Amyloid β Precursor Protein Regulates Male Sexual Behavior

Jin Ho Park,¹ Paul J. Bonthius,^{1,2} Houn-Wei Tsai,¹ Stefan Bekiranov,¹ and Emilie F. Rissman^{1,2}

¹Department of Biochemistry and Molecular Genetics, and ²Neuroscience Graduate Program, University of Virginia School of Medicine, Charlottesville, Virginia 22908

Sexual behavior is variable between individuals, ranging from celibacy to sexual addictions. Within normal populations of individual men, ranging from young to middle aged, testosterone levels do not correlate with libido. To study the genetic mechanisms that contribute to individual differences in male sexual behavior, we used hybrid B6D2F1 male mice, which are a cross between two common inbred strains (C57BL/6J and DBA/2J). Unlike most laboratory rodent species in which male sexual behavior is highly dependent upon gonadal steroids, sexual behavior in a large proportion of these hybrid male mice after castration is independent of gonadal steroid hormones and their receptors; thus, we have the ability to discover novel genes involved in this behavior. Gene expression arrays, validation of gene candidates, and transgenic mice that overexpress one of the genes of interest were used to reveal genes involved in maintenance of male sexual behavior. Several genes related to neuroprotection and neurodegeneration were differentially expressed in the hypothalamus of males that continued to mate after castration. Male mice overexpressing the human form of one of these candidate genes, amyloid β precursor protein (*APP*), displayed enhanced sexual behavior before castration and maintained sexual activity for a longer duration after castration compared with controls. Our results reveal a novel and unexpected relationship between APP and male sexual behavior. We speculate that declining APP during normal aging in males may contribute to the loss of sexual function.

Introduction

One of the most highly conserved and evolutionarily important behaviors is copulation. In most laboratory rodents male sexual behavior (MSB) is dependent upon gonadal hormones and their corresponding steroid receptors. This association between mating and gonadal steroids is a product of evolution since small short-lived mammals in the temperate zone restrict their mating to discreet times of the year (Bronson, 1989). Thus coordination of sperm production and mating behaviors is critical. In other vertebrates, particularly larger mammals, the role of gonadal steroids in MSB is less predictable and highly variable. Humans are an excellent example of a species in which individual differences in sexual behavior are prominent. Among normal men, testosterone levels in plasma do not correlate with reported sexual activity (Bagatell et al., 1994). Even castration or anti-androgen drug therapies produce wide and unpredictable variations in sexual activity ranging from no decline to a gradual waning in sexual activity (Phoenix et al., 1973; Miceal and Wilson, 1974).

One model organism for examining the genetics underlying these differences in behavior is the B6D2F1 hybrid male mice

produced by crossing two common mouse strains, C57BL/6J and DBA/2J (McGill and Manning, 1976; Clemens et al., 1988). Approximately 30% of all B6D2F1 males retain the ability to display ejaculatory reflexes for as many as 25 weeks after castration (herein after referred to as maters). Thus within populations of these hybrids there is both behavioral and genetic diversity. After castration, maters and non-maters do not differ in concentrations of plasma testosterone (T), hypothalamic nuclear estrogen receptors or neural aromatase activity (Clemens et al., 1988; Sinchak et al., 1996). Blockade of androgen or estrogen receptors or aromatase enzyme which catalyzes the conversion of androgens to estrogens do not extinguish the expression of sexual behavior in maters (Park et al., 2009). To investigate the genetic bases for male sexual behavior gene expression analyses were conducted using tissue from brain regions essential for male copulatory behavior, including the medial preoptic nucleus (mPOA) and bed nucleus of the stria terminalis (BNST).

One of the top candidate genes identified from the gene expression analyses was amyloid β precursor protein (*APP*). APP is normally involved in cell survival and neuroprotection; however, when APP processing is altered, an increase in A β 42 and N-APP occurs which impairs synaptic plasticity and induces aberrant neuronal and axonal degeneration (for review, see Kim and Tsai, 2009). Aberrant trafficking of APP may play a role in the pathogenesis of Alzheimer's disease (for review, see Selkoe, 1999; Sinha and Lieberburg, 1999). Relative expression of *APP* was upregulated in the maters when compared with the non-maters. To test the hypothesis that increased APP would enhance male sexual behavior, transgenic mice that overexpress human APP were examined.

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Correspondence should be addressed to Dr. Jin Ho Park, Psychology Department, University of Massachusetts, Boston, 100 Morrissey Boulevard, Boston, MA 02125. E-mail: JinHo.Park@umb.edu.

H.-W. Tsai's current address: Department of Biological Sciences, California State University at Long Beach, Long Beach, CA 90840.

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Materials and Methods

Animals. Male B6D2F1 hybrid mice (*Mus musculus*) were produced in the Jordan Hall Vivarium at the University of Virginia by crossing C57BL/6J females with DBA/2J males. Three different cohorts of male hybrid mice were used for the experiments. One cohort ($n = 35$) was used for the microarray study, another cohort ($n = 40$) for the quantitative reverse transcription (qRT)-PCR, and a third for the Western blots ($n = 48$). Hybrid B6D2F1 male mice were weaned at 20–21 d of age, single-sex group housed until the beginning of each of the experiments (between 50 and 80 d of age), and individually housed afterward for the rest of the experiments. All animals were maintained on a 12 h light/dark cycle (lights off at 12:00 P.M. EST). All of the mice received food (Harlan Diet 8604) and water *ad libitum* in the University of Virginia Animal Care Facility.

C57BL/6J females were used as stimulus females for MSB testing. Females were ovariectomized and injected subcutaneously with 0.5 μ g of estradiol (dissolved in sesame oil) 48 h before testing. Three to 5 h before testing, stimulus females were injected subcutaneously with 0.83 μ g of progesterone. The females were group-housed in the same colony room as the experimental males.

APP transgenic mice (B6.129S2-Tg(APP)^{8.9Btla}/J; Jackson Laboratory; stock #5301; $n = 10$) were developed by transfecting a 650 kb YAC transgene containing entire human APP gene (and ~350 kb flanking sequence) into 129S2/SvPas-derived D3 embryonic stem cells (Lamb et al., 1993). Founder animals were backcrossed to C57BL/6J for 11 generations. These mice express all mRNA and protein isoforms of the wild-type human amyloid β (A4) precursor protein, APP, and the expression pattern of the various protein isoforms of human APP mimics endogenous mouse gene expression patterns. There is a ~70% increase of total APP levels in brain extracts of the APP transgenic mice when compared with controls (Lamb et al., 1993). C57BL/6J males from our colony ($n = 10$) were used as controls. All animal procedures were conducted in accordance with our animal protocol, approved by the University of Virginia Committee on Animal Care and Use.

Behavioral testing. MSB was tested under dim red lights during the dark phase of a light/dark cycle (Wersinger et al., 1997). All males were habituated 1 h before the introduction of the stimulus female in an 18 $\times$ 40 $\times$ 11 cm clear Plexiglas testing cage containing the male's home cage bedding. Tests began with the introduction of a hormone-treated stimulus female. Once the male mounted, the test continued to a criterion of a successful ejaculatory reflex or for 120 min, whichever occurred first. If the stimulus female became unreceptive during testing she was replaced with a receptive female.

All tests were videotaped and scored by an observer blind to the classification of the individuals. During each behavioral test, the behavioral components recorded were as follows: mount latency (ML; time from the introduction of a receptive female to the first mount), intromission latency (IL; time from the introduction of a receptive female to the first intromission), and ejaculation latency (EL; interval between the first intromission and ejaculation).

All males received sexual experience before castration. Males that continued to copulate after castration were considered to be "maters" if they demonstrated the ejaculation reflex on at least three of the last four behavioral tests, including the last test. Males used in the gene expression study were tested between weeks 22 and 28 postcastration, for the qRT-PCR experiment testing was performed between weeks 14 and 20 postcastration and males used for protein studies were tested on weeks 16–22 postcastration. APP transgenic mice and controls had 4 weekly MSB tests before castration and were retested for 12 weeks after castration.

Gene expression microarray. Brains were rapidly dissected and frozen in dry ice and stored at -80°C . Brains were cut into 120- μ m-thick coronal sections with a Bright cryostat. Based on the mouse brain atlas (Franklin and Paxinos, 1997), the brain areas containing the mPOA and BNST were dissected bilaterally from ~8 single sections. Samples were homogenized at room temperature in QIAzol Lysis Reagent (Qiagen) and stored at -80°C until ready to be processed for RNA isolation. Qiagen kits were used to collect total RNA from brain areas containing both the mPOA and BNST from maters ($n = 5$) and non-maters ($n = 5$). Sufficiently high

quality RNA was generated from each individual animal; thus providing five biological replicates for both groups.

The University of Virginia Biomolecular Facility (BMF) hybridized the mRNA samples to Affymetrix GeneChip Mouse Genome 430 2.0 Arrays that contains probe sets that monitor >39,000 transcripts. The BMF performed a number of quality control checks on the samples including the purity and concentration of the RNA. A number of quality control analyses were performed on the array data including intensity histogram plots, calculation of background levels, and fraction of probes sets detecting RNA. Normalized unscaled SE (NUSE) and relative log expression (RLE) plots, which are sensitive indicators of hybridization quality (Gentleman et al., 2005) were generated. The data were further checked for reproducibility by generating MvA plots which are pairwise scatter plots of $M = \text{difference of log intensities}$ (y -axis) versus $A = \text{average log intensities}$ (x -axis) for all replicates. All replicates compared well to each other; therefore all 10 arrays were included in the analysis. A principle components analysis also revealed that the maters and non-maters were well separated from each other, supporting the fact that the data were reproducible within each group and there was a significant change in global gene expression patterns when comparing maters and non-maters (supplemental Fig. S1, available at www.jneurosci.org as supplemental material).

Differentially expressed genes were identified by first quantile normalizing all arrays together (Bolstad et al., 2003) and then estimating relative log₂ RNA abundance using the GCRMA (Wu et al., 2004) package in the Bioconductor software suite (Gentleman et al., 2005). Modified t tests on the log₂ expression estimates using the limma package were performed (Smyth, 2004). The false discovery rate (FDR) (Benjamini and Hochberg, 1995; Benjamini and Yekutieli, 2001; Smyth, 2004) for each gene was estimated (i.e., the estimated rate of false-positives divided by the total positives derived from the t test p -values) to correct for the fact that ~40,000 statistical tests were performed. A list of differentially expressed genes was derived by applying an FDR cutoff of 5%.

Quantitative RT-PCR. Total RNA was isolated from mouse brain tissues using RNeasy Lipid Tissue Mini kit (Qiagen) as described by the manufacturer's protocol. The quantity (A_{260}) and quality (A_{260}/A_{280}) of RNA were determined (Bio-Rad SmartSpec Plus spectrophotometer). The cDNA templates were prepared using SuperScript Reverse Transcriptase (Invitrogen). The reverse transcription reaction consisting of 1 μ g of total RNA, 500 ng of oligo (dT)_{12–18}, 500 μ M each dNTP, 10 mM DTT, 40 U of RNaseOUT RNase inhibitor, and 200 U of SuperScript RT was incubated at 37°C for 1 h and then heat-inactivated at 70°C for 15 min.

Real-time PCR was performed using ABI Prism 7300 Real-Time PCR System with Sequence Detection Software version 1.2.3 (Applied Biosystems). Separate β -actin endogenous control reactions were used to normalize RNA input. Oligonucleotide primers were designed using Primer Express version 2.0 and synthesized by Invitrogen (supplemental Table S3, available at www.jneurosci.org as supplemental material). The real-time PCR conditions were 95°C for 3 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. After the last PCR cycle, a dissociation melting curve stage was run according to software protocol. Target and endogenous control genes were measured in triplicate for each cDNA sample during each real-time run to avoid intersample variance. For each RNA sample a no-reverse transcriptase reaction was run in parallel to cDNA synthesis, and measured by qRT-PCR to control for contamination and genomic amplification. Each qRT-PCR was verified for a single PCR product of expected size with the dissociation melting curve stage, and some samples were checked with gel electrophoresis.

Normalization and quantifications of the genes of interest, and β -actin mRNA were performed with the comparative cycle thresholds (C_T) method as described in the ABI PRISM 7700 sequence detection system user bulletin (#2). Validation experiments were conducted to test for equally efficient target and endogenous control gene amplification as described in the user bulletin. All of the primers were at between 90 and 110% efficient for all amplifications.

Western blot analysis. Maters ($n = 10$), non-maters ($n = 6$) and sexually experienced gonad intact ($n = 6$) hybrid mice were killed, and the mPOA and BNST were collected from each. Males were tested biweekly

for sexual behavior between 10 and 20 weeks after castration; those that ejaculated on at least 5 of the 6 tests, including the last test, were considered maters and those that did not ejaculate on any of the tests were non-maters.

For protein extraction, brain tissues were thawed and homogenized in cold RIPA buffer (0.05 M Tris, 0.9% NaCl, 5 mM EDTA, pH = 7.4) with a protease inhibitor (Sigma). Tissue was homogenized, centrifuged and total protein concentrations were determined by BCA (bicinchoninic acid) Protein Assays (Pierce Chemical Co.). Samples were subjected to electrophoresis on 14% polyacrylamide-SDS gels and transferred to nitrocellulose. Membranes were blocked for 1 h then rinsed and incubated with APP antibodies (Millipore) overnight at 4°C. After rinsing, blots were incubated for 1 h in a horseradish peroxidase (HRP)-conjugated sheep anti-mouse IgG secondary antibody (1:10,000; Jackson Laboratory), followed by detection on x-ray film (Kodak X-OMAT) with SuperSignal West Pico Chemiluminescent Substrate (Pierce Chemical Co.). Later, blots were reprobbed with antibody against β -actin (1:50,000; Sigma-Aldrich Corp.), and after rinsing, the blots were incubated for 1 h in an HRP-conjugated sheep anti-mouse IgG secondary antibody (1:10,000; Jackson Laboratory) followed by chemiluminescent detection. The intensities of each of the candidate proteins and β -actin were measured and analyzed by densitometry with ImageQuant (Molecular Dynamics). Levels of each of the candidates were normalized to those of β -actin in each sample and a standard which was run on every gel. The standard used to normalize between blots was the protein concentration of the frontal cortex of a randomly selected hybrid mouse.

Statistical analysis. Chi-square tests were used to compare differences in the proportion of males displaying copulatory behavior between groups. Repeated ANOVAs were used to analyze the number of mounts and intromissions, as well as mount, intromission and ejaculation latencies of all mice. One-way ANOVAs were used to analyze mRNA levels and protein levels between groups. *Post hoc* comparisons were conducted using the Fisher Protected Least Significant Difference test where appropriate. Survival analyses were performed using the Mantel-Cox log-rank test to generate Kaplan-Meier curves which showed the percentage of mice displaying MSB at a specific time point during a behavioral test. Any test in which an animal never mounted was a censored data point of 7200 s indicating that the animal did not mount for at least the first 7200 s of the test. Any test in which an animal did not mount was dropped from the dataset for latency to intromit and ejaculate. The latency to intromit in any test in which an animal mounted but did not intromit was a censored data point calculated as 7200 s minus the latency to mount, and the test was dropped from the dataset for latency to ejaculate only. The latency to ejaculate in any test in which an animal intromitted but did not ejaculate, was a censored data point calculated as 7200 s minus latency to intromit starting from when the female was introduced. Observed differences were considered significant if $p < 0.05$. Statistical tests were run using the Statview program (Statview 5; SAS Institute).

Results

Five biological replicates for both groups were processed by Affymetrix gene expression arrays. Brain is a very complex tissue with diverse cell types and mRNA populations so it is important to apply rigorous statistical analysis of significance. A total of 532 differentially expressed genes were found after applying a 5% false discovery rate cutoff (Benjamini and Hochberg, 1995; Benjamini and Yekutieli, 2001; Smyth, 2004). Of these, expression of 267 genes was downregulated and expression of 265 genes was upregulated in maters relative to non-maters (supplemental Table S1, available at www.jneurosci.org as supplemental material). This list of candidates and their corresponding fold changes were entered into Pathway Express (Khatri and Drăghici, 2005). Seven of the 12 most impacted pathways were neurophysiological with 6 involved in neurodegenerative disease (Table 1). The list of candidate genes was further narrowed by genotype data from recombinant inbred mouse lines generated from the B6D2F1 hybrid mice (labeled BXD1–100). Previously (Coquelin, 1991) two

Table 1. The top 12 biological pathways impacted by changes in gene expression between maters and controls calculated using Pathway Express

Rank	Pathway name	Impact factor	% pathway genes	p-value
1	Phosphatidylinositol signaling system	35.28	2.74	1.73E-14
2	Alzheimer's disease	5.961	13.636	0.017940817
3	Type II diabetes mellitus	5.665	2.222	0.02309521
4	Axon guidance	4.94	4.545	0.042498314
5	Long-term depression	4.703	2.778	0.051714987
6	Huntington's disease	4.504	10.714	0.06089983
7	Dentatorubropallidolysian atrophy	4.399	7.143	0.066351677
8	Neurodegenerative disorders	4.006	9.091	0.091139607
9	Regulation of actin cytoskeleton	3.825	4.02	0.105273951
10	Tight junction	3.615	4.274	0.12422161
11	Amyotrophic lateral sclerosis (ALS)	3.029	5.882	0.194858467
12	MAPK signaling pathway	2.863	3.03	0.220566528

The impact factor is associated with an estimate of the degree to which the output products of the pathway are perturbed by changes in gene expression. The % pathway genes is the percentage of pathway genes that are differentially expressed, and the p-value is the probability that the list of 532 genes would be found in each respective pathway by chance.

Table 2. Candidate genes identified that may mediate persistent copulation in long-term orchidectomized B6D2F1 hybrid mice along with the pathways to which they have been associated

Gene	Description	Pathway
<i>PTEN</i>	Phosphatase and tensin homolog	Inositol phosphate metabolism
<i>IMPA1</i>	Inositol (<i>myo</i>)-1(or 4)-monophosphatase 1	Inositol phosphate metabolism
<i>SCA1A</i>	Sodium channel, voltage-gated, type I, alpha	
<i>MAPT</i>	Microtubule-associated protein tau	Neurodegenerative disease pathway; Alzheimer's disease; Mapk pathway
<i>APP</i>	Amyloid b (A4) precursor protein	Neurodegenerative disease pathway; Alzheimer's disease
<i>SOD1</i>	Superoxide dismutase 1	Neurodegenerative disease pathway

Table 3. mRNA levels as determined by quantitative RT-PCR for tissues from the mPOA and BNST of castrated non-maters and maters

	Castrated maters (% $\pm$ SE)	Castrated non-maters (% $\pm$ SE)	F value	p value
<i>APP</i>	106.7 $\pm$ 5.6	89.2 $\pm$ 4.8	$F_{(1,15)} = 4.968$	$p < 0.05$
<i>MAPT</i>	112.4 $\pm$ 4.4	95.0 $\pm$ 6.4	$F_{(1,15)} = 5.390$	$p < 0.05$
<i>SOD1</i>	71.5 $\pm$ 5.4	105.9 $\pm$ 15.9	$F_{(1,15)} = 5.523$	$p < 0.05$
<i>IMPA1</i>	76.7 $\pm$ 4.9	102.4 $\pm$ 9.4	$F_{(1,15)} = 6.914$	$p < 0.05$
<i>SCN1a</i>	54.6 $\pm$ 10.6	113.9 $\pm$ 30.0	$F_{(1,14)} = 4.207$	$p = 0.0595$
<i>PTEN</i>	70.5 $\pm$ 7.9	106.2 $\pm$ 14.7	$F_{(1,14)} = 5.181$	$p < 0.05$

recombinant lines of B6D2F1 mice that displayed mating after castration and four lines that ceased mating after surgery were identified. A total of 152 alleles at specific loci were identical among the two mater strains but differed from those in the four non-mating strains (supplemental Table S2, available at www.jneurosci.org as supplemental material). Cross referencing the unique genes from the BXD recombinant mice that mated with the genes from our microarray analysis, two additional genes were identified. The six genes that are strong candidates which may play an important role in mediating persistent copulation in long-term castrated B6D2F1 hybrid mice are listed in Table 2.

Quantitative real-time PCR validated differences revealed in the bioinformatic analysis. Relative mRNA expression of *APP* and *MAPT* was greater in maters compared with non-maters, and *SOD1*, *IMPA1*, *SCN1a*, and *PTEN* was greater in the non-maters compared with maters (Table 3). Western blots of APP protein from POA and BNST tissues showed that normal APP was $\sim$ 40%

Figure 1. Representative Western blots from mPOA and BNST tissues are shown. Three individuals are depicted in each group; non-maters, maters and sham castrates (gonad-intact) male B6D2F1 mice. In the graph relative amounts of APP protein are adjusted for loading controls (β -actin). The non-maters have less APP protein than males in either other group, $*p < 0.05$.

higher in maters than non-maters ($F_{(2,19)} = 5.496$, $p < 0.05$; Fig. 1). No differences in relative APP protein levels between sham castrated males and maters were noted. APP protein levels decline significantly after castration, but only in individuals that do not maintain their mating ability.

Both before and after castration, males overexpressing APP displayed increased facilitation of MSB compared with controls. At almost every time point tested, a higher percentage of MSB was demonstrated by APP overexpressing mice than MSB observed in controls, and at several time points, statistical significance was reached ($p < 0.05$; Fig. 2*a–c*). In addition, as revealed by the survival curves generated from the Mantel-Cox log-rank test, the survival curves for latencies to mount for APP transgenic mice were significantly different from those of wild-type controls both before and after surgery, and the survival curve for ejaculation latencies were significantly different from controls before castration ($p < 0.05$; Fig. 2*d–i*).

Discussion

These data reveal a previously unknown functional relationship between individual differences in MSB and the normal APP gene and protein. Among the B6D2F1 hybrid mice, greater APP expression and protein in the mPOA and BNST is correlated with MSB lasting months after castration. Males with low APP expression and protein stopped displaying MSB a few weeks after castration. This observation is more than correlational; MSB in mice overexpressing the human APP gene was enhanced compared with control males. The APP gene is best known for mutations that are associated with neurodegenerative diseases such as Alzheimer's. Mutations of APP eventually result in impairment of synaptic plasticity and induce aberrant neuronal and axonal degeneration (for review, see Kim and Tsai, 2009). The normal APP gene is involved in cell survival and neuroprotection. It is likely that individual differences in cell survival and neuroprotection in critical populations of neurons of the mPOA and BNST are responsible for differences in male sexual behavior. Because the transgenic mice tested in our study overexpress APP ubiquitously, one must be cautious in attributing APP action to only the mPOA and BNST until we validate that the relative increased levels of APP are site specific.

Several transgenic mouse lines that overexpress human APP are available (Quon et al., 1991; Kammesheid et al., 1992; Lamb

et al., 1993; Mucke et al., 1994; Higgins et al., 1995; Andr et al., 1996). In several of these lines, males tended to develop age-dependent cognitive decline (Van Dam et al., 2003), circadian activity disturbances (Vloeberghs et al., 2004), and increased aggression (Vloeberghs et al., 2006). Only one study investigated MSB in one of these transgenic lines (Vloeberghs et al., 2007), and this report is the first to examine MSB after castration in a transgenic mouse overexpressing human APP. In the one study that did investigate MSB in overexpressing APP mice, certain aspects of MSB, such as number of mounts, genital sniffing and licking, were not significantly different from wild-type controls; however, it is important to note that these mice, which exhibit a twofold overexpression of human APP, are a different transgenic line than the one we tested. The transgenic mouse line used in this study is known to overexpress human APP in brain at levels comparable to those noted in brains of castrated B6D2F1 hybrid males (Lamb et al., 1993). In addition, other aspects of MSB, such as frequencies of intromissions or ejaculations or latencies to mount, intromit or ejaculate were not reported (Vloeberghs et al., 2007).

Because APP affected MSB in gonad-intact mice, a relationship may exist between gonadal hormones, APP, and MSB. *In vitro*, estrogens increase secretion of soluble APP α (Xu et al., 1998) which regulates neuronal survival and neurite outgrowth. Perhaps estrogens reduce amyloid- β ($A\beta$) levels by promoting nonamyloidogenic processing and/or trafficking of APP (Greenfield et al., 2002). Most studies investigating the relationship between sex steroids and APP have focused on the abnormal accumulation of $A\beta$, a product of aberrant APP processing, which is believed to be the causative component of Alzheimer's disease pathogenesis. Anti-androgen treatments result in elevated plasma levels of $A\beta$, and a correlation is present between low T and elevated $A\beta$ levels (for review, see Pike et al., 2009). Particularly during aging, as androgens decline, abnormal production of $A\beta$ may occur as a result of dysfunctional APP gene. Other findings have indicated that androgen regulation of $A\beta$ involves an androgen receptor-dependent mechanism requiring upregulation of the $A\beta$ -catabolizing enzyme neprilysin (Yao et al., 2008). Because some of the B6D2F1 mice in the current study retain their ability to mate after castration and also have elevated APP, the degree to which this gene is regulated by steroid hormones is probably variable and may be thus related to observed variations in sexual behavior. Further tests are required to confirm the degree of neural protection and neural degeneration in the B6D2F1 mice.

One of the instructive aspects of our experiment is the use of several lines of genomic analyses to inform the selection of candidate gene pathways and genes. The bioinformatic analyses of gene expression arrays between castrated maters and non-maters yielded 532 genes that were differentially expressed, of which, 267 were downregulated and 265 were upregulated (supplemental Table S1, available at www.jneurosci.org as supplemental material). In conjunction with the data mined from the recombinant inbred lines of B6D2F1 mice, several other genes in addition to APP were found, and these were also associated with neurodegeneration and/or neuroprotection (Table 1). In this process, 532 candidates were reduced to six genes. While only one was directly tested here, the fact that expression of this gene was linked to the display of male sexual behavior is proof of principle and demonstrates that this is an instructive model for future gene expression studies.

It is highly likely that the other candidate genes discovered in this study, but not tested in transgenic mouse lines, regulate MSB. This is supported by our finding that the percentage of the cas-

Figure 2. Sexual behavior in APP overexpressing mice and controls. **a**, Percentage of APP overexpressing mice and wild-type controls that displayed mounting both before and after castration. **b**, **c**, Intromissive behaviors (**b**) and percentage of males displaying an ejaculatory reflex (**c**). **d–i**, Kaplan-Meier survival plots showing the percentage of APP transgenic mice displaying mounts (**d**, **g**), intromissions (**e**, **h**), and ejaculations (**f**, **i**) both before castration (**d–f**, respectively) and after castration (**g–i**, respectively). * $p < 0.05$, significantly different from the other group.

trated APP transgenic mice we tested was noticeably lower than previously reported in castrated B6D2F1 hybrid male mice 12 weeks after castration (Clemens et al., 1988; Park et al., 2009). Expression of phosphatase and tensin homolog (*PTEN*) was ~40% lower in maters compared with non-maters. Inactivation of *PTEN* constitutively activates the PKB/AKT pathway which promotes cell survival. Although complete deletion of *PTEN* in mice results in embryonic lethality and heterozygous deletion causes increased cancer incidence (Stiles et al., 2004), region-specific deletion of *PTEN* has been performed. Mice with *PTEN* knock-out limited to mature neurons in the cerebral cortex and hippocampus (Kwon et al., 2006a) display abnormal dendritic and axonal growth and synapse number, abnormal social interactions and inappropriate responses to sensory stimuli (Kwon et al., 2006b). These conditional *PTEN* mutant mice do not demonstrate MSB, and fail to impregnate female mice (Kwon et al., 2006b). Whether a localized deletion of *PTEN* in the MPOA rather than the cerebral cortex and hippocampus would affect MSB has yet to be determined.

Expression of superoxide dismutase 1 (*SOD1*) was significantly downregulated ~35% in maters compared with non-maters. *SOD1* deficient mice have less astrogliosis and neurodegeneration (Beni et al., 2006). *MAPT* encodes the protein tau, which stabilizes microtubules to promote their assembly by binding to tubulin (Hirokawa, 1994). Defects in *MAPT* cause frontotemporal dementia due to at-

rophy with behavioral changes including deterioration of cognitive capacities and loss of memory (for review, see Rademakers et al., 2004). Currently we are assessing MSB in transgenic mice overexpressing tau and predict a behavioral phenotype similar to that reported for APP overexpressors.

Cell death and/or neuroprotection is occurring after castration in brain areas essential for male reproductive behaviors. Neuroprotection is more often studied in conjunction with cognitive rather than sexual behaviors. A link between APP, impaired cognition and increased sexual behavior is hinted at in the clinical literature, but has not been directly tested. Several clinical studies have reported inappropriate behavior and sexual disinhibition among cognitively impaired older men (Wallace and Safer, 2009) and there is a correlation between erectile dysfunction and Alzheimer's disease (Zeiss et al., 1990). Many men experience loss of libido and/or erectile function, in addition to cognitive decline during aging (Lunenfeld, 2006). In clinical populations of testicular and prostate cancer patients, treatments routinely require use of anti-androgen therapies and up to 85% of these men report decreased libido and sexual intimacy (Wilt et al., 2008). The present data suggest that a portion of this variability may be due to alterations in APP function; however, further studies are required to delineate the potential relationship between the inhibitory sexual effects of anti-androgen therapy for testicular and prostate cancer and decreased APP. We propose a model whereby

APP gene regulation by androgens is reduced in certain individuals during normal aging. By virtue of a combination of other genetic, epigenetic and/or environmental factors, some males experience less of a decline in APP than others and it is these individuals that are most likely to retain their ability to display MSB. With increased longevity in normal and patient populations, new avenues for sex therapies that do not require steroid hormones are highly attractive for maintenance of normal sexual function.

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