Aggression in Knockout Mice

Randy J. Nelson and Silvana Chiavegatto

Introduction

Aggression is overt behavior with the intention of inflicting physical damage on another individual (Moyer 1968, 1971). The possibility for aggressive behavior exists whenever the interests of two or more individuals are in conflict (Svare 1983). Conflicts are most likely to arise over limited resources such as territories, food, and mates. In nature, the social interaction resolves which animal gains access to the contested resource. In many cases, a submissive posture or gesture on the part of one animal avoids the necessity of actual combat over a resource. Animals may also participate in psychological intimidation by engaging in threat displays or ritualized combat in which dominance is determined, but no physical damage is inflicted. To facilitate the study of aggressive interactions, the term "agonistic" was adopted to describe the entire behavioral repertoire of both aggressive and submissive actions within the context of a social interaction (Scott and Fredericson 1951). In the strict sense, however, submissive behaviors are not really aggressive behaviors; consequently, the physiologic mechanisms underlying the aggressive and submissive components of an agonistic interaction may be different (Leshner and Moyer 1975). Aggression and submission may represent the end points of a single behavioral continuum; alternatively, they may represent two independent, but interacting, dimensions of the behaving individual. This is not a trivial semantic issue, but rather a conceptual issue that influences the manner in which the neural and endocrine bases of agonistic behavior are studied (Schlinger and Callard 1990).

Many laboratory studies on aggression use domesticated house mice (*Mus musculus*), which are normally quite docile. Consequently, the mice must often be put into artificial situations that promote aggression, for example, they are housed in isolation or given an electrical shock. Female mice housed in groups will also attack a novel lactating female. More natural examples of laboratory-studied types of murine aggression include maternal aggression and male territorial aggression.

Randy J. Nelson, Ph.D., is a Professor in the Departments of Psychology, Neuroscience, and Biochemistry and Molecular Biology, and Silvana Chiavegatto, Ph.D., is a Postdoctoral Fellow in the Departments of Neurology and Psychology, at The Johns Hopkins University, Baltimore, Maryland.

Types of Aggression in Mice

Aggression has been divided into various types for ease of classification (Moyer 1968, 1971; Wilson 1975) (Table 1), and the different types of aggression appear to have different neuroendocrine bases. Maternal aggression, which serves to protect the offspring from intruders, appears to be mediated by hormonal changes associated with the production of offspring (Gammie and Nelson 1999). Specific relationships among blood concentrations of estrogens, progestins, and prolactin during the last days of pregnancy are correlated with the onset of maternal aggression (Bridges 1996).

Steroid hormones also underlie other types of aggressive behavior. Intermale aggression and territorial aggression, as well as sex- and rank-related aggression, all appear to be mediated by androgens (Bouissou 1983). These types of aggression are most commonly studied in the laboratory and are tested by the so-called resident-intruder paradigm (see below). Predatory aggression is another type of aggressive behavior that is performed in the context of obtaining food. The motivation and neuronal mechanisms underlying predatory aggression are likely different from the motivation and physiologic mechanisms underlying other types of aggression. Learned aggression and irritable aggression are often studied in the form of restraint aggression, which results after an animal is held motionless. Another type of agonistic behavior commonly studied in the laboratory has been called

Table 1 Types of murine aggression

Type of aggression	Reference[a]
Antipredatory	Wilson 1975, p. 343
Defensive	Wittenberger 1981, p. 614
Displaced	Immelmann and Beer 1989, p. 247
Fear (frustration)-induced	Immelmann and Beer 1989, p. 114
Intrasexual (usually male-male)	Wittenberger 1981, p. 617
Isolation-induced	Immelmann and Beer 1989, p. 114
Maternal (parental)	Olivier et al. 1995, p. 85
Predatory	Moyer 1968, p. 67
Sexual	Wilson 1975, p. 242
Territorial	Wilson 1975, p. 242

[a]See text.

fear-induced aggression, but this is more correctly termed "defense." Notably, the physiologic control of aggression in these contrived situations is likely to differ from the physiologic mechanisms underlying natural expressions of aggressive behavior. Tests of aggressive behavior in mice with targeted deletion of specific genes have been limited to isolation-induced and resident-intruder tests of males and maternal aggression of females (Table 2).

Types of Aggression Tests

Several models of aggression focus on the offensive components of agonistic interactions in knockout mice. Offensive behavior in this context is characterized by initiation on the part of the aggressive animal that often leads to damage to the opponents (Krsiak 1974). It follows a defined temporal course, occurring in episodic fashion with epochs of intense aggressive behavior alternating with relative quiescence (Miczek 1983).

In male mice, isolation for several weeks induces an extensive repertoire of natural agonistic behaviors (Miczek and Krsiak 1979). These singly housed mice are allowed to interact with nonaggressive group-housed male mice in an unfamiliar or neutral arena (isolation-induced aggression paradigm) or in their home cage (resident-intruder paradigm). During these confrontations, the attacker engages in pursuit, sideways threat, attack bites, and tail rattles, in addition to several nonagonistic activities such as grooming, rearing, and walking. Each occurrence of these behavioral elements can be measured in terms of frequency, as well as the onset and termination of specific behaviors from both live observations and video records. Because isolated mice (test animals) display more attacks as a resident (territorial aggression), which permits detection of both increases and decreases in aggression among the genotypes, the resident-intruder is the most extensive aggression test used in knockout mice (see Aggressive Phenotype of Various Knockout Mice below and Table 2). One important problem in conspecific confrontation is control of the stimuli situation, that is, the variation in the behavior of the "non-test" intruder. Because the aggressive behavior of residents responds to the movements of the opponent (i.e., changes in social investigation), the previous experience of the intruder can confound the results. A previously defeated or naive intruder can elicit different reactions from the resident. This potential problem can be reduced, however, when group-housed intruders are determined before the onset of the study to be nonaggressive (unpublished observations).

In female mice, aggressive behavior is generally observed only when parturient females are approached by strange intruders during the first part of the lactating period. This so-called maternal aggression wanes as the young approach weaning (Olivier et al. 1995). Maternal aggression in females is characterized by short latency attacks of high intensity, mostly directed toward the head/neck region of the opponent and usually without the introductory threatening behaviors typically displayed by male animals confronted with an intruder.

Table 2 Types of aggression in examined knockout mice

Type of aggression	Reference[a]
Isolation-induced (fear/frustration-induced)	Alleva et al. 1998 Demas et al. 1999 DeVries et al. 1997 Nelson et al. 1995 Ogawa et al. 1997 Sallinen et al. 1998
Resident-intruder (territorial/intersexual)	Cases et al. 1995 Chen et al. 1994 De Felipe et al. 1998 Demas et al. 1999 DeVries et al. 1997 Konig et al. 1996 Ledent et al. 1997 Nelson et al. 1995 Ogawa et al. 1997 Saudou et al. 1994 Stork et al. 1997
Maternal	Gammie and Nelson 1999 R. Hen, Columbia University, personal communication, 2000

[a]See text.

Brain Regions Associated with Aggression

Although an exhaustive discussion of the neural mechanisms underlying aggression is beyond the scope of this review, a brief review of the brain regions associated with aggression is presented below to help understand the effects of targeted gene disruption on aggressive behavior.

Several brain regions have been identified to be involved in the inhibition of aggression; the earliest example was provided by Klüver and Bucy (1939), who demonstrated that removal of the temporal lobes resulted in a syndrome of passivity. Lesions of limbic areas, including the olfactory bulbs, lateral septum, medial accumbens, dorsal and median raphe, and amygdala, enhance defensiveness and predation but not social aggression (Albert and Walsh 1984). Although the brain systems that mediate aggression appear to remain fairly constant among mammals, many details of the regulatory pathways appear to be species specific (Grisolía 1997).

In cats, two distinct neural circuits involving the hypothalamus and periaqueductal gray matter (PAG[1]) have been identified, which subserve defensive rage and predatory attack, respectively (Siegel et al. 1999). In rats and mice, a single area, largely coincident with the intermediate hypothalamic area, is crucial for the expression of offensive aggression (Siegel et al. 1999). Variations in the attack response of rats and mice in natural settings appear to be due primarily to environmental variables.

In rats, the amygdala provides excitatory input to parts of the hypothalamus mediating aggression (Grisolía 1997). As noted, ablation of the PAG permanently abolishes defensive rage in cats but not rats (Kruk 1991). In both rat and cat models of aggression, electrical stimulation of the medial amygdala, or the so-called "rage centers" in the hypothalamus, induces immediate attack behavior (or sham-rage) directed at cage-mates or the experimenter (Grisolía 1997). Stimulation of the PAG induces defensive reactions (e.g., freezing, flight) in rats. Although significant advances have been made in our knowledge of the circuitry underlying the neural basis of aggression in some species, additional mechanisms underlying aggressive behavior remain unspecified and require additional research. Studies of knockout mice, in conjunction with additional pharmacologic studies, will be useful to fill in the gaps in knowledge about the neural regulation of aggression.

Pharmacology of Aggression

Our overall understanding of the neurotransmitters regulating aggression is incomplete. The precise identification of specific transmitters at key synapses along the pathways associated with the expression and modulation of aggressive behavior has been limited by deficits in the knowledge of the neural mechanisms underlying aggression, the range in experimental conditions, and behavioral approaches (Siegel et al. 1999). Furthermore, because of marked species differences in the mechanisms underlying aggression, it is not obvious that similar neurotransmitters will be involved in the expression of specific types of aggression. This is important in postulating pharmacologic controls of human aggression based on different animal models.

Although experimental data indicate a role for noradrenaline (Haller et al. 1998), acetylcholine (Bell et al. 1985), γ-aminobutyric acid (Adams et al. 1993), and dopamine (Maeda et al. 1985) in aggression, increasing evidence points to serotonin (5-hydroxytryptamine [5-HT[1]]) as the major neurotransmitter modulating intermale as well as maternal aggressive behavior (Bell and Hobson 1994; De Almeida and Lucion 1997; Olivier and Mos 1992; Olivier et al. 1995). Depletion of 5-HT increases aggression in a variety of species in several different social situations (reviewed in Vergnes et al. 1986). Aggressive behavior in response to a wide variety of stimuli can be suppressed by 5-HT or any treatment that elevates 5-HT (Sanchez and Hyttel 1994). In a recent study in our laboratory, we found 5-HT involvement in the aggressive phenotype of mice lacking the gene encoding neuronal nitric oxide synthase (nNOS-/-) (Chiavegatto et al. 1999). Importantly, 5-HT does not simply inhibit aggression; rather, 5-HT appears to influence many different types of "risky" behavior, which includes aggression (Kraemer et al. 1997).

The early literature on the inhibitory effects of 5-HT on aggressive behavior was based on nonselective 5-HT agonists and antagonists, which has generated several instances of opposing and often controversial results (reviewed in Bell and Hobson 1994; Muehlenkamp et al. 1995). This confusion considerably complicates the interpretation of the role of 5-HT receptor subtypes in aggression. Recent studies have indicated that male-typical offensive aggression is reduced, in the absence of anxiolytic actions, by a combination of 5-HT_{1A} and $_{1B}$ agonists (Cologer-Clifford et al. 1996). These data are consistent with the dose-dependent decrease in male rodent aggression by the $5\text{-HT}_{1A/B}$ agonist eltoprazine and by the selective 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (Olivier et al. 1995; White et al. 1991) as well as the enrichment of 5-HT_{1A} and $_{1B}$ subtypes in rodent limbic areas. Taken together, these findings strongly suggest that the serotonergic system plays an important role in the aggressive behavior through 5-HT_{1A} and/or 5-HT_{1B} receptors subtypes.

Gene Targeting Technology

How to "Knock out" a Specific Gene

To inactivate or knock out a gene, molecular biologists typically rearrange the nucleotide sequence that encodes for the gene under investigation (Aguzzi et al. 1994; Soriano 1995; Tonegawa 1995). Because the chromosomes of mice are paired, both copies of each gene must be inactivated. The creation of a mouse with a targeted disruption (i.e., knockout) of a specific gene is an arduous task that combines several low-probability events. The gene in question must be identified, targeted, and marked precisely. This has been accomplished for an astounding number of murine genes during the past several years (Soriano 1995; Stark and Gudkov 1999). Next, mouse embryonic stem (ES[1]) cells must be harvested and cultured. A mutated form of the gene is created and introduced into the cultured ES cells by either microinjection or electroporation transfection (Tonegawa 1995). Homologous recombination will then incorporate a very small number of the altered genes into the DNA of the

[1]Abbreviations used in this article: 5-HT, 5-hydroxytryptamine; 7-NI, 7-nitroindazole; α-CaMKII, α-calcium-calmodulin-dependent kinase II; CNS, central nervous system; eNOS, endothelial isoform of nitric oxide synthase; ERKO, estrogen receptor knockout; ES, embryonic stem; IL-6, interleukin-6; iNOS, inducible isoform of nitric oxide synthase; MAOA, monoamine oxidase A; NCAM, neural cell adhesion molecule; NK-1, neurokinin-1; nNOS, neural isoform of nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; OT, oxytocin; PAG, periaqueductal gray matter; SP, substance P; WT, wild-type.

ES cells (Boggs 1990). The mutated ES cells are inserted into otherwise normal mouse embryos (blastocysts) and then implanted into surrogate mothers (Soriano 1995). If the mutated stem cells are incorporated into the germ lines of the resultant chimeric mouse, then some of the gametes will contain the mutant gene. Chimeric mice are then bred with wild-type (WT[1]) mice; some of the offspring produced will be heterozygous for the mutation. If the heterozygous mice are interbred, then approximately one fourth of their offspring will be homozygous for the mutation. These homozygous mice can be interbred to produce pure lines of mice with the gene in question inactivated (Sedivy and Sharp 1989; Stark and Gudkov 1999). The product for which the gene typically encodes will be missing from the progeny (Nelson 1997). Behavioral performance is typically compared among WT (+/+), heterozygous (+/–), and homozygous (–/–) mice in which the gene product is produced normally, usually produced at reduced levels, or completely missing, respectively, although this assumption must be tested directly.

Advantages and Disadvantages of the Knockout Technology in Behavioral Studies

This genetic technique offers new opportunities to study the mechanisms of behavior; however, as with all behavioral techniques, there are some potential limitations (reviewed in Crawley 1999; Nelson 1997; Nelson and Young 1998). For example, the products of many genes are essential to normal function, and inactivating the gene may prove lethal or induce gross morphologic or physiologic abnormalities that can complicate interpretation of discrete behavioral effects. Behavioral tests study the effects of the *missing* gene (and gene product), not the effects of the gene directly. This conceptual shortcoming can be overcome in the same way that it is overcome in other types of ablation studies, by collecting converging evidence using a variety of pharmacologic, lesion, and genetic manipulations. Because mammalian genome mapping is currently focused on mice (*Mus musculus*), standardized behavioral testing of mice should be adopted. Because most of the animal studies of aggression have been conducted on mice, this problem is not as great as it is for studies of learning or mating behavior in which the vast majority of studies have been conducted on rats.

Another limitation of the interpretation of behavioral data from knockout mice is the possibility that compensatory or redundancy mechanisms are activated when a gene is missing. For example, in nNOS–/– mice, there is a 20% increase in the expression of the endothelial isoform of nitric oxide synthase (Burnett et al. 1996). A compensatory mechanism may spare behavioral function and cloud interpretation of the normal contribution of the gene to behavior.

The availability of "inducible" or "conditional" knockouts, in which a specific gene can be inactivated at any point during development, or only inactivated in tissue-specific cells, should provide an important tool to bypass the problem of ontogenetic interactions (Nelson 1997). These conditional knockouts are currently being developed for a variety of genes (e.g., Cohen-Tannoudji and Babinet 1998; Gu et al. 1994; Kühn et al. 1995; No et al. 1996). Region- or cell-specific promoters are combined with genes that can be activated at any time by specific events induced by the investigators (e.g., exposure to tetracycline, ecdysone, or interferon) (Gu et al. 1994; Kühn et al. 1995; No et al. 1996). These substances serve as activators that terminate expression of a gene by binding to a promoter transgenically attached to the gene. Restricted gene activation can also be accomplished with a Cre-lox bacteriophage site-directed recombination method in which DNA recombination sites flank-targeted genes, and these sites bind to the site-specific recombinase Cre (Gu et al. 1994). Ecdysone can also be used to inactivate a gene during any point of pregnancy because it easily crosses the placenta-blood barrier (No et al. 1996). The widespread availability of inducible knockouts should prove extremely useful in murine behavioral studies because they will provide a method of studying genetic influences on behavior in the absence of ontogeny issues that currently obscure interpretation of knockout mouse behavior. The aggressive behavioral phenotypes of mice with targeted disruptions of specific genes tested to date are described below.

In contrast to the disadvantages described above are several important advantages to using knockout mice in behavioral research: (1) Disabling a gene is often a very precise and "clean" ablation; (2) the effects of the gene product can be abolished without the side-effects of drugs; and (3) genetic manipulations may be the only way to determine the precise role of many endogenous factors on behavior. The use of new inducible knockouts, in which the timing and placement of the targeted gene disruption can be controlled, will be an extremely important tool in murine behavioral biology research.

Aggressive Phenotype of Various Knockout Mice

Monoamine Oxidase A

Monoamine oxidase A (MAOA[1]) is responsible for the degradation of norepinephrine and 5-HT. Because 5-HT concentrations would be expected to be elevated in MAOA knockout mice, one would also predict that aggression would be reduced in these animals. Contrary to expectation, MAOA knockout mice exhibit high levels of offensive aggression despite elevated 5-HT concentrations (Cases et al. 1995). However, these mice are not healthy; they tremble, have difficulty righting, and have an elevated fear response before adulthood. Compared with controls in adulthood, these knockout mice spend an increased amount of time in the center of an open field arena—typically indicative of reduced fear responses (Cases et al. 1995). To attribute behavioral changes to a specific missing gene, it is critical to assess sensorimotor, anxiety, endocrine, and other parameters when

evaluating the aggressive behavior of knockout mice (Crawley 1999; Crawley and Paylor 1997; Nelson 1997).

5-Hydroxytryptamine$_{1B}$ Receptor

Additional evidence for a role of 5-HT in aggression has come from the use of knockout mice missing genes that either directly or indirectly affect 5-HT concentrations or metabolism. The 5-HT$_{1B}$ receptor, which is the rodent homologue of the human 5HT$_{1D}$ receptor, is expressed in a variety of brain regions, including the basal ganglia, central gray, hippocampus, and raphe nuclei (Maroteaux et al. 1992), either as an autoreceptor on serotonergic projections or as a heteroreceptor modulating the release of other neurotransmitters. Knockout mice that lack functional expression of the 5-HT$_{1B}$ receptor gene (5-HT$_{1B}$ –/–) have been generated (Saudou et al. 1994). In a resident-intruder paradigm, these mutant mice attack the intruder more quickly, more often, and more intensely than WT controls. Lactating female 5-HT$_{1B}$ –/– mice also attack unfamiliar male mice more rapidly and violently (R. Hen, Columbia University, personal communication, 2000). Even when they are housed with littermates, there is increased evidence of aggression among the mutants (Scearce-Levie et al. 1999). It is notable, however, that administration of the nonselective 5-HT$_{1B}$ agonist eltoprazine (termed "serenic") (Olivier et al. 1990) can significantly reduce aggressive behavior in 5-HT$_{1B}$ –/– as well as WT mice (R. Hen, personal communication, 2000). This suggests that whereas the 5-HT$_{1B}$ receptor contributes to aggression, it is not the sole 5-HT receptor subtype modulating this behavior. In particular, 5-HT$_{1A}$ receptor activation, which is also induced by eltoprazine, can also influence aggressive behavior.

α-Calcium-Calmodulin Kinase II

Homozygous mutant mice missing the gene for α-calcium-calmodulin-dependent kinase II (α-CaMKII[1]) display reduced offensive and defensive aggression (Chen et al. 1994). α-CaMKII mediates presynaptic transmitter release and activates tryptophan hydroxylase, the rate-limiting enzyme in 5-HT synthesis (Silva et al. 1992). 5-HT release is reduced in the dorsal raphe of mice missing the gene for α-CaMKII (Chen et al. 1994). Because murine aggressive behavior can also be motivated by fear, studies investigating knockout mice typically evaluate the mutants' response to fearful stimuli—α-CaMKII mutant mice display reduced fear when tested in a number of tasks (Chen et al. 1994). These findings emphasize the importance of testing a variety of behaviors when evaluating phenotypes of mutant mice.

Nitric Oxide

Nitric oxide (NO[1]) is very labile, with a half-life of <5 sec. Consequently, many studies have manipulated NO indirectly by affecting the synthetic enzyme nitric oxide synthase (NOS), which transforms arginine into NO and citrulline. Three distinct isoforms of NOS have been discovered: (1) one in the endothelial tissue of blood vessels (eNOS[1]); (2) an inducible form in macrophages (iNOS[1]); and (3) one in neural tissue (nNOS[1]) (Nelson et al. 1997). Suppression of NO formation either by elimination of arginine or by use of N-methyl-L-arginine, a potent NOS inhibitor, affects all three isoforms of NOS.

Neural Isoform of Nitric Oxide Synthase

nNOS is localized in high densities within emotion-regulating brain regions (Nelson et al. 1997). To examine the specific behavioral role of NO in neurons, homologous recombination was used to create nNOS–/– mice. Informal observation of the nNOS–/– mice revealed high levels of aggressiveness among male cage mates. Male nNOS–/– residents engaged in three to four times more aggressive encounters than WT mice in the intruder-resident model (Nelson et al. 1995). Nearly 90% of the aggressive encounters were initiated by the nNOS–/– animals. Similar results were obtained in diadic (paired) or group encounters in neutral arenas. In all test situations, male nNOS–/– mice rarely displayed submissive behaviors.

As mentioned above, behavioral studies of mice with targeted deletion of specific genes have met with the criticism that the gene product is not only missing during the testing period but is also missing throughout development when critical ontogenetic processes, including activation of compensatory mechanisms, may be affected. To address this criticism, mice were treated with 7-nitroindazole (7-NI[1]), which specifically inhibits nNOS formation in vivo (Demas et al. 1997). Mice treated with 7-NI displayed substantially increased aggression in two different tests of aggressiveness compared with control animals (Demas et al. 1997). Drug treatment did not affect nonspecific locomotor activities. Importantly, NOS activity in brain homogenates was reduced >90% in 7-NI-treated mice. Similarly, immunohistochemical staining for citrulline revealed a dramatic reduction in 7-NI-treated animals (Demas et al. 1997), suggesting that NO formation was virtually eliminated in these experimental animals. Because 7-NI treatment in WT mice increased aggression to the levels displayed by nNOS–/– mice, it appears that nNOS is an important mediator of aggression in male mice.

Plasma androgen concentrations can affect the display of aggressive behavior. There were no differences between nNOS–/– and WT mice in blood testosterone concentrations either before or after agonistic encounters. However, recent data on castrated nNOS–/– males suggest that testosterone is necessary, but not sufficient, to promote increased aggression in these mutants (Kriegsfeld et al. 1997). Castrated nNOS–/– mice displayed low levels of aggression that were equivalent to the aggression observed among castrated WT males. Androgen replacement therapy restored the elevated levels of aggression in nNOS–/– mice.

Importantly, inappropriate aggressiveness was never observed among female nNOS–/– mice in these test situations. However, when aggressive behavior was examined in female nNOS–/– mice in the context of maternal aggression when WT females are highly aggressive toward an intruder, nNOS–/– dams were very docile (Gammie and Nelson 1999). All other components of maternal care were normal in nNOS–/– females. There were no sensorimotor deficits among the mutant mice of either sex to account for the changes in aggressive behavior. Taken together, these results suggest that NO from neurons has important, but opposite, effects in the mediation of aggression in male and female mice.

Endothelial Isoform of Nitric Oxide Synthase

NO from the endothelial tissue could contribute to aggressive behavior. Mice with targeted disruption of the gene encoding eNOS–/– have recently been examined (Huang et al. 1995; Shesely et al. 1996). Because NO was originally identified as endothelium-derived relaxing factor (Furchgott and Vanhoutte 1989; Ignarro 1990) and eNOS is localized in the endothelial lining of vascular smooth muscle, blood pressure was the first phenotype investigated in these mutant mice (Huang et al. 1995; Shesely et al. 1996). eNOS–/– mice exhibit approximately a 35% increase in basal blood pressure relative to WT mice (110 and 81 mmHg, respectively; Huang et al. 1995).

Because nNOS–/– mice display elevated levels of aggressive behavior compared with WT mice, we recently investigated aggression in eNOS–/– mice (Demas et al. 1999). Anecdotal observations indicated that these knockout animals were very docile. Animals were tested using two behavioral paradigms. First, animals were tested using the resident-intruder paradigm. eNOS–/– mice displayed virtually no aggressive encounters and a dramatic decreased duration of agonistic encounters relative to WT mice when a WT intruder was placed in their home cage. Second, mice were tested in a neutral arena with a WT stimulus male. In this paradigm, eNOS-/- mice displayed many fewer attacks and a greatly increased latency to attack the stimulus male relative to WT mice (Demas et al. 1999). Pharmacologic normalization of blood pressure did not affect the absence of aggression in eNOS–/– mice. These data, in combination with the nNOS–/– data, suggest that the two isoforms of NOS may normally act to increase (eNOS–/–) and decrease (nNOS–/–) aggressive behavior in vivo. Thus, WT mice with normal concentrations of both isoforms of NOS display only moderate levels of aggression.

Oxytocin

Oxytocin (OT[1]) has been reported to mediate aggressive and affiliative behaviors in several species (Young et al. 1998). The behavioral role of OT has been established with physiologic manipulations that potentially affected blood pressure, which may have indirectly affected the behaviors under study. To provide converging evidence of the physiologic role of OT in aggressive behavior, WT, heterozygous (OT–/+), and homozygous (OT–/–) mutant mice were tested in two aggression paradigms (DeVries et al. 1997). In general, there was no significant difference in aggressiveness between WT and OT–/+ mice. However, there were significant reductions in the duration of aggressive behaviors among OT–/– animals, especially in agonistic encounters within neutral arenas. The OT–/– mice did not exhibit any sensorimotor deficits or display any altered general anxiety levels that may have accounted for the observed reduction in aggressive behavior (Young et al. 1998).

Neural Cell Adhesion Molecule

The neural cell adhesion molecule (NCAM[1]) is thought to be important during development and in adult neural plasticity (Goridis and Brunet 1992; Scholey et al. 1990). Testosterone concentrations are highly correlated with aggression in rodent species; castrated mice rarely display aggressive behavior, and testosterone replacement restores aggression to precastration values (reviewed in Gandelman 1980). Both homozygous (NCAM–/–) and heterozygous (NCAM+/–) mice deficient in the neural cell adhesion molecule display elevated aggression when tested in the resident-intruder paradigm (Stork et al. 1997). Studies of aggression in mice with targeted disruption of specific genes often fail to include measurements of testosterone, thereby complicating interpretation of the results from these studies. Pre- and posttest testosterone values in NCAM–/– mice are comparable with values seen in WT animals (Stork et al. 1997). Importantly, c-*fos* mRNA concentrations were elevated in limbic areas (i.e., septum, preoptic area, lateral hypothalamus, amygdala, and dorsal raphe) in NCAM–/– mice relative to WT animals after the interaction with the intruder (Stork et al. 1997). This finding suggests that NCAM–/– mice may experience a heightened "emotional" response to the presentation of a threatening stimulus, thereby leading to an increase in aggressive behavior. Converging evidence for this hypothesis was found when measuring pre- and posttest values of corticosterone in these mice. Although basal concentrations of corticosterone did not differ between genotypes, both NCAM–/– and NCAM+/– mutants exhibit a dramatic posttest increase in corticosterone concentrations relative to WT mice (Stork et al. 1997). Investigators studying aggression in knockout mice should consider not only the transmitter systems most likely affected by the genetic mutation but also the endocrine profiles of these mice.

Estrogen Receptor

Male mice with targeted disruption of the gene for the estrogen receptor, or estrogen receptor knockout (ERKO[1]), display reduced aggression in a number of testing situations

(Ogawa et al. 1997). Conversely, females exhibit increased levels of aggression toward other female mice relative to WT females, and ERKO females elicit aggression from WT males (Ogawa et al. 1996). Because estrogen is essential for the normal sexual differentiation of the central nervous system (CNS[1]) of male (and possibly female) mammals during development (Arnold 1996), studies of adult behavior in ERKO are complicated by the inability to dissociate genetic from ontogenetic causes of behavior.

Adenosine A_{2a} Receptor

The adenosine A_{2a} receptor is particularly abundant in basal ganglia, blood vessels, and platelets, and it stimulates adenylyl cyclase. It is a major target of caffeine, the psychoactive drug most widely used by humans. Mice with targeted disruption of the A_{2a} receptor (A_{2a}–/–) were generated. Anecdotal observations indicated that wounds due to bites were more common among the group-housed male A_{2a}–/– mice compared with WT males (Ledent et al. 1997). The A_{2a}–/– mice displayed an increased number of attacks and tail rattles, as well as a decreased latency to attack the intruder. In general, the A_{2a}–/– residents engaged in three to five times more aggressive encounters than the WT residents. These results are consistent with the inhibition in fighting and associated agonistic behaviors in isolated male mice treated with adenosine analogues (Palmour et al. 1989). The biochemical mechanism of adenosine receptor action with respect to aggression is unknown but may include neuromodulatory effects on the release of other neurotransmitters.

Interleukin-6

Interleukin-6 (IL-6[1]) is a cytokine released by activated immune cells that also affects brain function. In one study, aggressive and affiliative behaviors exhibited during agonistic encounters by transgenic male mice that either did not express IL-6 (IL-6 –/–) or overexpressed (NSE-hIL-6) IL-6 in the CNS were investigated (Alleva et al. 1998). Compared with WT controls, IL-6 –/– mice displayed more aggression. In contrast, NSE-hIL-6 mice displayed a tendency to be more involved in affiliative-type social interactions, displaying a higher frequency and duration of social behaviors such as anogenital, nose-to-nose, and whole body sniffing. IL-6 –/– mice showed a clear tendency to exhibit less affiliative interactions compared with WT mice. In any case, dopamine levels were modified in a number of brain regions in these IL-6 –/– mice (Alleva et al. 1998). Taken together, these results suggest that IL-6 may be involved in mediating specific features of social behavior.

Adrenergic α_{2C}-Receptors (α_{2C}-R)

Although all of the changes induced by pharmacologic manipulations of noradrenaline suggest a central role of this system in aggressive behavior, the participation of α_2-adrenoceptors is not clear. In the rat CNS, α_{2C}-R immunoreactivity is observed in cortex, septum, amygdala, hippocampus, raphe, striatum, and hypothalamic nuclei (Rosin et al. 1996), regions known to be involved in aggressive behaviors. Isolation-induced aggression was studied in two genetically engineered mouse strains, one with targeted inactivation of the α_{2C}-R (α_{2C}–/–) and the other with tissue-specific overexpression of α_{2C}-R (α_{2C}-OE) (Sallinen et al. 1998). These mutant mice have subtle alterations in their brain dopamine and 5-HT metabolism (Link et al. 1995). The α_{2C}–/– mice showed a reduced latency to attack, whereas the α_{2C}-OE mice, with a threefold targeted overexpression of α_{2C}-R, were associated with increased onset of fighting compared with their respective WT mice. However, no significant differences in the number of attacks were detected. A more ethologic approach of analysis, including the total time spent in aggressive behavior, would likely provide a more sensitive evaluation.

Neurokinin-1 Receptor

The peptide neurotransmitter substance P (SP[1]) modulates sensitivity to pain by activating neurokinin-1 (NK-1[1]) receptors, which are found throughout the brain (notably within the limbic system and hypothalamus). It has been suggested that SP plays a role in emotional behavior (Maeno et al. 1993). Male mice with targeted disruption of the gene encoding the NK-1 receptor were assessed in two resident-intruder test sessions after a previous training session (De Felipe et al. 1998). Even though the WT mice have low attack latency and display more attacks with repeated exposure to an intruder (previous fighting experience), the NK-1–/– mice remained considerably less aggressive than WT mice throughout testing. These data using knockout animals suggest a facilitatory role for SP in aggression. Consistent with this finding, defensive rage in cats provoked by medial amygdaloid-induced hypothalamic stimulation is blocked by a NK-1 antagonist either peripherally or injected in the medial hypothalamus (Shaikh et al. 1993).

Enkephalin

The endogenous opioid system appears to play a role in pain perception, anxiety, and aggression (Mansour et al. 1995). Enkephalins are endogenous opioid peptides that are derived from a preproenkephalin precursor protein. Enkephalinergic neurons are present in many regions involved in modulating emotional behaviors including the amygdala, hypothalamus, septum, cingulate, and entorhinal cortex (Nieuwenhuys

1985). Mutant enkephalin-deficient mice (enk–/–) were often aggressive, especially when housed individually (Konig et al. 1996). When evaluated in the resident-intruder test, the enk–/– mice scored higher in fighting and had a shorter attack latency in the first trial after two training sessions. In the second test trial, aggressive behavior did not differ significantly between enk–/– and enk+/+ mice. It is possible that the scoring system used to *estimate* the intensity of fighting and/or the 4-min duration of the test was not sufficiently sensitive to detect differences in aggression. These data are consistent with the results of several studies suggesting that opioids have inhibitory effects on aggression (Khantzian 1974; Shaikh et al. 1988). Because there is evidence suggesting that opioid peptides inhibit the release of SP in the hypothalamus (Micevych et al. 1984), it would be interesting to examine whether the increased aggression observed in the enk–/– mice is modulated by a facilitatory action of SP. These animals also exhibit a heightened supraspinal response to painful stimuli and increased anxiety as measured by the open field test (Konig et al. 1996).

Others

Both male and female mice that lack a functional tailless protein, an orphan nuclear receptor, are dramatically aggressive (Monaghan et al. 1997), although they have serum testosterone concentrations in the normal sex-specific range. These adult mutant mice show a reduction in the size of rhinencephalic and limbic structures, including the olfactory, infrarhinal, and entorhinal cortex, amygdala, and dentate gyrus. Tailless female –/– mice were more aggressive than WT males and engaged in 96% more aggression than WT females in resident and grouped aggression tests (unpublished observations), a rare trait in female mice.

Summary

Typically, altered behaviors of knockout mice are often sufficiently obvious or unusual that they catch the attention of animal care personnel, who then notify the investigators. Because of the large numbers of knockout mice being generated and because there are many neurobiologic pathways underlying aggression, it is likely that many knockout mice will exhibit increased aggression. Institutional staff may want to consider the ethical concern and implement standard behavioral analyses of knockout mice to preclude injury of group-housed mice.

Currently, dramatic behaviors including increased aggression, altered maternal care, seizures, impaired motor coordination, and sensory abilities are commonly reported for knockout mice (reviewed in Nelson and Young 1998). Although it has been noted that "... it is difficult ... to recognize minor neurologic abnormalities in mice" (Dutch-Belgian Fragile X Consortium 1994; p. 25), presumably additional aggressive behavioral changes of knockout mice, both dramatic and subtle, await discovery by behavioral biologists. We have presented a few examples for which aggressive behavior has been altered after deletion of a specific gene. Thousands of genetic knockouts are being created, but few are examined for specific behavioral changes. This new genetic research tool has the potential to improve our understanding of aggressive behavior dramatically.

Acknowledgments

We were supported during the preparation of this review by US Public Health Service grants MH 57535 and MH 57760 and a grant from Fundação de Amparo a Pesquisa de Estado de Sao Paulo-Brazil (98/10355-9).

References

Adams DB, Boudreau W, Cowan CW, Kokonowski C, Oberteuffer K, Yohay K. 1993. Offense produced by chemical stimulation of the anterior hypothalamus of the rat. Physiol Behav 53:1127-1132.

Aguzzi A, Brandner S, Sure U, Ruedi D, Isenmann, S. 1994. Transgenic and knock-out mice: Models of neurological disease. Brain Pathol 4:3-20.

Albert DJ, Walsh ML. 1984. Neural systems and the inhibitory modulation of agonistic behavior: A comparison of mammalian species. Neurosci Biobehav Rev 8:5-24.

Alleva E, Cirulli F, Bianchi M, Bondiolotti GP, Chiarotti F, De Acetis L, Panerai AE. 1998. Behavioural characterization of interleukin-6 overexpressing or deficient mice during agonistic encounters. Eur J Neurosci 10:3664-3672.

Arnold AP. 1996. Genetically triggered sexual differentiation of brain and behavior. Horm Behav 30:495-505.

Bell R, Hobson H. 1994. 5-HT1A receptor influences on rodent social and agonistic behavior: A review and empirical study. Neurosci Biobehav Rev 18:325-338.

Bell R, Warburton DM, Brown K. 1985. Drugs as research tools in psychology: Cholinergic drugs and aggression. Neuropsychobiology 14:181-192.

Boggs SS. 1990. Targeted gene modification for gene therapy of stem cells. Int J Cell Clon 8:80-96.

Bouissou MF. 1983. Androgens, aggressive behaviour and social relationships in higher mammals. Horm Res 18:43-61.

Bridges RS. 1996. Biochemical basis of parental behavior in the rat. In: Rosenblatt JS, Snowden CT, editors. Parental Care: Evolution, Mechanisms, and Adaptive Significance. San Diego: Academic. p 215-237.

Burnett AL, Nelson RJ, Calvin D, Demas GE, Klein SL, Kriegsfeld LJ, Dawson TM, Snyder SH. 1996. Nitric oxide-dependent penile erection in mice lacking neural nitric oxide synthase. Mol Med 2:288-296.

Cases O, Seif I, Grimsby J, Gaspar P, Chen K, Pournin S, Muller U, Aguet M, Babinet C, Shih JC. 1995. Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Science 268:1763-1766.

Chen C, Rainnie DG, Greene RW, Tonegawa S. 1994. Abnormal fear response and aggressive behavior in mutant mice deficient for α-calcium-calmodulin kinase II. Science 262:81-94.

Chiavegatto S, Dawson TM, Dawson VL, Nelson RJ. 1999. Serotonin is involved in the aggressive behavior of male nNOS null mice. (Abstract). Soc Neurosci Abs 25: 437.

Cohen-Tannoudji M and Babinet C. 1998. Beyond "knock-out" mice: New perspectives for the programmed modification of the mammalian genome. Mol Hum Reprod 4:929-938.

Cologer-Clifford A, Smoluk SA, Simon NG. 1996. Effects of serotonergic 1A and 1B agonists in androgenic versus estrogenic systems for aggression. Ann NY Acad Sci 20:339-342.

Crawley JN. 1999. Behavioral phenotyping of transgenic and knockout mice: Experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. Brain Res 835:18-26.

Crawley JN, Paylor R. 1997. A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. Horm Behav 31:197-211.

De Almeida RM, Lucion AB. 1997. 8-OH-DPAT in the median raphe, dorsal periaqueductal gray and corticomedial amygdala nucleus decreases, but in the medial septal area it can increase maternal aggressive behavior in rats. Psychopharmacology 134:392-400.

De Felipe C, Herrero JF, O'Brien JA, Palmer JA, Doyle CA, Smith AJ, Laird JM, Belmonte C, Cervero F, Hunt SP. 1998. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. Nature 392:394-407.

Demas GE, Eliasson MJL, Dawson TM, Dawson VL, Kriegsfeld LJ, Nelson RJ, Snyder SH. 1997. Inhibition of neuronal nitric oxide synthase increases aggressive behavior in mice. Mol Med 3:611-617.

Demas GE, Kriegsfeld LJ, Blackshaw S, Huang P, Gammie SC, Nelson RJ, Snyder SH. 1999. Elimination of aggressive behavior in male mice lacking endothelial nitric oxide synthase. J Neurosci 19:R30:1-5.

DeVries AC, Young WS, Nelson RJ. 1997. Reduced aggressiveness in mice with targeted disruption of the gene for oxytocin. J Neuroendocrinol 9:363-368.

Dutch-Belgian Fragile X Consortium. 1994. Fmr1 knockout mice: A model to study fragile X mental retardation. Cell 78:23-33.

Furchgott RF, Vanhoutte PM. 1989. Endothelium-derived relaxing and contracting factors. FASEB J 3:2007-2018.

Gammie SC, Nelson RJ. 1999. Maternal aggression is reduced in neuronal nitric oxide synthase-deficient mice. J Neurosci 19: 8027-8035.

Gandelman R. 1980. Gonadal hormones and the induction of intraspecific fighting in mice. Neurosci Biobehav Rev 4:133-140.

Goridis C, Brunet JF. 1992. NCAM: Structural diversity, function and regulation of expression. Semin Cell Biol 3:189-197.

Grisolía JS. 1997. Temporal lobe mechanisms and violence. In: Grisolía JS, Sanmartin J, Luján JL, Grisolía S, editors. Violence: From Biology to Sociology. Excerpta Medica International Congress. Series 1135. Amsterdam: Elsevier Science. p 43-52.

Gu H, Marth JD, Orban PC, Mossmann H, Rajewsky K. 1994. Deletion of a DNA polymerase gene segment in T cells using cell type-specific gene targeting. Science 265:103-106.

Haller J, Makara GB, Kruk MR. 1998. Catecholaminergic involvement in the control of aggression: Hormones, the peripheral sympathetic, and central noradrenergic systems. Neurosci Biobehav Rev 22:85-97.

Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC. 1995. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature 377:239-242.

Ignarro LJ. 1990. Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu Rev Pharmacol Toxicol 30:535-560.

Immelman K, Beer C. 1989. A Dictionary of Ethology. Cambridge MA: Harvard University Press.

Khantzian EJ. 1974. Opiate addiction: A critique of theory and some implications for treatment. Am J Psychother 28:59-70.

Klüver H, Bucy PC. 1939. Preliminary analysis of functions of the temporal lobes in monkeys. Arch Neurol Psychiatry 42:979-1000.

Konig M, Zimmer AM, Steiner H, Holmes PV, Crawley JN, Brownstein MJ, Zimmer A. 1996. Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. Nature 383:535-538.

Kraemer GW, Schmidt DE, Ebert MH. 1997. The behavioral neurobiology of self-injurious behavior in rhesus monkeys. Current concepts and relations to impulsive behavior in humans. Ann NY Acad Sci 836:12-38.

Kriegsfeld LJ, Dawson TM, Dawson VL, Nelson RJ, Snyder SH. 1997. Aggressive behavior in male mice lacking the gene for nNOS is testosterone-dependent. Brain Res 769:66-70.

Krsiak M. 1974. Behavioral changes and aggressivity evoked by drugs in mice. Res Commun Chem Pathol Pharmacol 7:237-257.

Kruk MR. 1991. Ethology and pharmacology of hypothalamic aggression in the rat. Neurosci Biobehav Rev 15:527-238.

Kühn R, Schwenk F, Aguet M, Rajewsky K. 1995. Inducible gene targeting in mice. Science 269:1427-1429.

Ledent C, Vaugeois JM, Schiffmann SN, Pedrazzini T, El Yacoubi M, Vanderhaeghen JJ, Costentin J, Heath JK, Vassart G, Parmentier M. 1997. Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A_{2a} receptor. 1997. Nature 388:674-678.

Leshner AI, Moyer JA. 1975. Androgens and agonistic behavior of mice: Relevance to aggression and irrelevance to avoidance-of-attack. Physiol Behav 11:671-676.

Link RE, Stevens MS, Kulatunga M, Scheinin M, Barsh GS and Kobilka BK. 1995. Targeted inactivation of the gene encoding the mouse alpha$_{2C}$-adrenoceptor homolog. Mol Pharmacol 48: 48-55.

Maeda H, Sato T, Maki S. 1985. Effects of dopamine agonists on hypothalamic defensive attack in cats. Physiol Behav 35:89-92.

Maeno H, Kiyama H, Tohyama M. 1993. Distribution of the substance P receptor (NK-1 receptor) in the central nervous system. Brain Res Mol Brain Res 18:43-58.

Mansour A, Watson SJ, Akil H. 1995. Opioid receptors: Past, present and future. Trends Neurosci 18:69-70.

Maroteaux L, Saudou F, Amlaiky N, Boschert U, Plassat JL, Hen R. 1992. Mouse 5HT1B serotonin receptor: Cloning, functional expression, and localization in motor control centers. Proc Natl Acad Sci U S A 89:3020-3024.

Micevych PE, Yaksh TL, Go VL. 1984. Studies on the opiate receptor-mediated inhibition of K+-stimulated cholecystokinin and substance P release from cat hypothalamus in vitro. Brain Res 290:87-94.

Miczek KA. 1983. Ethological analysis of drug action on aggression and defense. Prog Neuropsychopharmacol Biol Psychiatry 7:519-524.

Miczek KA, Krsiak M. 1979. Drug effects on agonistic behavior. In: Thompson T, Dews PB, editors. Advances in Behavioral Pharmacology. Vol 2. New York: Academic Press. p 87-162.

Monaghan AP, Bock D, Gass P, Schwager A, Wolfer DP, Lipp HP, Schutz G. 1997. Defective limbic system in mice lacking the tailless gene. Nature 390:515-517.

Moyer KE. 1968. Kinds of aggression and their physiological basis. Commun Behav Biol 2A:65-87.

Moyer KE. 1971. The Physiology of Hostility. Chicago: Markham.

Muehlenkamp F, Lucion A, Vogel WH. 1995. Effects of selective serotonergic agonists on aggressive behavior in rats. Pharmacol Biochem Behav 50:671-674.

Nelson RJ. 1997. The use of genetic "knock-out" mice in behavioral endocrinology research. Horm Behav 31:188-196.

Nelson RJ, Demas GE, Huang P, Fishman MC, Dawson V, Dawson TM, Snyder SH. 1995. Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase. Nature 378:383-386.

Nelson RJ, Kriegsfeld LJ, Dawson VL Dawson TM. 1997. Effects of nitric oxide on neuroendocrine function and behavior. Front Neuroendocrinol 18:463-491.

Nelson RJ, Young KA. 1998. Behavioral effects of targeted disruption of specific genes. Neurosci Biobehav Rev 22:453-462.

Nieuwenhuys R. 1985. Chemoarchitecture of the Brain. Berlin: Springer-Verlag. p 1-246.

No D, Yao TP, Evans RM. 1996. Ecdysone-inducible gene expression in mammalian cells and transgenic mice. Proc Natl Acad Sci U S A 93:3346-3351.

Ogawa S, Lubahn DB, Korach KS, Pfaff DW. 1997. Behavioral effects of estrogen receptor gene disruption in male mice. Proc Natl Acad Sci U S A 94:1476-1481.

Ogawa S, Taylor JA, Lubahn DB, Korach KS, Pfaff DW. 1996. Reversal of sex roles in genetic female mice by disruption of estrogen receptor gene. Neuroendocrinology 64:467-470.

Olivier B, Mos J. 1992. Rodent models of aggressive behavior and serotonergic drugs. Prog Neuropsychopharmacol Biol Psychiatry 16:847-870.

Olivier B, Mos J, Rasmussen D. 1990. Behavioural pharmacology of the serenic, eltoprazine. Drug Metabol Drug Interact 8:31-83.

Olivier B, Mos J, van Oorschot R, Hen R. 1995. Serotonin receptors and animal models of aggressive behavior. Pharmacopsychiatry 28(Suppl 2):80-90.

Palmour RM, Lipowski CJ, Simon CK, Ervin FR. 1989. Adenosine analogs inhibit fighting in isolated male mice. Life Sci 44:1293-1301.

Rosin DL, Talley EM, Lee A, Stornetta RL, Gaylinn BD, Guyenet PG, Lynch KR. 1996. Distribution of α_{2C}-adrenergic receptor-like immunoreactivity in the rat central nervous system. J Comp Neurol 372:135-165.

Sallinen J, Haapalinna A, Viitamaa T, Kobilka BK, Scheinin M. 1998. Adrenergic alpha$_{2C}$ -receptors modulate the acoustic startle reflex, prepulse inhibition, and aggression in mice. J Neurosci 18:3035-3042.

Sanchez C, Hyttel J. 1994. Isolation-induced aggression in mice: Effects of 5-hydroxytryptamine uptake inhibitors and involvement of postsynaptic 5-HT1A receptors. Eur J Pharmacol 264:241-247.

Saudou F, Amara DA, Dierich A, LeMeur M, Ramboz S, Segu L, Buhot MC, Hen R. 1994. Enhanced aggressive behavior in mice lacking 5-HT$_{1B}$ receptor. Science 265:1875-1878.

Scearce-Levie K, Chen JP, Gardner E, Hen R. 1999. 5-HT receptor knockout mice: Pharmacological tools or models of psychiatric disorders. Ann NY Acad Sci 868:701-715.

Schlinger BA, Callard GV. 1990. Aggressive behavior in birds: An experimental model for studies of brain-steroid interactions. Comp Biochem Physiol 97A:307-316.

Scholey AB, Rose SP, Zamani MR, Bock E, Schachner M. 1990. A role for the neural cell adhesion molecule in a late, consolidating phase of glycoprotein synthesis six hours following passive avoidance training of the young chick. Neuroscience 55:499-509.

Scott JP, Fredericson E. 1951. The causes of fighting in mice and rats. Physiol Zool 23:273-309.

Sedivy JM, Sharp PA. 1989. Positive genetic selection for gene disruption in mammalian cells by homologous recombination. Proc Natl Acad Sci U S A 86:227-231.

Shaikh MB, Shaikh AB, Siegel A. 1988. Opioid peptides within the midbrain periaqueductal gray suppress affective defense behavior in the cat. Peptides 9:999-1004.

Shaikh MB, Steinberg A, Siegel A. 1993. Evidence that substance P is utilized in medial amygdaloid facilitation of defensive rage behavior in the cat. Brain Res 625:283-94.

Shesely EG, Maeda N, Kim HS, Desai KM, Krege JH, Laubach VE, Sherman PA, Sessa WC, Smithies O. 1996. Elevated blood pressures in mice lacking endothelial nitric oxide synthase. Proc Natl Acad Sci U S A 93:13176-13181.

Siegel A, Roeling TA, Gregg TR, Kruk MR. 1999. Neuropharmacology of brain-stimulation-evoked aggression. Neurosci Biobehav Rev 23:359-389.

Silva AJ, Paylor R, Wehner JM., Tonegawa S. 1992. Impaired spatial learning in α-calcium-calmodulin kinase II mutant mice. Science 257: 206-211.

Soriano P. 1995. Gene targeting in ES cells. Ann Rev Neurosci 18:1-18.

Stark GR, Gudkov AV. 1999. Forward genetics in mammalian cells: Functional approaches to gene discovery. Hum Mol Genet 8:1925-1938.

Stork O, Welzl H, Cremer H, Schachner M. 1997. Increased intermale aggression and neuroendocrine response in mice deficient for the neural cell adhesion molecule (NCAM). Eur J Neurosci 9:1117-1125.

Svare B. 1983. Hormones and Aggressive Behavior. New York: Plenum.

Tonegawa S. 1995. Mammalian learning and memory studied by gene targeting. Ann NY Acad Sci 758:213-217.

Vergnes M, Depaulis A, Boehrer A. 1986. Parachlorophenylalanine-induced serotonin depletion increases offensive, but not defensive aggression in male rats. Physiol Behav 36:653-658.

White SM, Kucharik RF, Moyer JA. 1991. Effects of serotonergic agents on isolation-induced aggression. Pharmacol Biochem Behav 39:729-36.

Whittenberger JF. 1981. Animal Social Behavior. Boston: Duxbury Press.

Wilson EO. 1975. Sociobiology. Cambridge: Harvard University Press.

Young WS, Shepard E, DeVries AC, Zimmer A, LaMarca ME, Ginns EI, Amicol J, Nelson RJ, Hennighausen L, Wagner K-U. 1998. Targeted reduction of oxytocin expression provides insights into its physiological roles. Adv Exp Med Biol 449:231-240.