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Behavioral Disturbances in Estrogen-Related Receptor alpha-Null Mice

Huxing Cui¹, Yuan Lu¹, Michael Z. Khan¹, Rachel M. Anderson², Latisha McDaniel¹, Hannah E. Wilson^{1,5}, Terry C. Yin¹, Jason J. Radley^{2,4}, Andrew A. Pieper^{1,3,4}, and Michael Lutter^{1,4,*}

¹Department of Psychiatry, University of Iowa, Carver College of Medicine, Iowa City, IA 52242, USA

²Department of Psychology, University of Iowa, Carver College of Medicine, Iowa City, IA 52242, USA

³Department of Neurology, University of Iowa, Carver College of Medicine, Iowa City, IA 52242, USA

⁴Interdisciplinary Neuroscience Program, University of Iowa, Carver College of Medicine, Iowa City, IA 52242, USA

SUMMARY

Eating disorders, such as anorexia nervosa and bulimia nervosa, are common and severe mental illnesses of unknown etiology. Recently, we identified a rare missense mutation in the transcription factor estrogen-related receptor alpha (ESRRA) that is associated with the development of eating disorders. However, little is known about ESRRA function in the brain. Here, we report that *Esrra* is expressed in the mouse brain and demonstrate that *Esrra* levels are regulated by energy reserves. *Esrra*-null female mice display a reduced operant response to a high-fat diet, compulsivity/behavioral rigidity, and social deficits. Selective *Esrra* knockdown in the prefrontal and orbitofrontal cortices of adult female mice recapitulates reduced operant response and increased compulsivity, respectively. These results indicate that *Esrra* deficiency in the mouse brain impairs behavioral responses in multiple functional domains.

Graphical Abstract

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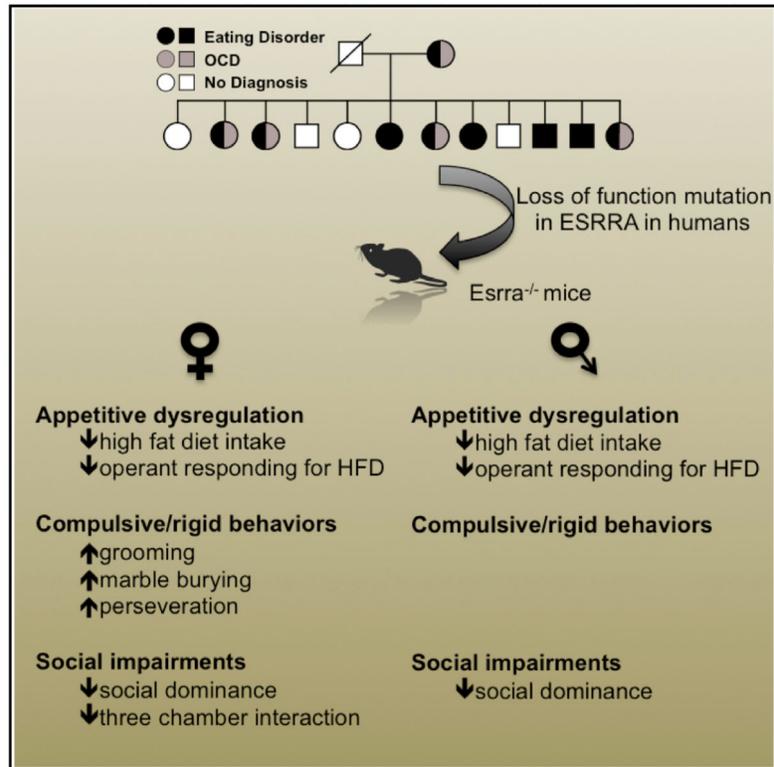
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*Correspondence: michael-lutter@uiowa.edu.

⁵Present address: West Virginia University, MD/PhD Program, Robert C. Byrd Health Science Center, P.O. Box 9104, Morgantown, WV 26506-9104, USA

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, two figures, and one table and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2015.03.032>.



INTRODUCTION

While eating disorders (EDs) are known to result from a complex interaction of environmental stress and genetic vulnerability (Kaye et al., 2013), the specific neural substrates that mediate development of EDs remain poorly understood. To elucidate molecular pathways that lead to the development of EDs, we previously conducted next-generation sequencing of two large families with multiple members affected by EDs (Cui et al., 2013). In that study, we identified rare missense mutations in the estrogen-related receptor alpha (*ESRRR*) and histone deacetylase 4 (*HDAC4*) genes that significantly increase the risk of having an ED (Cui et al., 2013). Specifically, the *ESRRR*^{R188Q} mutation reduces induction of known *ESRRR*-target genes in vitro, while the *HDAC4*^{A786T} mutation increases transcriptional repression of *ESRRR* by *HDAC4*. As both mutations result in decreased *ESRRR* transcriptional activity, we hypothesized that loss of *ESRRR* activity confers specific susceptibility to developing an ED.

While *ESRRR* is known to be expressed in the CNS, relatively little is known about its function in neurons. *Esrra* is also required for induction of mitochondrial biogenesis in the brain, in response to the peroxisome proliferator-activated receptor gamma agonist rosiglitazone (Strum et al., 2007). Furthermore, parkin, a protein associated with the development of Parkinson's disease, regulates *Esrra* levels through ubiquitination and degradation of *Esrra* by the proteasome, which decreases expression of the *Esrra*-target genes monoamine oxidase A and B (Ren et al., 2011). Recently, *Esrra* has also been implicated in mediating increased expression of the *Fdnc5* and *Bdnf* genes in mouse

hippocampus following endurance exercise (Wrann et al., 2013). Taken together, these findings suggest that tight control of *Esrra* levels in the CNS may regulate multiple metabolic processes critical to neuronal function. In the present study, we examine *Esrra* function in mouse brain by using genetic methods to examine the behavioral consequences of loss of *Esrra* expression.

RESULTS

To study the role of *Esrra* in the brain, we performed immunohistochemistry to determine its expression pattern in the whole mouse brain. *Esrra* is abundantly expressed in several cortical regions, including orbitofrontal cortex, medial prefrontal cortex (mPFC), and cingulate cortex (Figures 1A–1C). Additionally, *Esrra* is highly expressed in the primary and secondary somato-sensory cortex, piriform cortex, retrosplenial granular cortex, hippocampus, thalamus, mammillary nucleus, cerebellum, and several brainstem nuclei involved in transmission of sensory information (Figures S1A–S1C). Moderate levels of expression are observed in dorsolateral aspects of the striatum, septum, global pallidus, lateral hypothalamic area, and some midbrain structures including tegmentum and substantia nigra. Very limited expression was observed in the bed nuclei of stria terminalis, amygdala, and the remainder of the hypothalamus, regions that have been traditionally associated with a role in feeding regulation. Double-immunohistochemistry staining of *Esrra* and glial fibrillary acid protein failed to reveal colocalization of *Esrra* in glia (data not shown).

Upregulation of *Esrra* expression has been observed in peripheral tissues following calorie restriction (Ranhotra, 2009). Thus, we examined whether *Esrra* expression in brain would also be increased in this same condition. Ten days of 60% calorie restriction significantly increased the intensity of *Esrra* staining throughout the brain, including the cortex (Figures 1D, 1E, S1D, and S1E), indicating that *Esrra* activity may be involved in the response to energy balance. To quantify this effect, we performed quantitative real-time PCR to measure the relative abundance of *Esrra* mRNA in the cortex. Consistent with immunohistochemical analysis, 60% calorie restriction induced a significant increase in *Esrra* mRNA abundance in the cortex compared to that in mice housed under ad lib feeding conditions (2.48 ± 0.59 versus 4.93 ± 0.50 , $n = 6/\text{group}$, $p = 0.0098$).

After determining *Esrra* expression in several mouse brain regions and that *Esrra* expression is regulated by metabolic status, we examined the behavioral consequences of *Esrra* loss. A previous study reported that chow-fed male and female *Esrra*-null mice were ~15% smaller than wild-type littermates and displayed reduced weight gain on a high-fat diet (HFD), which is consistent with the clinical diagnosis of anorexia nervosa where body weight is maintained at or below 85% of ideal body weight (Luo et al., 2003). We also verified that *Esrra*-null mice were ~15% smaller than wild-type littermates and consumed less chow and HFD (data not shown); however, these phenotypes were identified in animals on an ad libitum diet. As the magnitude of ad libitum food intake primarily reflects intra-meal satiety independent of any component of behavioral effort (Perello et al., 2010), we utilized instrumental responding as a more sensitive measure of motivation to obtain a HFD.

Following a fixed-ratio training session, wild-type and *Esrra*-null mice were advanced to a progressive-ratio schedule in which obtaining each successive HFD pellet required an increased number of lever presses. Effortful responding for a HFD was then assessed during both caloric restriction and ad libitum feeding conditions by measuring the total number of lever presses and total number of pellets earned during the 2-hr session. During caloric restriction, *Esrra*-null mice exhibited reduced total lever presses and number of reward obtained compared to wild-type littermates (Figures 1F and 1G). Following re-feeding, total lever presses and reward obtained decreased for both groups as expected, and no significant differences were noted between treatment groups. At the end of testing, we measured fasting glucose and body composition to control for differences in energy reserves that might account for the decreased responding in *Esrra*-null mice. Fasting glucose levels (Figure S1F) and body composition (Figure S1G) were not different for both groups, indicating that reduced effortful responding observed in *Esrra*-null mice was not secondary to differences in energy reserves.

In separate cohorts of female mice, we performed a battery of behavioral tasks relevant to mental illness including locomotor activity, depression/anxiety, compulsivity/behavioral rigidity, and impairments in social functioning. *Esrra*-null mice have recently been reported to have reduced locomotion in both treadmill exercise and voluntary activity in a metabolic cage assessment (Perry et al., 2014). As locomotor activity varies greatly depending on the context, we measured activity under three conditions: voluntary wheel running, home-cage activity, and exploration of a novel environment. When given free access to a running wheel for 5 days, *Esrra*-null mice exhibited normal circadian patterns of activity but also a reduction in total revolutions (Figure 2A). Using the Laboras system, we assessed home-cage activity and found a modest reduction in locomotion in *Esrra*-null mice compared to wild-type littermates (Figure 2B). Finally, we evaluated exploration of a novel environment by measuring distance traveled in the elevated-plus-maze model of anxiety-like behavior and found no significant difference between the groups (Figure 2C). This finding is important as the majority of behavioral tests that we performed involved activity within a novel environment.

We assessed anxiety- and depression-like behaviors using the elevated-plus-maze and forced-swim test models. Female *Esrra*-null mice do not display any differences in overall activity or time on the open arm in the elevated-plus-maze (Figures 2C and 2D); however, they did exhibit reduced immobility in the forced-swim test (Figure 2E). Several tasks modeling aspects of perseveration and behavioral rigidity in mice have been used to study related neuropsychiatric disorders, such as autism and obsessive-compulsive disorder, including compulsive grooming (Shmelkov et al., 2010; Welch et al., 2007; Xu et al., 2013), marble burying (Angoa-Pérez et al., 2013), and reversal learning (Kim et al., 2011). Using a vibration-plate based system to quantify 24-hr home-cage grooming, we observed that loss of *Esrra* increases grooming in female mice (Figure 2F). The fact that chronic fluoxetine treatment has been shown to reduce repetitive grooming in several other genetic models of compulsivity (Shmelkov et al., 2010; Welch et al., 2007) led us to assess whether this treatment could also ameliorate the increased repetitive grooming in female *Esrra*-null mice. Separate groups of *Esrra*-null female mice were tested for baseline grooming and then randomized into two equal groups that received either fluoxetine or saline vehicle.

Following 1 week of treatment, no effect of fluoxetine was noted (saline $88.2\% \pm 12.1\%$ versus fluoxetine $93.0\% \pm 8.9\%$, $n = 6/\text{group}$, $p = 0.71$). We also assessed behavioral compulsivity in *Esrra*-null mice using the marble-burying test. Consistent with the compulsive grooming results, female *Esrra*-null mice showed a significantly greater degree of marble-burying behaviors after 30 min than wild-type littermates (Figure 2G). Mice were trained for 4 days to find an escape hole in the Barnes maze to assess hippocampal-dependent memory formation. On the fifth day, a probe trial was performed showing that both wild-type and *Esrra*-null mice spent more time in the area of the escape hole without a significant difference noted between the groups. Following this initial training, mice were next subjected to an additional 4 days of training in which the escape hole was relocated to the opposite position. Another probe trial was then performed, and time spent at the old and new target was measured. While both groups learned the location of the new escape hole, *Esrra*-null mice spent significantly more time at the original target (Figure 3H), an indication of behavioral rigidity.

Several tasks modeling social dysfunction in mice have been designed, including the three-chamber social interaction, social dominance, and novel object recognition. Using this battery of tasks, we also found that *Esrra*-null mice displayed reduced preference for interaction with a novel social target (Figure 2I), increased social subordination (Figure 2J), and no differences in novel object recognition as compared with wild-type littermate mice. Together these behavioral findings indicate that *Esrra*-null mice exhibit behavioral deficits in multiple domains including decreased motivation for HFD, perseveration/ behavioral rigidity, and impaired social functioning.

We next interrogated the neural circuits underlying the behavioral phenotypes exhibited by *Esrra*-null mice by abrogating expression of this gene in a region-specific manner in wild-type female mice. Using viral-mediated gene transfer to selectively knockdown expression of *Esrra* by short hairpin RNA (shRNA)-mediated silencing (Figures S2A–S2D), we selected several cortical regions implicated in the development of EDs and ED-related behaviors, including cingulate cortex, orbitofrontal cortex, and medial prefrontal cortex (mPFC) (Frank, 2013). While knockdown of *Esrra* in the cingulate and orbitofrontal cortex had no effect on HFD-induced weight gain in female mice, knockdown in the mPFC reduced weight gain (Figure 3A) via reduced intake of a HFD (Figure 3B). We tested a separate cohort of mice to assess whether knockdown of *Esrra* in the mPFC disrupted operant responding for HFD. Consistent with our observations in *Esrra*-null mice, knockdown of *Esrra* in mPFC was sufficient to reduce total lever presses and reward earned (Figure 3C). Subsequent analyses also examined the capacity of knockdown of *Esrra* within selected cortical fields to reproduce other behavioral phenotypes observed in null mutants. Increased grooming was observed only after knockdown of *Esrra* in orbitofrontal cortex, whereas no differences in grooming were observed following either cingulate or mPFC knockdown (Figure 3D). Reduced immobility was observed only after knockdown of *Esrra* in the cingulate cortex (Figure 3E). Together, these findings identify specific neural substrates accounting for the behavioral alterations observed in *Esrra*-null mice.

Finally, we determined whether there was a sexual dimorphism in any of the changes we observed in female *Esrra*-null mice by conducting all of the testing on male mice as well.

Table 1 summarizes the differences in behavioral phenotypes in female and male *Esrra*-null mice. Similar to female mice, male *Esrra*-null mice display reduced locomotor activity, decreased operant responding, and reduced immobility in the forced-swim test, but do not exhibit deficits in the compulsive/behavioral rigidity battery of tasks or the three chamber social interaction test.

DISCUSSION

In the current study, we examine the forebrain distribution and function of *Esrra*, a gene identified in human genetic studies of EDs. We report that *Esrra*-null mice display behavioral impairments relevant, including maintaining body weight at 15% below normal, impaired behavioral responses to caloric restriction, compulsivity/behavioral rigidity, and impaired social functioning. Furthermore, knockdown of *Esrra* expression within selected cortical subfields distinguished regional specificity for circuitry underlying behavioral deficits identified in *Esrra*-null mutants. These findings show that *Esrra* dysfunction in select forebrain regions impairs behaviors relevant to psychiatric disorders and offer insight into the potential role of *Esrra* dysfunction in the development of eating disorders, such as anorexia nervosa.

Esrra expression in brain is increased during calorie restriction and loss of *Esrra* by conventional knockout or shRNA-mediated knockdown in the mPFC results in decreased consumption of a HFD and decreased operant responding for a HFD. These results suggest that increased expression of *Esrra* is important for mediating behavioral responses to negative energy balance. As the *ESRRA*^{R188Q} mutation that was found to be associated with an increased risk of developing anorexia nervosa results in decreased transcriptional activity (Cui et al., 2013), our results suggest that loss of *ESRRA* activity in humans may predispose to the development of anorexia nervosa by impairing behavioral responses to calorie restriction.

Identifying the neural circuit that mediates the reduced effortful responding for HFD after fasting is another question of great clinical relevance. *Esrra* activity in the mPFC seems to be at least partially required for this effect, as viral-mediated knockdown of *Esrra* expression in the mPFC produces a similar reduction in responding (Figure 3C). Stimulation of glutamatergic neurons in the mPFC by pharmacological (Mena et al., 2013) or optogenetic (Land et al., 2014) approaches potently induces food intake in rodents. However, the site of this action is unclear as mPFC neurons project to multiple sites, including the amygdala, lateral-perifornical hypothalamic area, and nucleus accumbens that have been implicated in this behavioral response (Land et al., 2014; Mena et al., 2013). Recently developed chemogenetic and optogenetic approaches to the study of feeding may ultimately be required to deconstruct these circuits.

Additionally, the behavioral deficits we observe in *Esrra*-null mice might have important implications for other symptoms associated with the development of eating disorders. Neuropsychological testing has consistently found that patients with EDs display increased perseveration and behavioral rigidity (Roberts et al., 2010). Patients with EDs display deficits in social functioning as well including insecure attachment, reduced emotional

awareness, increased avoidance of emotion, and increase sense of social inferiority (Caglar-Nazali et al., 2014).

Our findings may also be relevant to the basis of sex differences observed in neuropsychiatric disorders. Many psychiatric disorders, including EDs, major depression, and most anxiety disorders, predominantly affect women although the neural basis of this sexual dimorphism remains unclear. Our data suggest that females may be more susceptible to the loss of *Esrra* function, as female knockout mice exhibited more behavioral impairments than male mice. The mechanism for the observed sex differences remains unclear. There is an estrogen response element in the promoter of the *Esrra* gene, and previous studies have indicated that estrogen receptor signaling affects expression of *Esrra* in peripheral tissues (Bonnelye et al., 2002; Liu et al., 2003), although we did not observe any change in *Esrra* mRNA levels in mPFC 4 weeks after ovariectomy in young adult female mice (data not shown). Alternatively, estrogen receptors alpha and beta have been localized to dendritic spines in the hippocampus and may have a direct, non-genomic signaling effect on synaptic plasticity (Sala and Segal, 2014). Delineating the roles of *Esrra* and estrogen receptor activity will have important implications in understanding sex-based differences in mental illness.

Several limitations of the current study should be noted. *Esrra*-null mice have reduced locomotor activity, which can confound the interpretation of certain behavioral tasks like operant responding and the forced-swim test. This hypolocomotor phenotype appears to be most severe for endurance activity, such as treadmill exercise (Perry et al., 2014) or voluntary wheel running (Figure 2A). The phenotype is less severe for home-cage activity (Figure 2B) and absent in the exploration of a novel environment (Figure 2C), which represents the majority of our tasks. Also, we have tried to select a balanced set of tasks to control for differences in locomotor activity. For instance, the increased activity of *Esrra*-null mice in the forced-swim test, marble-burying test, and grooming assay is in the opposite direction of an expected hypolocomotor effect.

Finally, there are limitations with the two methods used to decrease *Esrra* activity. We selected the *Esrra*-null mouse because the *ESRRA*^{R188Q} mutation that predisposes to the development of eating disorders in human populations decreases transcriptional activity of *Esrra* in vitro (Cui et al., 2013). While we believe that the *Esrra*-null mouse is a reasonable first approach to testing our hypothesis that reduced *Esrra* activity increases the risk of developing ED-related behaviors, we cannot be certain that the R188Q mutation does not induce an unanticipated change in the activity of the *Esrra* protein. Additionally, the use of shRNA-mediated gene knockdown has been questioned for possible off-target effects (Baek et al., 2014). While this concern is offset by our limited use of shRNA in the context of mapping behavioral deficits originally identified in *Esrra*-null mice, the use of more targeted genetic models, such as conditional deletion of *Esrra* expression with a Cre-lox approach, will further expand our understanding of the genetic necessity of *Esrra* within discrete neural circuits.

In conclusion, we show that loss of the transcription factor *Esrra* results in behavioral deficits in multiple functional domains, including maintenance of a low body weight,

reduced effortful responding for HFD after calorie restriction, compulsivity/behavioral rigidity, and impaired social functioning. These findings offer insight into the neurobiological function of *Esrra* and have potential implications for understanding the neurobiological basis of eating disorders.

EXPERIMENTAL PROCEDURES

Animals

Esrra-null and C57BL/6J mice were obtained from Jackson Laboratory and backcrossed more than five generations onto C57BL/6J. All animal procedures were performed in accordance with University of Iowa Institutional Animal Care and Use Committee guidelines. Mice heterozygous for *Esrra*^{+/-} alleles were bred to generate wild-type (*Esrra*^{+/+}) and *Esrra*-null (*Esrra*^{-/-}) littermate mice. Mice were weaned and genotyped at 3–4 weeks of age. See Supplemental Experimental Procedures for housing and animal husbandry conditions.

Behavioral Studies

Twelve- to 16-week-old animals were used for behavioral studies. Some mice were used after completing body-weight monitoring and had previously been exposed to a high-fat diet (42.8% calories from fat, TD.88137, Harlan-Teklad). No behavioral differences, however, were noted between chow- and HFD-fed animals and groups (data not shown). Separate cohorts of mice not exposed to a HFD were used for wheel running and operant responding, respectively, so that no mouse included in the other behavioral tasks had been exposed to increase physical activity or calorie restriction. See Supplemental Experimental Procedures for full methodology for behavioral experiments.

Immunohistochemistry

Immunohistochemistry was performed as previously reported (Cui et al., 2012). Rabbit monoclonal anti-*Esrra* antibody was used (04-1134; Millipore) at 1/1,000 dilution. The specificity of anti-*Esrra* antibody was confirmed by both immunohistochemistry (Figure S1B) and immunoblotting (Figure S1C) in *Esrra*-null mice. Quantification of immunohistochemistry was performed as previously described (Yin et al., 2014).

Viral Preparation

An shRNA sequence targeting nucleotides 1203–1223 of mouse *Esrra* (NM_007953) or scramble control were commercially prepared (Vector Laboratories). See Supplemental Experimental Procedures for full description of viral preparations.

Viral Delivery

Twelve-week-old female C57BL/6J (The Jackson Laboratory, #00664) mice were randomly assigned into two groups to receive either adeno-associated virus 2 (AAV) encoding both *mEsrra*-shRNA and GFP or AAV encoding scrambled sequence and GFP as control into the following regions: mPFC (AP +1.8 mm, ML +0.8 mm, DV -2.8 mm, 10-degree angle), lateral orbitofrontal (AP +2.8 mm, ML +1.7 mm, DV -2.0 mm, 10-degree angle), and

cingulate (AP +0.2 mm, ML +0.6 mm, DV -1.7 mm, 10-degree angle). See Supplemental Experimental Procedures for surgical procedures.

Data Analysis

Data are presented as mean \pm SEM. GraphPad Prism 6 software (GraphPad) were used to perform all statistical analyses. For feeding and wheel-running studies, comparisons between groups were made by two-way ANOVA with time as the repeated measure. The chi-square test was used to determine significance in the social dominance test. Student's *t* test was used for all other measures. $p < 0.05$ was considered to be statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Esrra expression in the mouse brain is regulated by energy status
- Loss of Esrra reduces response to a high-fat diet
- Esrra-null mice display behavioral compulsivity and social impairments
- Esrra knockdown in mPFC and OFC recapitulates feeding and grooming deficits

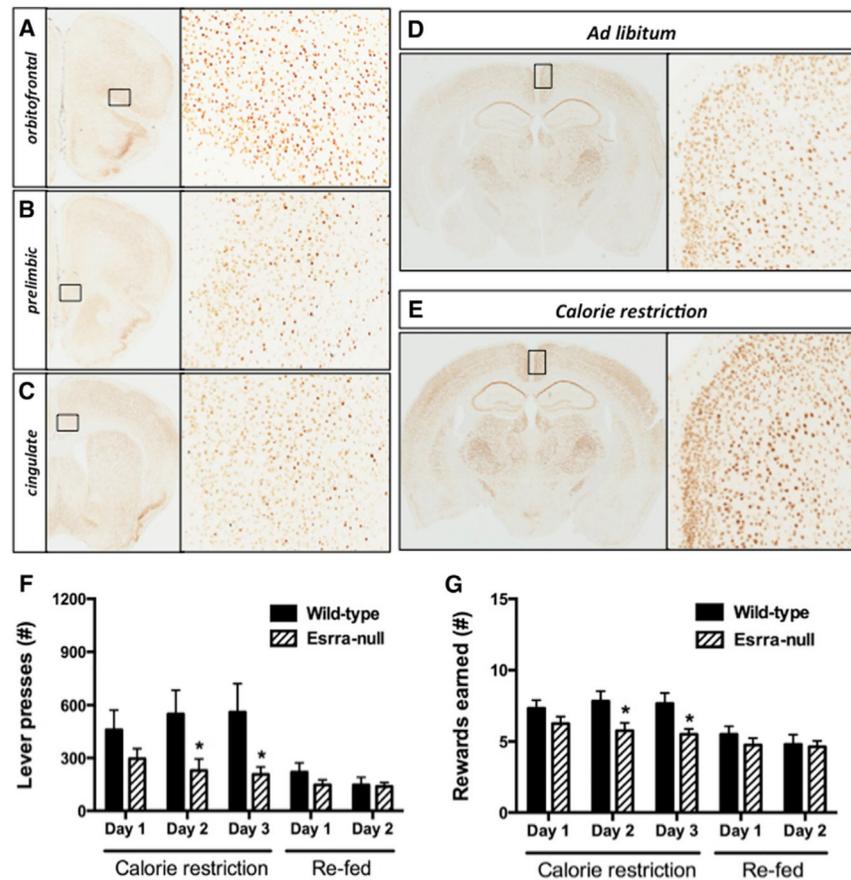


Figure 1. Esrra-Null Mice Display Impaired Behavioral Responses to Calorie Restriction

(A–C) Expression of *Esrra* in 14-week-old C57BL/6/J mice in lateral orbitofrontal, mPFC, and cingulate cortex.

(D and E) 60% calorie restriction for 10 days increases *Esrra* expression in mouse brain. Magnification shows retrosplenial region of cingulate cortex.

(F and G) Wild-type ($n = 7$) and *Esrra*-null ($n = 6$) littermate mice were trained to lever press for a HFD pellet on a fixed ratio and then tested for total lever presses (F) and reward earned (G) on a progressive ratio under calorie restriction and ad lib feeding conditions.

Data are presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ indicate significant differences between groups (Student's *t* test). See also Figure S1.

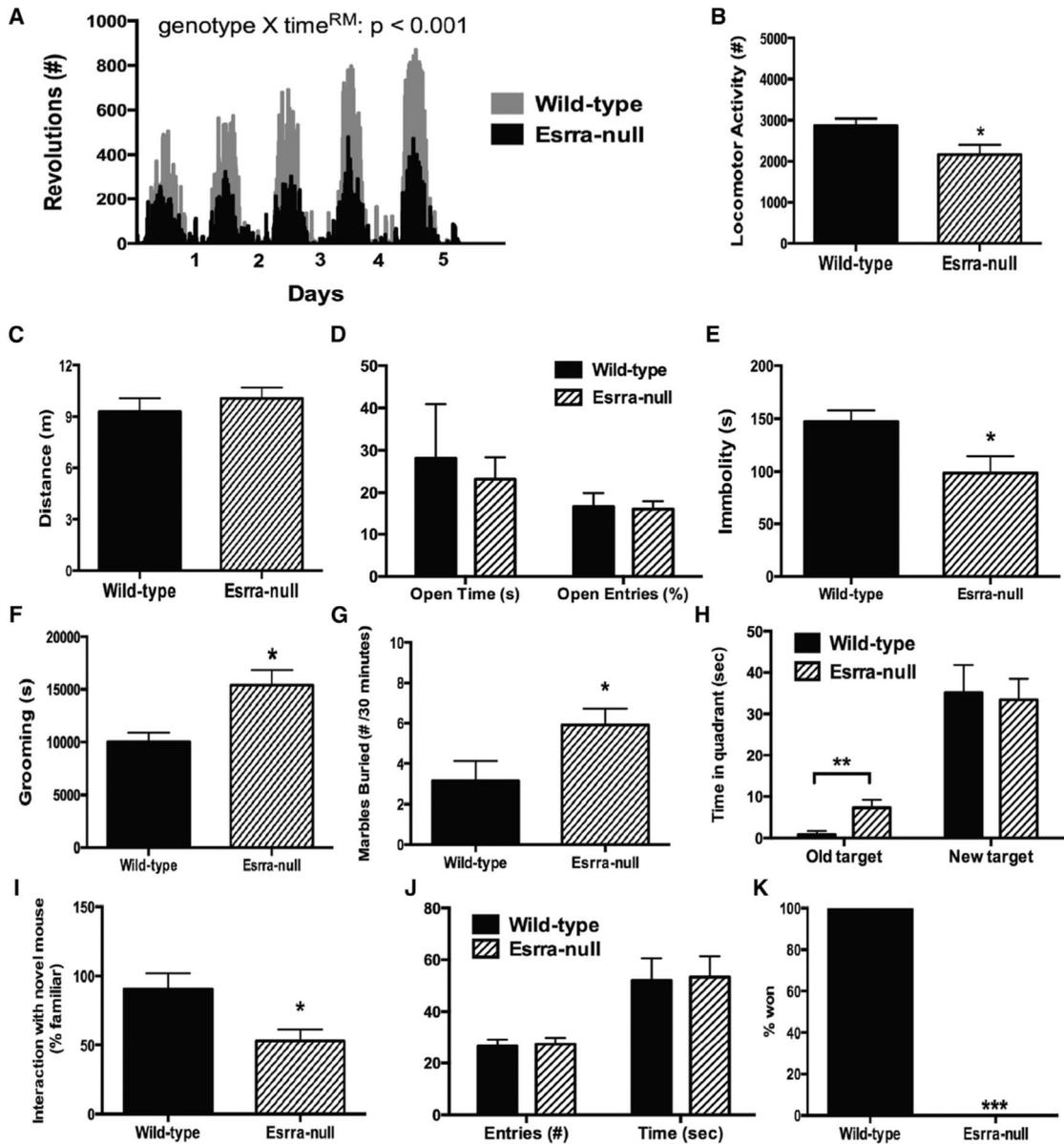


Figure 2. Behavioral Deficits in Esrra-Null Mice

(A) Voluntary wheel-running activity for female wild-type (WT) and Esrra-null mice for 5 days (n = 3 WT, 6 Esrra null).

(B) Time spent active in home cage as measured by vibration plate (n = 17 WT, 5 Esrra null).

(C and D) Distance moved and time spent on the open arm of the elevated-plus-maze (n = 8 WT, 14 null).

(E) Time immobile in the forced-swim test (n = 8 WT, 14 null).

(F) Home-cage grooming as measured by vibration plate (n = 17 WT, 5 Esrra null).

(G) Number of marbles buried within 30 min (n = 7 WT, 12 Esrra null).

(H) Time spent near old target and new target in Barnes maze (n = 5 WT, 6 Esrra null).

(I) Time spent with novel mouse in three-chamber test (n = 8 WT, 10 Esrra null).

(J) Time spent with novel object (n = 9 WT, 11 Esrra null).

(K) Percentage of victories in the social dominance test (n = 24 unique pairings).

Data presented as mean \pm SEM with *p < 0.05, **p < 0.01 indicating significant differences between groups (two-way ANOVA [A] or Student's t test [B–I and K]), except for (J), which is presented as a percentage of total victories with ***p < 0.001 indicating significant differences between groups (chi-square test).

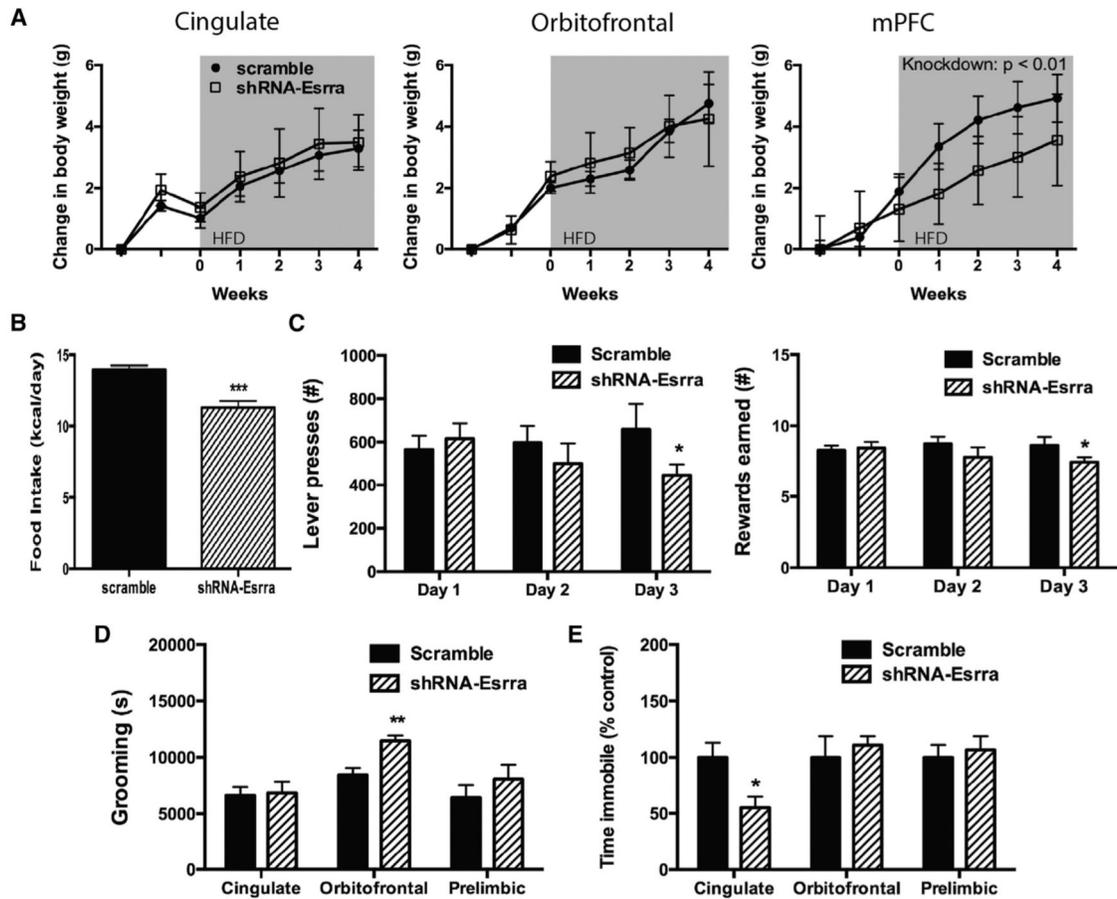


Figure 3. Knockdown of Esrra Expression by shRNA in Cortex Produces Region-Specific Behavioral Deficits

(A) Body-weight gain in female C57BL/6J mice on a HFD after AAV delivery into cingulate ($n = 10$ scramble and 9 shRNA-Esrra), orbitofrontal ($n = 9$ scramble and 10 shRNA-Esrra), or mPFC ($n = 9$ scramble and 11 shRNA-Esrra).

(B) Intake of a HFD after Esrra knockdown in mPFC.

(C) A separate cohort of mice received AAV into mPFC and was tested for total lever presses and reward earned.

(D and E) Mice from (A) were tested for home-cage grooming (D) and immobility in the forced-swim test (E).

Data presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ indicate significant differences between groups (ANOVA [A] or Student's *t* test [B–E]). See also Figure S2.

Table 1
Summary of Behavioral and Electrophysiological Phenotypes in Female and Male Esrra-Null Mice

	Female^a	Male^b
Operant responding		
Lever presses	decrease	decrease
Rewards	decrease	decrease
Wheel running	decrease	no difference
Home-cage activity	decrease	decrease
Elevated plus maze		
Distance traveled	no difference	no difference
Time on open arm	no difference	no difference
Forced-swim test	decrease	decrease
Grooming	increase	no difference
Marble burying	increase	no difference
Reversal learning	decrease	no difference
Three chamber test	decrease	no difference
Social dominance	decrease	decrease
Novel object	no difference	no difference

See also Table S1.

^aResults for female mice from Figures 1 and 2.

^bResults for male wild-type and Esrra-null littermate mice (n = 6–11/group) tested under identical protocols.