SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Sexually dimorphic gene expression in specific regions of the hypothalamus, BNST, and MeA. (Related to Figure 1.)

Representative coronal sections labeled for mRNA or protein expression are shown.

(A, B) More Gabrg1 expression in the POA of the male than the female.

(C-F) More Glra3 expression in the POA and BNSTmpm of the male than the female.

(G-J) More Gpr165 expression in the POA and BNSTmpl of the male than the female.

(K-R) More Greb1 expression in the POA, BNSTmpm, MeApd, and VMHvl of the male than the female.

(S-V) More Cart expression in the BNSTmpm and PMV of the male than the female.

(W-B') More Ecel1 expression in the POA, BNSTmpm, and MeApd of the male than the female.

(C'-F') More Rps6ka6 in the BNSTmpm, MeApd, and MeApv (arrowheads) of the male than the female.

(G', H') More Chodl expression in the BNSTmpm of the male than the female.

(I', J') More Pak3 expression in the BNSTmpm of the male than the female.

(K'-N') More Nnat expression in the VMHvl of the female than the male. More Nnat expression in the PMV of the male than the female.

(O'-R') More Dgkk expression in the VMHvl of the female than the male. More Dgkk expression in the PMV of the male than the female.

(S'-L'') More ERα expression in the POA, BNSTmpm, MeApd, VMHvl, and PeriV of the female than the male.

$n \geq 3$; scale bars = 75 μm.

Figure S2: Summary of sex differences in gene expression. (Related to Figure 4.)

(A) RT-qPCR quantitation of sex differences in gene expression normalized to the neuronal marker Synapsin I. The majority of sex differences we have identified represent sexual dimorphisms in mRNA expression per cell.

1 = no sex difference in gene expression (dashed lines).

N.D. = not determined.

n = 3/sex; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

(B) Genes (individual rows) with sex differences in gene expression in adult brain regions are represented as orange cells. Also shown is the non-dimorphic expression of genes within these regions (gray cells). Our screen selected for genes enriched in the hypothalamus compared to the rest of the brain and this appeared to be the case for all transcripts, except Pak3 which was expressed broadly in most brain regions, albeit at lower levels compared to its expression in the hypothalamus. Expression in other brain regions is not shown in this figure, but can be accessed from the AIBS website (http://www.brain-map.org/) (Ng et al., 2009). Note that many dimorphically transcribed genes were not expressed (white cells) in nuclei with overt dimorphism in the expression of other genes.

Figure S3: Control of sexually dimorphic gene expression by testosterone in males. (Related to Figure 4.)

Adult castrate males were supplemented with vehicle or testosterone for two weeks. Their brains were micro-dissected to obtain tissue from the POA, BNST, VMHvl, PMV, and MeA, and processed for RT-qPCR for genes whose expression changed upon castration in males (Figure 4

in main text). Testosterone supplementation restores expression of the vast majority of genes to levels that resemble those seen in intact males. Fold change indicated by dashed lines represents no difference in gene expression between castrates treated with vehicle or testosterone.

Cv = Castrate receiving vehicle (Circulating testosterone below level of detection).

Ct = Castrate receiving testosterone (Circulating testosterone = 14.7 ± 3.7 nM).

$n \geq 3$ for each cohort. * $p < 0.04$, ** $p < 0.01$, *** $p < 0.001$.

Figure S4: Cckar, Irs4, and Sytl4 are not required for various components of male-typical behaviors. (Related to Figure 5.)

(A-D, I-N) No difference between Null (*Cckar*$^{-/-}$) and Control (*Cckar*$^{+/+}$ or *Cckar*$^{-/+}$) males in various parameters of mating (A-D) and territorial aggression and urine marking (I-N). $n \geq 8$/cohort.

(E-H, O-T) No difference between Null (*Irs4*$^{-/Y}$) and Control (*Irs4*$^{+/Y}$) mice in various parameters of mating (E-H) and territorial aggression and urine marking (O-T). $n \geq 10$/genotype.

(U-Z) No difference between Null (*Sytl4*$^{-/Y}$) and Control (*Sytl4*$^{+/Y}$) mice in territorial aggression and urine marking. $n \geq 10$/genotype for aggression; $n \geq 7$/genotype for urine marking.

Perimeter = perimeter of cage floor; Center = rest of cage floor.

Figure S5: Brs3, Cckar, Irs4, and Sytl4 are not required for various components of female-typical behaviors. (Related to Figures 6, 7.)

(A-G) No difference between Null (*Brs3*$^{-/-}$) and Control (*Brs3*$^{-/+}$) females in various parameters of pup retrieval, maternal aggression, and sexual receptivity. $n \geq 3$/genotype.

(H-N) No difference between Null (*Sytl4*$^{-/-}$) and Control (*Sytl4*$^{-/+}$) females in various parameters of pup retrieval, maternal aggression, and sexual receptivity. n ≥ 5/genotype.

(O) No difference between Null (*Irs4*$^{-/-}$) and Control (*Irs4*$^{-/+}$) females in sexual receptivity. n ≥ 7.

(P-U) No difference between Null (*Cckar*$^{-/-}$) and Control (*Cckar*$^{+/+}$ or *Cckar*$^{-/+}$) females in various parameters of pup retrieval and maternal aggression. n ≥ 5/cohort.

Figure S6: Sexual differentiation of brain regions expressing Brs3, Cckar, Irs4, or Sytl4 appears unaffected in mice mutant for these genes. (Related to Figures 5-7.)

We assessed sexual differentiation of the POA, BNSTmpm, VMHvl, and MeApd with in situ hybridization for ERα, which is normally downregulated in males compared to females in these brain regions. We assessed sexual differentiation of the PMV with in situ hybridization for Dgkk, which is normally upregulated in males compared to females in this region. Representative coronal sections labeled for mRNA expression are shown.

(A, B) No difference between Sytl4 mutant and WT males in ERα expression in the BNSTmpm.

(C-F) No difference between Brs3 mutant and WT males in ERα expression in the BNSTmpm and MeApd.

(G-J) No difference between Irs4 mutant and control females in ERα and Dgkk expression in the VMHvl and PMV, respectively.

(K-R) No difference between Cckar mutant and WT females in ERα expression in the POA, BNSTmpm, MeApd, and VMHvl.

n ≥ 2/genotype. Scale bars = 100 μm.

SUPPLEMENTAL TABLE LEGENDS

Table S1: Lists of genes with sex differences in hypothalamic expression, genes upregulated in the hypothalamus compared to the whole brain reference sample, genes screened by in situ hybridization, and primers used in RT-qPCR. (Related to Figure 1.)

The average fold change in gene expression and the primers used to generate cDNA for synthesizing probes for in situ hybridization are also described where relevant.

Table S2: Brs3 regulates circulating testosterone titers, intromission, and aggression. (Related to Figure 5.)

Brs3 mutants develop a late onset mild obesity, starting at 13 weeks of life (Ladenheim et al., 2008). Our behavioral assays were conducted at 10-15 weeks of life, well before we could discern a notable difference in weight between WT and Brs3 mutants. We did not observe gross motor deficits or abnormal gait during behavioral testing of $Brs^{-/Y}$ mice (Null), who were also similar to their WT male littermates (Control) in general motor activity and social interactions such as grooming (XX, AW, unpublished observations). Sexual differentiation of regions in which Brs3 was expressed in a dimorphic manner appears unaffected in Brs3 mutants (Figure S5C-F).

$Brs^{-/Y}$ mice exhibit a male repertoire of behaviors: they mate with females, they attack intruder males as previously described (Yamada et al., 2000), and they deposit urine spots in their cages similar to $Brs^{+/Y}$ siblings. We find that Null males intromit females faster and they attack WT intruder males with a shorter latency compared to their Control littermates. We find elevated serum testosterone in Null males compared to their Control siblings. Although the mean serum testosterone of Brs3 mutant and WT males is within the normal range of circulating

testosterone, which can vary as much as 20-fold in male mice (Batty, 1978; Bartke et al., 1973), we cannot exclude the possibility that these behavioral phenotypes in mating and fighting result from the altered circulating testosterone. Regardless of the underlying mechanisms, our results show that Brs3 function is essential for male-typical displays of mating and aggression.

Table S3: *Sytl4* mutants are comparable to control males in maturation of the gonads and serum testosterone. (Related to Figure 5.)

A cohort of adult, socially naive WT and Sytl4 mutant males were analyzed at 9 weeks of age, a timepoint before any behavioral testing would have commenced. We find comparable levels of testosterone in these 2 groups. Moreover, the weight of the testes and seminal vesicles, a sensitive functional indicator of circulating testosterone, is also similar between the WT and mutants. $n \geq 4$/genotype.

Table S4: Irs4 does not affect female fertility, ability to wean, general social interactions and motor performance. (Related to Figure 6.)

When co-housed with WT males, Irs4 mutant females were no different than control ($Irs4^{-/+}$) females in delivering pups, fecundity, and weaning pups successfully (see Irs4_1 worksheet). A previous report suggested reduced fertility and an increase in the loss of entire litters in assays consisting of paired Irs4 null males and females co-housed for several months (Fantin et al., 2000). This likely reflects differences in the experimental design or reduced fertility in Irs4 mutant males.

Irs4 mutant females are no different than control siblings in tests of motor performance and general social interactions (see Irs4_2 worksheet; $n \geq 5$/genotype). The mutants are

comparable to controls in motivated motor performance such as staying on a rotarod or finding a hidden cookie upon overnight starvation. During non-aggressive social interactions with intruder males, the Irs4 mutant females sniff and groom the intruder in a manner similar to control females. Movement within the cage, as evaluated by the number of times midline crossings, is also similar between mutant and control Irs4 females.

SUPPLEMENTAL MOVIES

Movie S1 (Related to Figure 6.)

This movie shows a resident control mother (dark coat) displaying maternal aggression towards a WT male intruder (light coat). Note that the pups were removed prior to insertion of the male into the cage.

Movie S2 (Related to Figure 6.)

This movie shows a resident Irs4 mutant mother (dark coat) interacting with but not attacking a WT male intruder (light coat). Note that the pups were removed prior to insertion of the male into the cage.

Movie S3 (Related to Figure 7.)

This movie shows a resident WT male (dark coat) mounting and intromitting a control female (light coat) in estrus.

Movie S4 (Related to Figure 7.)

This movie shows a resident WT male (dark coat) mounting a Cckar mutant female (light coat) in estrus. The female is not receptive and does not permit the male to intromit following either bout of mounting.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animals

Mice were group-housed by sex at weaning and housed as described previously (Wu et al., 2009). Adult (9-24 weeks of age) mice were used for all studies. Null mutants for *Cckar* were obtained from matings between heterozygous parents. For X-linked genes (*Brs3*, *Irs4*, and *Sytl4*), null females and males were obtained from matings between heterozygous females and hemizygous males, or in the case of null males, from matings between heterozygous females and WT males. WT or heterozygous siblings generated from these crosses were used as control animals for all behavioral studies. Mice used to obtain tissue for mRNA, in situ hybridization, and behavior were on a C57Bl/6J background except WT intruder males for aggression (129/Sv), WT stud males for testing female sexual behavior (hybrid 129/Sv and C57Bl/6J), and mice bearing mutant alleles of *Cckar* (129/Sv) and *Sytl4* (mixed 129/Sv and C57Bl/6J). Male and female mice were surgically castrated and supplemented with hormones as described previously (Wu et al., 2009). Briefly, the hormones were resuspended in sterile sesame oil (Sigma) and mice received the hormone or equivalent volume of vehicle subcutaneously. For testosterone supplementation, we dosed the males with 75 µg (50 µL oil) of testosterone propionate (Sigma) on alternate days. To induce receptivity in females, we injected 10 µg (100 µL oil) and 5 µg (50 µL oil) of 17β-estradiol benzoate (Sigma) 48 and 24 hours preceding the test, respectively; on the day of the test, we injected 50 µg of progesterone (Sigma; resuspended in 50 µL oil) 4-6 hours prior to the test.

Behavioral assays

Animals were tested for behaviors only after they were 10 weeks of age and assays were initiated ≥1 hour after onset of the dark cycle. All experimental mice, males and females, were always exposed to intruders they had not encountered previously, and each assay was separated by ≥ 2 days. For male mice, singly housed residents were tested 3 times each for sexual behavior for 30 min with a WT estrus intruder and for aggression with a WT group-housed male intruder for 15 min. Performance in urine marking was tested once for 60 min in a fresh cage following social experience. A separate cohort of socially naive males was tested for ultrasonic vocalizations once for 3 min each to a WT male and female intruder introduced separately into the cage. Once behavioral testing commenced, it was completed within 3 weeks at which point the males were sacrificed and blood was collected to determine serum hormone levels as described previously (Wu et al., 2009). There can also be changes in testosterone titers across the day within a male. This is unlikely to impact our results since we did all testing within the same 2-3 hour window, and WT and mutant males were used randomly within this timeframe to remove bias resulting from the time of the test. Assays were performed after lights out since mice are nocturnal, and blood collection was done at a time at which testing would have commenced.

To test for sexual receptivity, females were castrated, and, subsequent to estrus induction with estrogen and progesterone, inserted singly into the home cage of a sexually experienced WT male for 30 min each in 3 assays. Pup retrieval and maternal aggression were tested in experimental females impregnated by a WT male and singly housed 3-5 days prior to parturition. At 2, 4, and 6 days after parturition, the dam was removed briefly from the cage and 3-4 pups were scattered across the cage floor away from the nest. The dam was returned to the cage and her pup retrieval ability was tested for 15 min. To test for maternal aggression, pups of postnatal

age 8, 10, and 12 days were removed and a group-housed adult WT male intruder was inserted into the cage for 15 min. The pups were returned to the mother at the end of each assay.

For the rotarod test, we followed standard procedures described previously (Moldovan et al., 2011). In brief, the mice were acclimated once to an accelerated rotarod set-up (Ugo Basile) and then tested twice for 5 min each. We monitored the time each mouse could successfully remain on the rotarod, and its performance was assessed by the average of these two tests. For the cookie finding test, we followed a previously described protocol (Wysocki et al., 1982). Briefly, mice maintained on unrestricted food were starved for 18 hours and then placed into a fresh cage containing a cookie buried under the bedding. Their behavior was recorded for 3 min following which the assay was terminated. Each mouse was tested twice in this cookie finding assay and its performance was assessed by the average of these two tests.

Determination of estrous cyclicity

Daily vaginal smears were obtained from group housed females, and the cytological characteristics of the smear were imaged using brightfield optics. An experimenter blind to the genotypes independently scored the stage of the estrous cycle.

Microarray studies

MEEBO arrays, whose probe set provides ~84% gene coverage of the mouse genome, were printed at the UCSF Center for Advanced Technology. Sexually naive C57Bl/6J mice 10 weeks of age were singly housed for 7 days prior to tissue collection in order to reduce variability in gene expression resulting from social interactions. Freshly dissected brains were sectioned into 1

mm coronal slices using a tissue chopper (EMS). The hypothalamus, including the BNST, was identified by landmarks and coordinates from the mouse brain atlas (Paxinos and Franklin, 2003), dissected in ice-cold d-PBS (free of Ca^{++} and Mg^{++}) with the aid of a dissecting stereomicroscope, and immediately frozen on dry ice. For each experiment, 4 males and 4 females were dissected to provide hypothalamic tissue that was pooled by sex; in addition, the whole brain (extending from the olfactory bulbs to the medulla oblongata) and pituitary were taken from 1 male and female each and pooled into a single "whole brain reference" sample. mRNA was extracted in 3 steps: Trizol (Invitrogen) extracted RNA was column purified (Micro-to-Midi columns, Invitrogen), followed by mRNA purification (Fast Track, Invitrogen). The mRNA was incubated with DNase I (DNase I, Amplification Grade, Invitrogen) to remove residual genomic DNA and the DNase I was subsequently heat-inactivated. For each experiment, 0.5 µg of hypothalamic mRNA from each sex and 2 µg of whole brain reference mRNA was reverse transcribed (StrataScript, Stratagene) with random nonamers and oligo dT and labeled with amino-allyl dUTP (Ambion). The hypothalamic samples were conjugated with Cy3 and the whole brain reference sample was coupled to Cy5 using protocols supplied by the manufacturer (Amersham). Each hypothalamic sample from males and females was split and hybridized onto 2 arrays and the whole brain reference sample was hybridized onto all 4 arrays. Thus, each experiment consisted of 2 technical replicates for both sexes, and we performed 3 such independent experiments (biological repeats). In an independent experiment, we used the T7 polymerase based amplification method (Amino allyl message AmpII, Invitrogen) to amplify and label mRNA from male and female hypothalamic tissue as well as the whole brain reference. We used 10 µg of the amplified Cy3-coupled hypothalamic sample of each sex and 20 µg of the Cy5-coupled whole brain reference and performed 2 technical replicates as described above. All

arrays were hybridized and washed using standard protocols. The arrays were subsequently dried and scanned with an Axon slide scanner 4000B in an ozone-reduced environment. Array images were analyzed with the Genepix 4.0 software to obtain hybridization intensity data for each printed spot on the array for both channels (Cy3 and Cy5).

Diverse computations with different assumptions about the distribution of the underlying data have been employed to interrogate microarray data (Smyth and Speed, 2003; Quackenbush, 2002; Holloway et al., 2002; Hoffmann et al., 2002; Ding and Wilkins, 2004). We reasoned that large sex differences in gene expression would withstand scrutiny by different mathematical approaches. Accordingly, we employed distinct schemes to compile a masterlist of genes whose expression revealed a consistent sexual dimorphism across several analyses. To obtain fold changes in gene expression, the male (m) versus female (f) expression analysis was performed either on the intensity (I) data for each sex (I_m/I_f) or on intensity data that were first compared to the whole brain reference (wbr) intensity obtained within the same array $[(I_m/I_{wbr,m})/(I_f/I_{wbr,f})]$. $I_{wbr,m}$ and $I_{wbr,f}$ refer to the Cy5 intensity of the whole brain reference sample for the array on which male and female Cy3-coupled cRNA samples were hybridized, respectively. This ratio of ratios can minimize variability from technical artifacts such as subtle differences in printing or hybridization. We performed normalization on these ratios (Global; Quantile) as well as on the intensity datasets (Quantile; Ubiquitin; Intensity Sum). The Matlab manorm function was used to execute Global normalization, which assumes that the majority of transcripts on an array are not different between the two samples and adjusts the calculated ratios accordingly. The Matlab quantilenorm function was used to perform Quantile normalization, which assumes that the same range of values exist among the samples and adjusts the range of raw intensities or ratios

accordingly. For "Ubiquitin Normalization", we assumed that the intensity of the hybridization for Ubiquitin C (Ubc), an ubiquitously expressed gene that is printed on 260 spots distributed across each array, would not be different between the samples being compared. Accordingly, we separately performed normalizations analogous to the Global and Quantile schemes described above. The "Intensity Sum Normalization" assumes that the overall intensity of the hybridization signal should be similar since equal amounts of labeled cRNA are loaded onto the reaction. Technical variability can result in different overall intensities, and the Intensity Sum Normalization introduces a correction so that the sum of all intensities on an array equals the average of the sum of all intensities of all arrays.

Lists of gene expression were generated with or without normalizing the fold changes obtained by ratiometric or direct intensity comparisons. The cutoff criteria for inclusion on the lists were: $\geq$ 1.2 fold sex difference in expression or $\geq$ 2 fold upregulated expression in the hypothalamus; absolute intensity $\geq$ 500; and, $p \leq 0.16$. The p value was determined using the one sample t-test with log transformed ratios. Subsequent to the generations of these lists, we generated masterlists of genes whose expression revealed upregulation in either sex or the hypothalamus (compared to the whole brain reference sample) such that the expression of any gene on the list was upregulated subsequent to $\geq$1 normalization scheme and ratiometric comparison (Table S1). We subsequently compiled a list-of-lists that included 70 genes which fulfilled all the criteria mentioned above, showed a sex difference, and were upregulated in the hypothalamus (Table S1). Our in situ hybridization analysis of these 70 genes identified 13 genes to be dimorphic at the cellular resolution afforded by this approach. We reasoned that genes expressed at low copy number or in small subsets of neurons within the hypothalamus

would be missed with our approach. We therefore also screened our hypothalamus upregulated list for genes whose expression was not apparently sexually dimorphic from our microarray intensity data but that were expressed in small sets of neurons in the hypothalamus as determined by the data displayed on the Allen Institute for Brain Science (AIBS) website (http://www.brain-map.org/) (Table S1) (Ng et al., 2009). The studies by the AIBS have been largely restricted to the male brain, and we therefore screened this list of 14 additional genes by in situ hybridization on adult male and female C57Bl/6J mice as described in the methods. This screen identified 3 additional genes (*Cckar, Dgkk, Gabrg1*) to be expressed in a sexually dimorphic pattern.

RT-qPCR

We collected 200 μm thick coronal slices from acutely dissected 10-12 week old brains of C57Bl/6J mice using a vibrating microtome (Leica) into a dish containing ice-cold d-PBS (free of Ca^{++} and Mg^{++}). The POA, BNST, VMH, PMV, and MeA were dissected from these slices using a dissecting stereomicroscope and immediately frozen on dry ice. Total RNA was extracted with Trizol, treated with DNase I as described above, and an equal quantity of RNA was subjected to first strand cDNA synthesis (SuperScript III) using random hexamers as well as oligo-dT primed reactions. qPCR was performed using the primers listed in Table S1 on an ABI 7900 machine and the 2XSYBR master mix (Fermentas). The sex difference in gene expression was calculated using the standard $2^{-\Delta\Delta Ct}$ method with Synapsin I as the reference gene. One sample t-test was performed on log transformed ratios to determine significance. All primer pairs used for qPCR are listed in Table S1.

Histology

We analyzed gene expression by ISH on 100 μm thick serial coronal sections that spanned the entire rostrocaudal extent of the hypothalamus and MeA (1 mm rostral to bregma through 3.5 mm caudal to bregma) of age-matched socially naive mice. Sex differences in gene expression in forebrain areas not included in this series, such as the dimorphism reported in the nucleus accumbens for CART expression in rats (Fagergren and Hurd, 1999), were not evaluated in our studies. In situ hybridization was performed as described previously (Wu et al., 2009). Probes for in situ hybridization were generated from subcloned RT-PCR products obtained using primers listed in Table S1. Hybridization was visualized with the histochemical substrates BCIP and NBT subsequent to incubation with an alkaline phosphatase conjugated sheep anti-digoxygenin (Roche, 1:2,000) antibody. The mRNA labeling was imaged using brightfield optics and quantified using NIH ImageJ software. Fluorescent staining and imaging of immunolabeled sections (65 μm thick) has been described previously (Wu et al., 2009; Shah et al., 2004). The primary antisera used for fluorescent immunolabeling is rabbit anti-ERα (Millipore, 1:10,000). All brain regions were identified using standard landmarks defined by the mouse brain atlas (Paxinos and Franklin, 2003).

For ISH studies on castrate mice, adult males or females were surgically castrated and gene expression was compared with unoperated or sham-operated controls. These comparisons were performed 2-3 weeks following surgery, a timepoint by which sexually dimorphic behaviors are lost.

For comparison between the sexes or between castrate and control mice, ISH labeled sections were imaged with a 5X objective and brightfield optics. These color images were

converted into grayscale (Adobe Photoshop), the region of interest (ROI) was outlined, and labeling intensity was quantitated (NIH ImageJ). We imaged and analyzed every histological section containing the ROI in this manner, including the POA, periV, BNST, VMHvl, PMV, MeApd, and MeApv. In parallel, we also imaged and quantitated for each histological section the staining intensity in an adjacent region that contained no labeled cells, thereby providing us with an estimate of the background labeling (noise) for each probe. The pixel values representing noise were subtracted from the pixel values obtained from imaging the ROI, thereby yielding an estimate of the corrected signal for that region. The pixel values obtained by summing the corrected signal across a given brain region provides an estimate of the level of gene expression for that area. The fold-change in gene expression between different experimental conditions (male:female, male:castrate male, and female:castrate female) was log transformed and we performed the one sample t-test to determine statistical significance.

REFERENCES

Bartke, A., Steele, R. E., Musto, N., and Caldwell, B. V. (1973). Fluctuations in plasma testosterone levels in adult male rats and mice. Endocrinology *92*, 1223–1228.

Batty, J. (1978). Plasma levels of testosterone and male sexual behaviour in strains of the house mouse (Mus musculus). Anim Behav *26*, 339–348.

Ding, Y., and Wilkins, D. (2004). The effect of normalization on microarray data analysis. DNA Cell Biol *23*, 635–642.

Fagergren, P., and Hurd, Y. L. (1999). Mesolimbic gender differences in peptide CART mRNA expression: effects of cocaine. Neuroreport *10*, 3449–3452.

Fantin, V. R., Wang, Q., Lienhard, G. E., and Keller, S. R. (2000). Mice lacking insulin receptor substrate 4 exhibit mild defects in growth, reproduction, and glucose homeostasis. Am. J. Physiol. Endocrinol. Metab *278*, E127–E133.

Hoffmann, R., Seidl, T., and Dugas, M. (2002). Profound effect of normalization on detection of differentially expressed genes in oligonucleotide microarray data analysis. Genome Biol *3*, Research0033.

Holloway, A. J., van Laar, R. K., Tothill, R. W., and Bowtell, D. D. L. (2002). Options available--from start to finish--for obtaining data from DNA microarrays II. Nat. Genet *32 Suppl*, 481–489.

Ladenheim, E. E., Hamilton, N. L., Behles, R. R., Bi, S., Hampton, L. L., Battey, J. F., and Moran, T. H. (2008). Factors contributing to obesity in bombesin receptor subtype-3-deficient mice. Endocrinology *149*, 971–978.

Moldovan, M., Alvarez, S., Pinchenko, V., Klein, D., Nielsen, F. C., Wood, J. N., Martini, R., and Krarup, C. (2011). Na(v)1.8 channelopathy in mutant mice deficient for myelin protein zero is detrimental to motor axons. Brain *134*, 585–601.

Ng, L., Bernard, A., Lau, C., Overly, C. C., Dong, H.-W., Kuan, C., Pathak, S., Sunkin, S. M., Dang, C., Bohland, J. W., et al. (2009). An anatomic gene expression atlas of the adult mouse brain. Nat. Neurosci *12*, 356–362.

Paxinos, G., and Franklin, K. B. J. (2003). The Mouse Brain in Stereotaxic Coordinates: Compact Second Edition, Second Edition 2nd ed. (Academic Press).

Quackenbush, J. (2002). Microarray data normalization and transformation. Nat. Genet *32 Suppl*, 496–501.

Shah, N. M., Pisapia, D. J., Maniatis, S., Mendelsohn, M. M., Nemes, A., and Axel, R. (2004). Visualizing sexual dimorphism in the brain. Neuron *43*, 313–319.

Smyth, G. K., and Speed, T. (2003). Normalization of cDNA microarray data. Methods *31*, 265–273.

Wu, M. V., Manoli, D. S., Fraser, E. J., Coats, J. K., Tollkuhn, J., Honda, S.-I., Harada, N., and Shah, N. M. (2009). Estrogen masculinizes neural pathways and sex-specific behaviors. Cell *139*, 61–72.

Wysocki, C. J., Nyby, J., Whitney, G., Beauchamp, G. K., and Katz, Y. (1982). The vomeronasal organ: primary role in mouse chemosensory gender recognition. Physiol. Behav. *29*, 315–327.

Yamada, K., Ohki-Hamazaki, H., and Wada, K. (2000). Differential effects of social isolation upon body weight, food consumption, and responsiveness to novel and social environment in bombesin receptor subtype-3 (BRS-3) deficient mice. Physiol. Behav *68*, 555–561.