Published in final edited form as:

Biol Psychiatry. 2010 January 15; 67(2): 168–174. doi:10.1016/j.biopsych.2009.08.017.

Effects of Estradiol on Learned Helplessness and Associated Remodeling of Hippocampal Spine Synapses in Female Rats

Tibor Hajszan^{1,2}, Klara Szigeti-Buck^{1,3}, Nermin L Sallam¹, Jeremy Bober¹, Arpad Parducz², Neil J MacLusky⁴, Csaba Leranth^{1,5}, and Ronald S Duman^{3,6}

¹Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine; New Haven, CT 06520, USA

²Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, H-6726 Szeged, Hungary

³Department of Pharmacology, Yale University School of Medicine; New Haven, CT 06520, USA

⁴Department of Biomedical Sciences, Ontario Veterinary College, Guelph, Ontario, Canada N1G 2W1

⁵Department of Neurobiology; Yale University School of Medicine; New Haven, CT 06520, USA

⁶Department of Psychiatry; Yale University School of Medicine; New Haven, CT 06520, USA

Abstract

Background—Despite the fact that women are twice as likely to develop depression as men, our understanding of depression neurobiology in females is limited. We have recently reported in male rats that development of helpless behavior is associated with a severe loss of hippocampal spine synapses, which is reversed by treatment with the antidepressant, desipramine. Considering the fact that estradiol has a hippocampal synaptogenic effect similar to those of antidepressants, the presence of estradiol during the female reproductive life may influence behavioral and synaptic responses to stress and depression.

Methods—Using electron microscopic stereology, we analyzed hippocampal spine synapses in association with helpless behavior in ovariectomized female rats (n=70), under different conditions of estradiol exposure.

Results—Stress induced an acute and persistent loss of hippocampal spine synapses, while subchronic treatment with desipramine reversed the stress-induced synaptic loss. Estradiol supplementation given either prior to stress or prior to escape testing of nonstressed animals both increased the number of hippocampal spine synapses. Correlation analysis demonstrated a statistically significant negative correlation between the severity of helpless behavior and hippocampal spine synapse numbers.

Conclusions—These findings suggest that hippocampal spine synapse remodeling may be a critical factor underlying learned helplessness and, possibly, the neurobiology of depression.

Corresponding Author: Tibor Hajszan, MD, PhD, Yale University Dept of OBGYN, 333 Cedar St LSO212, New Haven CT 06520, Phone 203-737-4766, Fax 203-785-7684, tibor.hajszan@yale.edu.

Disclosures: The authors report no biomedical financial interests or potential conflicts of interest.

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 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.

Keywords

depression; stress; synaptic plasticity; desipramine; estrogen; stereology

Introduction

Major depressive disorder is a devastating illness (1) with an estimated lifetime prevalence of 17% in the United States (2). Despite intensive research on depression neurobiology and antidepressant mechanisms, current clinical management of the disease remains limited (3). Evidence for hippocampal atrophy in depressed patients (4-6), as well as derailment of many hippocampus-related functions in depression (7-9) indicates that the hippocampus is critically involved in the disease (10). Based on considerable evidence (11-17), it has been postulated for many years that stress and depression are associated with the loss of hippocampal dendritic spines and spine synapses (18-20), contributing to hippocampal dysfunction, although there are many reports that do not support this hypothesis (21-23). We have recently demonstrated that the number of spine synapses, determined by electron microscopy, in certain hippocampal areas is a valuable neuroanatomical marker for helpless behavior (24). We have found in male rats that helpless behavior is associated with a severe loss of these synapses across the hippocampal circuitry, which is reversed in animals that respond to antidepressant treatment with decreased helplessness (24). This acute stress-induced spine synapse remodeling does not occur in cortical areas that are not related to stress/depression, such as the motor cortex (24).

It has been reported that contrary to males, females are resistant to the shrinkage of CA3 apical dendrites in response to chronic stress or corticosterone administration (16;25;26), suggesting that spine synapse remodeling induced by stress/helplessness may be different in the hippocampus of females vs. males. In addition, the principal ovarian estrogen, estradiol, has a hippocampal synaptogenic effect similar to those of antidepressants (17;27). As a result, the presence of estradiol during the female reproductive life may, to a large extent, influence behavioral and synaptic responses to stress and depression. Indeed, clinical studies have shown that women are twice as likely to develop depression as men (28); and sex differences have also been observed in various animal models of depression (29;30). Unfortunately, there is much less known about synaptic remodeling in female stress/depression models; and studies at the ultrastructural level, in particular, are currently not available in the female. Therefore, to investigate potential interactions between the synaptic effects of estradiol and helpless behavior, we used electron microscopic stereology to analyze spine synapses in the female rat hippocampus in association with behavioral changes in the learned helplessness (LH) model of depression and antidepressant response, under different conditions of estradiol exposure.

Materials and Methods

Adult female Sprague-Dawley rats (n=70, 200-250 g; Charles River Laboratories, Wilmington, MA) were kept under standard laboratory conditions. The animals were group housed, maintained on a 12/12-h light/dark cycle, with tap water and rodent chow available *ad libitum*. The animal protocol was approved by the Institutional Animal Care and Use Committee of Yale University School of Medicine. All rats were ovariectomized a week before stress exposure. Ovariectomy prevents the cyclic change of serum estradiol levels and the resultant fluctuation in the number of hippocampal spine synapses (27). Moreover, the ovariectomized rat is an excellent subject for our studies, because stress and depression are associated with sexual dysfunction (31), ovarian dysfunction (32), and decreased fertility (33).

Learned helplessness

A standard LH paradigm was used as previously described (24). Briefly, testing was conducted in commercial shuttle boxes (Med Associates, St. Albans, VT). Inescapable footshock (IES) was administered in one side of shuttle boxes (60 scrambled footshocks, 0.85 mA intensity, 15 s average duration, 60 s average inter-shock interval). This schedule of IES presentation has been shown to be effective in inducing helpless behavior in rats (24;34). Nonstressed (NS) control animals were exposed to the chambers but did not receive footshock. Helpless behavior was evaluated by analyzing performance in an active escape paradigm, consisting of 30 trials of escapable footshock (0.65 mA intensity, 35 s maximum duration, 90 s average inter-trial interval). An initial five fixed ratio 1 trials, during which one shuttle crossing terminated the shock, was followed by 25 trials, when two shuttle crossings were required to terminate shock (fixed ratio 2 trials). Shock was terminated automatically if the response requirement was not met within 35 s. Escape latencies were recorded automatically for each trial by a computer. Escape latency represented the time it took for animals to escape shock; while in the case of escape failures, escape latency was set at 35 s. IES administration and active escape testing was conducted in a dimly lit room between 1000-1600 h.

Electron microscopic stereology

The number of spine synapses in CA1 stratum radiatum (CA1sr), CA3 stratum lucidum and radiatum (CA3sl/sr), and dentate gyrus stratum moleculare (DGsm) was calculated as previously described (24). Spine synapses in these areas represent important “relay stations” along the hippocampal trisynaptic loop and, therefore, they critically influence hippocampal signal flow and cellular activity. For more detailed rationale of selecting these areas for analysis, please see our previous paper (24). Briefly, animals were perfusion fixed through the ascending aorta with a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde dissolved in phosphate buffer. Brains were dissected out and postfixed overnight in the same fixative without glutaraldehyde. Throughout the hippocampus, 100- μ m thick serial sections were cut in the coronal plane using a vibratome and systematically sorted into ten groups. One randomly selected group of sections was embedded in Durcupan (Electron Microscopy Sciences, Fort Washington, PA) between slides and coverslips. Using these embedded sections, the volume of sampling areas (CA1sr, CA3sl/sr, and DGsm) was estimated utilizing the Cavalieri Estimator module of the Stereo Investigator® system (MicroBrightField, Williston, VT) mounted on a Zeiss Axioplan 2 light microscope. Thereafter, 20 sampling sites for electron microscopic analysis were localized in each sampling area using a systematic-random approach, as previously described (24). At each sampling site, digitized electron micrographs (Figure 1) were taken for the physical disector in a Tecnai-12 transmission electron microscope (FEI Company, Hillsboro, OR) furnished with a Hamamatsu digital camera system (Hamamatsu Photonics, Hamamatsu, Japan), at a final magnification of 11,000 $\times$. This sampling technique provided 20 disectors for each of CA1sr, CA3sl/sr, and DGsm, i.e., 60 disectors per brain altogether. Prior to spine synapse counting, the pictures were coded for blind analysis. Asymmetric spine synapses were counted according to the rules of the disector technique (35). Synapsing spines were identified by the presence of postsynaptic densities, as well as by the absence of mitochondria, microtubules, and synaptic vesicles. The average volumetric density of spine synapses (synapse/ μ m³) within each sampling area was then determined. Finally, the volumetric density of spine synapses was multiplied by the volume of the sampling area, determined earlier, to arrive at the total number of spine synapses. For more details of this technique, please see our previous paper (24).

Treatment group sizes for synapse counting

Because electron microscopic stereology is a time-consuming and labor-intensive method, we performed careful statistical power calculations to minimize the number of animals to be

analyzed. In our earlier studies (17;24), standard deviation for spine synapse counts has consistently been in the range of 5-10% of the mean. Thus, assuming 10% standard deviation and $n=4$ animals per group in the experimental design described below, ANOVA can detect about 25% change in the number of spine synapses with the desired 80% power at $\alpha=0.05$. Because our initial observations (17;24) suggest that modifications in the number of spine synapses will potentially be larger than 25%, we set initial treatment group sizes at $n=4$.

Experimental design and statistics

Six experimental groups were designed to examine the effects of acute stress on hippocampal spine synapses, and whether it is affected by treatments with the antidepressant, desipramine (DMI), or the estrogen, estradiol benzoate (EB) (Table 1). Animals ovariectomized for a week were exposed to the shuttle boxes either with (4 groups) or without (2 groups) receiving footshock stress. A week after stress exposure, four females were randomly selected from each group for electron microscopic stereology and sacrificed when the remaining rats underwent active escape testing. As a result, our synapse counts represent the number of spine synapses at the beginning of active escape testing. Animals in the IES/DMI group received antidepressant treatment for six days, from stress exposure until escape testing (10 mg/kg DMI dissolved in distilled water; given i.p. twice daily, in a volume of 1 ml/kg). We have used the same DMI treatment schedule in our previous male study for successfully reversing both helpless behavior and hippocampal spine synapse loss (24). Estradiol supplementation was given either prior to footshock stress (EB/IES group) or prior to escape testing of nonstressed females (NS/EB group) in the form of two s.c. injections 24 h apart (each injection contained 40 $\mu\text{g/kg}$ EB dissolved in sesame oil, given in a volume of 1 ml/kg). Testing of escape performance was performed 48 h (NS/EB group) or 9 days (EB/IES group) following the second hormone dose. This estradiol supplementation schedule for ovariectomized rats has been utilized in several past studies to simulate normal proestrus levels of estradiol, and has also been demonstrated to reproduce the number of CA1 spine synapses observed during proestrus (27).

Data from individual animals were used to determine group means ($\pm$ S.E.M. for escape latency and $\pm$ S.D. for spine synapses). Escape latency measurements were examined by two-way repeated measures ANOVA (treatment as between factor $\times$ time as within factor); while spine synapse counts were analyzed by two-way ANOVA (treatment $\times$ sampling area). We used the conservative Tukey post-hoc test for individual comparison of spine synapse counts. However, because behavior naturally shows greater variability, the less conservative Holm-Sidak post-hoc test was applied in the case of escape latency. Correlation between escape latencies and number of spine synapses was analyzed with the Pearson product moment test. A criterion for statistical confidence of $p<0.05$ was adopted.

Results

Two-way repeated measures ANOVA found significant treatment ($F_{5,40}=12.950$, $p<0.001$), time ($F_{5,200}=115.858$, $p<0.001$), and treatment $\times$ time interaction effects ($F_{25,200}=2.494$, $p<0.001$) on escape latencies. When compared with nonstressed, oil-treated controls, acute stress exposure significantly increased mean escape latency in oil-treated animals at both 24 h (IES1d vs. NS/OIL group) and a week (OIL/IES vs. NS/OIL group) after stress. The escape performance of IES1d and OIL/IES females was similar, indicating that escape deficits develop acutely, within 24 h of stress exposure, and that rats do not show any signs of behavioral recovery within the first week. Estradiol supplementation of nonstressed animals significantly decreased mean escape latency (NS/EB vs. NS/OIL group). Administering estradiol prior to stress exposure also significantly decreased mean escape latency (EB/IES vs. OIL/IES group), as the escape performance of stressed, estradiol-treated females (EB/IES group) was identical

with that of nonstressed, oil-treated controls (NS/OIL group). A six-day treatment of stressed, oil-treated rats with the tricyclic antidepressant, desipramine, significantly decreased mean escape latency (IES/DMI vs. OIL/IES group), bringing escape latency levels of DMI-treated animals (IES/DMI group) to that of nonstressed, estradiol-supplemented females (NS/EB group). Escape latency measurements and their statistical analysis are summarized in Figure 2a.

Two-way ANOVA revealed significant treatment ($F_{5,54}=262.940$, $p<0.001$), sampling area ($F_{2,54}=142.455$, $p<0.001$), and treatment $\times$ sampling area interaction effects ($F_{10,54}=9.923$, $p<0.001$) on the number of hippocampal spine synapses. In general, significant decreases in escape latency were associated with significant increases in the number of spine synapses in all hippocampal areas analyzed. The only exception was the DGsm area, where spine synapse numbers a week after of stress exposure (in the OIL/IES group) were at the level of nonstressed, oil-treated controls (NS/OIL group), in contrast to the significantly better escape performance of NS/OIL females. Because acute stress exposure uniformly decreased the number of spine synapses across hippocampal areas 24 h following stress (in the IES1d group), spine synapse growth in DGsm a week later (in the OIL/IES group) is likely the initial sign of spontaneous synaptic recovery. Spine synapse counts and their statistical analysis are summarized in Figure 2b.

Mean escape latencies and hippocampal spine synapse numbers across all three areas analyzed showed a significant negative linear correlation ($r = -0.973$, $p=0.001$, Figure 3a), suggesting that escape latency decreases as the number of hippocampal spine synapses increases. The strongest correlations were in CA1sr ($r = -0.985$, $p<0.001$, Figure 3b) and CA3sl/sr ($r = -0.992$, $p<0.001$, Figure 3c), becoming weaker but still statistically significant in DGsm ($r = -0.899$, $p=0.015$, Figure 3d). The weaker correlation in DGsm results primarily from the spontaneous recovery of spine synapses following stress exposure, which did not occur in the other two areas. The recovery of DGsm spine synapses does not seem to be reflected in escape performance, perhaps because it represents a relatively small contribution to the combined number of spine synapses from all analyzed hippocampal areas.

Discussion

These observations provide strong ultrastructural evidence for remodeling of hippocampal spine synapses in association with behavioral changes in the female LH model of depression. These synaptic changes are similar to what we have observed in the male LH model (24). Footshock stress induces an acute and persistent loss of hippocampal spine synapses, except for the DGsm area in untreated females, where spine synapses spontaneously recover to pre-stress levels within a week of stress exposure. By contrast, we have not observed this spontaneous spine synapse recovery in the male LH model (24). In line with our previous demonstration of synaptogenic effects of fluoxetine in the nonstressed female hippocampus (17), subchronic treatment with the tricyclic antidepressant, desipramine, reverses the stress-induced synaptic loss.

The role of estradiol

Estradiol supplementation in nonstressed animals and estradiol treatment prior to acute stress exposure both increase hippocampal spine synapse numbers and improves escape performance (see Figure 2). However, judging the true influence of estradiol given prior to stress is limited due to the 9-day delay between estradiol treatment and escape testing. It is conceivable that hippocampal spine synapse numbers in estradiol-treated, stressed females (EB/IES group) are higher initially, but then they decline during the 9-day delay to the level of nonstressed, oil-treated controls (NS/OIL group) due to lack of estradiol (27). Despite the limitation, this design led to an important situation, because these two treatment groups (EB/IES and NS/OIL),

differing in both stress exposure and estradiol supplementation, displayed identical spine synapse numbers and identical escape performance, suggesting a potentially critical role of hippocampal spine synapse remodeling in helpless behavior. Another factor that may confound the interpretation of our findings is the locomotor-activating effect of estradiol in female rats (36), which probably contributes to the behavioral observations in the nonstressed NS/EB group. On the other hand, the antidepressant-like action of estradiol given prior to footshock stress (EB/IES group) cannot be explained with locomotor activation. First, stress appears to interfere with estradiol's locomotor activating effect (37), mostly negating it, which is further supported by our own recent observation that estradiol given after footshock stress does not improve escape performance (Hajszan T, MacLusky NJ, Leranath C, Duman RS, unpublished observation). Most importantly, the escape performance of EB/IES animals was tested nine days after the last estradiol injection, a period long enough to completely clear injected estradiol from the circulation (38). As a result, at the time of escape testing, EB/IES females were not under the influence of estradiol and hence, the locomotor activating effect could not be present.

Our finding of an antidepressant-like effect from estradiol is in line with the well-documented relevance of decreasing estradiol levels to elevated risks for developing depressive symptoms in both women (39;40) and laboratory animals (41). It has been reported that gene polymorphisms affecting estradiol synthesis, metabolism, and signaling increase the occurrence of depression in young/midlife women (42;43). In the postmenopausal period, serum concentrations of estradiol are inversely associated with depressed mood (44;45). From the therapeutic perspective, women with mood disorders seem to benefit from estradiol administration both in young/midlife (46), and in the peri/postmenopausal period (47;48). It also has to be mentioned, however, that there are an equally large number of clinical studies reporting that hormone therapy does not affect depression severity (49-51). Furthermore, the increased prevalence of depression in women exists only during the reproductive years, beginning with puberty and lasting until menopause (39;52). Some recent work with the mouse forced-swim test model (53;54) also suggests that the presence of ovarian hormones amplifies the risk of mood disorders, seemingly contradicting our findings. (However, the forced-swim test is primarily a screening test for antidepressant efficacy (55) rather than a depression model per se.) In fact, there are two characteristic peaks of depression prevalence in women; the first is postpartum depression, the second is perimenopausal depression (39;52). Both of these peaks are associated with large drops (postpartum) or excessive fluctuations (perimenopausal) of serum estradiol levels (52), indicating that it is the drop in serum levels rather than the presence of estradiol that increases the risk of depression. It has to be noted, however, that the ovariectomized animals as used in our study are not appropriate to model perimenopausal hormone fluctuations. Nevertheless, the controversy about the role of ovarian hormones in stress/depression remains to be further investigated.

The involvement of estradiol in affective behavior is not surprising considering its impact on many mechanisms thought to be important in depression neurobiology. In addition to its hippocampal synaptogenic effects, estradiol influences neurogenesis (56), expression of brain-derived neurotrophic factor (57;58), and monoaminergic neurotransmission (59;60). Apart from hippocampal spine synapse remodeling, however, these estrogen-related mechanisms show questionable correlations to helpless behavior, and even their relevance to depressive symptoms is intensively debated (61-63).

Relevance of hippocampal synaptic remodeling to stress and depression

The negative correlation between escape latency and hippocampal spine synapse numbers provides strong evidence supporting the hypothesis that depressive behavior is linked to spine synapse remodeling (18-20;24). It is most impressive and significant that the correlation exists irrespective of several conditions, such as footshock stress exposure, estradiol supplementation

and antidepressant treatment, as both stressed and nonstressed, estradiol-supplemented and -nonsupplemented, as well as antidepressant-treated and -nontreated animals can show identical escape performance, (e.g., EB/IES vs. NS/OIL, and NS/EB vs. IES/DMI groups), given that their hippocampal spine synapse numbers are also identical.

Our observations are in line with the earlier study of Shors et al. (22) reporting dendritic spine loss in the female hippocampus in response to acute stress. On the other hand, our results are in conflict with most other studies (16;25;26) indicating that females are resistant to the shrinkage of CA3 apical dendrites in response to stress or corticosterone administration. We have already noted in our previous paper (24) that direct, electron microscopic analysis of spine synapses may not reproduce the effects observed via studying dendritic structure or synaptic markers [e.g., (24) c.f. (12;13)]. Although differences in experimental design may partially explain the varying observations, this is not applicable in the case of our NS/OIL (ovariectomized, oil-treated, nonstressed animals) and NS/EB groups (ovariectomized, estradiol-supplemented, nonstressed females). These two groups received treatments that are well established and have been used in many earlier light microscopic studies that examined the effects of estradiol on hippocampal morphology. Nevertheless, there are striking differences between these earlier light microscopic reports (64;65) and our present ultrastructural findings. This is an exciting phenomenon suggesting differentiated roles for dendritic, molecular, and synaptic plasticity in mood regulation. Our findings also suggest that remodeling of dendritic spines, synaptic vesicle proteins, and spine synapses is regulated independently from each other. Indeed, earlier evidence indicates that formation of new spine synapses does not necessitate the emergence of new spines and axonal boutons; and spine synapse loss does not necessarily imply the loss of these structures (66;67).

How could remodeling of hippocampal spine synapses contribute to changes in helpless behavior? The hippocampus is strongly involved in learning and memory (68), regulating the stress response (8;69;70), and modulating the mesolimbic dopaminergic system and motivation circuitries (71-73), which all seem to be important contributors to the mechanisms of LH (24;74;75). The very synapses that we analyzed in this study represent the vast majority of connections between hippocampal principal cells and as such, they serve as an important morphological correlate of hippocampal activity. Loss of these synapses compromises hippocampal signal flow and activity by decreasing the amount of excitatory input that principal cells receive. Deteriorating hippocampal activity in turn may critically contribute to the joint development of cognitive problems, dysregulation of the stress response, and loss of motivation, common traits in helpless animals and in human depression (18;74).

In summary, our findings demonstrate that the hippocampal synaptic loss associated with helpless behavior can fully be reversed in female rats by treatment with the antidepressant, desipramine. Although the detailed role of synaptic plasticity in mood regulation remains to be further investigated, the observed strong association of reliable synapse counts with escape performance supports our hypothesis that hippocampal spine synapse remodeling critically contributes to the modulation of helpless behavior. Based on its excellent face, predictive, and construct validity, learned helplessness is considered one of the best animal models of depression and antidepressant response (76;77). Our findings in this model thus indicate that hippocampal spine synapse remodeling may represent a neuroanatomical marker that predicts the risk of depression and the effectiveness of antidepressant treatment.

Acknowledgments

This work was supported by NIH grants MH074021 (Hajszan), MH025642 (Duman), MH045481 (Duman), and ES014893 (Leranth); a NARSAD Young Investigator Award (Hajszan); a Hungarian Ministry of Health grant ETT476/2006 (Parducz); a Hungarian National Office for Research and Technology grant RET08/2004 (Parducz); a

Hungarian Scientific Research Fund grant OTKA75954 (Parducz); and by the Connecticut Mental Health Center and the Veterans Administration Center for Posttraumatic Stress Disorder.

References

1. Belmaker RH, Agam G. Major depressive disorder. *N Engl J Med* 2008;358:55–68. [PubMed: 18172175]
2. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, et al. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry* 1994;51:8–19. [PubMed: 8279933]
3. Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, et al. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am J Psychiatry* 2006;163:28–40. [PubMed: 16390886]
4. Sheline YI, Sanghavi M, Mintun MA, Gado MH. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *J Neurosci* 1999;19:5034–5043. [PubMed: 10366636]
5. Frodl T, Meisenzahl EM, Zetzsche T, Born C, Groll C, Jager M, et al. Hippocampal changes in patients with a first episode of major depression. *Am J Psychiatry* 2002;159:1112–1118. [PubMed: 12091188]
6. MacMaster FP, Mirza Y, Szeszko PR, Kmiecik LE, Easter PC, Taormina SP, et al. Amygdala and hippocampal volumes in familial early onset major depressive disorder. *Biol Psychiatry* 2008;63:385–390. [PubMed: 17640621]
7. Gallassi R, Di Sarro R, Morreale A, Amore M. Memory impairment in patients with late-onset major depression: the effect of antidepressant therapy. *J Affect Disord* 2006;91:243–250. [PubMed: 16499971]
8. McEwen BS. Mood disorders and allostatic load. *Biol Psychiatry* 2003;54:200–207. [PubMed: 12893096]
9. O'Brien JT, Lloyd A, McKeith I, Gholkar A, Ferrier N. A longitudinal study of hippocampal volume, cortisol levels, and cognition in older depressed subjects. *Am J Psychiatry* 2004;161:2081–2090. [PubMed: 15514410]
10. Campbell S, Macqueen G. The role of the hippocampus in the pathophysiology of major depression. *J Psychiatry Neurosci* 2004;29:417–426. [PubMed: 15644983]
11. Law AJ, Weickert CS, Hyde TM, Kleinman JE, Harrison PJ. Reduced spinophilin but not microtubule-associated protein 2 expression in the hippocampal formation in schizophrenia and mood disorders: molecular evidence for a pathology of dendritic spines. *Am J Psychiatry* 2004;161:1848–1855. [PubMed: 15465982]
12. Iwata M, Shirayama Y, Ishida H, Kawahara R. Hippocampal synapsin I, growth-associated protein-43, and microtubule-associated protein-2 immunoreactivity in learned helplessness rats and antidepressant-treated rats. *Neuroscience* 2006;141:1301–1313. [PubMed: 16814933]
13. Reines A, Cereseto M, Ferrero A, Sifonios L, Podesta MF, Wikinski S. Maintenance treatment with fluoxetine is necessary to sustain normal levels of synaptic markers in an experimental model of depression: Correlation with behavioral response. *Neuropsychopharmacology*. 2007 In Press.
14. Sousa N, Lukoyanov NV, Madeira MD, Almeida OF, Paula-Barbosa MM. Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience* 2000;97:253–266. [PubMed: 10799757]
15. Tata DA, Marciano VA, Anderson BJ. Synapse loss from chronically elevated glucocorticoids: relationship to neuropil volume and cell number in hippocampal area CA3. *J Comp Neurol* 2006;498:363–374. [PubMed: 16871536]
16. Watanabe Y, Gould E, Cameron HA, Daniels DC, McEwen BS. Phenytoin prevents stress- and corticosterone-induced atrophy of CA3 pyramidal neurons. *Hippocampus* 1992;2:431–435. [PubMed: 1308199]
17. Hajszan T, MacLusky NJ, Leranath C. Short-term treatment with the antidepressant fluoxetine triggers pyramidal dendritic spine synapse formation in rat hippocampus. *Eur J Neurosci* 2005;21:1299–1303. [PubMed: 15813939]

18. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron* 2002;34:13–25. [PubMed: 11931738]
19. Manji HK, Quiroz JA, Sporn J, Payne JL, Denicoff K, Gray NA, et al. Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. *Biol Psychiatry* 2003;53:707–742. [PubMed: 12706957]
20. Hajszan T, MacLusky NJ. Neurologic links between epilepsy and depression in women: is hippocampal neuroplasticity the key? *Neurology* 2006;66:S13–22. [PubMed: 16567737]
21. Diamond DM, Campbell AM, Park CR, Woodson JC, Conrad CD, Bachstetter AD, Mervis RF. Influence of predator stress on the consolidation versus retrieval of long-term spatial memory and hippocampal spinogenesis. *Hippocampus* 2006;16:571–576. [PubMed: 16741974]
22. Shors TJ, Chua C, Falduto J. Sex differences and opposite effects of stress on dendritic spine density in the male versus female hippocampus. *J Neurosci* 2001;21:6292–6297. [PubMed: 11487652]
23. Donohue HS, Gabbott PL, Davies HA, Rodriguez JJ, Cordero MI, Sandi C, et al. Chronic restraint stress induces changes in synapse morphology in stratum lacunosum-moleculare CA1 rat hippocampus: a stereological and three-dimensional ultrastructural study. *Neuroscience* 2006;140:597–606. [PubMed: 16600515]
24. Hajszan T, Dow A, Warner-Schmidt JL, Szigeti-Buck K, Sallam NL, Parducz A, et al. Remodeling of hippocampal spine synapses in the rat learned helplessness model of depression. *Biol Psychiatry* 2009;65:392–400. [PubMed: 19006787]
25. Galea LA, McEwen BS, Tanapat P, Deak T, Spencer RL, Dhabhar FS. Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. *Neuroscience* 1997;81:689–697. [PubMed: 9316021]
26. Liu HH, Payne HR, Wang B, Brady ST. Gender differences in response of hippocampus to chronic glucocorticoid stress: role of glutamate receptors. *J Neurosci Res* 2006;83:775–786. [PubMed: 16498614]
27. Woolley CS, McEwen BS. Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. *J Neurosci* 1992;12:2549–2554. [PubMed: 1613547]
28. Kessler RC, McGonagle KA, Swartz M, Blazer DG, Nelson CB. Sex and depression in the National Comorbidity Survey. I: Lifetime prevalence, chronicity and recurrence. *J Affect Disord* 1993;29:85–96. [PubMed: 8300981]
29. Leuner B, Mendolia-Loffredo S, Shors TJ. Males and females respond differently to controllability and antidepressant treatment. *Biol Psychiatry* 2004;56:964–970. [PubMed: 15601607]
30. Dalla C, Edgecomb C, Whetstone AS, Shors TJ. Females do not Express Learned Helplessness like Males do. *Neuropsychopharmacology* 2008;33:1559–1569. [PubMed: 17712351]
31. Williams K, Reynolds MF. Sexual dysfunction in major depression. *CNS Spectr* 2006;11:19–23. [PubMed: 16871134]
32. Bethea CL, Centeno ML, Cameron JL. Neurobiology of stress-induced reproductive dysfunction in female macaques. *Mol Neurobiol* 2008;38:199–230. [PubMed: 18931961]
33. Williams KE, Marsh WK, Rasgon NL. Mood disorders and fertility in women: a critical review of the literature and implications for future research. *Hum Reprod Update* 2007;13:607–616. [PubMed: 17895237]
34. Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci* 2002;22:3251–3261. [PubMed: 11943826]
35. Sterio DC. The unbiased estimation of number and sizes of arbitrary particles using the disector. *J Microsc* 1984;134:127–136. [PubMed: 6737468]
36. Colvin GB, Sawyer CH. Induction of running activity by intracerebral implants of estrogen in ovariectomized rats. *Neuroendocrinology* 1969;4:309–320. [PubMed: 4894216]
37. Walf AA, Frye CA. Antianxiety and antidepressive behavior produced by physiological estradiol regimen may be modulated by hypothalamic-pituitary-adrenal axis activity. *Neuropsychopharmacology* 2005;30:1288–1301. [PubMed: 15756306]
38. Woolley CS, McEwen BS. Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *J Comp Neurol* 1993;336:293–306. [PubMed: 8245220]

39. Deecher D, Andree TH, Sloan D, Schechter LE. From menarche to menopause: exploring the underlying biology of depression in women experiencing hormonal changes. *Psychoneuroendocrinology* 2008;33:3–17. [PubMed: 18063486]
40. Soares CN, Zitek B. Reproductive hormone sensitivity and risk for depression across the female life cycle: a continuum of vulnerability? *J Psychiatry Neurosci* 2008;33:331–343. [PubMed: 18592034]
41. Walf AA, Frye CA. A review and update of mechanisms of estrogen in the hippocampus and amygdala for anxiety and depression behavior. *Neuropsychopharmacology* 2006;31:1097–1111. [PubMed: 16554740]
42. Kravitz HM, Janssen I, Lotrich FE, Kado DM, Bromberger JT. Sex steroid hormone gene polymorphisms and depressive symptoms in women at midlife. *Am J Med* 2006;119:S87–93. [PubMed: 16949393]
43. Mill J, Kiss E, Baji I, Kapornai K, Daroczy G, Vetro A, et al. Association study of the estrogen receptor alpha gene (ESR1) and childhood-onset mood disorders. *Am J Med Genet B Neuropsychiatr Genet* 2008;147B:1323–1326. [PubMed: 18449864]
44. Almeida OP, Lautenschlager N, Vasikaram S, Leedman P, Flicker L. Association between physiological serum concentration of estrogen and the mental health of community-dwelling postmenopausal women age 70 years and over. *Am J Geriatr Psychiatry* 2005;13:142–149. [PubMed: 15703323]
45. Freeman EW, Sammel MD, Lin H, Nelson DB. Associations of hormones and menopausal status with depressed mood in women with no history of depression. *Arch Gen Psychiatry* 2006;63:375–382. [PubMed: 16585466]
46. Young EA, Kornstein SG, Harvey AT, Wisniewski SR, Barkin J, Fava M, et al. Influences of hormone-based contraception on depressive symptoms in premenopausal women with major depression. *Psychoneuroendocrinology* 2007;32:843–853. [PubMed: 17629629]
47. Rasgon NL, Altshuler LL, Fairbanks LA, Dunkin JJ, Davtayan C, Elman S, Rapkin AJ. Estrogen replacement therapy in the treatment of major depressive disorder in perimenopausal women. *J Clin Psychiatry* 2002;63:45–48. [PubMed: 11995778]
48. Cohen LS, Soares CN, Poitras JR, Prouty J, Alexander AB, Shifren JL. Short-term use of estradiol for depression in perimenopausal and postmenopausal women: a preliminary report. *Am J Psychiatry* 2003;160:1519–1522. [PubMed: 12900318]
49. Morrison MF, Kallan MJ, Ten Have T, Katz I, Tweedy K, Battistini M. Lack of efficacy of estradiol for depression in postmenopausal women: a randomized, controlled trial. *Biol Psychiatry* 2004;55:406–412. [PubMed: 14960294]
50. Almeida OP, Lautenschlager NT, Vasikaran S, Leedman P, Gelavis A, Flicker L. A 20-week randomized controlled trial of estradiol replacement therapy for women aged 70 years and older: effect on mood, cognition and quality of life. *Neurobiol Aging* 2006;27:141–149. [PubMed: 16298249]
51. Resnick SM, Maki PM, Rapp SR, Espeland MA, Brunner R, Coker LH, et al. Effects of combination estrogen plus progestin hormone treatment on cognition and affect. *J Clin Endocrinol Metab* 2006;91:1802–1810. [PubMed: 16522699]
52. Steiner M, Dunn E, Born L. Hormones and mood: from menarche to menopause and beyond. *J Affect Disord* 2003;74:67–83. [PubMed: 12646300]
53. LaPlant Q, Chakravarty S, Vialou V, Mukherjee S, Koo JW, Kalahasti G, et al. Role of nuclear factor kappaB in ovarian hormone-mediated stress hypersensitivity in female mice. *Biol Psychiatry* 2009;65:874–880. [PubMed: 19251249]
54. Autry AE, Adachi M, Cheng P, Monteggia LM. Gender-specific impact of brain-derived neurotrophic factor signaling on stress-induced depression-like behavior. *Biol Psychiatry* 2009;66:84–90. [PubMed: 19358977]
55. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* 1977;229:327–336. [PubMed: 596982]
56. Tanapat P, Hastings NB, Reeves AJ, Gould E. Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. *J Neurosci* 1999;19:5792–5801. [PubMed: 10407020]

57. Cavus I, Duman RS. Influence of estradiol, stress, and 5-HT_{2A} agonist treatment on brain-derived neurotrophic factor expression in female rats. *Biol Psychiatry* 2003;54:59–69. [PubMed: 12842309]
58. Scharfman HE, MacLusky NJ. Estrogen and brain-derived neurotrophic factor (BDNF) in hippocampus: complexity of steroid hormone-growth factor interactions in the adult CNS. *Front Neuroendocrinol* 2006;27:415–435. [PubMed: 17055560]
59. Amin Z, Canli T, Epperson CN. Effect of estrogen-serotonin interactions on mood and cognition. *Behav Cogn Neurosci Rev* 2005;4:43–58. [PubMed: 15886402]
60. Prange-Kiel J, Rune GM, Leranath C. Median raphe mediates estrogenic effects to the hippocampus in female rats. *Eur J Neurosci* 2004;19:309–317. [PubMed: 14725625]
61. Zazpe A, Artaiz I, Labeaga L, Lucero ML, Orjales A. Reversal of learned helplessness by selective serotonin reuptake inhibitors in rats is not dependent on 5-HT availability. *Neuropharmacology* 2007;52:975–984. [PubMed: 17141811]
62. Greenwood BN, Strong PV, Foley TE, Thompson RS, Fleshner M. Learned helplessness is independent of levels of brain-derived neurotrophic factor in the hippocampus. *Neuroscience* 2007;144:1193–1208. [PubMed: 17161541]
63. Henn FA, Vollmayr B. Neurogenesis and depression: etiology or epiphenomenon? *Biol Psychiatry* 2004;56:146–150. [PubMed: 15271582]
64. Gould E, Woolley CS, Frankfurt M, McEwen BS. Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J Neurosci* 1990;10:1286–1291. [PubMed: 2329377]
65. Woolley CS, Gould E, Frankfurt M, McEwen BS. Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J Neurosci* 1990;10:4035–4039. [PubMed: 2269895]
66. Bastrikova N, Gardner GA, Reece JM, Jeromin A, Dudek SM. Synapse elimination accompanies functional plasticity in hippocampal neurons. *Proc Natl Acad Sci U S A* 2008;105:3123–3127. [PubMed: 18287055]
67. Yankova M, Hart SA, Woolley CS. Estrogen increases synaptic connectivity between single presynaptic inputs and multiple postsynaptic CA1 pyramidal cells: a serial electron-microscopic study. *Proc Natl Acad Sci U S A* 2001;98:3525–3530. [PubMed: 11248111]
68. Vertes RP, Kocsis B. Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. *Neuroscience* 1997;81:893–926. [PubMed: 9330355]
69. Sala M, Perez J, Soloff P, Ucelli di Nemi S, Caverzasi E, Soares JC, Brambilla P. Stress and hippocampal abnormalities in psychiatric disorders. *Eur Neuropsychopharmacol* 2004;14:393–405. [PubMed: 15336301]
70. Herman JP, Mueller NK. Role of the ventral subiculum in stress integration. *Behav Brain Res* 2006;174:215–224. [PubMed: 16876265]
71. Cooper DC, Klipec WD, Fowler MA, Ozkan ED. A role for the subiculum in the brain motivation/reward circuitry. *Behav Brain Res* 2006;174:225–231. [PubMed: 16870273]
72. Taepavarapruk P, Floresco SB, Phillips AG. Hyperlocomotion and increased dopamine efflux in the rat nucleus accumbens evoked by electrical stimulation of the ventral subiculum: role of ionotropic glutamate and dopamine D1 receptors. *Psychopharmacology (Berl)* 2000;151:242–251. [PubMed: 10972471]
73. Lisman JE, Grace AA. The hippocampal-VTA loop: controlling the entry of information into long-term memory. *Neuron* 2005;46:703–713. [PubMed: 15924857]
74. Willner P. Animal models of depression: an overview. *Pharmacol Ther* 1990;45:425–455. [PubMed: 2405444]
75. Winter C, von Rümohr A, Mundt A, Petrus D, Klein J, Lee T, et al. Lesions of dopaminergic neurons in the substantia nigra pars compacta and in the ventral tegmental area enhance depressive-like behavior in rats. *Behav Brain Res* 2007;184:133–141. [PubMed: 17698212]
76. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci* 2002;23:238–245. [PubMed: 12008002]
77. Willner P, Mitchell PJ. The validity of animal models of predisposition to depression. *Behav Pharmacol* 2002;13:169–188. [PubMed: 12122308]

Figure 1.

High power representative electron micrograph taken from the CA1 stratum radiatum of a stressed rat. Arrowheads point to examples of spine synapses established between dendritic spines (s) and axonal boutons (b). The asterisk indicates a stubby spine as it emerges from a dendritic shaft (d) and establishes an asymmetric synapse with a large bouton. Scale bar, 500 nm.

Figure 2.

Effects of footshock stress, estradiol supplementation, and antidepressant treatment on escape latency (panel a) and number of hippocampal spine synapses (panel b). **Panel a and insert:** This chart traces the change of escape latencies during active escape testing. Each tracing point represents average escape latencies from bins of five consecutive trials. The insert depicts mean escape latencies, representing the average of all 30 trials. * $p < 0.001$; # $p < 0.05$ (Holm-Sidak test). **Panel b:** * $p < 0.001$; # $p < 0.01$ (Tukey test). **Abbreviations:** CA1sr, CA1 stratum radiatum; CA3sl/sr, CA3 stratum lucidum and radiatum; DGsm, dentate gyrus stratum moleculare; IES1d, ovariectomized, sesame oil vehicle (OIL)-treated animals analyzed 24 h after inescapable footshock (IES) ($n=8$ for escape testing and $n=4$ for synapse analysis); OIL/IES

and EB/IES, treatment groups of ovariectomized females treated with OIL (n=8 for escape testing and n=4 for synapse analysis) or estradiol benzoate (EB) (n=7 for escape testing and n=4 for synapse analysis), respectively, prior to IES; NS/EB and NS/OIL, treatment groups of ovariectomized, nonstressed (NS) rats treated with EB (n=7 for escape testing and n=4 for synapse analysis) or OIL (n=8 for escape testing and n=4 for synapse analysis), respectively, prior to active escape testing; IES/DMI, ovariectomized, OIL-treated females that were administered desipramine (DMI) for six days after IES (n=8 for escape testing and n=4 for synapse analysis).

Figure 3.

Scatter plots of mean escape latencies as functions of spine synapse numbers in all three analyzed hippocampal areas combined (**panel a**), in CA1 stratum radiatum (CA1sr, **panel b**), CA3 stratum lucidum and radiatum (CA3sl/sr, **panel c**), and dentate gyrus stratum moleculare (DGsm, **panel d**). Regression lines were computed with the least squares estimator. The Pearson product moment test provided the correlation coefficients (r) and the probability values (p). See Figure 2 for abbreviations.

Schedule of Treatments and Tests

Table 1

Group	Day 1	Days 5-6	Day 8	Days 9-11	Days 12-13	Day 14	Day 15
NS/OIL	OVX ¹	OIL ²	NS ³	DW ⁴	OIL+ DW	DW	perfusion (n=4) AE ⁵ (n=8)
NS/EB	OVX	OIL	NS	DW	EB ⁶ + DW	DW	perfusion (n=4) AE (n=7)
OIL/IES	OVX	OIL	IES ⁷	DW	OIL+ DW	DW	perfusion (n=4) AE (n=8)
EB/IES	OVX	EB	IES	DW	OIL+ DW	DW	perfusion (n=4) AE (n=7)
IES1d	OVX	OIL		DW	OIL+ DW	IES+ DW	perfusion (n=4) AE (n=8)
IES/DMI	OVX	OIL	IES	DMI ⁸	OIL+ DMI	DMI	perfusion (n=4) AE (n=8)

¹ Ovariectomy;

² Oil vehicle treatment (sesame oil, 1 ml/kg, s.c.);

³ Non-stress exposure (the animals were placed in the shuttle box but did not receive footshock);

⁴ Distilled water vehicle treatment (1 ml/kg, twice daily, i.p.);

⁵ Active escape testing;

⁶ Estradiol treatment (estradiol benzoate in sesame oil, 40 µg/kg, s.c.);

⁷ Stress exposure (60 inescapable footshocks in the shuttle box);

⁸ Antidepressant treatment (desipramine in distilled water, 10 mg/kg, twice daily, i.p.).