Stress, 1999, Vol. 3(2), pp. 163-171
Reprints available directly from the publisher
Photocopying permitted by license only

Acute Stress Persistently Enhances Estrogen Levels in the Female Rat

TRACEY J. SHORS*, JANE PICKETT, GWENDOLYN WOOD† and MARTIN PACZYNSKI

Department of Psychology and Center for Neuroscience Rutgers University

(Received April 12, 1999; Revised July 13, 1999; In final form July 15, 1999)

Here we tested whether exposure to either tailshock or swim stress alters ovarian hormone levels, estrogen and progesterone, in females and whether the effects are persistent. Adrenal hormone levels were also measured in males and females. Estradiol levels were elevated in unstressed females during proestrus relative to females in other stages of estrous, and exposure to the stressors enhanced estradiol beyond basal levels. For females stressed during diestrus 2, estradiol levels were elevated immediately after stressor cessation and up to 24 hrs. Exposure to tailshock, but not swim-stress, transiently enhanced progesterone in females stressed during the stage of proestrus and estrus. Glucocorticoid levels were elevated in response to both stressors and were supraelevated in females under both basal and stress conditions relative to males, particularly in blood from females exposed to acute swim stress. These results indicate that exposure to a relatively acute stressful event immediately and persistently enhances serum estradiol and are discussed in the context of reports that exposure to the same stressors immediately and persistently impairs associative learning in the female rat.

Keywords: corticosterone, progesterone, ovarian hormones, learning, classical conditioning, adrenal glands, sex differences, Alzheimer's disease, shock, swim

INTRODUCTION

It has been reported that exposure to an acute stressor of either swim stress or intermittent tailshocks enhances associative learning in male rats (Beylin & Shors, 1998; Servatius & Shors, 1994; Shors, Weiss, & Thompson, 1992; Shors & Servatius, 1995; Shors & Servatius, 1997; Shors & Mathew, 1998c). In contrast, exposure to the same stressor dramatically impairs the same type of learning in females (Shors, 1998; Shors, Lewczyk, Paczynski, Mathew, & Pickett, 1998b; Wood & Shors, 1998). The stress-induced impairment in females is dependent on the presence of ovarian hormones and the availability of estrogen receptors (Wood and Shors, 1998). In order to account for these effects of stress on learning, we considered the possibility that exposure to the stressor altered the release of ovarian hormones, estrogen and progesterone (Shors, 1998). Several studies had reported that stress can elevate ovarian steroid levels and induce pseudopregnancy, a condition associated with elevated noncycling levels of ovarian hormones (Bow-

* Send correspondence to : Tracey J. Shors, Ph.D., Department of Psychology, Busch Campus, Rutgers University, Piscataway, NJ 08854–8020 ; phone (732) 445–6968 ; fax (732) 445–2263 ; e-mail: SHORS@rci.rutgers.edu
† Laboratory of Neuroendocrinology, Rochefeller University, NY, NY.

man and Miller, 1996; Garland et al., 1987; MacNiven et al., 1992). It was also reported that exposure to cold swimming increases serum estradiol (Cardenas, 1992). However, in response to a chronic stressor of restraint for 6h/day for 21 days, female rats demonstrated decreased levels of estradiol (Galea et al., 1997). Thus, exposure to stressful events can enhance or reduce estrogen, presumably depending on the length and type of stressful experience.

Without specifying a direction, we hypothesized that exposure to an acute stressor of either swim or tailshock stress would alter the release of ovarian hormones, estrogen and/or progesterone. In the first experiment, we tested this hypothesis by measuring serum levels of estradiol and progesterone in females during the 4 stages of estrous and in response to one of 2 stressors : tailshock and swim stress. We also measured serum corticosterone in male and female rats exposed to each stressor, as well as unstressed controls of both sexes.

Results from the first experiment suggested that exposure to an acute stressor elevates estrogen levels immediately after its cessation. In the second experiment, we characterized the effect over a 24-hr period after stressor cessation. Females in diestrus 2 were exposed to the stressor and sacrificed immediately, 3, 6 or 24 hrs after stressor cessation. Blood serum samples were analyzed for circulating adrenal and ovarian hormone levels and compared to those from unstressed controls.

MATERIALS AND METHODS

Experiment 1 : Immediate Effects of Stress on Adrenal and Ovarian Hormones

Animals

A total of 101 females and 29 male adult (60–70 days) Sprague Dawley rats were colony bred and housed 4–5 per cage with littermates (12:12 light cycle, lights on at 7:00 AM). Stages of estrous were determined by daily lavage between 9:00 and 11:00 AM each day for at least two, consecutive 4–5 day cycles prior to stressor exposure. Daily vaginal cell smears were fixed in 95% ethanol, rinsed in H_2O, and stained in a slightly basic aqueous 1% solution of Toluidine Blue and again dehydrated with 95% EtOH (Everitt, 1989). Early proestrus is marked by pink staining epithelial cells, late proestrus and estrus by masses of dark blue staining cornified cells, diestrus 1 by the presence of many dark staining leukocytes with scattered epithelial cells, and diestrus 2 by only sparse cell distribution.

Stressor Procedure

Exposure to the stressors occurred between 12:00–2:00 PM. For the tailshock stress, male rats and females in different stages of the estrous cycle [males (n = 5), proestrus (n = 7), estrus (n = 7), diestrus 1 (n = 7), and diestrus 2 (n = 11)] were restrained in Plexiglas containers and 30, 1 mA, 1 s tail stimulations were delivered at a rate of 1/min for 30 min. Rats were smeared again, immediately sacrificed and trunk blood was collected. For swim stress, male rats and females [males (n = 12), proestrus (n = 3), estrus (n = 7), diestrus 1 (n = 2), and diestrus 2 (n = 11)] in different stages of estrous were placed in room-temperature water bath (19–20°C) for 20 min and sacrificed immediately after stressor cessation : serum hormone levels of stressed subjects were compared to that of unstressed rats [males (n = 12), proestrus (n = 17), estrus (n = 15), diestrus 1 (n = 8), and diestrus 2 (n = 9)]. Unstressed rats remained in their home cages until decapitation. Trunk blood was collected in test tubes with heparin (volume 0.1 ml) and centrifuged for 20 minutes at 3000 rpm. Serum aliquots were stored at –80°C.

Hormone Assays

Corticosterone, progesterone and estradiol (labeled with ^{125}I) were measured using solid-phase RIA kits (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA). Interassay variability for corticosterone was < 5.8% and assay sensitivity was 5.7 ng corticosterone/ml. Crossreactivity with progesterone (100,000 ng/ml added) was 0.42%, and there was no crossreactivity with estradiol at that concentration.

For the estradiol assay, interassay variability was < 8.1% and assay sensitivity was 8 pg/ml with no cross-reactivity with either corticosterone or progesterone. For progesterone, the interassay variability was < 3.9% and the assay sensitivity was 20 pg/ml. Crossreactivity with corticosterone was 0.9% and not detectable with added estradiol. The samples were coded and run in duplicate. Hormones within a rat were assayed simultaneously and 4 assays per hormone were conducted for Experiment 1.

Experiment 2 : Persistent Effects of Stress on Ovarian and Adrenal Hormones

A total of 78 females were housed as described in Experiment 1. Females in diestrus 2 were either exposed to the swim stressor or not and sacrificed at the following time points : 0 hrs (n = 7 stressed females; n = 6 unstressed females), 3 hrs (n = 8 stressed females; n = 9 unstressed females), 6 hrs (n = 9 stressed females; n = 9 unstressed females), and 24 hrs (n = 14 stressed females; n = 16 unstressed females). To avoid the relatively high basal levels of glucocorticoids observed in the afternoon due to diurnal variation (see results of Experiment 1), rats were lavaged, stressed and sacrificed before 12 PM. Trunk blood was collected and hormone assays were conducted as described. Hormone measures within a rat were assayed simultaneously and 4 assays per hormone were conducted for Experiment 2.

RESULTS

Experiment 1 : Immediate Effects of Stress on Adrenal and Ovarian Hormones

In general, basal levels of glucocorticoid levels were relatively high, but did correspond to the peak response observed in the later afternoon (~2 PM) on other serum analyzed and reported with this assay (Coat-A-Count Rat Corticosterone, Diagnostic Products Corp., Los Angeles, CA). Using analysis of variance, there was a significant interaction between sex (male versus female) and the type of stressor (swim or tailshock) [$F(2,122) = 16.17$; $p<0.001$]. Under all conditions, females released more corticosterone than males (Figure 1). Using Newman Keuls post-hoc analysis, it was determined that unstressed females had higher corticosterone levels than unstressed males ($p < 0.005$); swim stressed females had higher corticosterone levels than swim stressed males ($p < 0.001$); and tailshock stressed females had higher corticosterone levels than tailshock stressed males ($p < 0.001$). Exposure to both stressors, swim and tailshock, produced a significant elevation in plasma corticosterone in both sexes compared to plasma obtained from unstressed rats [$F(1,122) = 73.47$; $p<0.001$]. In females, there was no interaction between the stressor exposure and stages of estrous on corticosterone levels [$F(6,87) = 1.00$; $p = 0.43$], and the stage of estrous alone did not influence the basal level of corticosterone [$F(3,87) = 0.10$; $p = 0.96$]. Within females, exposure to the swim stress procedure enhanced corticosterone levels more than exposure to tailshock stress ($p < 0.001$). Within males, corticosterone levels in response to either swim or tailshock were elevated relative to unstressed male controls ($p < 0.001$; $p < 0.05$, respectively), but the response to the two different stressors was not significantly different ($p = 0.35$).

In terms of percentage, the increase in corticosterone levels after stress was similar for females exposed to tailshock stress (130% of baseline) as males exposed to tailshock stress (140%). However, the increase in corticosterone levels in females exposed to swim stress (194%) was supraelevated relative to males exposed to swim stress (147%).

As expected, estradiol levels in the unstressed females differed depending on the stage of estrous [$F(3,91) = 5.14$; $p < 0.005$] (Figure 2). Post-hoc analysis using Newman Keuls confirmed that estradiol levels were higher in blood extracted from females in proestrus than diestrus 1 ($p < 0.001$) and estrus ($p < 0.05$), nearly significant when compared to those in diestrus 2 ($p=0.07$), and higher in those in estrus than diestrus 1 ($p = 0.05$).

Exposure to the stressful event enhanced estradiol [$F(1,91) = 4.25$; $p<0.05$] (Figure 2). There was no interaction between exposure to the stressors and

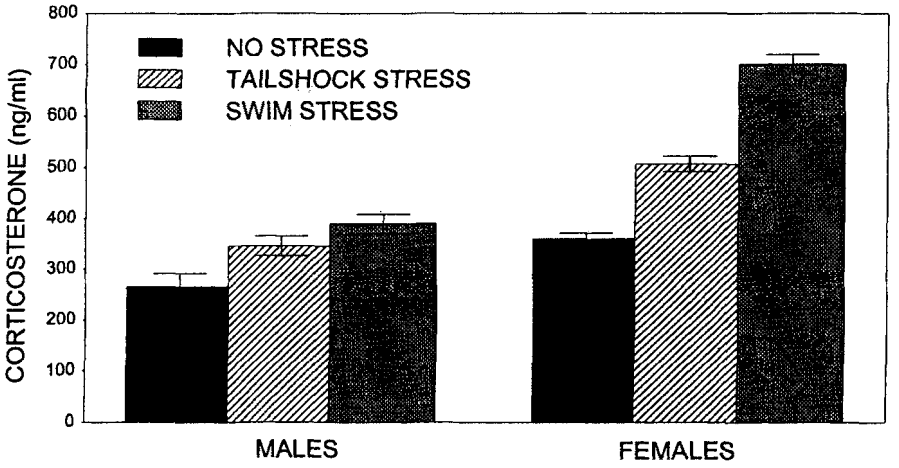

FIGURE 1 *Serum corticosterone (ng/ml) measured immediately after either tailshock or swim stress in males and females.* Because there was no difference in corticosterone levels across stages of estrous, data are collapsed. Corticosterone levels were higher in females than males under stressed and unstressed conditions, and particularly elevated in response to swim stress in females

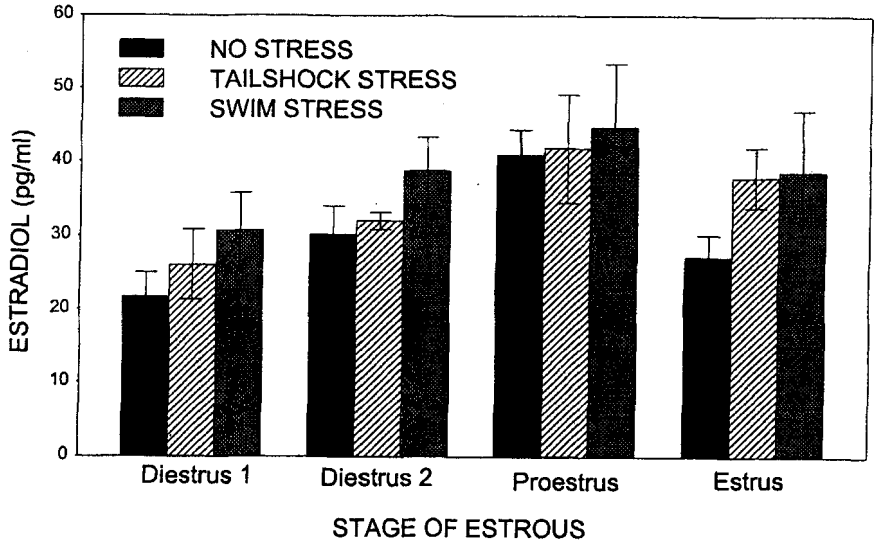

FIGURE 2 *Serum estradiol levels (pg/ml) measured immediately after either tailshock or swim stress during four stages of estrous.* Under unstressed conditions, estradiol levels were highest in proestrus. Overall, estradiol was enhanced in response to the stressors

stages of estrous [F(3,91)=0.52; p=0.67], nor was there a difference in estradiol levels in rats exposed to the stressor of swim versus tailshock (p = 0.97). Thus, there was an overall increase in estradiol in response to stress collapsing across the stages of estrous.

Levels of progesterone did not differ across the estrous cycle [F(3, 89)=0.71; p=0.55] (Figure 3). This was not surprising, given the short period of time in which progesterone peaks and the fact that we only monitored blood levels once. There was an interaction

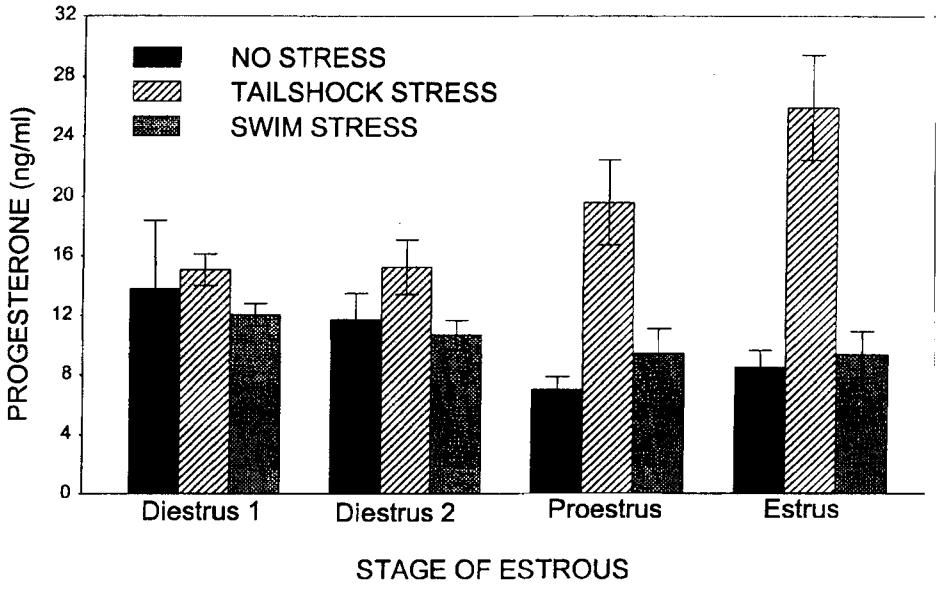

FIGURE 3 *Serum progesterone levels (ng/ml) measured immediately after either tailshock or swim stress during four stages of estrous.* Exposure to tailshock stress enhanced the levels of progesterone, but only for females stressed during proestrus and estrus

between the stage of estrous and exposure to stress [F(6,89)=2.79; p=0.01]. Specifically, stress enhanced serum progesterone when female rats were stressed during the stage of estrus ($p<0.05$) and proestrus ($p<0.05$). However, only tailshock stress enhanced progesterone ($p<0.001$), whereas swim stress had no effect (p=0.69).

Collapsing across the estrous cycle, there was a positive correlation between the levels of progesterone and corticosterone in the female rats (r=0.38). There was no correlation between levels of estrogen and corticosterone (r=0.11) or progesterone and estrogen (r=0.11). Examining either stressed rats or unstressed rats alone, there were no within animal correlations between the various hormone levels.

Experiment 2 : Persistent Effects of Stress on Ovarian and Adrenal Hormones

All stressed females were exposed to swim stress during diestrus 2 and sacrificed and sampled either within diestrus 2 (time 0, 3, 6), or 24 hrs later in proestrus. There was a significant interaction between manipulation of stress and time since stressor cessation on corticosterone [$F(3,70) = 31.45$, $p<0.001$] (Figure 4). As expected, exposure to 20 min of inescapable swim stress resulted in an immediate increase in plasma corticosterone levels ($p < 0.001$). Plasma corticosterone returned to basal levels within 3 hrs ($p = 0.31$), and remained there 6 hrs ($p = 0.07$) and 24 hrs after stressor cessation ($p = 0.90$).

In unstressed females, there was an overall increase in estradiol levels across time [$F(3,70) = 4.13$; $p < 0.01$] (Figure 5). Using a Newman Keuls post-hoc analysis, it was determined that estradiol was elevated 6 hrs ($p < 0.05$) and 24 hrs after diestrus 2 ($p < 0.05$). The 24 hr time point corresponds to proestrus and thus estradiol levels in unstressed females were highest during proestrus, as expected. Overall, exposure to swim stress enhanced estradiol levels relative to basal levels [$F(3,70) = 13.63$; $p < 0.01$]. That is, there was a main effect of stress on estradiol collapsing across the time points after stressor exposure in diestrus 2. There was no interaction between the stressor exposure and time since stressor exposure ($p>0.05$). Thus, as in Experiment 1, there was an increase in estradiol in response to

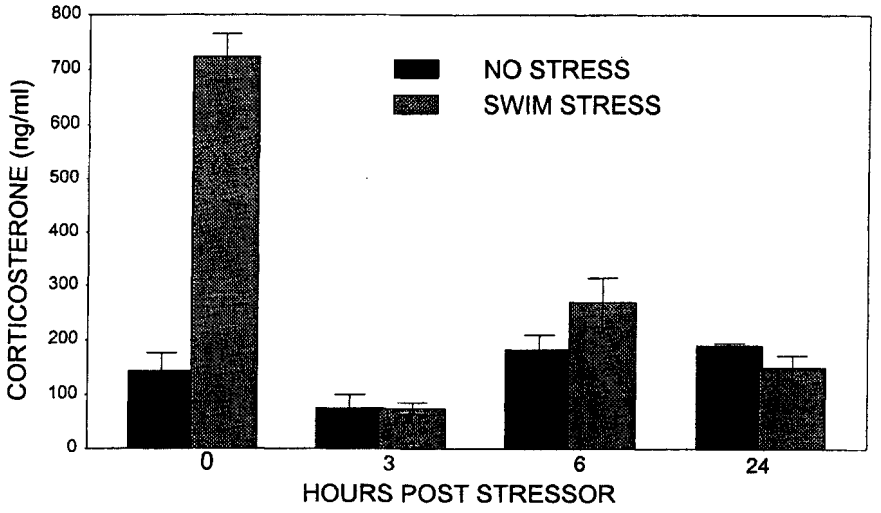

FIGURE 4 *Serum corticosterone levels (ng/ml) measured immediately, 3 hrs, 6 hrs, and 24 hrs after cessation of swim stress.* Serum corticosterone levels were enhanced immediately after stressor exposure, but returned to basal levels within 3 hrs of stressor cessation

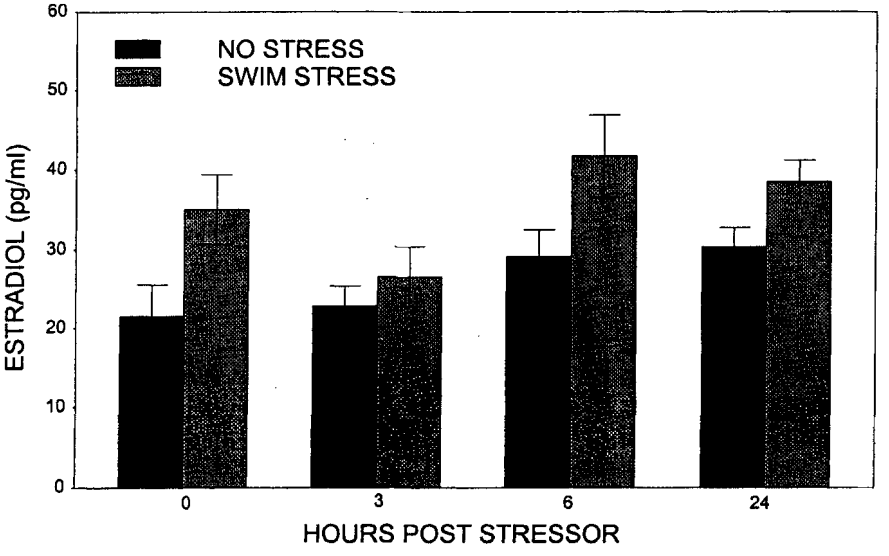

FIGURE 5 *Serum estradiol levels (pg/ml) measured immediately, 3 hrs, 6 hrs, and 24 hrs after swim stress.* Swim stress enhanced serum estradiol levels up to 24 h after stressor cessation

stress, except in this case the effect was evident collapsing across time rather than across the estrous cycle.

Progesterone levels fluctuated significantly over the transition from diestrus 2 to proestrus [$F(3,70) = 7.83$; $p < 0.001$]. There was no interaction between stress and stage of estrous [$F(3,70) = 2.42$; $p=0.07$], and as in the Experiment 1, swim stress did not enhance the release of progesterone when females were stressed during diestrus 2 [$F(1,70) = 0.02$; $p = 0.88$] (data not shown).

For the females stressed in diestrus, there were significant positive correlations between the levels of corticosterone and progesterone (r=0.36) and corticosterone and estrogen (r=0.33), but no relationship between estrogen and progesterone (r=0.04). Examining the stressed rats alone, there was an even stronger correlation between corticosterone and progesterone (r=0.59) and a modest correlation between corticosterone and estrogen (r=0.24). Again, there was no relation between estrogen and progesterone (r=0.16). Under unstressed conditions alone, the positive correlation between corticosterone and progesterone was not evident (r=0.08), although a positive correlation existed between corticosterone and estrogen (r=0.36).

DISCUSSION

In summary, these experiments document changes in ovarian hormones in response to an acute stressful event of either inescapable swim or intermittent tailshocks. Plasma estradiol levels were elevated immediately after stressor cessation (Figure 2). In rats stressed during diestrus 2, estradiol levels were elevated over time and up to 24 hrs later (Figure 5). Despite the fact that the degree of enhancement of estradiol was small, it is considered that these changes represent physiological responses to environmental events that should have functional consequences. We previously reported that exposure to the stressors of either swim stress or tailshock dramatically impaired associative learning in females 24 hrs after stressor cessation (Shors & Wood, 1998; Shors et al., 1998; Shors, 1998). Ovariectomy as well as the blockade of estrogen receptors prevented the impaired performance, suggesting that the effect of stress on learning was dependent on the presence of estrogen (Wood & Shors, 1998). The present results support our hypothesis that a stress-induced change in estrogen levels *contributes* to the impaired performance. However, the effect of stress on learning occurred primarily in rats exposed to stress during diestrus and tested in proestrus (as mimicked in Experiment 2), whereas exposure to the stressor enhanced estrogen when collapsing across stages -- not in any particular stage (Results from Experiment 1). Thus, we would amend the hypothesis to propose that the effect of stress on associative learning is regulated by a stress-induced elevation of estrogen that occurs while estrogen levels are rising during the transition from diestrus to proestrus. It is also noted that the increase in estrogen was more evident after swim stress than tailshock, yet both are known to dramatically impair associative learning in females (Shors et al., 1998). Thus, other factors in addition to estrogen are most likely involved.

In agreement with past studies (Akinci & Johnston, 1993; Atkinson & Waddell, 1997; Galea et al., 1997; Gaskin & Kitay, 1970; Kitay, 1961, 1963; Kant et al, 1983), females exhibited elevated basal and stress levels of glucocorticoids relative to males. Of the two stressors, swim-stress provoked a more intense adrenal corticosterone response than tailshock in females. Apparently, the increase in female levels relative to males under unstressed and stressed conditions is not due to decreased clearance, since the half-life of corticosterone is actually shorter in the female (Kitay, 1961). Moreover, it has been reported that castration has an inhibitory effect on the stress-induced release of glucocorticoids in males while ovariectomy only slightly reduced the stress-induced release in females (Gaskin & Kitay, 1970). Thus, the perceived supraelevation in female corticosterone levels relative to those in males would simply be an illusion based on a testosterone-induced decrease in corticosterone levels in males.

In the present study, the adrenal response to each of the acute stressors, tail shock and swim-stress, followed a typical pattern of rapid corticosterone elevation that had dissipated within 6 hrs of stressor cessation (Figure 4). Previous studies have reported that corticosterone is preferentially elevated in proestrus (Atkinson & Waddell, 1997; Galea et al., 1997), and that exposure to stress elevates corticosterone especially during proestrus (Carey, Deterd, de Koning, Helmerhorst, & de Kloet, 1995; Viau & Meaney, 1991). In the present experiments, we did not detect differences in corticosterone levels across stages of estrous, nor a supraelevation in females stressed during proestrus relative to other stages. It is

unclear why we did not observe these effects as others have. Certainly, the predicted changes in estrogen were observed across the stages and there was a positive correlation between estrogen and corticosterone in experiment 2. In order to detect a difference between the cycles, it may be necessary to either increase the group size or to sample more frequently within a cycle and within animals.

The present results indicate that exposure to a relatively acute stressful event (20–30 min) rapidly induces an increase in estradiol levels in the blood (Figure 2 and 5). This effect may be dependent on the presence of glucocorticoids, at least initially, since both estradiol and corticosterone were enhanced immediately afterward (Figure 1). Although compelling, it is unclear how these 2 systems would interact. There is evidence for glucocorticoid receptors in the ovaries (Hillier & Tetsuka, 1998). Provided that progesterone could be a stimulus, there is also evidence for the release of progesterone from the adrenal glands (Fajer, Holzbauer, & Newport, 1971). Based on the positive correlation between corticosterone and estrogen in stressed rats and the supraelevation of corticosterone in stressed females, it is tempting to conclude that the stress-induced release of glucocorticoids actually caused the enhanced release of estrogen. Consistent with this interpretation, the stress-induced release of glucocorticoids was most evident after swim stress relative to tailshock, as was the stress-induced release of estrogen. If related at all, the direction of a such a cause-and-effect could be reversed, since estrogen is known to increase corticosterone production by the adrenals (Kitay, 1963).

The effect of stress on estradiol was not only rapidly induced but also persistently expressed. Relative to stage-specific unstressed controls, females stressed during diestrus 2 had elevated levels of estradiol up to 24 hr after the stressor has ceased (Figure 5). Apparently, the long-term increase is not directly or solely due to the stress-induced increase in glucocorticoids, since corticosterone levels returned to baseline well before estradiol.

There are numerous studies addressing the role of estrogen in cognitive function and little consensus as to whether estrogen enhances, impairs or has no effect on learning (Bucci, Chiba, & Gallagher, 1995; Luine, Richards, Wu & Beck, 1998; Miles, Green, Sanders, & Hines, 1998; Packard, 1998; Shors et al., 1998; Wood & Shors, 1998;). In previous studies, we reported that females in proestrus learn more rapidly than females in any other stage and more rapidly than males, but females in proestrus are also most impaired by stress (Shors et al., 1998). In contrast, others have reported that performance in the Morris water maze is poor during proestrus (Warren & Juraska, 1997). In light of the present results, it is possible that proestrus females are impaired in water maze performance due to supraelevated levels of estrogen induced by the swim procedure. Interestingly, females in proestrus performed optimally on the visible platform task, a version of the water maze in which exposure to swim stress is minimal and easily escapable.

Estrogen is known to assist in the prevention of bone loss, heart disease, and even Alzheimer's disease (Henderson, Paganini-Hill, Emaluel, Dunn, & Buckwalter, 1994; MacGregor & Jordan, 1998). The present results should be considered instructive to the practice of estrogen therapy in post-menopausal women, in that stressful life experience may further enhance blood levels of estrogen beyond levels optimal for effective performance (Shors, 1998).

Acknowledgements

supported by grants to TJS from the NIMH (R01 MH59970–01) and NSF (IBN-9511027)

References

Akinci, M.K. & Johnston, A.R. (1993). Sex differences in the effects of acute swim stress on binding of GABAa receptors in mouse brain. *Journal of Neurochemistry*, **60**, 2212–2216.

Atkinson, H.C. & Waddell, B.J. (1997). Circadian variation in basal plasma corticosterone and adrenocorticotropin in the rat : sexual dimorphism and changes across the estrous cycle. *Endocrinology*, **9**, 3842–3948.

Beylin, A.V. & Shors, T.J. (1998). Stress enhances excitatory trace eyeblink conditioning and opposes acquisition of inhibitory conditioning. *Behavioral Neuroscience*, **6**, 1327–1338.

Bucci, D.J., Chiba, A.A., & Gallagher, M. (1995). Spatial learning in male and female Long-Evans rats. *Behavioral Neuroscience*, **109**, 180–183.

Carey, M.P., Deterd, C.H., de Koning, J., Helmerhorst, F., & de Kloet, E.R. (1995). The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat. *Journal of Endocrinology*, **144**, 311–321.

Everitt, J.W. (1989). *Neurobiology of Reproduction in the Female Rat; Monographs on Endocrinology*. Berlin : Springer-Verlag.

Fajer, A.B., Holzbauer, M., & Newport, H.M. (1971). The contribution of the adrenal gland to the total amount of progesterone produced in the female rat. *Journal of Physiology*, **214**, 115–126.

Galea, L.A.M., McEwen, B.S., Tanapat P, Deak T, Spencer R.L., & Dhabhar F.S. (1997). Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. *Neuroscience*, **81**, 689–697.

Gaskin, J.H. & Kitay, J.I. (1970). Adrenocortical function in the hamster : sex differences and effects of gonadal hormones. *Endocrinology*, **87**, 779–786.

Henderson, V.W., Paganini-Hill, A., Emaluel, C.K., Dunn, M.E., & Buckwalter, J.G. (1994). Estrogen replacement therapy in older women. *Archives of Neurology*, **51**, 896–900.

Hillier, S.G & Tetsuka, M. (1998). An anti-inflammatory role for glucocorticoids in the ovaries? *Journal of Reproductive Immunology*, **39**, 21–27.

Kant, G.J., Lenox, RH, Bunnell, B.N., Mougey, E.H., Pennington, L.L., & Meyerhoff, J.L. (1983). Comparison of stress response in male and female rats : pituitary cyclic AMP and plasma prolactin, growth hormone and corticosterone. *Psychoneuroendocrinology*, **8**, 421–428.

Kitay, J.I. (1961). Sex differences in adrenal cortical secretion in the rat. *Endocrinology*, **68**, 818–824.

Kitay, J.I. (1963). Pituitary adrenal function in the rat after gonadectomy and gonadal hormone replacement. *Endocrinology*, **73**, 253–260.

Luine, V.N., Richards, S.T., Wu, V.Y. & Beck, K.D. (1998). Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. *Hormones and Behavior*, **34**, 149–162.

MacGregor, J.I. & Jordan, V.C. (1998). Basic guide to the mechanisms of antiestrogen action. *Pharmacological Review*, **50**, 151–196.

Miles, C., Green, R., Sanders, G., and Hines, M. (1998) Estrogen and memory in a transexual population. *Hormones and Behavior* **34**, 199–208.

Packard, M.G. (1998). Posttraining Estrogen and memory modulation. *Hormones and Behavior*, **34**, 126–139.

Servatius, R.J. & Shors, T.J. (1994). Exposure to inescapable stress persistently facilitates associative and nonassociative learning in rats. *Behavioral Neuroscience*, **108**, 1101–1106.

Shors, T.J. (1998). Stress and sex effects on associative learning : For better or for worse. *The Neuroscientist*, **4**, 353–364.

Shors, T.J., Lewczyk C, Paczynski, M., Mathew, P.R., & Pickett, J. (1998). Stages of estrous mediate the stress-induced impairment of associative learning in the female rat. *Neuroreport*, **9**, 419–423.

Shors, T.J. & Mathew, P.R. (1998). NMDA receptor antagonism in the basolateral but not central nucleus of the amygdala prevents the induction of facilitated learning in response to stress. *Learning and Memory*, **5**, 220–230.

Shors, T.J. & Servatius, R.J. (1995). Stress-induced sensitization and facilitated learning require NMDA receptor activation. *Neuroreport*, **6**, 677–680.

Shors, T.J. & Servatius, R.J. (1997). The contribution of stressor intensity, duration, and context to the stress-induced facilitation of associative learning. *Neurobiology of Learning and Memory*, **67**, 92–96.

Shors, T.J., Weiss, C., & Thompson, R.F. (1992). Stress-induced facilitation of classical conditioning. *Science*, **257**, 537–539.

Viau, V. & Meaney, M.J. (1991). Variations in the hypothalamic-pituitary-adrenal response to stress during the estrous cycle in the rat. *Endocrinology*, **129**, 2503–2511.

Warren, S.G. & Juraska, J.M. (1997). Spatial and nonspatial learning across the rat estrous cycle. *Behavioral Neuroscience*, **111**, 259–266.

Wood, G.E. & Shors, T.J. (1998). Stress facilitates classical conditioning in males but impairs conditioning in females through activational influences of ovarian hormones. *Proceedings of the National Academy of Sciences*, **95**, 4066–4071.