Stress, December 2005; 8(4): 259–264

Taylor & Francis
Taylor & Francis Group

Social isolation-induced changes in the hypothalamic–pituitary–adrenal axis in the rat

M. SERRA[1,2,3], M.G. PISU[3], I. FLORIS[1], & G. BIGGIO[1,2,3]

[1]*Department of Experimental Biology,* [2]*Center of Excellence for Neurobiology of Drug Dependence, University of Cagliari, Cagliari, Italy, and* [3]*C.N.R. Institute of Neuroscience, Pisa 56127, Italy*

(Received 1 July 2005; revised 23 November 2005; accepted 28 November 2005)

Abstract
Social isolation of rats both reduces the cerebrocortical and plasma concentrations of 3α-hydroxy-5α-pregnan-20-one (3α,5α-TH PROG) and 3α,5α-tetrahydrodeoxycorticosterone and potentiates the positive effects of acute stress and ethanol on the concentrations of these neuroactive steroids. We now show that social isolation decreased the plasma level of adrenocorticotropin (ACTH), moreover, intracerebroventricular administration of corticotropin releasing factor (CRF) induced a marked increase in the plasma corticosterone level in both isolated and group-housed rats, but this effect was significantly greater in the isolated rats (+121%) than in the group-housed rats (+86%). In addition, in isolated rats, a low dose of dexamethasone had no effect on the plasma corticosterone concentration, whereas, a high dose significantly reduced it; both doses of dexamethasone reduced plasma corticosterone in group-housed rats. Furthermore, the corticosterone level after injection of dexamethasone at the high dose was significantly greater in the isolated animals than in the group-housed rats. These results suggest that social isolation increased sensitivity of the pituitary to CRF and impaired negative feedback regulation of the hypothalamic–pituitary–adrenal (HPA) axis.

Keywords: *Ethanol, HPA axis, corticosterone, rat, social isolation, stress*

Introduction

Long-term social isolation after weaning markedly affects the behavior of rats. Isolated animals are aggressive, neophobic and highly reactive to human handling. They appear nervous and show both an anxiety-like profile in the elevated plus-maze test and increased locomotor activity in response to novel situations (Hatch et al. 1963; Parker and Morinan 1986; Wongwitdecha and Marsden 1996). Social isolation is thus thought to be stressful for these normally gregarious animals and their abnormal reactivity to environmental stimuli when reared under this condition is thought to be a product of prolonged stress. Although, the underlying mechanisms remain poorly understood, similar social conditions are thought to contribute to the etiology of psychiatric disorders such as schizophrenia, depression and anxiety in humans (Heim and Nemeroff 2001).

Several acute stressful stimuli, as well as ethanol, increase the brain and plasma concentrations of neuroactive steroids, which are endogenous steroids that affect the excitability of central neurons in a manner independent of nuclear hormone receptors (for review see Biggio and Purdy 2001). Some of these compounds, such as 3α-hydroxy-5α-pregnan-20-one (allopregnanolone or 3α,5α-TH PROG) and 3α,5α-tetrahydrodeoxycorticosterone (3α,5α-TH DOC), are among the most potent positive allosteric modulators of type A receptors for the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Majewska et al. 1986; Belleli and Lambert 2005). Thus, their acute administration in pharmacological doses elicits anxiolytic, anticonvulsant and sedative-hypnotic effects in rodents; moreover, physiological

Correspondence: M. Serra, Department of Experimental Biology, Chair of Pharmacology, University of Cagliari, Cagliari 09100, Italy. Tel: 39 070 675 4136. Fax: 39 70 675 4166. E-mail: mserra@unica.it

ISSN 1025-3890 print/ISSN 1607-8888 online © 2005 Taylor & Francis
DOI: 10.1080/10253890500495244

or pharmacologically induced changes in the levels of 3α,5α-TH PROG are implicated in the regulation of $GABA_A$ receptor plasticity in addition to modulation of receptor function (for reviews see Biggio and Purdy 2001; Smith 2004).

We found that social isolation of rats for 30 days immediately after weaning, in the absence of any additional stressor, resulted in a decrease in the cerebrocortical and plasma concentrations of 3α, 5α-TH PROG and 3α,5α-TH DOC compared with the corresponding values for group-housed animals, an effect prevented by handling of the animals twice daily (Serra et al. 2000). The molecular mechanism responsible for the persistent decrease in the abundance of neuroactive steroids induced by social isolation in rats remains unclear. The observations that adrenalectomy both markedly reduces the brain content of neuroactive steroids (Purdy et al. 1991; Khisti et al. 2002; O'Dell et al. 2004) and prevents the increase in the plasma and brain concentrations of these compounds induced by acute stress (Barbaccia et al. 1997) suggest that adrenal steroidogenesis plays an important role in maintaining the abundance of neuroactive steroids in both brain and plasma. An altered regulation of the hypothalamic–pituitary–adrenal (HPA) axis might thus contribute to the reduction in the amounts of neuroactive steroids apparent in isolated animals. We have previously shown that the increases in the brain and plasma concentrations of 3α,5α-TH PROG and 3α,5α-TH DOC induced by foot shock (Barbaccia et al. 1996, 1997) used in this instance as a novel acute stressor were markedly greater on a percentage basis in socially isolated rats (395% and 292%, respectively) than in group-housed animals (78% and 107%, respectively; Serra et al. 2000). These results suggest that social isolation induced a change in regulation of the HPA axis rather than a decrease in secretory capability *per se*. This conclusion is consistent with the notion of development during exposure to chronic stress of a "facilitatory trace", characterized by hyperresponsiveness of the HPA axis to new stimuli (Akana et al. 1992). To examine the mechanism responsible for the reduction in the basal concentrations of neuroactive steroids and the increased sensitivity of the production of these steroids to stress induced by social isolation, we have now investigated the effect of social isolation on neuroendocrine state.

Materials and methods

Animals

Male Sprague-Dawley CD rats at 30 days of age, immediately after weaning, were housed for 30 days either in groups of 6–8 per cage or individually in smaller cages. They were maintained under an artificial 12-h-light, 12-h-dark cycle (lights on at 07:00 h) at a constant temperature of 23° ± 2°C and 65% humidity. All experiments were performed between 08:30 h and noon. Food and water were freely available at all times. Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Treatments

Corticotrophin releasing factor (CRF). Seven days before the end of the 30-day period, a polyethylene cannula (SP-10 PE) was implanted into the right lateral ventricle of rats anesthetized with equithesin (propylene glycol 20%, ethanol 10%, pentobarbital 0.2 M, 0.3 ml/kg, i.p.). At the end of the 30-day period, CRF (500 ng in 5 μl of physiological saline) or saline was injected into the lateral cerebral ventricle of the experimental animals with the use of a 10-μl microsyringe and an injection cannula inserted into the guide cannula. The animals were killed 30 min later. Correct placement of the cannula was verified histologically.

Dexamethasone. Rats were injected intraperitoneally (i.p.) with dexamethasone (3 or 500 μg/kg body weight) or physiological saline (0.9%) vehicle and killed 150 min later.

Extraction and assay of corticosterone

Rats were killed by decapitation with a guillotine. Blood was collected from the trunk of killed rats into heparinized tubes and centrifuged at 900*g* for 20 min at room temperature. The resulting plasma was frozen at −80°C until assayed for steroids. Corticosterone was extracted from the plasma with ethyl acetate (recovery of 70–80% as monitored by addition of a trace amount of [^{3}H]corticosterone) and then quantified by radioimmunoassay, as described previously (Serra et al. 2000), with specific antibodies (ICN, Costa Mesa, CA).

Adrenocorticotrophin hormone (ACTH) radioimmunoassay

Blood was collected from the trunk of killed rats into prechilled (4°C) tubes containing EDTA and then centrifuged within 60 min at 900*g* for 10 min in a refrigerated centrifuge (4°C). The resulting plasma was frozen (−80°C) until assayed for radioimmunoassay with a kit obtained from ICN, Costa Mesa.

Results

We postulated that an altered regulation of the HPA axis might contribute to the reduction in the amounts

of neuroactive steroids found in isolated animals. Consistent with this hypothesis, we found that the basal concentration of ACTH in plasma was significantly decreased in isolated rats (1023 ± 148 pg/ml) compared with group-housed rats (1495 ± 210 pg/ml) (data are means ± SD of values from 36 animals, $P < 0.01$, Student's *t*-test).

We previously found that foot-shock stress or ethanol injection increased the cerebrocortical and plasma concentrations of neuroactive steroids by a greater percentage in isolated rats than in group-housed animals (Serra et al. 2000, 2003). This indicates a hyperresponsiveness of the HPA axis in isolated rats, and indirectly suggests that in spite of the decreased basal plasma level of ACTH, full secretory capacity of the pituitary corticotrophs is maintained in these animals. Therefore, we examined the effect of central administration of CRF on the plasma concentration of corticosterone (Figure 1). CRF was injected into the lateral ventricle to gain access to the primary capillary plexus of the hypothalamo–pituitary portal system, although there may have also been central actions of CRF leading to HPA axis activation. CRF induced a marked increase in the plasma corticosterone concentration in both isolated and group-housed rats, but this effect was significantly greater ($P < 0.01$) in the isolated animals (+121%) than in the group-housed rats (+86%).

Next, we investigated the effect of intraperitoneal injection of dexamethasone on the basal concentration of corticosterone in the plasma of socially isolated rats. As shown in Figure 2, we found that the plasma corticosterone concentration in group-housed rats was significantly reduced by injection of the low or high dose of dexamethasone (− 38 and − 81% of basal values, as given in Figure 2 legend). In isolated rats, however, the low dose of dexamhasone had no effect on the plasma corticosterone concentration, whereas the high dose significantly reduced it (− 51%). The plasma corticosterone concentration after injection of dexamethasone at the high dose was nevertheless significantly greater in the isolated animals than in the group-housed rats (Figure 2).

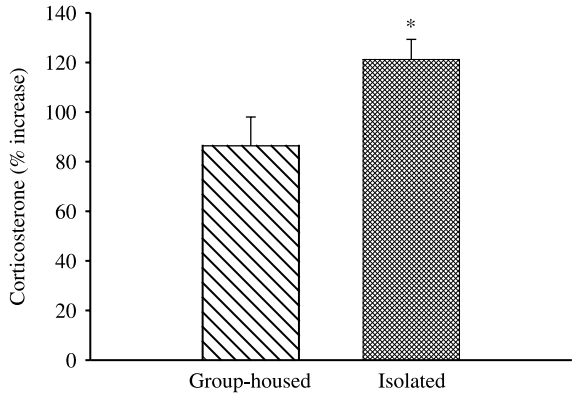

Figure 1. Effect of exogenous CRF on the plasma concentration of corticosterone in socially isolated rats. Rats were housed in groups or in isolation for 30 days. Data represent the percentage increase in the plasma concentration of corticosterone in the rats given intracerebroventricular (i.c.v.) CRF, relative to the corresponding values for control (saline-injected) rats and are means ± SEM of values from 14 animals. Basal values: group-housed rats, 99 ± 11 ng/ml; isolated rats, 127 ± 16 ng/ml. $\star P < 0.01$ vs group-housed rats (Student's *t*-test).

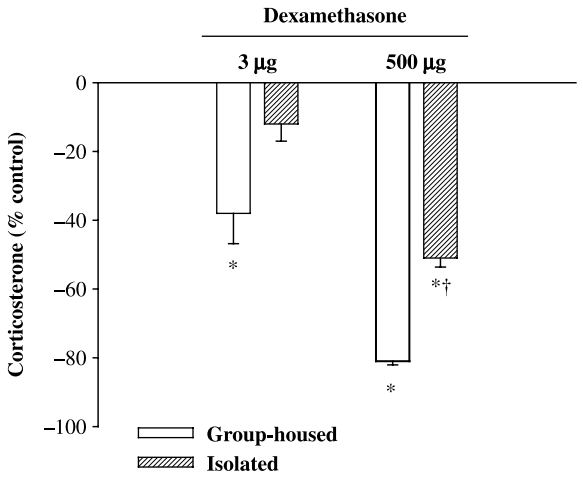

Figure 2. Effect of i.p. dexamethasone on the plasma concentration of corticosterone in socially isolated rats. Rats were housed in groups or in isolation for 30 days. Data represent the plasma concentration of corticosterone expressed as a percentage of the corresponding values for control (saline-injected) rats and are means ± SEM of values from 20 animals. Basal values: group-housed rats, 113 ± 10 ng/ml; isolated rats, 131 ± 14 ng/ml. $\star P < 0.01$ vs corresponding control rats; $^{\dagger}P < 0.01$ vs corresponding group-housed rats (two-way analysis of variance followed by Newman-Keuls test).

Discussion

We have shown that social isolation decreased the plasma concentration of ACTH, evidently increased sensitivity of the pituitary to CRF and impaired glucocorticoid negative feedback regulation of corticosterone secretion.

A decrease in the plasma concentration of ACTH, despite the continuous presence of the stressor, has been described for animals exposed to various chronic stressful stimuli and several mechanisms for this effect, in addition to a reduction in pituitary responsiveness to modulators of ACTH secretion (CRF, AVP), have been proposed (Keller-Wood and Dallman 1984; Rivier and Vale 1987; Hauger et al. 1988). Rivier and Vale (1987) suggested that both a decrease in the readily releasable pool of ACTH and the negative feedback exerted by corticosterone may account for the diminished responsiveness of the HPA axis of rats exposed to chronic intermittent electroshock.

In contrast, we have previously shown that social isolation increases the responsiveness of the HPA axis to new stimuli. Thus, the increases in the brain and plasma concentrations of 3α,5α-TH PROG and 3α,5α-TH DOC induced by foot shock (Barbaccia et al. 1996, 1997), used in this instance as a novel acute stressor, or by systemic injection of ethanol (Van Doren et al. 2000), were markedly greater on a percentage basis in socially isolated rats than in group-housed animals (Serra et al. 2000, 2003). The enhanced effects of acute stress and ethanol on the brain and plasma concentrations of neuroactive steroids in isolated rats may be related to an abnormal reactivity of the HPA axis that develops as an adaptive response to chronic stress. Abnormalities in the behavioral response of isolated rats to distinct challenges have been associated with functional changes in the endocrine response, although differences in social isolation procedures or test environments among studies have led to apparently discrepant results. For example, the basal level of corticosterone in plasma was found to be either unchanged (Morinan and Leonard 1980; Viveros et al. 1988; Haller and Halàsz 1999), increased (Rivier and Vale 1987; Greco et al. 1990; Genaro et al. 2004; Sandstrom and Hart 2005) or decreased (Miachon et al. 1993; Sanchez et al. 1998; Chida et al. 2005) in socially isolated animals.

Given that CRF is the main stimulator of ACTH release (Axelrod and Reisine 1984), and the stimulatory effect of ethanol on the corticotrophs requires the release of endogenous CRF (Lee et al. 2004), either enhanced CRF release in response to stress and to ethanol, or an increased pituitary sensitivity to CRF might be responsible for the exaggerated response of isolated rats to a novel stress.

The results presented suggest that the enhanced corticosteroid secretion apparent in response to a novel acute stress in socially isolated rats may be attributable, at least in part, to an increased sensitivity of the pituitary corticotrophs to CRF, although, an augmented release of CRF and AVP from the hypothalamic paraventricular nucleus or an increased POMC primary transcript level (Lee et al. 2004) cannot be ruled out.

Studies on HPA axis sensitivity during chronic stress have generated apparently contradictory findings as a result of the large variation in the intensity and duration of exposure to stressors and in the doses of administered CRF. Pituitary–adrenocortical responses to CRF have been found to be unaffected by chronic stress associated with immobilization (Hashimoto et al. 1988; Culman et al. 1991) or crowding (Bugajski et al. 1994) in rats, whereas chronic shock-avoidance stress resulted in an attenuated ACTH response to CRF (Odio and Brodish 1990). In contrast, the ACTH response to intravenous administration of CRF was significantly increased in rats stressed by cold adaptation (Uehara et al. 1989) or by social defeat (Buwalda et al. 1999). The latter study also showed that levels both of the glucocorticoid receptor in the hippocampus and hypothalamus and of the mineralocorticoid receptor in the hippocampus were significantly decreased in the stressed animals, resulting in reduced feedback inhibition of the HPA axis (Buwalda et al. 1999). Expression of glucocorticoid and mineralocorticoid receptors in the brain has been found not to be markedly affected by social isolation in rats (Holson et al. 1991; Olsson et al. 1994; Weiss et al. 2004; Filipovic et al. 2005). Nevertheless, it is possible that the increased responsiveness of socially isolated rats to acute stimuli is attributable in part to decreased negative feedback by corticosterone. The negative feedback exerted by corticosterone on its own release after exposure of animals to stress is mediated by glucocorticoid receptors in the pituitary, hypothalamus and hippocampus (Keller-Wood and Dallman 1984). A gradual decrease in the number of glucocorticoid receptors in specific brain areas in response to social isolation might result in a reduced effectiveness of feedback inhibition of the HPA axis, thereby leading to an increased ACTH response.

The data obtained with the dexamethasone suppression test suggest that the chronic mild stress associated with social isolation impairs negative feedback. Several studies have demonstrated a pituitary rather than a brain site of action in the suppression of HPA axis activity if moderate amounts of dexamethasone are administered (De Kloet et al. 1975; Miller et al. 1992; Cole et al. 2000). Low doses of the synthetic steroid in the drinking water were previously found to induce selective activation of glucocorticoid receptors in the pituitary, with mineralocorticoid and glucocorticoid receptors in the brain being unaffected; in contrast, high doses of dexamethasone activate glucocorticoid receptors in the brain (Miller et al. 1992). Although, we used a different route (i.p.) of administration of dexamethasone, we selected a low and a high dose of the steroid, in an attempt to examine separately the effects of glucocorticoid receptor activation in the pituitary and in the brain (hypothalamus, hippocampus, cerebral cortex). The low dose of dexamethasone, which effectively reduced the plasma corticosterone level in group-housed rats, presumably by acting primarily at the glucocorticoid receptors in the anterior pituitary, failed to affect the plasma level of corticosterone in isolated animals. Moreover, the partial suppression of corticosterone secretion induced by the high dose of dexamethasone in the isolated rats is suggestive of a partial down-regulation of glucocorticoid receptors in brain areas responsible for feedback inhibition. This hypothesis is supported by the results of studies showing that most procedures for the induction of chronic stress

result in down-regulation of glucocorticoid receptors in both the hippocampus and hypothalamus as well as in a consequent hyperresponsiveness of the HPA axis (Makino et al. 1995; Kitraki et al. 1999).

Acknowledgements

Our studies were supported by grants from RAS (Prevenzione ed Educazione Sanitaria 2004) and by GIO.I.A. Foundation (Pisa, Italy).

References

Akana SF, Scribner KA, Bradbury MJ, Strack AM, Walker C-D, Dallman MF. 1992. Feedback sensitivity of the rat hypothalamo–pituitary–adrenal axis and its capacity to adjust to exogenous corticosterone. Endocrinology 131:585–594.

Axelrod J, Reisine TD. 1984. Stress hormones: Their interaction and regulation. Science 224:452–459.

Barbaccia ML, Roscetti G, Trabucchi M, Mostallino MC, Concas A, Purdy RH, Biggio G. 1996. Time-dependent changes in rat brain neuroactive steroid concentrations and $GABA_A$ receptor function after acute stress. Neuroendocrinology 63:166–172.

Barbaccia ML, Roscetti G, Trabucchi M, Purdy RH, Mostallino MC, Concas A, Biggio G. 1997. The effects of inhibitors of GABAergic transmission and stress on brain and plasma allopregnanolone concentrations. Br J Pharmacol 120: 1582–1588.

Belleli D, Lambert J. 2005. Neurosteroids: Endogenous regulators of the GABA(A) receptor. Nat Rev Neurosci 6:565–575.

Biggio G, Purdy RH, editors. 2001. Neurosteroids and brain function international review of neurobiology. vol. 46. San Diego: Academic Press.

Bugajski J, Gadek-Michalska A, Borycs J, Bugajski AJ. 1994. Effect of corticotropin releasing hormone on the pituitary–adrenocortical activity under basal and social stress conditions. J Physiol Pharmacol 45:593–601.

Buwalda B, de Boer SF, Schmidt ED, Felszeghy K, Nyakas C, Sgoifo A, Van der Vegt DJ, Tilders FJ, Bohus B, Koolhaas JM. 1999. Long-lasting deficient dexamethasone suppression of hypothalamic–pituitary–adrenocortical activation following peripheral CRF challenge in socially defeated rats. J Neuroendocrinol 11:513–520.

Chida Y, Sudo N, Kubo C. 2005. Social isolation stress exacerbates autoimmune disease in MRL/lpr mice. J Neuroimmunol 158:138–144.

Cole MA, Kim PJ, Kalman BA, Spencer RL. 2000. Dexamethasone suppression of corticosteroid secretion: Evaluation of the site of action by receptor measures and functional studies. Psychoneuroendocrinology 25:151–167.

Culman J, Kopin IJ, Saavedra JM. 1991. Regulation of corticotropin-releasing hormone and pituitary–adrenocortical response during acute and repeated stress in the rat. Endocr Regul 25:151–159.

De Kloet ER, Wallach G, McEwen BS. 1975. Differences in corticosterone and dexamethasone binding to rat brain and pituitary. Endocrinology 95:598–609.

Filipovic D, Gavrilovic L, Dronjak S, Radojcic MB. 2005. Brain glucocorticoid receptor and heat shock protein 70 levels in rats exposed to acute, chronic or combined stress. Neuropsychobiology 5:107–114.

Genaro G, Schmidek WR, Franci CR. 2004. Social condition affects hormone secretion and exploratory behavior in rats. Braz J Med Biol Res 37:833–840.

Greco AM, Gambardella P, Sticchi R, D'Aponte D, De Franciscis P. 1990. Chronic administration of imipramine antagonizes deranged circadian rhythm phases in individually housed rats. Physiol Behav 8:67–72.

Haller J, Halàsz J. 1999. Mild social stress abolished the effects of isolation on anxiety and chlordiazepoxide reactivity. Psychopharmacology 144:311–315.

Hashimoto K, Suemaru S, Takao T, Sugawara M, Makino S, Ota Z. 1988. Corticotropin-releasing hormone and pituitary–adrenocortical responses in chronically stressed rats. Regul Pept 23:117–126.

Hatch A, Balazs T, Wiberg GS, Grice HC. 1963. Long-term isolation stress in rats. Science 142:507–510.

Hauger RL, Millan MA, Lorang M, Harwood JP, Aguilera G. 1988. Corticotropin-releasing factor receptors and pituitary adrenal responses during immobilization stress. Endocrinology 123:396–405.

Heim C, Nemeroff CB. 2001. The role of childhood trauma in the neurobiology of mood and anxiety disorders: Preclinical and clinical studies. Biol Psychiatry 49:1023–1039.

Holson RR, Scallet AC, Ali SF, Turner BB. 1991. "Isolation stress" revisited: Isolation-rearing effects depend on animal care methods. Physiol Behav 49:1107–1118.

Keller-Wood ME, Dallman MF. 1984. Corticosteroid inhibition of ACTH secretion. Endocr Rev 5:124.

Khisti RT, Kralic JE, Van Doren MJ, Morrow AL. 2002. Adrenalectomy attenuates increase in cortical allopregnanolone and behavioral effects induced by acute ethanol administration. Alcohol Clin Exp Res 26:103A.

Kitraki E, Karandrea D, Kittas C. 1999. Long-lasting effects of stress on glucocorticoid receptor gene expression in the rat brain. Neuroendocrinology 69:331–338.

Lee S, Selvage D, Hansen K, Rivier C. 2004. Site of action of acute alcohol administration in stimulating the rat hypothalamic–pituitary–adrenal axis: Comparison between the effect of systemic and intracerebroventricular injection of this drug on pituitary and hypothalamic responses. Endocrinology 145:4470–4479.

Majewska MD, Harrison NL, Schartz RD, Barker JL, Paul SM. 1986. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232:1004–1007.

Makino S, Smith MA, Gold PW. 1995. Increased expression of corticotropin-releasing hormone and vasopressin messenger ribonucleic acid (mRNA) in the hypothalamic paraventricular nucleus during repeated stress: Association with reduction in glucocorticoid receptor mRNA levels. Endocrinology 139:3299–3309.

Miachon S, Rochet T, Mathian B, Barbagli B, Claustrat B. 1993. Long-term isolation of Wistar rats alters brain monoamine turnover, blood corticosterone, and ACTH. Brain Res Bull 32:611–614.

Miller AH, Spencer RL, Pulera M, Kang S, McEwen BS, Stein M. 1992. Adrenal steroid receptor activation in rat brain and pituitary following dexamethasone: Implications for the dexamethasone suppression test. Biol Psychiatry 32:850–869.

Morinan A, Leonard BE. 1980. Some anatomical and physiological correlates of social isolation in the young rat. Physiol Behav 24:637–640.

O'Dell LE, Alomary AA, Vallee M, Koob GF, Fitzgerald RL, Purdy RH. 2004. Ethanol-induced increases in neuroactive steroids in the rat brain and plasma are absent in adrenalectomized and gonadectomized rats. Eur J Pharmacol 26:241–247.

Odio MR, Brodish A. 1990. Effects of chronic stress on *in vivo* pituitary–adrenocortical responses to corticotropin releasing hormone. Neuropeptides 15:143–152.

Olsson T, Mohammed AH, Donaldson LF, Henriksson BG, Seckl JR. 1994. Glucocorticoid receptor and NGFI-a gene expression are induced in the hippocampus after environmental enrichment in adult rats. Mol Brain Res 23:349–353.

Parker V, Morinan A. 1986. The socially-isolated rat as a model for anxiety. Neuropharmacology 25:663–664.

Purdy RH, Morrow AL, Moore Jr, PH, Paul SM. 1991. Stress-induced elevations of γ-aminobutyric acid type A receptor-active steroids in the rat brain. Proc Natl Acad Sci USA 88: 4553–4557.

Rivier C, Vale W. 1987. Diminished responsiveness of the hypothalamic–pituitary–adrenal axis of the rat during exposure to prolonged stress: A pituitary-mediated mechanism. Endocrinology 121:1320–1328.

Sanchez MM, Aguado F, Sanchez-Toscano F, Saphier D. 1998. Neuroendocrine and immunocytochemical demonstrations of decreased hypothalamo–pituitary–adrenal axis responsiveness to restraint stress after long-term social isolation. Endocrinology 139:579–587.

Sandstrom NJ, Hart SR. 2005. Isolation stress during the third postnatal week alters radial arm maze performance and corticosterone levels in adulthood. Behav Brain Res 56: 289–296.

Serra M, Pisu MG, Littera M, Papi G, Sanna E, Tuveri F, Usala L, Purdy RH, Biggio G. 2000. Social isolation-induced decreases in both the abundance of neuroactive steroids and $GABA_A$ receptor function in rat brain. J Neurochem 75:732–740.

Serra M, Pisu MG, Floris I, Cara V, Purdy RH, Biggio G. 2003. Social isolation-induced increase in the sensitivity of rats to the steroidogenic effect of ethanol. J Neurochem 85:257–263.

Smith SS, editor. 2004. Neurosteroid effects in the central nervous system. Methods & New Frontiers in Neuroscience. New York: CRS Press.

Uehara A, Habara YY, Kuroshima A, Sekiya C, Takasugi Y, Namiki M. 1989. Increased ACTH response to corticotropin-releasing factor in cold-adapted rats *in vivo*. Am J Physiol 257: E336–E339.

Van Doren MJ, Matthews DB, Janis GC, Grobin AC, Devaud LL, Morrow AL. 2000. Neuroactive steroid 3α-hydroxy-5α-pregnan-20-one modulates electrophysiological and behavioral actions of ethanol. J Neurosci 20:1982–1989.

Viveros MP, Hernandez R, Martinez I, Gonzalez P. 1988. Effects of social isolation and crowding upon adrenocortical reactivity and behavior in the rat. Rev Esp Fisiol 44:315–321.

Weiss IC, Pryce CR, Jongen-Rêlo AL, Nanz-Bahr NI, Feldon J. 2004. Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behav Brain Res 152:279–295.

Wongwitdecha N, Marsden CA. 1996. Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. Behav Brain Res 75:27–32.