Hypothalamic destruction and mouse aggression

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Lesions of the medial preoptic-anterior hypothalamic continuum (MPAH) virtually eliminate the display of aggressive behavior in most male mice. The absence of fighting after MPAH damage is not accompanied by signs of impaired testicular functioning. Apparently, neural elements originating in and/or passing through MPAH are essential for the display of aggressive behavior in most male mice.

In many strains of mice, males are highly aggressive. If two males which have been previously isolated from each other are placed together, vigorous fighting and wounding usually occur. The males will repeatedly strike and bite at each other's flanks and rear, and in particularly fierce encounters the males may remain in biting contact for many seconds—twisting, tumbling, and wrestling with each other about the test area.

Aggressive behavior in males is partly dependent upon the presence of male sex hormones (androgens) secreted by the testes. Castration of males prior to puberty will usually prevent the spontaneous occurrence of fighting, but the propensity for fighting can be easily aroused in castrates by the administration of androgens (Beeman, 1947; Edwards, 1969).

In a variety of species other than the mouse, hormone-dependent social behaviors seem particularly vulnerable to disruption by damage to the medial preoptic-anterior hypothalamic (MPAH) continuum of the forebrain. In this study, we extend the generality of this observation, in reporting that bilateral lesions of the MPAH virtually eliminate the display of intermale aggression in mice.

METHOD

Male mice of the Swiss-Webster albino strain were commercially obtained (Marland Farms, N.J.) at about 60 days of age. Upon arrival, males were housed individually in stainless steel pans (4 × 10 × 5 in. deep) with ad-lib food and water unless otherwise noted.

After 1-2 months of isolation, males were tested on a number of separate occasions over a period of several weeks for the display of aggressive behavior. A single test for aggression consisted of pairing a male with a "stimulus male" mouse of the same strain in a neutral pan identical in construction to the home pan of the experimental mouse. Stimulus males were drawn from a population of males rendered nonaggressive by castration and group housing. An attack was scored if the experimental male repeatedly

lunged at and bit the stimulus male. Once an attack was scored, the mice were separated and returned to their home pans. If no attack occurred within 5 min of starting, the test was scored negative and the males returned to their respective home pans. All tests were conducted during the first few hours of the dark phase of a 14/10-h light/dark cycle. Once a male attacked on three consecutive tests, it was randomly assigned to one of two surgical groups. Males in one group ($n = 15$) were operated upon with the intention of bilaterally lesioning the medial preoptic area; males in the other group ($n = 12$) were sham operated. Details of surgery follow.

Each mouse was injected with atropine sulfate and anesthetized with sodium pentobarbital supplemented with ether. Once anesthetized, the mouse's head was stabilized with the skull level in a Kopf stereotaxic frame. Lesion coordinates were: AP, 4.0 mm anterior to stereotaxic zero, 0.2 or 0.3 mm to each side of the midline, and 4.9 or 5.0 mm down from the surface of the skull. Lesions were made by passing 1-mA anodal dc current between the electrode and a rectal probe for 6 or 7 sec on each side. The electrode was constructed of 31-ga stainless steel wire insulated except at the 0.5-mm blunt tip. Sham-operated mice were treated identically, except that the electrode was only lowered to within 2 mm of the site on each side and no current was passed. After surgery, a topical antibiotic was applied to the incision and the skin was sutured. Each mouse was then returned to a freshly cleaned pan, but food and water were withheld until the morning after surgery. Following this procedure, recovery from surgery is uneventful (although a high mortality rate occurs when current is passed at the same coordinates for longer than 7 sec), and mice return to normal or near-normal body weight within a week after surgery. Average body weight at the time of surgery for the 15 lesioned males was 42 g (range: 37-46 g).

Beginning 1 week after surgery, each mouse was tested for aggressive behavior once every other day for a total of seven tests. Tests were as described earlier, but experimental animals were caged so that the surgical condition of each male was unknown to the person scoring the test.

After the completion of testing, each mouse was killed by cervical dislocation. The seminal vesicles were quickly removed and, after fluid was expressed from them, weighed. This was done in order to evaluate the possibility that a change in aggressiveness subsequent to lesioning might be secondary to a fall in testicular androgen secretion which could occur if lesioning decreased hypothalamic gonadotrophin secretion. Androgens are essential for the maintenance of seminal vesicle tissue, and a decrease in androgen secretion would be reflected in a decrease in seminal vesicle weight. After removal of seminal vesicles, each mouse was perfused intracardially with physiological saline followed by 10% Formalin. Brains were then removed and hardened in 10% Formalin and later in a 30% sucrose-Formalin solution.

Brains were blocked for the microtome according to the angle described in the atlas of Montemurro and Dukelow (1972), and frozen 50- μ coronal sections were cut through the extent of the

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brain damage. Every other section was saved, mounted, and stained with thionin.

With the help of a Bausch and Lomb microprojector, the lesioned area for each brain was drawn freehand onto a histology sheet showing drawings of coronal sections prepared from the plates of Montemurro and Dukelow (1972). The lesion was defined as the area of evacuation and/or gliosis. Each lesion was drawn through its entire anterior-posterior extent, and these drawings were made without reference to the behavioral data.

RESULTS

Histology

All of our lesions damaged part of the medial preoptic anterior hypothalamic continuum, and one of the largest of the lesions is shown in Figure 1A. Between individuals, lesions were variable in size, but in every case some damage to the most medial aspect of the MPAH was evident. Lesions never extended so ventral as to damage the suprachiasmatic nucleus. In two mice, only unilateral damage was evident, and a composite of these two lesions is shown in Figure 1B.

Aggressive Behavior after MPAH Lesions

Damage to MPAH virtually eliminated aggressive behavior in most males. Ten of 15 lesioned males never fought after surgery. Included among those lesioned mice which never fought after surgery were those two males in which only unilateral MPAH damage was evident. Two other lesioned males fought in only 1 of the 7 post-surgical tests. In contrast, 10 of 12 sham-operated males continued to fight after surgery. Each male was given a fight score equal to the percent of postoperative tests on which it attacked the stimulus male. Mean fight score for the sham-operates was significantly higher than the mean fight score for the MPAH-lesioned males (Table 1); the basis for this difference clearly lies in the fact that most of the lesioned males did not fight at all, while most of the sham-operates fought on most of the postoperative tests.

Lesioned and sham-operated groups did not differ with respect to seminal vesicle weight (Table 1).

DISCUSSION

In most cases, bilateral damage to the MPAH virtually eliminated aggressive behavior in male mice.

Figure 1. Representative frontal sections through the medial preoptic-anterior hypothalamic continuum, showing (A) one of the largest of the bilateral lesions which eliminated aggressive behavior, and (B) a composite of the unilateral lesions of two individual males which never fought after surgery. Sections have been drawn from the Montemurro and Dukelow atlas (1972).

Only three mice were exceptions to this rule, fighting on 57%, 57%, and 100% of their tests. The size and locus of lesions for these males were not remarkably different from those of other males which never fought after MPAH lesions, indicating that not every male is equivalently susceptible to the aggression disrupting effect of MPAH damage.

Two males with unilateral damage never fought after surgery. Both unilateral lesions (Figure 1B) included damage to the most medial aspect of the caudal preoptic area just underneath the anterior commissure, as well as damage to the tissue immediately lateral to the top of the third ventricle in more caudal sections. Since the completion of this study, we have made unilateral MPAH lesions in four additional male mice selected for their aggressiveness. In each instance, the unilateral hypothalamic destruction eliminated fighting.

In cases of unilateral damage, assessing the relationship between brain damage and behavior change is ipso facto not complicated by the usual asymmetry of bilaterally placed lesions. Thus, the zone of tissue described the the two unilateral lesions which eliminated aggressive behavior represents, in this study, the smallest and most localized estimate of the site at which damage to MPAH is likely to eliminate aggressive behavior in male mice.

MPAH lesions did not affect seminal vesicle

Table 1
Medial Preoptic-Anterior Hypothalamic (MPAH) Lesions, Aggressive Behavior, and Seminal Vesicle Weight

Surgical Group	n	Percent Fighting	Fight Score		Seminal Vesicle Weight	
			Mean	SEM	Mean	SEM
MPAH Lesion	15	33.3*	16.2	7.8**	75.9	3.6
Sham Operated	12	83.3	60.7	11.3	75.9	5.5

Note—Proportions from which percentages were derived were analyzed by chi-square; fighting scores were analyzed by *t* test (two-tailed). *Significantly different from sham-operated group at beyond the .02 level. **Significantly different from sham-operated group at beyond the .01 level.

weights. Thus, the elimination of aggressive behavior by MPAH damage can be interpreted without regard to a possible influence of brain damage upon gonadotrophin secretion and testicular function.

In rats, the medial preoptic area is populated with cells which concentrate testosterone (Sar & Stumpf, 1972). In the mouse, neural elements of the preoptic area may be importantly involved in the activation of aggressiveness by androgenic hormones. The fact that damage to this region eliminates aggressive behavior in most males is consistent with such a notion. However, implanting testosterone propionate into the preoptic area is apparently only marginally effective in restoring the aggressive behavior of castrates (Owen, Peters, & Bronson, 1974), suggesting either that the preoptic area is not an important site of action for the aggression promoting effect of testosterone, or that if it is, androgenic activation of this region alone is not sufficient for the arousal of aggression.

Efferents of the MPAH have been described in detail (Conrad & Pfaff, 1976). However, our results do little to detail the neuroanatomy of aggressiveness save to indicate that cell bodies of and/or axons of passage through the MPAH are essential for the display of aggression in most males. Intracerebral injection of some amino acids will cause degeneration of neurons with cell bodies near the site of injection, but spares axons terminating in or passing through the same area from some other point of origin (Simson, Gold, Standish, & Pellett, 1977). This procedure could be used to determine the extent to which cells *originating* in MPAH are important in the display of aggressive behavior in male mice.

Brain damage which has been reported to attenuate aggressive behavior in male mice includes: removal of the olfactory bulbs (Ropartz, 1968); lesions of the septal region (Slotnick & McMullen, 1972); ventro-

medial hypothalamic lesions (Olivier & Wiepkema, 1974); and, in the present study, damage to MPAH. Whether or not the superficially similar behavioral effect of some of these disparate lesions reflects a disruption in some common neural system remains an interesting challenge for future research.

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