid injected ranged from 24 to 36 μl per animal bilaterally. During surgery a stable level of anesthesia was maintained with a combination of isoflurane (1.5%) and i.v. infusion of fentanyl (7–10 mg/kg/h).

Social behavior observations

All behavioral observations were collected with The Observer software (Noldus, Sterling, VA, USA) by trained observers demonstrating an inter-observer reliability >90%. Each mother–infant pair was observed in their home cage on six separate occasions for a total of 10 min per session. Each session consisted of a 5-min observation of each mother–infant pair, followed by another rotation of 5-min observation. The average infant age during this phase was 4.5 months. At 6.5 months of age, the infants were observed in dyadic interactions using a round-robin design consisting of lesion/control, lesion/lesion and control/control dyads. Twenty-minute dyadic interactions took place in a test cage (120 cm W×120 cm D×120 cm H). For each trial, two infants were placed in separate compartments. An opaque divider was then raised, allowing the infants free access to each other. Alternating 5-min focal animal samples were taken of each infant in a predetermined random order. In addition to frequency and duration of discrete behavior, observers recorded the second subject's identity and the direction of the interaction (initiate or receive). Each infant participated in 20 dyads and was observed as the focal animal for a total of 200 min.

Responsiveness testing

Responsiveness testing took place when the animals were 8.5 months old. All objects and food rewards were placed directly inside the home cage. The following categories of behavior were recorded: object exploration, vocalizations, facial expressions, and latency to take the food (except for trial 1). All infants received five daily trials for 4 days as follows. Trial 1, novel object: the experimenter hung a novel object (simple or complex) to the cage ceiling with a clip and a 4-inch chain. The animal was free to explore the object for 60 s. Trial 2, food only: the experimenter presented a piece of banana in a plastic bowl (3.5 inches in diameter) placed in the center of the front of the cage floor. This bowl was constantly present in the animals' home cage outside the testing schedule so that animals were familiar with this object. The trial lasted for 30 s, after which the bowl was removed. Trial 3, fear object (complex or simple): the experimenter presented a fear stimulus at the center of the front of the cage floor and placed a piece of banana in a plastic bowl right next to the object, towards the back of the cage. The trial lasted for 60 s, after which the object and the bowl were removed. Trial 4, fear object: same as trial 3, except that a different version (simple or complex) of the object presented in trial 3 was presented. Trial 5, food only: this trial was the same as trial 2.

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