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Foot Shock Induces Time and Region Specific Adrenergic Receptor Changes in Rat Brain

ROBERT M. COHEN,[1] MARTIN R. COHEN, CATHERINE A. McLELLAN

Section on Clinical Brain Imaging, Laboratory of Cerebral Metabolism
National Institute of Mental Health, U.S. Public Health Service
Department of Health and Human Services, Bethesda, MD 20205

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COHEN, R. M., M. R. COHEN AND C. A. McLELLAN. *Foot shock induces time and region specific adrenergic receptor changes in rat brain.* PHARMACOL BIOCHEM BEHAV 24(6) 1587–1592, 1986.—Rats were subjected to 1 hr or 2 hr of electric foot shock for 1 day or 7 days and adrenergic receptor binding was evaluated in the hypothalamus, brainstem and cortex. β-Adrenergic receptor density in the hypothalamus was dramatically reduced following 1 hr of shock. Following repeated shock, α_2-adrenergic receptors in the cortex and brainstem were observed to increase. Cortical α_2-adrenergic receptors were more sensitive to stress than the α_2-adrenergic receptors of the brainstem, alterations in the latter only reaching statistical significance following 7 days of shock and 24 hr of recovery. α_1- and β-adrenergic receptors in the brainstem and cortex were relatively resistant to stress induced changes. The significance of type of stress, duration of stress, and strain of rat for understanding the current data are discussed in the context of prior reports of stress induced receptor changes.

Stress Foot shock α-Adrenergic receptors β-Adrenergic receptors Cortex Hypothalamus Brain stem Adaptation

STRESS has been postulated to be a psychologically meaningful concept for understanding the processes by which environmental events can impact on animals and man to effect physical, psychosomatic and psychiatric illness [1, 3, 20]. Neurochemically, acute stress increases the release of catecholamines and pituitary hormones in the periphery and centrally produces effects in a large number of neurotransmitter pathways including the catecholamine, indoleamine, opiate and gabaergic [1, 5, 8, 10]. Adaptation to repeated stress involves alterations in a variety of parameters in many of these same pathways, e.g., neurotransmitter synthesizing enzyme activities and levels of neurotransmitters [5, 8, 10].

Recently, adaptive changes in adrenergic receptors during acute and repeated stress have been reported [5, 7, 23, 24, 27, 28, 31, 33]. Acute and repeated immobilization stress of 2.5 hr duration leads to changes in α_2- and β-adrenergic receptors that are dependent both on the anatomic region and time selected for examination [5, 7, 27, 31, 33]. In another popular stress paradigm, electric foot shock, decreases in β-adrenergic receptor density and in norepinephrine stimulated adenylate cyclase activity in rat cortex were observed after repeated but not acute stress [25]. β-Adrenergic receptor changes in other regions have not been examined in the foot shock paradigm, nor have α-adrenergic receptor changes been evaluated. As electric foot shock has been an extremely popular paradigm for both the study of stress and learned helplessness, findings of similar receptor changes in response to this stressor compared to those already observed with 2.5 hr of restraint would support the importance of receptor alterations in the overall neurochemical and behavioral adaptation to stress.

METHOD

Six-to-twelve-week-old male Sprague-Dawley rats (175–200 g), obtained from Taconic Farms, Germantown, NY, were housed 6 to a cage under a 12 hr light and dark cycle with food and water ad lib. The animals were subjected to electric foot shock as described by Sherman and Allers [21] as sufficient to induce learned helplessness. Animals were placed in modular test cages in which the floors were electric grids and received randomized shocks of 0.7 milliamps for 10 sec as delivered by a programmable solid state shocker/distributor (Coulbourn Instruments). Following the various periods of acute or repeated stress with or without 24 hr of allowed recovery, animals were decapitated, brains removed and then dissected into hypothalamus, cortex and brainstem portions approximating the region inclusive of the

[1]Requests for reprints should be addressed to Dr. Robert M. Cohen, Chief, Section on Clinical Brain Imaging, NIMH, 9000 Rockville Pike, Building 10, Room 4N317, Bethesda, MD 20205-1000.

TABLE 1

STRESS-INDUCED ADRENERGIC RECEPTOR CHANGES (±S.E.)

	Control		Stress		Probability for differences beyond chance between control and stress groups	
	B_{max} (femtomoles/mg protein)	K_D (nM)	B_{max} (femtomoles/mg protein)	K_D (nM)	In Slopes and/or intercepts (B_{max})	Slopes (K_D)
[^{3}H]Dihydroalprenolol						
Cortex						
1×1 hr	86.3 ± 8.7	(0.90 ± 0.14)	85.9 ± 9.2	(0.84 ± 0.15)	NS	NS
7×1 hr	90.9 ± 9.2	(0.94 ± 0.12)	87.1 ± 3.6	(0.79 ± 0.05)	NS	NS
7×1 hr + 24 hr			91.3 ± 8.2	(0.94 ± 0.14)	NS	NS
Brainstem						
7×1 hr	56.0 ± 7.9	(1.19 ± 0.44)	57.9 ± 8.4	(1.81 ± 0.95)	NS	NS
Hypothalamus						
1×1 hr	57.1 ± 5.5	(0.94 ± 0.20)	26.5 ± 3.9	(0.88 ± 0.14)	<0.005	NS
1×2 hr	47.5 ± 7.7	(0.74 ± 0.25)	30.0 ± 4.0	(0.91 ± 0.28)	<0.02	NS
7×1 hr + 24 hr	40.6 ± 10.2	(1.08 ± 0.42)	62.0 ± 9.6	(1.12 ± 0.27)	<0.02	NS
[^{3}H]WB4101						
Cortex						
1×2 hr	222.3 ± 34.2	(0.83 ± 0.19)	216.2 ± 14.6	(0.80 ± 0.08)	NS	NS
7×1 hr	250.6 ± 34.8	(0.88 ± 0.18)	247.2 ± 28.4	(0.81 ± 0.14)	NS	NS
7×1 hr + 24 hr			272.7 ± 27.9	(0.86 ± 0.13)	NS	NS
[^{3}H]Clonidine						
Cortex						
1×2 hr	52.1 ± 5.3	(1.32 ± 0.23)	95.0 ± 2.9	(1.78 ± 0.09)	<0.001	NS
7×1 hr	65.8 ± 2.4	(2.77 ± 0.21)	93.9 ± 4.1	(4.00 ± 0.41)	<0.003	(0.004)<0.03
7×1 hr + 24 hr			68.1 ± 5.2	(2.78 ± 0.44)	NS	NS
Brainstem						
1×1 hr	48.3 ± 7.4	(3.22 ± 0.86)	50.4 ± 4.8	(2.17 ± 0.34)	NS	NS
2×1 hr	30.7 ± 2.7	(2.81 ± 0.36)	35.8 ± 4.6	(1.89 ± 0.39)	NS	NS
7×1 hr	27.8 ± 0.6	(3.50 ± 0.30)	32.4 ± 4.1	(2.90 ± 0.70)	NS	NS
7×1 hr +24 hr			76.8 ± 7.1	(8.80 ± 2.40)	<0.05	NS

The stress regimen is indicated by mxnh where m is equal to the number of days of stress and n is equal to the number of hours of stress per day. Where a group of animals were given a 24 hr stress-free period (+24 hr) prior to sacrifice, the comparison control group is taken to be the same as that which is used for the stress group sacrificed immediately following stress. In comparisons where slopes do not significantly differ significance levels in the first probability column reflect the significance of B_{max} changes. In comparisons where slopes differ a separate test for intercepts (B_{max}) is listed in parentheses.

medulla and pons. All experiments were conducted with 6 animals assigned to a group.

α- and β-adrenergic receptor binding assays were performed as previously described [6]. In brief, brain regions were weighed and homogenized in 1 percent (w/v) Tris-HCl (50 mM, pH 7.6) at 4°C. The homogenates were centrifuged at 18,500 × g for 10 min at 4°C. The pellets were suspended in 50 vol of buffer and recentrifuged. The resulting pellets were resuspended in 10 vol of buffer and used in the assay procedures. The protein concentrations of the tissue homogenates were determined by the method of Lowry *et al.* [11], and incubations carried out with 0.25–0.6 mg of protein at 25°C. All ligand binding assays were performed in triplicate and in parallel for both total and nonspecific binding except for determinations of hypothalamic binding which were made in duplicate. α_1-Adrenergic receptor binding was measured using 20 min incubation times with [^{3}H](2,6-dimethoxyphenoxyethyl)aminomethyl-1,14-benzodioxane ([^{3}H]WB4101) as the radioactive ligand. α_2-Adrenergic receptor binding was measured using [^{3}H]clonidine as the radioactive ligand with incubation times of 30 min. Specific α-adrenergic receptor binding was defined as the difference between the amount of radioactive ligand bound in the presence and absence of 10 μM phentolamine and represented 80 percent of the total radioactive ligand bound. Reasonable arguments can be made for the choice of alternative radioactive ligands for the study of adrenergic receptor binding. Our choice was based on the following reasoning and the knowledge that no one radioactive ligand is sufficient to characterize all properties of either α_1- or α_2-adrenergic receptor binding, e.g., agonist and antagonist forms. Almost all prior work relating α_2-adrenergic receptor adaptation to antidepressants and stress have been conducted with clonidine [1, 7, 10, 12, 27, 35]. These receptor changes have been associated with func-

tional changes (locomotor and self stimulation) in response to clonidine challenge. We wished to be able to directly compare these alterations with those that might occur with respect to the induction of learned helplessness. Similarly, WB4101 was chosen as it has been studied with respect to antidepressant administration and stress. At the time these studies were initiated its relative lack of selectivity with respect to other α_1-adrenergic receptor ligands such as prazosin was not well recognized. For the β-adrenergic receptor binding assay, incubations were carried out for 30 min with [^{3}H]dihydroalprenolol ([^{3}H]DHA) as the radioactive ligand. Specific binding was defined as the difference between the amount bound in the presence and absence of 10 μM d,l-propanolol, and represented 70 percent of the total radioactive ligand bound. All incubations were terminated by the addition of 7 ml of cold 0.9 percent saline, rapidly filtered through Whatman glass fiber filters (GF/C for α and GF/B for β) and subsequently washed with 2 additional 7 ml vol of cold 0.9 percent saline. Filters were counted by liquid scintillation spectrometry.

Homogenates of brain regions from each of the six animals from each treatment group were pooled for Scatchard determinations utilizing a 0.2 to 4 $\times$ K_D range of ligand concentrations. B_{max}'s and K_D's were formulated from estimates of the least squares analyses of Scatchard Plots. The determination of individual animal binding was performed to support those group differences observed by Scatchard analyses of the pooled samples as such analyses do not provide a direct measure of interindividual variance. These determiniations were made at a single concentration of ligand approximating 2 $\times$ K_D. The statistical significance of observed treatment and control differences in parameters were determined with the use of appropriate F and 2-tailed *t*-tests.

RESULTS

The results of foot shock on brain adrenergic receptor binding as determined by Scatchard analyses are collated in Table 1. β-Adrenergic receptors in rat cerebral cortex remained unchanged after 1 hr of foot shock and following 7 days of 1 hr of foot shock per day whether or not animals were allowed a 24 hr period without stress prior to sacrifice. In a separate experiment under identical conditions, the examination of individual cortices revealed a small but statistically nonsignificant decrease in binding as a result of 7 days of footshock and 24 hr of recovery (control=5816±1093 dpm, stress=5333±538 dpm). Seven days of repeated 1 hr of shock was also not sufficient to induce changes in β-adrenergic receptor binding in the brainstem (Table 1). However, β-adrenergic receptor binding in the hypothalamus was markedly reduced by a single 1 hr period of foot shock (Table 1). This represented a statistically significant 54.3 percent change in B_{max} ($p<0.005$) while the affinity constant was not significantly altered. A similar but somewhat less robust reduction of 37 percent was observed in the hypothalamus after 2 hr of foot shock. However, animals receiving 7 days of 1 hr of foot shock per day, but allowed a 24 hr period free of stress prior to sacrifice demonstrated a 53 percent increase in B_{max} compared to controls with no K_D change (Table 1).

Alterations in cortical α_1-adrenergic receptor binding did not occur with a single 2 hr period of foot shock (Table 1). Nor were changes apparent in animals given 7 days of 1 hr of foot shock per day and sacrificed immediately or following a 24 hr period free of stress.

Whereas α_1-adrenergic receptor binding appeared unaffected by foot shock, α_2-adrenergic receptor binding was. After 2 days of 1 hr of foot shock per day a significant increase in cortical α_2-adrenergic receptor binding was observed (Table 1). A comparison of individual cortical binding confirmed the increase in binding observed in the Scatchard analysis (control=1375±83 dpm; stress=1703±152 dpm; $p<0.01$). A 43 percent increase was also observed at the seventh day of footshock; however, the cortical α_2-adrenergic receptor binding of animals sacrificed 24 hr after the seventh day of foot shock did not differ from controls. In the brainstem small, but statistically nonsignificant increases in α_2-adrenergic receptor binding were observed after 1 hr of foot shock, 2 days of 1 hr of foot shock per day, and following 7 days of foot shock (Table 1). However, animals sacrificed 24 hr following 7 days of repeated 1 hr foot shock showed significant increases in brainstem α_2-adrenergic receptor binding. The functional or physiological significance, however, of B_{max} changes in the presence of affinity changes are unknown. Thus the meaning of the 7 day changes in α_2-adrenergic receptor binding in both cortex and brainstem requires further delineation.

DISCUSSION

Comparison to Earlier Studies

It is important to note that, in general, prior studies of stress induced receptor changes have utilized either rather long periods of stress, 2.5 hr of restraint or 48 hr of food deprivation, or the exceedingly brief period of handling and pain requisite to a saline injection. Evaluations of receptor changes have then been made at the end of a single stress or for as long as 14 days following repeated stress as in the instance of immobilization. It is possible that the specific receptor changes reported were dependent upon the parameters of type of stress imposed, duration of stress period, and time of receptor evaluation compared to time of stress termination. This study, using 1 hr stress periods with receptor evaluations both immediately and 24 hr following repeated stress, together with the earlier studies, suggests that the duration of stress, the repeated or continuous nature of the stress, the number of repetitions of stress, the time chosen for evaluation in relation to the termination of stress, and the genetic strain of the organism examined are critical determinants of receptor alteration in response to stress. Some of these parameters appear to be equally important in determining decreases in cyclic adenosine 3′,5′-monophosphate responses to catecholamines as induced by restraint and foot shock stress [29].

Consistent with prior studies, both α_1- and β-adrenergic receptors in the cortex appear relatively resistant to alteration by stress. The largest alteration observed in β-adrenergic receptors in cortex reported to date are those of U'Prichard and Kvetnansky [33] and Stanford *et al.* [24]. The former reported a 38 percent decrease following 14 days of 2.5 hr/day immobilization while α_2-adrenergic receptor binding in animals allowed 24 hr for recovery were no different than controls. Changes were not observed following a single stress period. In the latter study a 36 percent decrease was observed 24 hr following the 14th day of brief 1 min handling and saline injection, but evaluations closer to the termination of stress were not made. Nomura *et al.* [16], using 90 min of tail shock, observed a 15 percent decrease in beta-adrenergic cortical binding only after 5 days. Stone and Platt [27] reported a maximum reduction of 7.5 percent in a

similar immobilization paradigm as that of U'Prichard and Kvetnansky [33]. The latter result is consistent with the 8.3 percent decrease we observed immediately following 7 days of foot shock, but not in animals allowed 24 hr to recover. With the number of animals studied, 6, however, this small change, not surprisingly, did not reach statistical significance. Repeated 1 hr electric foot shock did not induce β-adrenergic receptor changes in the brainstem either. This resistance to stress induced changes in brainstem β-adrenergic receptors has also been observed with Nomura's 5 day, 90 min tail shock procedure [16] and in Roman high avoiders subjected to 2.5 hr of immobilization, but is different from the reduction in density observed in Roman low avoiders [7]. In contrast, β-adrenergic receptors in the hypothalamus appear to be exceedingly sensitive to foot shock stress and to immobilization stress at least as evaluated by Torda *et al.* [31] and Cohen and Campbell [7]. These changes also appear sensitive to duration of individual stress periods, the number of repetitions and the time of evaluation. Nomura *et al.* [16] did not see changes in the hypothalamus with 5 days of tail shock, but did not evaluate possible changes following a single stress. Stone and Platt [27] observed somewhat smaller decreases in hypothalamic β-adrenergic receptor density. They reported a maximum reduction of 12 percent by day 7 of repeated immobilization. Changes, however, were apparent by day 4, at which time cortical and brainstem β-adrenergic receptors were unchanged. The authors suggested that their somewhat smaller changes compared to those previously reported were the probable result of the use of propranolol to determine specific binding. Isoproterenol is suggested to be the appropriate ligand displacer in both hypothalamus and brainstem [26]. As our studies were initiated prior to their report, our analyses were not performed with the probable displacing ligand of choice; however, we feel that this is an unlikely explanation for the quantitative differences. In our study, similar changes were not observed in the brainstem which are subject to the same potential problems as the hypothalamus. Alterations in the affinity constant of stressed animals did not occur. Nor were changes in nonspecific binding observed in the stress group, while equivalent total binding changes were observed even at very low concentrations of the labeled ligand DHA. The magnitude of the changes, however, could be related to strain. Torda *et al.* [31], and our own unpublished replication of the Torda study used Zivic-Miller derived Sprague-Dawley rats. This report used Sprague-Dawley rats obtained from Taconic Farms and the Stone and Platt [27] study used Sprague-Dawley rats obtained from Charles River. Our earlier report [7] of stress induced decreased hypothalamic β-adrenergic receptor density used Roman high and low avoiders which are Wistar derived. Finally, these findings are consistent with other data demonstrating differential hypothalamic sensitivity in response to stress induced norepinephrine and catecholamine turnover changes [9, 15, 30].

In both the foot shock and forced immobilization stress paradigms, a differential sensitivity of cortical α_2-adrenergic receptors compared to cortical α_1- and β-adrenergic receptors is observed. A differential sensitivity of α_2-adrenergic receptors has previously been observed in the rat cortex in response to pharmacologic treatments that enhance norepinephrine levels in the synapse [6,18]. The direction of these receptor changes has varied although this variability appears to be secondary to drug dose, duration of treatment and area of brain examined [22]. Our findings of an increase in cortical α_2-adrenergic receptors in response to foot shock stress is similar to the changes observed by U'Prichard and Kvetnansky [33] following a single day of 2.5 hr immobilization. With 14 days of repeated stress, these authors observed a return of receptor density to control levels. In contrast, a reduction in α_2-adrenergic receptor density at 7 days was observed by Lynch *et al.* [12] in a similar restraint paradigm as well as by Stanford *et al.* [24] who evaluated receptors 24 hr after 14 days of mild stress (handling plus saline injection). A reduction of α_2-adrenergic receptors in forebrain has also been observed in Roman high and low avoiders receiving 2.5 hr of repeated restraint [7] in the absence of significant α_1-adrenergic receptors (unpublished). And in still another paradigm, α_2-adrenergic receptor density in the basal hypothalamus was observed to increase by 100 percent following 5 days of starvation with α_1-receptors remaining unchanged [23]. The differences in the α_2 responses observed, as in the instance of the hypothalamic β-adrenergic receptor changes, may result from differences in the experimental parameters of the rat strain and duration of stress employed. This study and those of U'Prichard and Kvetnansky [33] and Torda *et al.* [31] used Sprague-Dawley rats, whereas the other observations have been made with Wistar or Wistar derived strains.

Theoretically one would expect that receptor alterations would be dependent on the length of the individual stress period. Acute stress with the immediate outpouring of catecholamines might be expected to induce a downregulation of catecholamine receptors. However, continued stress, past this point, without previous adaptation, would be expected to lead to catecholamine depletion and a return toward normal levels of receptors. Indeed, even an increase in receptor density might occur depending on how the time dependent levels of norepinephrine in the synapse are integrated at the receptor in the process of overall receptor adaptation. This might possibly explain finding more dramatic reductions in β-adrenergic receptors following 1 hr of shock than following a longer period when catecholamines may already be exhausted. In this respect it is not surprising to imagine that differential changes with regard to brain regions reflect specific patterns of norepinephrine turnover in response to stress [15, 30, 32], catecholamine metabolic pathway differences in cell bodies compared to axons, and regional differences in adrenergic receptor subtypes. For example, the delayed alterations in the α_2-adrenergic receptor observed in the brainstem compared to the cortex in response to at least some forms of stress could be the direct result of these considerations.

The Significance of Noradrenergic Pathway Changes Induced by Stress

Although the involvement of a considerable number of neurotransmitter pathways is likely in the physiological and behavioral responses to acute and repeated stress, there is reason to believe that noradrenergic pathways may play one of the more important roles [1, 3, 5, 8, 9]. Noradrenergic pathways make significant contributions to motivational, arousal, reinforcement and learning systems in animals [14]. Changes in noradrenergic pathways have been most closely linked to the pathophysiologic changes of stress, both in terms of mediation and predisposition [9].

In this context, Stone and Platt [27] reported that the reduction in the physiological effects of stress, i.e., the formation of gastric ulcers and change in eating behavior, was

positively related to β-adrenergic receptor density. This could either represent, as hypothesized by Stone, an adaptation that results in the organism's resistance to stress or a reflection of the important neurochemical parameters (e.g., presynaptic or other postsynaptic mechanisms) that actually determine the response to stress. For example, the continuing presence of releasable transmitter in some animals would sustain the stress-induced release of catecholamines that could not only cause the change in receptor number, but also prevent the formation of ulcers and the change in eating behavior. Experimental support for this possibility may be derived from the observations of Tsuda *et al.* [32]. These investigators demonstrated an increase in 3-methoxy-4-hydroxy-phenylethyleneglycol (MHPG-SO_4, the chief metabolite of norepinephrine in the rat brain) levels in all brain regions except the basal ganglia which paralleled stress-induced gastric ulceration during succeeding days of activity-stress. The same group, noting regionally selective time-related changes in catecholamine turnover, have postulated that these changes may be associated with changes in the "emotional" behavior of the animal in response to stress which begins with early vigorous struggling, but is followed by a quiescent period.

Of all the stress-induced behavioral effects observed, perhaps, the most notable description of behavioral changes following inescapable shock was made by Miller and Seligman [13]. Their "learned helplessness" animal model was based primarily on demonstrated deficits in the performance and learning of active avoidance behavior [17,19]. As similar behavioral deficits had been observed in psychiatric patients, particularly depressed patients, these investigators proposed that both the animals and the patients had learned the concept that nothing they did mattered. Other investigators, particularly Weiss *et al.* [34], have offered an alternative explanation in which a stress-induced reduction in brain catecholamine levels result in a problem with "motor activation." Their work has been supported by (1) the close association in the time course of norepinephrine changes in the cortex to the temporal course of the behavior deficit, and (2) the accurate prediction of the behavioral effects of pharmacologic manipulations with catecholamine enhancing and blocking agents. Most recently, Weiss *et al.* [35], has demonstrated that the infusion of clonidine into the locus ceruleus reverses the behavioral deficits induced by inescapable shock, suggesting that it is the lack of norepinephrine occupancy of the α_2-adrenergic receptor in the region of the locus ceruleus that results in the stress induced behavioral deficit. This data is consistent with the proposed role of noradrenergic neurons with cell bodies in the locus ceruleus in the integration of incoming sensory information with reinforcement, drive and motivation that ultimately results in the organism's choice of response [4]. Finally, antidepressants act both to prevent the behavioral deficits imposed by stress and alter adrenergic receptor number and function, as e.g., in response to clonidine administration [1, 9, 21, 22].

CONCLUSIONS

This study suggests that foot shock, over as short a period as 1 hr (a time, however, sufficient to induce "learned helplessness"), as in the instance of restraint, preferentially induces β-adrenergic receptor changes in the hypothalamus and α_2-adrenergic receptor changes. It is worth emphasizing, however, that our study and those of others measuring stress associated adrenergic receptor binding changes, are only measuring one parameter of noradrenergic pathway function. The meaning of these changes, even with respect to receptor function, are as yet poorly understood. Clearly, much more work needs to be done before we understand the totality of changes that the noradrenergic pathway undergoes with stress, and the import of these changes for the pathophysiology of stress. Nevertheless, conceptually, the data concerning receptor changes in response to stress are consistent with prior findings of discrete anatomic changes in both catecholamine levels and turnover following stress and the importance of the temporal and intensity parameters of the delivered stress to the determination of the physical and behavioral changes induced. These findings suggest the utility of applying autoradiographic receptor binding procedures to further delineate the specific neurotransmitter pathways that are involved in the response and adaptation to stress. For example, do the changes in β-adrenergic receptors found only after repeated stress, result from the induction of larger changes in the same anatomic regions, or do some previously unaffected anatomic regions become recruited? As receptor dependent measurements in man are possible by indirect pharmacologic challenge procedures, the newer positron emission tomographic methods and through post mortem examination, it is important that a body of knowledge be developed to help formulate the most appropriate receptor dependent questions to be asked and to interpret the information these findings would provide concerning the state of an organism. Thus, the specific form of the stress utilized for study in animals may not be as important as understanding the factors responsible for the homeostatic neurochemical mechanisms that occur in response to environmental perturbations and how these events lead to state transitions, i.e., neurochemical adaptation and consequent behavioral change [7]. For example, how do organisms adapt to intermittent stress compared to continuous stress? In this context, the apparent interaction of genetic strain, particularly those strains that have been behaviorally defined, with stress related receptor alterations, makes these studies of potential greater importance in the understanding of human normal and abnormal behavior [7].

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