Physiological Evidence for Genetically Mediated Sibling Recognition in Mice

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The kin selection theory predicts that individuals would behave differently toward one another, depending on their genetic relatedness. Kin discrimination has been demonstrated in mice from social behavior, and previous familiarity, as well as familiarity with the partner’s phenotype, has been postulated to represent proximate mechanisms. It has already been demonstrated that siblings’ reunion resulted in a decrease in pain sensitivity that is mediated by endogenous opioids. In this study, using a cross-transferring design, it is shown that genetic relatedness with the male partner, independently of postnatal association, is responsible for changes in nociceptive threshold. Conversely, previous association until weaning has no effect on pain sensitivity. These data suggest that endogenous opioids activity and social behavior represent indices of different processes: the recognition of related animals and the discrimination of familiar (and also usually related) subjects, respectively.

KEY WORDS: Mice; cross-transferring; sibling reunion; analgesia; kin recognition mechanisms.

INTRODUCTION

Adult male mice can distinguish between unrelated individuals and mice of a wide range of degrees of relatedness. Kareem and Barnard (1982) showed that adult male mice tested in single-sex pairs distinguished between full, half-siblings and unrelated males. Unfamiliar, unrelated males showed less passive body contact, and more investigatory and aggressive behavior, than full or half-sibling males. However, if animals were allowed to become familiar before testing, there was no longer any evidence of kin discrimination. Since full siblings were postnatally familiar, differences in social interaction between full and half-siblings might have reflected differences in the degree of familiarity between animals. Kareem’s studies (1983; Kareem and Barnard, 1982), however, also showed that males of the non-inbred CFLP strain were able to distinguish siblings without any postnatal association. Porter (1988) suggested that “indirect familiarization” might explain those cases of kin recognition that involved unfamiliar full siblings born in successive litters (Kareem and Barnard, 1982; Winn and Vestal, 1986, in wild house mice). Such discrimination of unfamiliar kin might be based on previous association with other relatives or with the organism’s own self-perceived phenotype.

All the above findings of kin discrimination in adulthood are based on the analysis of social interaction during brief social encounters. No evidence exists of the unobservable internal process of recognition.

In a previous study (D’Amato and Pavone, 1993) it has been demonstrated that sibling reunion induced analgesia in both members of the dyad. This change in pain reactivity was apparently mediated by endogenous opioids since this effect was blocked by injection of naloxone, an opioid antagonist. This modification at the neural level did not occur when social interaction involved nonrelated...
animals. This suggested that social reunion in kin was accompanied by a release of endogenous opioids that reached its maximum 120 min after the beginning of the social encounter.

This new measure of kin discrimination, based on modification of the activity of the nervous system, might shed light on the role of familiarity on kin discrimination. If familiarity plays the major role in kin discrimination, it is predicted that familiar mice, i.e., those sharing the same postnatal environment till weaning independently of being genetically related, differ from unfamiliar mice, these being genetically related or not. Otherwise, if genetic recognition occurs, whatever the specific recognition mechanism involved, it is predicted that full siblings separated after birth would behave as full siblings reared by the same mother.

METHODS

Outbred NMRI albino male mice were used in this study. They were maintained on a partially inverted 12-h light/12-h dark cycle (light on at 1 AM) at a constant temperature (21–24°C). Adult females, born in this laboratory from different pairs, were housed with a male of proven fertility for 15 days. Cages were inspected twice a day for live pups. The cross-transferring procedure was completed within 24–48 h from birth, using litters varying by no more than 24 h in their parturition times. Litters used consisted of between 10 and 14 pups. Each female received half of the litter of a second female, and half of her litter was given to a third female. Seven triplets of females exchanging pups in this way were set up. Each female's own pups were marked with odorless, waterproof mascara (cosmetic product). The pups were weaned at 40 days. At that age, males were individually marked with fur dye and housed in groups of five or six in 30 × 13 × 13-cm cages, which contained males of similar age but from different triplets.

The experiment was based on four experimental groups of male-male pairs. The first group consisted of familiar full siblings (FFS; males sharing parents and reared by their natural mother), the second group was unfamiliar full siblings (UFS; males sharing parents but reared by different mothers), the third group was familiar unrelated males (FUM; males conceived by different parents, but reared by the same dam), and the fourth group was unfamiliar, unrelated males (UUM; males from different triplets). The sample sizes of the four experimental groups were not the same because no more than two subjects per litter were included in each group to avoid confounding litter effects. Moreover, only subjects that were not wounded at all were used in this study. The scores of one subject in the FUM group were lost through technical problems.

Pain sensitivity (tail-flick test), during and following social interaction with a male partner, was evaluated when the subjects were about 100–110 days old, at the beginning of the dark phase. Two males were simultaneously introduced into a clean cage and left for a 2-h period. Tail-flick tests were conducted immediately before pairing the mice (Time 0) and then after 10, 20, 30, 60, and 120 min. In this test (D'Amour and Smith, 1941) the animal was restrained in a tube and radiant heat was focused on its tail. A flick of the tail stopped the heat stimulus, and the latency to flick was displayed by a digital timer. This value, expressed as seconds, was used as a measure of pain sensitivity. If no tail-flick occurred within 10 s, the test was interrupted to prevent tail injury, and the score for the animal was 10. Due to the repeated test procedure, each animal was tested in its tube, to avoid confounding effects due to odors of conspecifics.

A three-way ANOVA for repeated measures was conducted to analyze the effect of (1) relatedness (two levels: full-sibling and unrelated mice), (2) familiarity (two levels: familiar and unfamiliar mice), and (3) time course (six levels: Time 0, 10, 20, 30, 60, and 120 min).

RESULTS

Tail-flick latencies are reported in Table I. Related mice (FFS + UFS; familiar, plus unfamiliar, full siblings) showed an increase in tail-flick latency with time that did not occur in familiar, or unfamiliar, unrelated mice. The three-way ANOVA for repeated measures indicated no significant main effect of relatedness and familiarity, a significant effect of time \( [F(5/255) = 6.40, p < .001] \) and a significant relatedness × time interaction effect \( [F(5/255) = 2.91, p < .02] \). Simple effects analysis indicated a significant effect of relatedness at Time 120 \( [F(1/87) = 4.61, p < .05] \) and a significant effect of time for related mice \( [F(5/255) = 7.92, p < .001] \). Full siblings (FFS + UFS) showed an increase in pain threshold from 60 min of time onward (Student's \( t \) test for repeated measures: Time