Published in final edited form as:

Neuropeptides. 2010 August ; 44(4): 349–353. doi:10.1016/j.npep.2010.04.005.

CRF₂ null mutation increases sensitivity to isolation rearing effects on locomotor activity in mice

Jodi Gresack, Ph.D.¹, Susan Powell, Ph.D.¹, Mark Geyer, Ph.D.^{1,2}, Mary-Stenzel Poore, Ph.D.³, Sarah Coste, Ph.D.³, and Victoria Risbrough, Ph.D.^{1,2}

¹ Dept of Psychiatry, University of California, San Diego

² Veteran's Affairs Center of Excellence for Stress and Mental Health

³ Dept. of Molecular Microbiology and Immunology, Oregon Health and Sciences University

Abstract

Background—Developmental stressors are consistently reported to increase risk for certain neuropsychiatric disorders including schizophrenia, depression, and post-traumatic stress disorder. Recent clinical evidence supports a “double-hit” hypothesis of genetic vulnerability interacting with developmental challenges to modulate this risk. Early life stressor effects on behavior may be modulated in part by alterations in corticotropin releasing factor (CRF) signaling via two known receptors, CRF₁ and CRF₂. One extant hypothesis is that CRF₂ activation may modulate long term adaptive responses after homeostatic challenge. As such, loss of CRF₂ activity via genetic variance may increase sensitivity to the long-term effects of developmental stress.

Methods—We tested the hypothesis that CRF₂ function may mitigate the behavioral effects of isolation rearing, predicting that loss of CRF₂ function increases sensitivity to this developmental challenge. Using the behavioral pattern monitor (BPM), we examined exploratory behavior and locomotor patterns in adult CRF₂ wild-type (WT) and gene knockout (KO) mice reared socially or in isolation.

Results—Isolation housing produced robust increases in the amount of locomotor activity and investigatory holepoking, and altered the temporal distribution of activity in CRF₂ KO but not CRF₂ WT mice. Isolation housing significantly increased rearing behavior and altered spatial patterns of locomotor activity regardless of genotype.

Conclusions—Loss of CRF₂ function increased sensitivity to the effects of chronic social isolation on exploratory locomotor behavior. Thus, CRF₂ activation appears to mitigate isolation rearing effects on exploratory behavior. Further research assessing the interaction between CRF₂ function and developmental challenges is warranted.

Keywords

CRF; CRH; social isolation; locomotor activity; schizophrenia; isolation rearing; developmental stress

Corresponding author: Victoria Risbrough, Ph.D., Dept. of Psychiatry, University of California, San Diego, 9500 Gilman Dr. MC0804, La Jolla, CA 92093-0804, Tel: (619) 543-3582, Fax: (619) 543-2475.

All other authors have no conflicts to disclose.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Behavioral and endocrine response to homeostatic challenge are coordinated by the corticotropin releasing factor (CRF) family of peptides and their activation of two known receptors, CRF₁ and CRF₂ (Vale et al., 1981, Hauger et al., 2006). Both receptors are located in limbic and subcortical structures within the central nervous system, where they modulate many behaviors including exploration, sensorimotor gating, feeding, and both anxiety- and depressive-like behaviors (for review see Risbrough and Stein, 2006, Bale and Vale, 2004, Hauger et al., 2009). In animals, the CRF₁ receptor is well known for mediating the effects of stress on multiple behaviors, while CRF₂ plays a much more complex role, with evidence for both potentiating and attenuating effects on stress responses (for review see Hauger et al., 2009).

Recent data have also suggested that CRF signaling is important for the long-term effects of developmental stressors on adult traits (e.g. Heim and Nemeroff, 2001). For example, recent studies have shown that CRF₁ gene variation has been associated with vulnerability and resilience to depressive disorders in adults exposed to early life stress (Bradley et al., 2008, Liu et al., 2006, Polanczyk et al., 2009). Therefore, CRF signaling variance may influence the contribution of early life stress to the risk for adult anxiety and depressive disorders. Little is known however about the potential role that CRF₂ signaling plays during and after developmental challenges.

Developmental stressors (e.g. post-weaning social isolation rearing, post-weaning maternal deprivation, prenatal and postnatal immune challenges, prenatal chronic variable stress) are modeled in animals to understand mechanisms mediating different effects of homeostatic challenges on brain development and to identify potential risk factors (e.g. genetic susceptibility) that increase neuropsychiatric vulnerability (Powell and Geyer, 2002, Meyer and Feldon, 2009). Social deprivation during development, i.e. rearing rodents in social isolation from weaning, produces lasting alterations in brain and behavior throughout adulthood. These alterations include adaptations in CRF and HPA systems as well as limbic pathology (Fone and Porkess, 2008, Lukkes et al., 2009a, Voikar et al., 2005, Mueller and Bale, 2008, Serra et al., 2005)

CRF expression and CRF neuron activation are increased in the hypothalamus and hippocampus of isolated prairie voles (Grippe et al., 2007, Pournajafi-Nazarloo et al., 2009). Social isolation stress specifically modulates CRF₂ receptor signaling, although these effects are dependent upon isolation duration, species, and brain region measured. For example, social isolation of prairie voles increases or decreases CRF₂ receptor expression in the hippocampus and hypothalamus, respectively (Pournajafi-Nazarloo et al., 2009). In rats housed in social isolation during early life, CRF₂ receptor levels are increased in the dorsal raphe nucleus, leading to greater serotonin release in response to CRF (Lukkes et al., 2009b). In a similar study, isolation effects on social interaction were reversed by selective CRF₂ blockade, suggesting that alterations in CRF₂ signaling mediate some of the behavioral abnormalities produced by isolation rearing. However, the changes in CRF₂ function may instead be a compensatory response to isolation. This hypothesis is supported by data suggesting that CRF₂ plays a role in the long-term adaptation to stress (Coste et al., 2006, Waselus et al., 2009, Greetfeld et al., 2009, McEuen et al., 2008). Mice with CRF₂ null mutation have been suggested to exhibit “maladaptive” stress responses after acute and chronic stress (Coste et al., 2006, McEuen et al., 2008)

Based on these data, we hypothesized that CRF₂ activation modulates the enduring effects of environmental stressors such as isolation rearing. We previously reported that CRF₂ WT and KO mice exhibit similar reductions in context fear learning after isolation rearing (Gresack et

al., 2010), however a lack of gene effect in this study may have been due to floor effects in context fear deficits after isolation rearing in WT mice, precluding the ability to detect any further decline in cognitive performance in isolated KO mice. Hence to test our hypothesis further, we compared the effects of isolation rearing on exploratory locomotor activity in mice with and without the CRF₂ receptor. Locomotor hyperactivity has been commonly associated with social isolation in rats (Fone and Porkess, 2008, Powell and Geyer, 2002), in particular reducing habituation and increasing rearing behavior (Paulus et al., 1998, Powell et al., 2002). A reduction in the effects of isolation rearing due to the absence of CRF₂ would suggest that CRF₂ activation mediates isolation effects on exploration. If CRF₂ null mutation potentiates isolation effects, this result would suggest that CRF₂ plays a compensatory role in response to social isolation stress effects on exploration. To measure isolation effects, we used the behavioral pattern monitor (Risbrough et al., 2006), which characterizes a profile of the amount and pattern of exploratory locomotor activity.

Methods

Subjects

Male, heterozygously bred CRF₂ receptor wild-type (WT) and knockout (KO) mice (backcrossed >N14 to C57BL/6J mice; Coste et al., 2000) were used (n=37). On postnatal day 28, pups were weaned and housed in standard mouse cages in groups of either three per cage (social condition) or one per cage (isolate condition) for the duration of the study (final n=7–11/group). Each litter was represented in both social and isolate rearing conditions. Mice underwent locomotor activity testing in a novel environment as adults (5 mo of age). Before locomotor testing mice underwent startle reactivity and prepulse inhibition testing at 2, 3 and 4 mo of age (data not shown). All mice were housed in a temperature- and humidity-controlled room with a 12:12 h reverse light/dark cycle (lights off at 08:00) and food and water available *ad libitum*.

Apparatus

Locomotor activity was measured in 10 mouse Behavioral Pattern Monitors (BPM; San Diego Instruments, CA) as described previously (Risbrough et al., 2006; Geyer et al., 1986). By simultaneous monitoring of different responses in sequence and time, the mouse BPM assessed the quantity and aspects of the quality of movement patterns of the mice. Data were collected on the location of the mouse in successive X–Y coordinates, which can be used to produce region locations and locomotor paths. Each Plexiglas chamber contains a 30 by 60 cm holeboard floor enclosed in an outer box that minimizes outside light and noise. Subject activity was detected by sets of infrared photobeams 1 cm from the floor (2.5 cm apart along the length and the width of the chamber; 24 × 12 X–Y array) recording the location of the mouse every 0.1 sec. The subject's position was defined across 9 unequal regions (4 corners, 4 walls and center; Geyer et al. 1986). As a complementary measure of exploratory behavior, another set of 16 photobeams, 1.9 cm apart, located on the Y axis only, at the height of 6.9 cm from the floor, measured the amount of rearing. Additionally, there are 11 holes (3 holes in the floor and eight on the walls; 1.25 cm in diameter, 1.9 cm from the floor) equipped with photobeams to detect investigatory holepokes.

Locomotor Pattern Testing

At start of testing, mice were placed in the bottom left hand corner of the enclosure. The experimental session was initiated by an individual button press, and the session lasted 60 minutes. During testing the locomotor activity chambers were in complete darkness (no house light was on). The measures obtained were transitions across regions, total holepokes and rearings, as well as scaling measures. The spatial scaling exponent “d” (Paulus and Geyer 1991) quantifies the geometrical structure of the locomotor path. A value of 1 represents a path in a

straight line, 1.5 a meandering path, and 2 a highly circumscribed path. The spatial coefficient of variation (CV) is a measure of the X–Y pattern representing how predictable the pattern is across the 9-region transition matrix (with 40 permissible transitions). Spatial CV increases when the mouse preferentially repeats certain transitions between the 9 regions of the chamber (Geyer et al., 1986). Temporal CV assesses how uniformly the animal's time is spent across the 9 regions, with a high temporal CV representing a stronger preference for particular regions over others.

Statistical analyses

The above measures were analyzed by two-way analyses of variance (ANOVAs) with housing and genotype as between-subject variables, and time block as a within-subject variable. The activity data were analyzed using 6 blocks of 10 min, while spatial scaling parameters were analyzed over the summation of the 60 min session. Subsequent ANOVAs were used to examine housing in each genotype separately as appropriate (significant interaction or trend for an interaction). For spatial scaling measures (no within subject factor, i.e. time) a Tukey's post hoc test was conducted when appropriate. Habituation of locomotor activity over the 60 min session was calculated as a percent reduction in transitions from the first 10 min block to the last ten min block of the session: $100 - (\text{total transitions from 50–60 min} / \text{total transitions from 0–10 min}) \times 100$. Percent habituation of rearing and holepokes was calculated in the same manner. Alpha level was set to 0.05. The Biomedical Data Programs (BMDP) statistical software (Statistical Solutions Inc., Saugus, Mass., USA) was used for all analyses.

Results

Activity

Isolation rearing-induced hyperactivity effects were dependent upon genotype [Gene $\times$ Housing $F(1,33)=4.43$, $p<0.05$], with CRF₂ WT mice showing no isolation effect on number of transitions in the chamber, whereas isolated CRF₂ KO mice exhibited a robust increase in activity throughout the 60 min session compared to CRF₂ KO social housed mice [Figure 1 top panels, main effect of housing WT: $F(1,14)<1$, N.S., KO: $F(1,19)=13.16$, $p<0.01$]. There were no significant differences in activity level in simple comparisons across genotype in either housing condition. Although there was a main effect of block [$F(5,165)=95.59$, $p<0.001$], reflecting habituation to the chamber, this factor did not significantly interact with gene or housing. Nevertheless, to further explore effects of housing on habituation in CRF₂ KO mice, percent reduction in locomotor activity between the first 10-min block and the last 10-min block was calculated. CRF₂ KO isolates showed less habituation compared to CRF₂ KO socials (Table 1, simple comparison of housing in KO mice: $F(1,19)=10.23$, $p<0.005$).

Investigatory Behavior

Rearing behavior was increased significantly in mice housed in isolation regardless of CRF₂ genotype [Housing $F(1,33)=18.02$, $p<0.001$, no significant interaction with gene] (Figure 1, bottom panels). Isolation rearing significantly increased holepokes [main effect of Housing $F(1,33)=4.33$, $p<0.05$, Gene $\times$ Housing $F(1,33)=2.49$, $p=.12$]. Upon inspection of the data, however, this effect was likely driven by isolation effects mainly in CRF₂ KO mice, since there were no significant housing effects in CRF₂ WT mice according to post-hoc analysis [Figure 1 center panels, Main effect of housing WT: $F(1,14)<1$, N.S., KO: $F(1,19)=14.61$, $p<0.01$]. There was no effect of housing or genotype on percent habituation of either rearing or holepoking behavior. To further illustrate the effects of housing on overall exploratory behavior, the x–y position and holepoke frequency during the last 30 min block were plotted using representative mice in each group (mice were chosen based on having the most similar transitions and holepoke frequencies as the group average; Figure 2). These plots illustrate the

substantially higher level of region coverage and overall exploratory behavior in the CRF₂ KO mice housed in isolation compared to the other groups.

Locomotor Patterns

Mice housed in isolation exhibited more linear movements as described by significantly lower spatial d across the 60 min test session across genotypes [Table 1; Housing $F(1,33)=6.47$, $p<0.05$]. Isolation rearing, however, only affected temporal CV in CRF₂ KO mice [Gene $\times$ Housing: $F(1,33)=5.64$, $p<0.05$], with isolate KO mice exhibiting significantly lower temporal CV than KO mice reared socially ($p<0.05$ Tukey's post hoc test, Table 1), suggesting that isolation-reared CRF₂ KO mice exhibited less preference for a particular region of the chamber compared to socially reared KO mice. Housing had no effect on spatial CV measures, reflecting no change in the predictability of transitions between regions. There was no overall effect of CRF₂ genotype on any of the three measures quantifying locomotor patterns.

Discussion

Consistent with most previous reports, social isolation from weaning through adulthood had mild effects on exploratory behavior in male C57BL/6J mice (Varty et al., 2006, Voikar et al., 2005, but see Pietropaolo et al., 2008). Isolation rearing increased exploratory behavior as measured by rearing behavior and the linearity of locomotor paths (decreased spatial d) across genotypes. Loss of CRF₂ function produced a much more robust effect, with CRF₂ KO mice housed in isolation exhibiting hyperactivity (increased transitions), increased holepoking behavior, and decreased temporal CV. The increase in locomotor activity observed in isolated CRF₂ KO mice was likely due to less locomotor habituation. This interpretation is supported by a reduction in percent habituation (Table 1) in these mice and previous reports of a lack of CRF₂ KO effect under brief testing conditions (i.e. 10 min test, Gresack et al. 2010). The decrease in temporal CV suggests that the isolation-reared CRF₂ KO mice distributed their time more equally across the nine regions of the BPM chamber compared to socially housed CRF₂ KO mice (Geyer et al. 1986), which is consistent with their higher levels of locomotor activity (Figure 2). Taken together, these data suggest that the CRF₂ receptor may mitigate the long-term locomotor effects of isolation rearing, with loss of function potentiating the effects of this developmental stressor.

Similar to what we have published previously, isolation rearing did not affect amount of horizontal locomotor activity in C57BL/6J mice (Varty et al., 2006), although isolated mice did display increased vertical activity (rearing behavior) and more linear locomotor paths (decreased spatial d) as previously reported in isolated rats (Paulus et al., 1998). Similar to what has been reported in rats, there appear to be strain differences in the effects of isolation rearing on locomotor activity in mice, with C57BL/6J and ICR mice reared in isolation showing minimal effects on the amount of locomotion (present results, Varty et al., 2006, Koike et al., 2009, Voikar et al., 2005, Gresack et al., 2010, but see Pietropaolo et al., 2008.), while DBA/J and 129 strains exhibit more robust locomotor increases after isolation rearing (Varty et al., 2006, Voikar et al., 2005). These data suggest that genetic background is a critical feature in sensitivity to the effects of isolation rearing on exploratory locomotor activity.

In the present study, we showed that null mutation of the CRF₂ gene appears to unmask a robust increase in locomotor activity in isolation-reared mice on a C57BL/6J background while CRF₂ null mutation has no significant effect on locomotor activity on its own. These behavioral data support a functional interaction between isolation rearing and CRF₂ signaling in mice, similar to previous reports of social isolation rearing induced augmentation of CRF₂ function across other rodent species. Selective increases in hippocampal and dorsal raphe CRF₂ (but not CRF₁) gene expression have been observed in isolated prairie voles (Pournajafi-Nazarloo et al., 2009) and increased CRF₂ protein levels have been reported in rats after isolation rearing

(Lukkes et al., 2009b). These data indicate that CRF₂ gene expression may be particularly sensitive to isolation rearing effects in comparison to CRF₁.

The potential mechanism underlying increases in sensitivity to isolation effects on locomotor activity *per se* in CRF₂ KO mice is not known, though evidence suggests that the serotonergic system may be involved. In isolation-reared rats, CRF-induced reductions in 5-HT release in the nucleus accumbens are absent (Lukkes et al., 2009b), and this finding appears to be due to increased activity of CRF₂ in the dorsal raphe. Blockade of CRF₂ activation in the dorsal raphe increases social interaction in isolation-reared rats, suggesting that CRF₂ activation in this region contributes to increased anxiety-like behavior. Taken together with the present results, it is therefore possible that increased CRF₂ activity in isolation-reared rodents plays a role in reducing social and spatial exploration, with increased exploration being unmasked by CRF₂ inactivation. These findings support future studies to test the hypothesis that CRF₂ modulation of serotonin mediates these effects of isolation rearing in mice.

Although there is little evidence as yet regarding CRF₂ gene mutation effects in humans, a recent study suggests that a single nucleotide polymorphism in the CRF₂ gene is associated with increased suicide in bipolar subjects (De Luca et al., 2007). The function of this genetic mutation on CRF₂ activity is unknown however. CRF₂ SNPs have not been found to be associated with panic disorder (Tharmalingam et al., 2006) or suicide in schizophrenia subjects (De Luca et al., 2008). However we are not aware of studies examining the interaction between CRF₂ and known environmental stressors in a neuropsychiatric population. Such association study strategies have been successful with other HPA axis gene mutations in CRF₁ receptor gene and glucocorticoid regulator gene FKBP5 (Wasserman et al., 2008, Bradley et al., 2008; Binder et al., 2008). Our data, although preliminary, support expansion of research in environmental stressor interactions with the CRF system to include the CRF₂ receptor.

In conclusion, isolation rearing produced increased exploratory behavior in CRF₂ KO mice without having robust effects in WT mice. This increased exploration was characterized by reduced habituation, increased holepoke behavior, and a reduction in region preference in CRF₂ KO isolate mice. The increased sensitivity of CRF₂ KO mice to isolation suggests that CRF₂ activation may play a compensatory role in mitigating isolation stress effects on exploratory behavior. These data support the hypothesis that CRF₂ may play a role in long term adaptation to developmental stressors.

Acknowledgments

The authors would like to thank Christine Scott and Mahalah Buell for their technical assistance and Virginia Masten for her assistance with data analysis. These studies were supported by MH052885 and by the Veterans Affairs Center of Excellence for Stress and Mental Health and the VISN 22 Mental Illness Research, Education, and Clinical Center. Dr. Geyer owns an equity interest in San Diego Instruments.

References

- Bale TL, Vale WW. CRF and CRF receptors: role in stress responsivity and other behaviors. *Annu Rev Pharmacol Toxicol* 2004;44:525–57. [PubMed: 14744257]
- Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, Tang Y, Gillespie CF, Heim CM, Nemeroff CB, Schwartz AC, Cubells JF, Ressler KJ. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA* 2008;299:1291–1305. [PubMed: 18349090]
- Bradley RG, Binder EB, Epstein MP, Tang Y, Nair HP, Liu W, Gillespie CF, Berg T, Evces M, Newport DJ, Stowe ZN, Heim CM, Nemeroff CB, Schwartz A, Cubells JF, Ressler KJ. Influence of child abuse on adult depression: Moderation by the corticotropin-releasing hormone receptor gene. *Arch Gen Psychiatry* 2008;65:190–200. [PubMed: 18250257]

- Coste SC, Heard AD, Phillips TJ, Stenzel-Poore MP. Corticotropin-releasing factor receptor type 2-deficient mice display impaired coping behaviors during stress. *Genes Brain Behav* 2006;5:131–138. [PubMed: 16507004]
- Coste SC, Kesterson RA, Heldwein KA, Stevens SL, Heard AD, Hollis JH, Murray SE, Hill JK, Pantely GA, Hohimer AR, Hatton DC, Phillips TJ, Finn DA, Low MJ, Rittenberg MB, Stenzel-Poore MP. Abnormal adaptations to stress and impaired cardiovascular function in mice lacking corticotropin-releasing hormone receptor-2. *Nat Genet* 2000;24:403–409. [PubMed: 10742107]
- De Luca V, Tharmalingam S, Kennedy JL. Association study between the corticotropin-releasing hormone receptor 2 gene and suicidality in bipolar disorder. *European Psychiatry* 2007;22:282–287. [PubMed: 17532191]
- De Luca V, Tharmalingam S, Zai C, Potapova N, Strauss J, Vincent J, Kennedy J. Association of hpa axis genes with suicidal behaviour in schizophrenia. *J Psychopharmacol*. 2008 epub before print. 10.1177/0269881108097817
- Fone KC, Porkess MV. Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. *Neurosci Biobehav Rev* 2008;32:1087–1102. [PubMed: 18423591]
- Geyer MA, Russo PV, Masten VL. Multivariate assessment of locomotor behavior: Pharmacological and behavioral analyses. *Pharmacol Biochem Behav* 1986;25:277–288. [PubMed: 2875472]
- Greetfeld M, Schmidt MV, Ganea K, Sterlemann V, Liebl C, Müller MB. A single episode of restraint stress regulates central corticotrophin-releasing hormone receptor expression and binding in specific areas of the mouse brain. *Journal of Neuroendocrinology* 2009;21:473–480.
- Gresack JE, Risbrough VB, Scott CN, Coste S, Stenzel-Poore M, Geyer MA, Powell SB. Isolation rearing-induced deficits in contextual fear learning do not require CRF₂ receptors. *Behavioural Brain Research* 2010;209:80–84. [PubMed: 20097235]
- Grippe AJ, Gerena D, Huang J, Kumar N, Shah M, Ughreja R, Carter SC. Social isolation induces behavioral and neuroendocrine disturbances relevant to depression in female and male prairie voles. *Psychoneuroendocrinology* 2007;32:966–980. [PubMed: 17825994]
- Hauger RL, Risbrough V, Brauns O, Dautzenberg FM. Corticotropin releasing factor (crf) receptor signaling in the central nervous system: New molecular targets. *CNS Neurol Disord Drug Targets* 2006;5:453–479. [PubMed: 16918397]
- Hauger RL, Risbrough VB, Oakley RH, Olivares-Reyes JA, Dautzenberg FM. Role of CRF receptor signaling in stress vulnerability, anxiety, and depression. *NY Acad Sci* 2010;1179:120–43.
- Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: Preclinical and clinical studies. *Biol Psychiatry* 2001;49:1023–1039. [PubMed: 11430844]
- Koike H, Ibi D, Mizoguchi H, Nagai T, Nitta A, Takuma K, Nabeshima T, Yoneda Y, Yamada K. Behavioral abnormality and pharmacologic response in social isolation-reared mice. *Behav Brain Res* 2009;202:114–121. [PubMed: 19447287]
- Liu Z, Zhu F, Wang G, Xiao Z, Wang H, Tang J, Wang X, Qiu D, Liu W, Cao Z, Li W. Association of corticotropin-releasing hormone receptor1 gene snp and haplotype with major depression. *Neuroscience Letters* 2006;404:358. [PubMed: 16815632]
- Lukkes J, Vuong S, Scholl J, Oliver H, Forster G. Corticotropin-releasing factor receptor antagonism within the dorsal raphe nucleus reduces social anxiety-like behavior after early-life social isolation. *J Neurosci* 2009a;29:9955–9960. [PubMed: 19675229]
- Lukkes JL, Summers CH, Scholl JL, Renner KJ, Forster GL. Early life social isolation alters corticotropin-releasing factor responses in adult rats. *Neuroscience* 2009b;158:845–855. [PubMed: 19010398]
- Mceuen JG, Beck SG, Bale TL. Failure to mount adaptive responses to stress results in dysregulation and cell death in the midbrain raphe. *J Neurosci* 2008;28:8169–8177. [PubMed: 18701679]
- Meyer U, Feldon J. Epidemiology-driven neurodevelopmental animal models of schizophrenia. *Prog Neurobiol*. 2009 epub ahead of print. 10.1016/j.pneurobio.2009.10.018
- Mueller BR, Bale TL. Sex-specific programming of offspring emotionality after stress early in pregnancy. *J Neurosci* 2008;28:9055–9065. [PubMed: 18768700]
- Nemeroff CB. Neurobiological consequences of childhood trauma. *J Clin Psychiatry* 2004;65(Suppl 1): 18–28. [PubMed: 14728093]

- Neufeld-Cohen A, Evans AK, Getselter D, Spyroglou A, Hill A, Gil S, Tsoory M, Beuschlein F, Lowry CA, Vale W, Chen A. Urocortin-1 and -2 double-deficient mice show robust anxiolytic phenotype and modified serotonergic activity in anxiety circuits. *Mol Psychiatry*. 2009 epub ahead of print. 10.1038/mp.2009.115
- Paulus MP, Bakshi VP, Geyer MA. Isolation rearing affects sequential organization of motor behavior in post-pubertal but not pre-pubertal lister and sprague-dawley rats. *Behav Brain Res* 1998;94:271–280. [PubMed: 9722278]
- Pietropaolo S, Singer P, Feldon J, Yee B. The postweaning social isolation in c57bl/6 mice: Preferential vulnerability in the male sex. *Psychopharmacology* 2008;197:613–628. [PubMed: 18317735]
- Polanczyk G, Caspi A, Williams B, Price TS, Danese A, Sugden K, Uher R, Poulton R, Moffitt TE. Protective effect of *crhr1* gene variants on the development of adult depression following childhood maltreatment: Replication and extension. *Arch Gen Psychiatry* 2009;66:978–985. [PubMed: 19736354]
- Pournajafi-Nazarloo H, Partoo L, Sanzenbacher L, Paredes J, Hashimoto K, Azizi F, Carter SC. Stress differentially modulates mrna expression for corticotrophin-releasing hormone receptors in hypothalamus, hippocampus and pituitary of prairie voles. *Neuropeptides* 2009;43:113–123. [PubMed: 19185916]
- Powell SB, Geyer MA. Developmental markers of psychiatric disorders as identified by sensorimotor gating. *Neurotox Res* 2002;4:489–502. [PubMed: 12754162]
- Powell SB, Swerdlow NR, Pitcher LK, Geyer MA. Isolation rearing-induced deficits in prepulse inhibition and locomotor habituation are not potentiated by water deprivation. *Physiol Behav* 2002;77:55–64. [PubMed: 12213502]
- Risbrough VB, Masten VL, Caldwell S, Paulus MP, Low MJ, Geyer MA. Differential contributions of dopamine d1, d2, and d3 receptors to mdma-induced effects on locomotor behavior patterns in mice. *Neuropsychopharmacology* 2006;31:2349–2358. [PubMed: 16855533]
- Risbrough VB, Stein MB. Role of corticotropin releasing factor in anxiety disorders: A translational research perspective. *Horm Behav* 2006;50:550–561. [PubMed: 16870185]
- Serra M, Pisu MG, Floris I, Biggio G. Social isolation-induced changes in the hypothalamic-pituitary-adrenal axis in the rat. *Stress: The International Journal on the Biology of Stress* 2005;8:259–264.
- Tessner KD, Mittal V, Walker EF. Longitudinal study of stressful life events and daily stressors among adolescents at high risk for psychotic disorders. *Schizophr Bull*. 2009
- Tharmalingam S, King N, De Luca V, Rothe C, Koszycki D, Bradwejn J, Macciardi F, Kennedy JL. Lack of association between the corticotrophin-releasing hormone receptor 2 gene and panic disorder. *Psychiatr Genet* 2006;16:93–97. [PubMed: 16691126]
- Tyrka AR, Price LH, Gelernter J, Schepker C, Anderson GM, Carpenter LL. Interaction of childhood maltreatment with the corticotropin-releasing hormone receptor gene: Effects on hypothalamic-pituitary-adrenal axis reactivity. *Biol Psych* 2009;66:681–685.
- Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 1981;213:1394–1397. [PubMed: 6267699]
- Varty GB, Powell SB, Lehmann-Masten V, Buell MR, Geyer MA. Isolation rearing of mice induces deficits in prepulse inhibition of the startle response. *Behav Brain Res* 2006;169:162–167. [PubMed: 16406103]
- Voikar V, Polus A, Vasar E, Rauvala H. Long-term individual housing in C57BL/6J and DBA/2 mice: Assessment of behavioral consequences. *Genes Brain Behav* 2005;4:240–252. [PubMed: 15924556]
- Walker E, Mittal V, Tessner K. Stress and the hypothalamic pituitary adrenal axis in the developmental course of schizophrenia. *Annu Rev Clin Psychol* 2008;4:189–216. [PubMed: 18370616]
- Waselus M, Nazzaro C, Valentino RJ, Van Bockstaele EJ. Stress-induced redistribution of corticotropin-releasing factor receptor subtypes in the dorsal raphe nucleus. *Biol Psych* 2009;66:76–83.
- Wasserman D, Sokolowski M, Rozanov V, Wasserman J. The *CRHR1* gene: A marker for suicidality in depressed males exposed to low stress. *Genes, Brain and Behavior* 2008;7:14–19.

Figure 1.

CRF₂ KO mice exhibit greater sensitivity to isolation rearing effects on exploratory activity. Data depicted are mean $\pm$ SEM number of transitions (top panels), holepokes (center panels), and rearings (bottom panels) across CRF₂ receptor wild-type (WT, left panels) and knockout (KO, right panels) mice. * p <0.05 Post-hoc simple comparison of housing effects in KO mice for transitions and hole poke measures. + p <0.05 Main effect of housing across both genotypes on rearing behavior, see results for details.

Figure 2.

Locomotor exploration paths. Plots depict the x–y position (in black) and holepoke frequency (each red dot represents a holepoke event) during the last 30 min block of the 60 min session. Representative mice were chosen based on having the most similar transitions and holepoke frequency as their respective group average. Columns depict housing status (social or isolation) and rows depict genotype (CRF₂ WT or KO).

Table 1

Effect of isolation rearing on locomotor patterns.

Scaling Measure	Social-Reared	Isolation-Reared
	<i>Wild-type</i>	
Spatial <i>d</i>	1.55±0.02	1.46±0.04
Spatial CV	1.29±0.07	1.28±0.07
Temporal CV	1.88±0.214	1.97±0.27
%Locomotor Habituation	69.4±7.5	63.9±9.5
	<i>CRF₂ knockout</i>	
Spatial <i>d</i>	1.48±0.02	1.44±0.02
Spatial CV	1.26±0.05	1.23±0.04
Temporal CV	2.30±0.24	1.42±0.07*
%Locomotor Habituation	77.9±6.5	48.3±6.6*

* p<0.05 vs. Social-Reared CRF₂ KO, Tukey's post hoc test.

Note there was a main effect of isolation regardless of genotype on spatial *d*, see results for details.