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Deletion of the *Bax* Gene Disrupts Sexual Behavior and Modestly Impairs Motor Function in Mice

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ABSTRACT: **Cell death is a nearly ubiquitous feature of the developing nervous system, and differential death in males and females contributes to several well studied sex differences in neuron number. Nonetheless, the functional importance of neuronal cell death has been subjected to few direct tests. *Bax*, a pro-apoptotic protein, is required for cell death in many neural regions. Deletion of the *Bax* gene in mice increases neuron number in several areas and eliminates sex differences in cell number in the brain and spinal cord. Here, sexual and motor behaviors were examined in *Bax*−/− mice and their wild-type siblings to test the functional consequences of preventing *Bax*-dependent cell death. Animals were gonadectomized in adulthood and provided with ovarian hormones or with testosterone for tests of feminine and masculine sexual behaviors, respectively. Wild-type mice exhibited a sex difference in feminine sexual behavior, with high lordosis scores in females and low scores in males. This sex difference was eliminated by *Bax* deletion, with very low receptivity exhibited by both male and female *Bax*−/− mice. Masculine sexual behavior was not sexually dimorphic among wild-type mice, but mounts and pelvic thrusts were nearly eliminated in *Bax*−/− mice of both sexes. Motor strength and performance at low speeds on a RotaRod apparatus did not differ by sex or *Bax* gene status. However, *Bax*−/− animals exhibited impairments on the RotaRod at higher speeds. Thus, developmental cell death may be required for masculine and feminine sexual behaviors and the fine tuning of motor coordination.**
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INTRODUCTION

Development of the mammalian nervous system is characterized by an overproduction of neurons, followed by a period of naturally-occurring cell death during which 20–80% of the neurons initially produced die by apoptosis (Oppenheim, 1991). The phenomenon is so widespread that it has become newsworthy to identify a neural region that does *not* undergo a period of developmental cell death. Nonetheless, the functional consequences of developmental neuronal cell death have received little attention. Authors of a comprehensive review recently summed up the state-of-the art by noting that studies on the adaptive role of neural cell death are still in their infancy, and "so far have revealed few striking behavioral or functional phenotypes" (Buss et al., 2006).

In some neural regions, gonadal steroid hormones regulate the magnitude of cell death, and several of the best studied sex differences in the nervous system are due to hormone-regulated cell death (reviewed in Forger, 2006). For example, testosterone secreted by the perinatal testes decreases developmental cell death in the spinal nucleus of the bulbocavernosus (SNB), the sexually dimorphic nucleus of the preoptic area (SDN-POA), and the principal nucleus of the

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bed nucleus of the stria terminalis (BNSTp) of rats, but increases cell death in the anteroventral periventricular nucleus (AVPV; Nordeen et al., 1985; Murakami and Arai, 1989; Davis et al., 1996; Chung et al., 2000). As a result, adult male rats have more neurons in the SNB, SDN-POA, and BNSTp, whereas females have more neurons than males in the AVPV (Breedlove and Arnold, 1983; Guillamon et al., 1988; Dodson and Gorski, 1993).

One approach to studying the function of neuronal cell death is to examine the behavior of animals in which programmed cell death has been perturbed. The death of developing neurons is crucially controlled by interactions among members of the Bcl-2 family of proteins (Merry and Korsmeyer, 1997). Some family members prevent cell death (e.g., Bcl-2), whereas others promote death (e.g., *Bax*). Mice overexpressing Bcl-2 exhibit reduced developmental cell death and increased cell number in several neural regions (Martinou et al., 1994; Bonfanti et al., 1996; Zanjani et al., 1996; Zup et al., 2003). Bcl-2 overexpressors display no impairment of basic motor or sensory functions, but exhibit a deficiency in a complex motor task (Rondi-Reig et al., 1999).

The proapoptotic protein *Bax* is singularly important for neuronal cell death and deletion of the *Bax* gene causes a nearly complete suppression of cell death in many regions of the developing nervous system (White et al., 1998). Although the brains and behavior of *Bax*−/− mice are reported to be grossly normal, detailed behavioral analyses have been lacking. We recently observed an increase in cell number and an elimination of sex differences in cell number, in the SNB, AVPV, and BNSTp of adult *Bax*−/− mice (Forger et al., 2004; Jacob et al., 2005). As these neural regions are involved in the control of copulatory behaviors and neuroendocrine function (e.g., Sachs, 1982; Claro et al., 1995; Simerly, 1996), we asked whether sexual behaviors, and/or sex differences in behavior, might be disrupted in *Bax*−/− animals. Here we examined feminine and masculine sexual behaviors in adult *Bax*−/− mice and their wild-type (*Bax*+/+) littermates. Tests of motor strength, balance, and coordination were also performed.

METHODS

Animals and Study Design

Bax+/+ and *Bax*−/− mice were obtained by mating males and females heterozygous for a *Bax* gene deletion (*Bax*+/−; Jackson Labs, Bar Harbor, ME). Although originally generated on a mixed C57Bl/6 × 129 background (Knudson et al., 1995), the mice subsequently were backcrossed onto C57Bl/6J for eight generations. Animals were genotyped by PCR amplification of tail DNA, using previously published primer sequences (White et al., 1998). Mice were singly housed (except for the social experience manipulation described below) and maintained on a reversed 14:10 light–dark cycle (lights off at 11 a.m.). All behavior testing began 1 h after lights off and was conducted under red light illumination.

Two separate cohorts of mice were used: Cohort I consisted of mice tested for feminine sexual behavior, as well as balance and coordination on a RotaRod apparatus. Mice of Cohort II were tested for motor strength, balance and coordination, and masculine sexual behavior. Details on behavior tests and number of animals per group for each test are given below.

Feminine Sexual Behavior

Animals were gonadectomized at 4–6 months of age under isoflurane inhalation anesthesia and testing began 1 month later. All subjects were sexually naïve at the time of the first test. Groups consisted of 10 *Bax*+/+ males, 10 *Bax*+/+ females, 9 *Bax*−/− males, and 8 *Bax*−/− females. Testing was conducted weekly for 6 consecutive weeks, as several weeks of repeated testing is required for the induction of maximal sexual receptivity in mice (Thompson and Edwards, 1971; Mani et al., 1997; Kudwa et al., 2005). On each week, mice were injected subcutaneously with estradiol benzoate (EB, 20 μg in 0.1 mL sesame oil) followed 48 h later by progesterone (P, 500 μg in 0.1 mL oil). Testing with gonadally intact stud males of the CD1 strain began 3 h after the P injection and was conducted in a clear Plexiglas arena (18 × 38 cm^2) fitted with a mirror to allow the visualization of intromissions. Stud males were first habituated in the arena for 30 min prior to testing. An experimental animal was then introduced and the pair was observed for a total of 20 min or 20 mounts, whichever came first. A lordosis response was scored when the subject remained immobile, with the four paws grounded and rump elevated above the floor of the cage, in response to a mount attempt by the stud male. A lordosis quotient was calculated as LQ = (number of lordosis responses/number of mounts) × 100. Latency to the first mount was also scored. Following sex behavior testing, animals of this cohort were tested on a RotaRod apparatus (see below).

Masculine Sexual Behavior

Sexually-naïve animals of Cohort II were gonadectomized at 2–3 months of age and a Silastic™ capsule (1.02 mm ID × 2.16 mm OD) packed with 5 mm crystalline testosterone (T) was placed subcutaneously between the shoulder blades at the time of surgery. Groups consisted of 10 *Bax*+/+ males, 10 *Bax*+/+ females, 10 *Bax*−/− males and 10 *Bax*−/− females. Masculine sexual behavior tests began 1 month after surgery; in the interim, subjects were tested on the RotaRod apparatus and Hang Test (see below). Four masculine sexual behavior tests were conducted on consecu-

tive weeks. For each test, the subject was placed in a neutral, 18 × 38 cm^2, Plexiglas arena with a receptive, sexually experienced stimulus female from our C57Bl/6J breeding colony that had been ovariectomized and injected with EB (20 μg in 0.1 mL sesame oil) followed by P (500 μg in 0.1 mL), as above. Animals were observed for 40 min and the number of mounts, mounts with pelvic thrusting, and ejaculations were scored. Low levels of masculine sexual behavior were observed in all groups during the first three tests. A "social housing" paradigm, in which animals are exposed to male or female conspecifics for short periods of time prior to testing, has previously been used to increase masculine sexual behavior in mice (Rissman et al., 1997; Wersinger and Rissman, 2000; Burns-Cusato et al., 2004). Therefore, following the third test, animals were housed for 2 days with a female mouse; the fourth test commenced 1 week later. Data from 2 animals (one *Bax−/−* male and one *Bax−/−* female) that died or showed signs of illness during testing were removed from analysis. Animals were killed 1 week after the last sex behavior test and seminal vesicles collected as an indirect measure of circulating androgen levels.

Hang Test

Neuromuscular strength was tested using a Hang Test as previously described (Crawley, 1999; Karl et al., 2003). Animals (n = 10 in each of the four groups) were allowed to grasp the lid of their cage for a few seconds before the cage lid was inverted and shaken gently from side to side. The latency of the animals to fall off was recorded up to 60 s. Each animal received two trials separated by at least 10 min. Animals with no neurological impairment are expected to remain on the wire lid throughout the test.

RotaRod Test

Motor coordination and balance were tested using an accelerating RotaRod apparatus (Columbus Instruments). Mice were first acclimated by allowing them to balance for 90 s on a portion of the rod textured with thin rubber strips while the rod slowly rotated. Virtually all animals remained on the rod throughout the practice session. For test trials, animals were placed on nontextured portions of the rod (3 cm diameter), which gradually accelerated from 0 to 11 rpm or 0 to 22 rpm Mice received four trials at the lower speed and, 1 week later, six trials at the higher speed. Individual trials were separated by at least 5 min. Latency to fall was noted at each trial, up to a maximum of 300 s. Both cohorts of mice were tested on the RotaRod, with similar results; data from the two cohorts are combined in the analysis below (n = 20 *Bax+/+* males, 20 *Bax+/+* females, 19 *Bax−/−* males and 18 *Bax−/−* females).

Statistics

Lordosis quotients in feminine sex behavior tests and latency to fall in the RotaRod tests were analyzed by two-way, repeated measures ANOVAs with sex and genotype as between-subjects factors and test session as the within-subjects factor. Significant main effects and interactions were followed by pairwise comparisons using Fisher's LSD. The proportion of animals in each group passing the Hang Test, and the proportion of animals exhibiting masculine sexual behaviors (mounts and pelvic thrusts), were compared using Fisher's Exact Tests. Data on latency to fall in the Hang Test, and mount and thrust frequencies in masculine sex behavior tests were not normally distributed and were therefore analyzed by the Mann–Whitney U test. Means ± SEMs are reported; probabilities <0.05 (two-tailed) were considered significant.

RESULTS

Feminine Sexual Behavior

Across the 6 weekly behavior tests, we found significant main effects of sex ($F_{1,32} = 7.5$, $p < 0.01$) and genotype ($F_{1,32} = 37.5$, $p < 0.0005$), as well as a sex-by-genotype interaction ($F_{1,32} = 5.9$, $p < 0.05$) on LQ. We also observed a significant effect of test-session ($F_{5,160} = 4.1$, $p < 0.002$) and a test-by-sex interaction ($F_{5,160} = 3.0$, $p < 0.05$). LQs were low among all groups during the first two tests (Fig. 1). Receptivity increased in subsequent tests in wild-type (*Bax+/+*) females, with LQs hovering around 80% for Tests 3 through 6 (Fig. 1). In contrast, LQs of wild-type males remained low throughout testing. LQs were

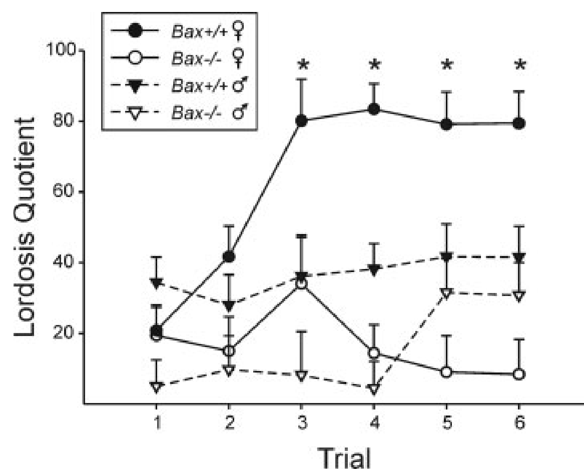

Figure 1 Mean (± SEM) lordosis quotients (LQs) of wild-type (*Bax+/+*) and Bax knockout (*Bax−/−*) mice over 6 weekly sex behavior tests. Animals were gonadectomized and treated with estradiol plus progesterone prior to testing. Female wild-type mice display high LQs from weeks 3 through 6. LQs are suppressed in wild-type males and in *Bax−/−* mice of both sexes. (Number of animals per group: 10 *Bax+/+* males, 10 *Bax+/+* females, 9 *Bax−/−* males, and 8 *Bax−/−* females).

Table 1 Performance of *Bax*+/+ and *Bax*−/− Mice on Masculine Sexual Behavior Tests, and Seminal Vesicle Mass at Sacrifice

	Mounting (%)	Thrusting (%)	Mounts[a,b] (*n*)	Seminal vesicles[b] (mg)
Male *Bax*+/+($n = 10$)	40.0	30.0	9.3 ± 3.7 (4)	368 ± 38
Female *Bax*+/+($n = 10$)	70.0	60.0	14.8 ± 2.7 (7)	–
Male *Bax*−/−($n = 9$)	5.5	0	3.0 (1)	381 ± 21
Female *Bax*−/−($n = 9$)	0	0	–	–

[a] Includes only those animals exhibiting the behavior.
[b] Means ± SEMs are reported.

significantly higher in wild-type females than in wild-type males during each of weeks 3, 4, 5, and 6 (all *p*-values ≤ 0.005), confirming the sex difference in feminine sexual behavior reported previously (Edwards and Burge, 1971).

Bax−/− mice of both sexes showed low levels of feminine sexual behavior and no significant increase in behavior across test sessions. There also was no sex difference in LQ between female and male *Bax*−/− animals over all six tests combined ($p > 0.30$) or on any single test (all $p > 0.10$). Across all tests, LQs in *Bax*−/− animals were lower than in wild-type females ($p < 0.0005$), and did not differ significantly from LQs of wild-type males ($p > 0.05$). Thus, *Bax* gene deletion impaired feminine sexual behavior and eliminated the sex difference in this behavior.

Latency to the first attempted mount by the stud male was similar in all groups; there was no effect of sex or genotype and no sex-by-genotype interaction on this measure (all $p > 0.80$). Collapsing across the six behavior tests, mean (±SEM) latency to the first mount for each group was as follows: *Bax*+/+ males, 293 ± 63 s; *Bax*+/+ females, 283 ± 71 s; *Bax*−/− males, 306 ± 40 s; *Bax*−/− females, 293 ± 82 s.

Masculine Sexual Behavior

Low rates of masculine sex behavior were observed prior to social housing: five wild-type animals (three male; two female) and no *Bax*−/− animals displayed mounts or mounts with thrusts during the initial three tests. Increased masculine sexual responding was observed in Test 4, following a social housing intervention (brief exposure to conspecifics; see Methods). The percentage of mice in each group exhibiting mounts or mounts with thrusts in any of the four behavior tests is given in Table 1.

We did not observe a sex difference in masculine sexual behavior. If anything, the proportion of females exhibiting mounts or mounts with thrusts across all tests was higher than for males, although this was not statistically significant ($p > 0.16$ for both mounts alone and mounts with thrusts; Table 1). There also was no significant effect of sex on the number of mounts or mounts with thrusts, regardless of whether all animals are included ($p > 0.20$ for mounts and $p > 0.15$ for mounts with thrusts, Mann-Whitney U Test), or only those animals exhibiting behavior are considered ($p > 0.25$ for mounts alone; $p > 0.10$ for mounts with thrusts).

Wild-type mice exhibited higher rates of masculine sex behavior than did *Bax*−/− mice: Ten of twenty (50%) wild-type animals displayed mounts and/or mounts with thrusts in at least one test, in contrast to only 1 of 18 *Bax*−/− animals (5.5%; Table 1). No *Bax*−/− mouse displayed any masculine sexual behavior during the first three tests, and a single *Bax*−/− male exhibited mounts with no thrusting during the fourth and final test. The near absence of male copulatory behavior in *Bax* knockouts limits the number of valid statistical comparisons that can be made. Fisher's Exact Test confirms that the proportion of animals mounting ($p < 0.003$) or performing mounts with pelvic thrusts ($p < 0.002$) was significantly lower in *Bax*−/− than in *Bax*+/+ mice. If all animals are included in the analysis (whether or not they showed any behavior), Mann–Whitney U Test confirms that *Bax*−/− animals exhibited fewer overall mounts ($p < 0.002$) and mounts with thrusts ($p < 0.001$) than did *Bax*+/+ mice. Considering only those animals exhibiting behavior, wild-type animals mounted on average 12.8 times per test (males and females combined), compared to three mounts for the single *Bax*−/− mouse that showed any masculine sexual behavior.

Motor Behavior

Most mice remained on the cage lid throughout both Hang Tests, and the percent of mice successfully completing both tests did not differ significantly by sex (70% male versus 85% female; $p > 0.15$) or genotype (80% *Bax*+/+ versus 75% *Bax*−/−; $p > 0.25$). Mean latency to fall also did not vary by sex ($p > 0.20$) or genotype ($p > 0.70$; data not shown).

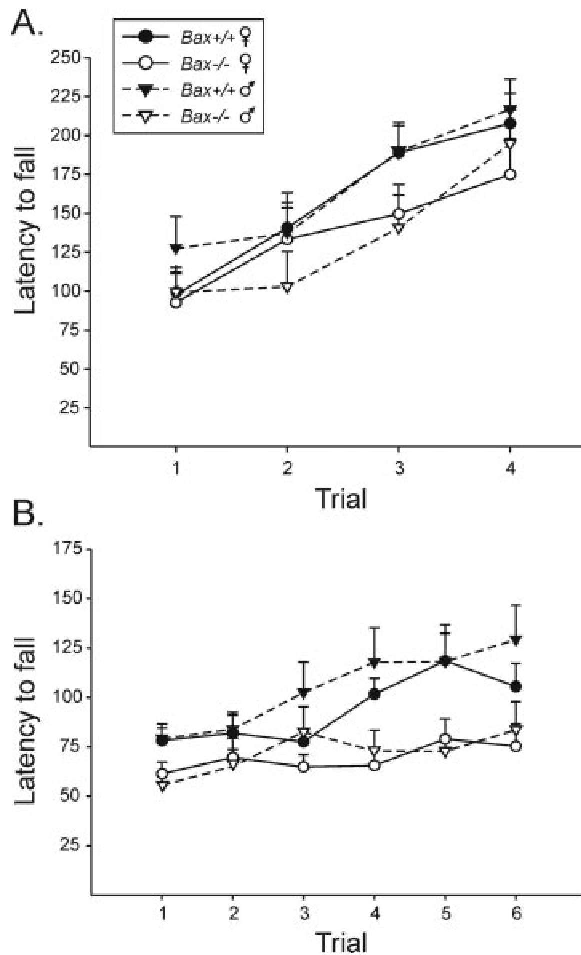

Figure 2 Performance of male and female *Bax*+/+ and *Bax*−/− mice on an accelerating RotaRod apparatus. (A) Mean (± SEM) latency to fall did not vary by sex or genotype at low rotation velocities (0–11 rpm). (B) *Bax*−/− mice were impaired relative to *Bax*+/+ animals at higher rotation velocities (0–22 rpm). (Number of animals per group: 20 *Bax*+/+ males, 20 *Bax*+/+ females, 19 *Bax*−/− males, and 18 *Bax*−/− females).

In the RotaRod test, there was no effect of sex on latency to fall at either speed ($p > 0.8$ and $p > 0.4$ for main effects of sex at low and high speeds, respectively). Mice of all groups improved with trial when tested at the lower speed [Fig. 2(A); $F_{3,216} = 29.12$, $p < 0.0005$]. There was a tendency for *Bax*−/− mice to fall with shorter latencies during low speed tests, but this was not significant ($F_{1,72} = 3.53$, $p < 0.07$). At higher speeds, *Bax*−/− mice were impaired relative to wild-type controls [Fig. 2(B); $F_{1,72} = 10.37$, $p = 0.002$]. Both genotypes again improved with trial at the higher speed ($F_{5,360} = 9.56$; $p < 0.0005$), but improvements were greater for wild-type than for *Bax* knockout animals ($F_{5,360} = 2.69$; $p < 0.03$ for trial-by-genotype interaction).

Although the motor deficit in *Bax* knockout animals was relatively subtle, we wondered whether decrements in sex behavior could be attributed to the motor impairment. To test this, mean latency to fall across all RotaRod trials was computed for each animal; this measure was greater in wild-type mice than in *Bax* knockouts (125.0 ± 6.6 s versus 96.8 ± 7.0 s for *Bax*+/+ and *Bax*−/−, respectively; $p < 0.005$, *t*-test). If analyses of feminine sex behavior are restricted only to *Bax*−/− females with mean RotaRod latencies above 125 s (i.e., above the mean score for wild-type mice), a clear deficit in sex behavior nonetheless remains ($F_{1,11} = 10.1$; $p < 0.01$, repeated measures ANOVA). We also computed Pearson correlation coefficients to test for a relationship between LQ and RotaRod performance of *Bax*−/− females. For these analyses, mean LQ for feminine sexual behavior tests 4–6 was determined for each animal and compared to mean latency to fall on the RotaRod during all trials (low plus high speed), or during only high speed trials. No significant correlations were found ($r = 0.368$, $p > 0.40$, and $r = 0.035$, $p > 0.90$ for all RotaRod trials and high speed trials, respectively). Thus, it is not likely that the motor deficit accounts for the feminine sex behavior deficit. A similar analysis for masculine sexual behavior was not possible, given the near absence of this behavior in *Bax*−/− animals.

DISCUSSION

Developing neurons display an unusual requirement for the *Bax* protein in programmed cell death. For most other cell types, *Bax* is functionally redundant with the related prodeath protein, Bak (Lindsten et al., 2000). Neurons lack full-length Bak, however, and instead express a splice variant (N-Bak) with altered function (Sun et al., 2001; Uo et al., 2005). Deletion of the *Bax* gene alone nearly abolishes apoptotic cells in the brain during the cell death period (White et al., 1998), and *Bax*−/− mice have more neurons than do Bax+/+ mice in many, but not all, neural regions (Deckwerth et al., 1996; Fan et al., 2001; Middleton and Davies, 2001; Sun et al., 2003; Forger et al., 2004; Jacob et al., 2005). Germ cells of the gonads and lymphoid cells also are affected in *Bax* knockout mice (Knudson et al., 1995; Perez et al., 1999). Despite the profound effect of *Bax* deletion on neuronal cell death, the brains of *Bax*−/− mice are grossly normal, and *Bax* knockouts are not distinguishable from wild-type littermates based on general behavior or external appearance (Knudson et al., 1995; and our own observations).

Upon closer inspection, however, we find that the absence of *Bax* severely impairs feminine and mascu-

line sexual behavior. As far as we know, this is the first study to examine sexual behavior in mice that are mutant for a cell death gene. Although it is possible that the behavioral impairments result from some as-yet-unknown function of *Bax* that is unrelated to cell death, the most likely interpretation is that developmental cell death is required for normal sexual behaviors in mice.

We also find a decrement in agility and coordination in *Bax*−/− mice based on performance on a RotoRod apparatus. Our motor findings are in accord with the selective deficits in performance of mice overexpressing the Bcl-2 protein under the control of the neural enolase promoter. Tests of vision, locomotor activity, motor strength, gait, and equilibrium were all normal in Bcl-2 over-expressors, but impairment on the RotaRod was noted, especially at high rotation velocities (Porciatti et al., 1999; Rondi-Reig et al., 1999, 2001). Bcl-2 transgenics also swam normally to a visible platform, but took longer to find a hidden platform than did wild-type mice (Rondi-Reig et al., 2001). Recently, a detailed analysis of neuromuscular development in *Bax* knockout mice was reported (Buss et al., 2006). Despite the fact that they have excess motoneurons, electrophysiological and behavioral analyses suggested that most parameters of motor function were completely normal in *Bax*−/− mice. *Bax* knockouts performed as well as controls on tests of motor coordination and balance (RotaRod, beam walking, and hole board walking) and performed better than controls on a test of grip strength (Buss et al., 2006). Muscle mass, muscle fiber density, and fiber composition also were indistinguishable between *Bax*+/+ and *Bax*−/− animals. Although the finding of normal performance on the RotaRod in *Bax*−/− animals appears to contradict the current report, a single latency measure was reported for each group by Buss and colleagues, in contrast to the multiple trials used here and by Rondi-Reig et al. (1999). Taken together, we conclude that motor and sensory functions develop relatively normally in mice with supernumerary neurons due to Bcl-2 overexpression or *Bax* deletion, but fine grained testing may reveal small differences (impairments *or* enhancements) in performance. In contrast, the effects of *Bax* deletion on sexual behaviors appear to be more robust.

Following gonadectomy in adulthood and treatment with EB and P, female mice more readily exhibit the lordosis posture in response to mount attempts than do males (Edwards and Burge, 1971). This sex difference was confirmed among wild-type mice in the present report, but eliminated by *Bax* deletion: *Bax*−/− females showed low LQs throughout 6 weeks of testing, and did not differ from knockout or wild-type males on this measure. *Bax*−/− females are poor breeders, and exhibit ovarian abnormalities in aging (Perez et al., 1999). Because *Bax* knockout females in the current study remained sexually unreceptive even after gonadectomy and replacement with ovarian hormones, we conclude that the reduced fertility may be due, in whole or in part, to behavioral effects of *Bax* deletion, irrespective of the ovaries.

Low levels of feminine sexual behavior could be due to many factors, including reduced motivation, altered sensory processing (especially of olfactory stimuli, see Bakker, 2003, for review), changes in responsiveness to gonadal hormones, or reduced attractivity of *Bax* knockouts to stud males. Although the data presented here do not allow us to discriminate between these possibilities, we note that stud males did readily attempt to mount *Bax*−/− animals of both sexes, and latency to first mount by the stud male did not differ across groups. In addition, the doses of EB and P used here were much higher than the minimum doses required for eliciting receptivity in wild-type C57BL/6 females (Mani et al., 1996), and similar to doses used in earlier work on sexual differentiation of mouse feminine sexual behavior (Edwards and Burge, 1971; Edwards, 1971; Vale et al., 1973). Thus, it is unlikely that the low level of receptivity shown by *Bax*−/− females was due to insufficient levels of steroid hormones.

Previous reports have been contradictory with respect to the question of whether male copulatory behavior is sexually dimorphic in mice. Females of several mouse strains show high levels of mounting and thrusting in response to a receptive female when treated with T or EB in adulthood (Edwards and Burge, 1971; Manning and McGill, 1974; Wersinger et al., 1997; but see Vale et al., 1973). In the present study, wild-type females exhibited masculine sexual behaviors at rates that were, if anything, (nonsignificantly) higher than in males. Taken together, we conclude that masculine sexual behavior is not sexually differentiated in C57Bl/6J mice, at least under the conditions tested here.

No *Bax*−/− animals displayed pelvic thrusts or intromissions and only one *Bax*−/− mouse mounted in any of four masculine sex behavior tests in the current study. Thus, a clear deficit in masculine sexual responding was identified in *Bax* knockouts. However, only 50% of our wild-type animals exhibited masculine sexual behaviors in tests with stimulus females, suggesting that some aspect of our test regimen may not have been optimal for eliciting the behavior. The mating arena, T capsules, and other aspects of our test protocol were all patterned on

those used previously (Wersinger and Rissman, 2000; Dominguez-Salazar et al., 2004; Kudwa et al., 2005). In addition, the percentage of wild-type mice exhibiting copulatory behaviors here is similar to that reported in a recent study also using C57Bl/6J mice (Bodo and Rissman, in press). Nonetheless, by testing the animals in their home cages, rather than in the presumably more stressful neutral arena (cf., Wee and Clemens, 1989), and/or by extending the length of each test (cf., Wersinger et al., 1997), it is possible that we would have observed higher response rates in both wild-type and *Bax* knockout animals.

Although the testes of *Bax−/−* adults are reduced in size (Knudson et al., 1995), the gonadal phenotype is unlikely to have contributed to the lack of masculine sexual behavior in *Bax* knockouts on several grounds. First, our tests were conducted on gonadectomized mice with equivalent hormone replacement. In addition, the testicular phenotype of *Bax−/−* animals appears confined to the seminiferous tubules; the androgen-producing Leydig cells are not affected (Rodriguez et al., 1997), and both anogenital distance (an indirect measure of developmental androgen exposure) and circulating T levels are normal in *Bax* knockouts (Forger et al., 2004). We propose instead that neural changes, resulting from the prevention of developmental cell death in the brains of *Bax−/−* mice, are responsible for the observed deficits in both masculine and feminine sexual behaviors.

The specific neural changes that might lead to impairments in the sexual behavior of *Bax* knockout mice are not known. We previously demonstrated that *Bax* gene deletion increases cell number in the BNSTp and AVPV, and eliminates the normal sex differences in neuron number in these regions (Forger et al., 2004). In general, sex differences in neuron number are thought to contribute to sex differences in behavior or function, although this has rarely been directly tested. It has been proposed that the "extra" cells in the BNSTp of male rats may serve to inhibit feminine sexual behaviors (Segovia and Guillamon, 1993), based primarily on the observations that BNST neurons send direct, inhibitory projections to the AVPV and ventromedial nucleus of the hypothalamus, regions crucial for ovulation and the expression of lordosis, respectively (López and Carrer, 1982; Polston et al., 2004). Our present finding, that feminine sexual behavior is suppressed in male and female *Bax−/−* mice, which have elevated cell number in the BNSTp (Forger et al., 2004), is consistent with this proposal.

On the other hand, the BNSTp is important for masculine sexual behavior, with lesions to this region disrupting copulation in male rats (Emery and Sachs, 1976; Claro et al., 1995). Thus, based on a simplistic model in which "more is better," one might have predicted good or even enhanced male copulatory behaviors in *Bax−/−* mice, which clearly was not observed. Normal sexual functioning in rodents is complex, requiring the processing of sensory cues and the integration of these cues with hormonal signals to trigger appropriate motor output (Blaustein and Erskine, 2002; Hull et al., 2002). Many more brain regions are involved than have been so far examined in *Bax−/−* animals. In addition, it is not known whether neurons rescued by *Bax* deletion are functional or project normally to targets (Sun et al., 2003; Jacob et al., 2005; Buss et al., 2006). Brain regions such as the AVPV, the BNSTp, and other regions involved in neuroendocrine function and/or sex behavior are heterogeneous with respect to neurochemistry and projection patterns, and *Bax* deletion does not affect the survival of all cell types equally (Forger et al., 2004). In future studies it may be possible to alter developmental neuronal cell death with regional and neurochemical specificity, to identify the functional consequences of perturbing the number of neurons in select brain regions expressing particular neurotransmitters and/or neuropeptides. Until that time, it will be difficult to pinpoint the neural basis for the observed disruptions in *Bax* knockout mice.

The widespread occurrence of programmed cell death during neural development suggests an essential role for this process. Observations of *Bax* knockout and Bcl-2 overexpressing mice indicate that a perturbation of neural cell death, while not incompatible with survival, may disrupt sexual behavior, and other complex behaviors requiring the integration of multiple neural systems. Because mutations in genes related to apoptosis are not likely to be passed to subsequent generations if they cause even small decrements in fertility, these findings may help to explain the remarkable conservation of the Bcl-2 gene family through evolution, with homologs of *Bcl-2* and *Bax* playing important roles in neuronal cell death in species ranging from nematodes to humans (Putcha and Johnson, 2004).

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