The Brain on Stress: Insight from Studies Using the Visible Burrow System

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Abstract

The discovery of adrenal steroid receptors outside of the hypothalamus in the hippocampus and other forebrain regions catalyzed research on the effects of stress upon cognitive function, emotions and self-regulatory behaviors as well as the molecular, cellular and neuroanatomical mechanisms underlying acute and chronic stress effects on the brain. Indeed, this work has shown that the brain is a plastic and vulnerable organ in the face of acute and chronic stress. The insight that Bob and Caroline Blanchard had in developing and interpreting findings using the Visible Burrow System model made an enormous contribution to the current view that the human brain is very sensitive to the social environment and to agonistic interactions between individuals. Their collaboration with Sakai and McEwen at The Rockefeller University extended application of the Visible Burrow System model to demonstrate that it also was a unique and highly relevant neuroethological model with which to study stress and adaptation to stressors. Those studies focused on the brain and systemic organ responses to stress and, in turn, described that the brain is also very responsive to changes in systemic physiology.

1. Introduction

Both the word and concept of “stress” were introduced by Hans Selye with an emphasis on physical stressors such as physical injury, heat and cold (1, 2) and later modified by the work of John Mason to include psychological stressors (3). One common response to this wide range of challenges was activation of the hypothalamic-pituitary-adrenal (HPA) axis, ultimately leading to an increase in glucocorticoid secretion by the adrenal cortex. Neuroendocrinology developed as a discipline that explored the hypothalamic control of pituitary function (4) and largely focused upon the feedback regulation of hormone secretion at the level of these two structures, without reference to the rest of the brain. Now there is a broader view of neuroendocrinology that emphasizes the bidirectional communication between brain and body and the recognition that the social as well as physical environment plays a powerful role in shaping individuals over the lifecourse via this bidirectional communication (5).

What led to this outlook? We contributed one key finding when we discovered glucocorticoid receptors outside of the hypothalamus using cellular uptake and nuclear retention of radiolabeled corticosterone; this binding was initially found in the hippocampal formation of the rat (6-8) and later observed in the primate as well (9). Subsequent discoveries by many investigators demonstrated the importance of adrenal steroids and other hormones in mediating both adaptation and damage in the hippocampus and other forebrain areas, but it was not until we collaborated with Bob and Caroline Blanchard that we came to appreciate the role of the social environment in the regulation of these endocrine and neuromodulatory effects.

2. Functional significance of adrenal steroid receptors in the brain

What is the function of such receptors in hippocampus? The initial focus for the hippocampus was on inhibitory behaviors and sleep (10-13). Then, Robert Sapolsky, as a student and then postdoctoral fellow in our laboratory, developed the “glucocorticoid cascade hypothesis” of stress and aging with McEwen and Lewis Krey; this concept placed an emphasis on wear and tear upon the brain and body, mediated by cortisol feedback on the hippocampus, over a stressful life and as part of the aging process (14). By that time, neural actions of adrenal steroids were shown by Reul and de Kloet to involve both Type I (mineralocorticoid) and Type II (glucocorticoid) receptors (15). Meanwhile, biphasic actions of adrenal steroids were recognized on memory (16, 17), long-term potentiation (18), appetite and locomotor activity during the diurnal cycle (see (19)) and later, on acquired immunity (20). Interestingly, the memory and immune-enhancing effects of acute stress were also shown to involve catecholamines, which are released by the sympathetic nervous system under stressful conditions (21, 22).

But there were other, fundamental actions of adrenal steroids yet to be discovered when Elizabeth Gould joined the McEwen lab and introduced the Golgi method to visualize neuronal structure. This led to discovery of glucocorticoid and stress-induced remodeling of hippocampal neurons, particularly in the CA3 region (23, 24), an effect that was later shown by Magariños to involve excitatory amino acid mediation (25-27). Gould, with Heather Cameron, also “re-discovered” adult neurogenesis in the dentate gyrus (28, 29), which had been shown previously by Altman (30) and Kaplan (31) but generally disregarded (32). Like hippocampal remodeling, dentate gyrus neurogenesis is also regulated by both glucocorticoids and excitatory amino acids (33).

However, regarding both dendritic remodeling and postnatal neurogenesis, there remained the task of showing generality across mammalian species. Both Gould and Magariños worked with Eberhard Fuchs on his tree shrew variant of a resident-intruder paradigm that examines the consequences of stressful social interactions. Gould and colleagues demonstrated postnatal neurogenesis both in tree shrew model and in macaque hippocampus (34, 35). The phenomenon of CA3 dendritic remodeling was also shown to occur in the tree shrew by Magariños and Fuchs (36) and in hibernating hamsters by Magariños and Paul Pevet (37).

But that gets ahead of the story, because, until we began to collaborate with Bob and Caroline Blanchard at the University of Hawaii, we had not fully appreciated the role of social behavior and social hierarchies as stressors, even though Mason had shown that the psychological stress of one monkey seeing another getting food was an enormously effective stressor as indicated by an elevation in cortisol (38).

3. Studies using the Visible Burrow System with the Blanchards

Around the same time that our lab was exploring the effects of steroid hormones on brain function, Bob and Caroline Blanchard were developing experimental methods to study rodent behavior in naturalistic settings. Rats are normally social animals, living in mixed-sex groups of up to several hundred animals in complex underground habitats consisting of interconnecting tunnel and burrow systems (39-41). Within these larger societies, rats form smaller single-sex and mixed-sex colonies of three to 20 animals. These underground habitats made the rat difficult to study in the wild, so the Blanchards attempted to recreate these conditions in the laboratory. Their initial studies simply housed mixed-sex groups of Long-Evans rats in a large open area (“colony” condition), where dominance hierarchies quickly formed among the males,

but not the females (42). They then attempted to create a more naturalistic laboratory model by providing the animals with a dirt substrate in which they could create their own burrows and tunnels. The males housed in the burrowing habitats showed significantly greater aggression toward intruders than the “colony” animals; furthermore, a significant percentage of subordinate animals died within four months of living in the habitats, whereas there was no mortality among the “colony” subordinates (43). The elevated mortality among subordinate animals was consistent with that observed by Barnett (40) and Calhoun (41), and appeared to be related more to the physiological effects of social subordination, as opposed to lethal wounding, as the frequency of fighting diminished with duration of housing.

While the burrowing habitats effectively recreated key environmental and behavioral features of rat colonies in the wild, the Blanchards soon realized that once the animals started burrowing, they were effectively “underground” and, therefore, just as difficult to observe as in the wild. Their next step—the “visible burrow system” or “VBS” —was a stroke of creative genius. Rather than having the rats dig their own tunnels and burrows, Bob and Caroline Blanchard built the burrows for them, equipping them with dark sides and limited light to simulate underground conditions for the rats, with clear plexiglas tunnels and chambers with clear tops to facilitate observation by the experimenters (44). The design of the VBS apparatus has evolved into the one diagrammed in Figure 1.

As in the wild, one of the key features of the VBS colony model is the use of mixed sex groups, generally consisting of 4-5 male and 2-3 female Long-Evans rats. Initial studies used singly housed males as controls, while subsequent iterations used male-female pairs, to allow for sexual interactions and reduce any adverse effects of social isolation. The rats are introduced into an open area, where they have access to food and water, as well as tunnels leading to several

other compartments (44). The males quickly form dominance hierarchies, with the dominant guarding access to food and water reservoirs and attempting, somewhat unsuccessfully, to limit access to the females as well. The hierarchies are usually established during the first few days of group housing, with the dominants and subordinates being readily identified by distinctive patterns of offensive or defensive wounding; the subordinates generally lose a significant amount of body weight during this period as well (45). The colonies are generally studied for periods of approximately two weeks, after which mortality among the subordinates becomes a problem.

Randall Sakai, at the time a post-doctoral fellow with McEwen, was key in initiating the VBS collaborations between the McEwen and Blanchard Laboratories in 1989. Our studies with the Blanchards using the VBS model have demonstrated profound consequences of chronic subordination stress. These changes suggest that subordination, a common and consistent feature of life for many animals living in social groups, may be a particularly relevant model for investigating the behavioral, neural and endocrine correlates of chronic stress (46). Over the years, we have systematically shown alterations in hormone activity and regulation, metabolic activity, reproductive function, neurochemistry and neuronal morphology in subordinate animals; furthermore, we have shown that there are costs associated with being the dominant as well (summarized in Table 1).

3.1. Effects on the HPA axis

Perhaps the most consistent findings in the VBS model have been the effects of social stress on various indices of HPA axis activity. After removal from the VBS, plasma corticosterone (CORT) levels were consistently higher in the subordinate animals, and often elevated in the dominant animals as well, when compared to controls (Blanchard et al. 1993,

1995; McKittrick et al. 1995). In the dominant animals, the increased CORT appears to be context-dependent, however, as the elevated CORT was seen only immediately upon removal from the VBS, but not after the animals have been returned to their home cage for an hour (47). In contrast, in the subordinates, the baseline elevations in CORT persisted for at least an hour after removal from the VBS.

In addition to changes in baseline and VBS context-dependent CORT levels, subordinates often exhibited a blunted response to a novel stressor, suggesting more complex dysfunction of the HPA axis. Close examination the stress response in individual animals, however, led us to conclude that the subordinate rats did not constitute a uniform group, but rather could be subdivided into “stress-responsive” (SRS) or “non-responsive” (NRS) subordinates, depending on their glucocorticoid response to the novel applied stressor (46)(Figure 2). This classification of subordinates proved useful in subsequent studies as the NRS group generally exhibited the most adverse consequences of subordination (cf. effects on testosterone, CBG, etc.). We have observed that NRS do not mount a normal stress response prior to 7 days after removal from the VBS, thus the NRS phenotype persists even during a recovery period and is unlikely that it is context dependent. The proportion of subordinate rats that exhibit a NRS stress response profile was smaller once commercially-bred Long Evans rats were used rather than the colony-bred rats used in the University of Hawaii studies perhaps suggesting a role for genetic factors or intensity of stress experienced while in the VBS. The relative contribution of these factors to the NRS phenotype has not yet been systematically examined in this model.

VBS housing led to changes in other determinants of HPA function within the hippocampus and other brain areas. In the hippocampus, subordinates showed lower levels of mRNA for both Type I (MR) and Type II (GR) glucocorticoid receptors compared to controls

(48); this was consistent with other studies indicating compensatory downregulation of these receptors in response to elevated levels of glucocorticoids. The corticotropin releasing hormone (CRH) system was also affected in both hypothalamic and extrahypothalamic brain regions. Non-responsive subordinates (NRS) expressed a significantly lower average number of CRH mRNA grains per cell in the paraventricular hypothalamic nucleus (PVN) compared with SRS, dominants, or cage-housed control rats (49)(Figure 3A). The number of cells containing CRH mRNA was also significantly lower in NRS than in SRS or dominants (Figure 3B). The changes in the PVN were limited to CRH, since arginine vasopressin (AVP) mRNA levels did not vary with behavioral rank in this area. The blunted CORT response to the novel stressor may be a direct consequence of diminished synthesis and release of CRH seen in the NRS animals. The relationship between lower CRH levels and an impaired ability to mount a CORT response to a novel stressor is supported by the observation that no changes in CRH mRNA in the PVN were seen in a VBS experiment in which all of the subordinates had a normal stress response (Choi et al. 2006).

In contrast to the PVN, in the central amygdala, CRH mRNA levels were increased in both groups of subordinates compared with control rats, whereas SRS exhibited higher levels than the dominants rats as well (49)(Figure 3C). However, in the medial amygdala, the number of cells expressing AVP mRNA was significantly greater in control rats compared with both groups of subordinates; in addition, the number of AVP-positive cells significantly correlated with plasma testosterone level. In another study, CRH levels were also increased in the bed nucleus of the stria terminalis in the subordinates (50). It has been postulated that extrahypothalamic CRH may play a role in mediating stress-induced behavioral changes (51).

The VBS studies with the Blanchards revealed manifestations of social stressors on

systemic regulation of HPA activity as well. Compared to controls, VBS-housed animals all showed a decrease in corticosteroid binding globulin (CBG), which plays a role in regulating the levels of free bioavailable CORT (52). There was a large reduction in plasma CBG levels of subordinate (-70%) and dominant (-40%) rats relative to control rats, and there was a significant correlation between plasma CBG level and available Type II (or GR) receptors in the spleen. Corticosteroid receptor levels in these adrenal-intact animals reflect the number of receptors that were unoccupied by endogenous hormone at the time of sacrifice. As a result of the reduced CBG, both subordinate and dominant rats had fewer available splenic Type II receptors than control rats, suggesting that a greater proportion of receptors were occupied and activated by endogenous hormone at the time of sacrifice in these animals than in control rats. These results suggest that a decrease in CBG levels as a result of chronic social stress led to greater access of free CORT hormone to Type II receptors in the spleen than is typically present in rats under basal or acute stress conditions. This increased stimulation of splenic Type II glucocorticoid receptors could, in turn, provide a mechanism by which chronic stress may have a greater impact than acute stress on splenic immune function (52). The glucocorticoid resistance associated with social stress may have a compensatory role by moderating glucocorticoid suppression of immune responses to pathogens and wounds resulting from aggressive social interactions (53).

3.2. Effects on hypothalamic-pituitary-gonadal (HPG) axis

It is well established that the hypothalamic-pituitary-gonadal axis can be suppressed in response to chronic stress. Decreased testosterone is one of the first signs of stress-induced decline in reproductive function in males (54). Subordinate animals consistently have suppressed testosterone production during chronic social stress (55-57). In studies using the

VBS model, plasma luteinizing hormone (LH) and plasma testosterone levels of subordinates are poorly correlated across time, relative to what occurs in both controls and dominants (54).

Plasma LH levels of subordinates remain similar to those of control through at least 1 week of social stress, and are lower than both the control and dominant groups by the end of 2 weeks of VBS housing. In contrast, plasma testosterone levels in subordinates are already lower than those of dominant rats by Day 2 of VBS housing, a time point at which subordinate plasma LH levels are still normal. Thus, the reduction in plasma testosterone in subordinates occurs prior to a decrease in LH suggesting that chronic stress may have influences on the HPG axis at multiple levels to affect testosterone production and reproductive function.

In collaboration with Matt Hardy at the Population Council, we found that the decrease in testosterone levels in subordinate rats may be explained, at least in part, by the failure of Leydig cells to increase 11β HSD oxidative activity to compensate for higher circulating glucocorticoids (54). High circulating levels of glucocorticoids inhibit the Leydig cell's production and secretion of testosterone. Thus, the Leydig cells are normally protected from high circulating plasma glucocorticoids by 11β HSD, which catalyzes the conversion of biologically active glucocorticoids into their inert 11-keto metabolites, thereby allowing for normal steroidogenesis to occur. Subordinate rats have reduced 11β HSD protein levels and reduced 11β HSD activity, in contrast to dominant and control animals who have normal plasma testosterone levels and similar levels of 11β HSD protein and enzyme activity (58). Of note, however, is that reductions in plasma testosterone may occur as early as Day 2 of VBS housing, and have been observed prior to elevated basal levels of corticosterone, again indicating that testosterone production is modulated by multiple factors.

3.3. Effects on serotonin systems

Animals housed in the VBS showed alterations in several neurotransmitter systems. One of the Blanchards' earliest studies of the VBS showed that defensive behavior in subordinate rats was associated with elevated 5HIAA/5HT ratios in several brain regions (59). Furthermore, the subordinates exhibited changes in 5HT_{1A} and 5HT_{2A} receptor binding that mimicked those seen in our laboratory and others in response to chronic stress (e.g. (60-62) (Figure 4).

Regarding HPA activity and serotonin, subordinate rats had elevated basal corticosterone (CORT) levels relative to dominants and individually housed controls. Stress responsive subordinates (SRS) showed reduced binding to 5-HT_{1A} receptors compared to controls throughout hippocampus and dentate gyrus (Figure 4). NRS showed decreased binding only in CA3 of hippocampus. In addition, dominants showed decreased 5-HT_{1A} binding in CA1, CA3, and CA4 compared to controls. Both responsive and nonresponsive subordinates showed increased binding to 5-HT₂ receptors in parietal cortex compared to controls. No changes were observed in binding to 5-HT_{1B} receptors (63). Moreover, both dominants and subordinates showed decreased binding to the serotonin transporter (5HTT) in Ammon's horn compared to controls, while 5HTT binding remained unchanged in dentate gyrus and raphe (47). The functional significance of these changes is unclear; however, alterations in serotonergic function are thought to be involved in several stress-associated disorders, such as major depression, PTSD and anxiety disorders.

3.4. Effects on catecholamine systems

The neurochemical consequences of chronic social stress were diverse and widespread, affecting not only the serotonergic system, but also noradrenergic and dopaminergic pathways as

well. Tyrosine hydroxylase (TH) mRNA levels measured with in situ hybridization were elevated in locus coeruleus (LC) of non-responsive subordinates (NRS) compared to singly or pair-housed controls; NRS also had higher TH levels than dominants (64). TH protein levels determined by immunautoradiography were also higher in LC of NRS and stress responsive subordinates (SRS) versus pair-housed controls (Figure 4). Since the activity of LC neurons is increased in response to stress (Jacobs et al., 1991), it is likely that the changes in TH allow an increase in the synthetic capacity of the noradrenergic neurons in order to compensate for stress-related demands upon the system.

The dopaminergic system also responded to the social stress of the VBS (65). For this study rats were exposed for 2 weeks to the VBS and classified as SRS and NRS. After a brief recovery period, half of the cohorts were subsequently returned to the VBS for two additional cycles of reexposure and recovery. NRS rats showed decreased dopamine transporter density after a single VBS exposure in the dorsolateral caudate putamen (DLCPu) and also showed decreased DA transporter density in the nucleus accumbens (Acb) after repeated VBS exposure. In addition, dopamine D2 receptor density was elevated after a single VBS exposure in the Acb of both subordinate groups (SRS and NRS) and after repeated VBS exposure in the DLCPu, dorsomedial CPu, and Acb of NRS compared with controls. No changes in dopamine D1 receptor binding were observed in any group. Stress has long been known to increase vulnerability to drug abuse and addiction (66) and these finding provide a possible mechanism by which long-lasting changes in mesolimbic dopamine activity induced by chronic stress contribute to this vulnerability.

3.5. Neurogenesis and neuronal morphology in the hippocampal formation

The proliferation of new cells and the structure of existing neurons in the hippocampal formation were also affected by social hierarchies in the VBS. Although neurogenesis in the dentate gyrus did not differ between dominants and subordinates, more new neurons were observed in the dentate gyrus of the dominant males compared with both subordinates and controls (67). Under the conditions of this particular VBS system, which used Sprague-Dawley rats, dominant and subordinate animals did not differ in basal, stress or recovery levels of CORT and they also did not differ in thymus, adrenal gland, and body weights, suggesting that variables other than stress were responsible for the changes in adult neurogenesis. The differences in brain structure persisted among the animals that had no access to the burrow system after the dominance hierarchy stabilized, suggesting that social status rather than living in a complex environment accounted for the effect of dominance on adult neurogenesis.

Chronic social stress also led to significant remodeling of the apical dendritic trees of the pyramidal neurons in the hippocampus. There was a decrease in the number of branch points and total dendritic length in the apical dendritic trees of CA3 pyramidal neurons in dominant animals compared to unstressed controls; subordinates also had a decreased number of dendritic branch points (47)(Figure 5). The similarity of the changes in serotonin transporters (5HTT) binding, summarized above, and dendritic arborization between both groups of VBS animals, despite apparent differences in stressor severity experienced by the animals, suggests that these changes may be part of the normal adaptive response to chronic social stress (47). Yet, because of the greater dendritic shrinkage in dominants compared to subordinates, in spite of the larger adrenals in subordinates, these findings took us away from the simplistic idea that CORT caused it all, and it also turned attention to the impact of the social environment, as will be discussed in the next section on the human brain.

Indeed, the mechanism for dendritic shrinkage has subsequently been shown to involve multiple interacting mediators, with excitatory amino acid receptors playing a major role, along with other mediators such as brain derived neurotrophic factor (BDNF), tissue plasminogen activator (tPA), corticotrophin releasing hormone (CRH) and others; a more complete discussion of these mechanisms may be found elsewhere (27, 68).

4. Systemic effects of social stressors

Although the majority of the effects of social stress discussed above are focused on the brain, even the earliest studies of this model showed widespread effects on systemic physiology. The most basic observation of increased mortality and morbidity among the subordinates (42) demonstrated the consequences of chronic social stress on the overall health and wellbeing of the organism. The alterations in splenic glucocorticoid receptors discussed above may contribute to impaired immune responses in these animals; indeed, the subordinate animals in the VBS appeared to be particularly vulnerable to mycoplasmic respiratory infections endemic to the colony in Hawaii (Blanchard and Blanchard, unpublished observations).

Sakai went on to establish a 12 VBS facility in his laboratory at the University of Cincinnati in 2000 and has replicated many of the salient effects of social stress documented in the VBS experiments at the University of Hawaii. In addition, the Cincinnati studies used rats obtained from a commercial vendor indicating that the VBS model may be generalized to rat populations other than the in-house colony maintained in Hawaii. The consistency of the data and generalizability of the model emphasize the value and broad utility of the VBS model developed by Bob and Caroline Blanchard in studying social stress. Sakai and his colleagues extended use of the VBS model into investigations of the metabolic consequences of chronic

stress, the role of androgens in social hierarchy formation and maintenance, and the influence of early life stress on social hierarchy development, among others.

Changes in body weight and metabolism were apparent early on (45). Although the decreases in body weight among colony animals were most dramatic in the versions of the VBS in which food was only available in the “surface” area, subordinate animals showed a rapid and sustained loss of up to 15% of their body weight, even when they were provided with food either during frequent “vacations” from the VBS, or within the burrows themselves (57, 69). Body weight of dominants was affected by VBS housing as well: the dominants typically lose a small but significant amount of weight in the VBS, and also fail to gain weight at the rate of the control animals (57). The metabolic basis of the weight loss differs between the two groups, as the subordinates lost both adipose and lean tissue, whereas the weight loss in the dominant animals was attributable to loss of adipose tissue alone (56). Leptin and insulin levels have been shown to co-vary with adiposity (70) and these two hormones were decreased in the subordinate animals and, to a lesser extent, in the dominants as compared to controls.

An important adaptation to the VBS set up in Sakai’s lab was outfitting each apparatus with sophisticated computerized monitoring systems to measure food intake and determine meal patterns for each animal in the colony (identified by a subcutaneously implanted microchip) during chronic stress (Figure 1). Food intake is decreased in all animals upon introduction to the VBS; however, dominants quickly returned to their basal level of intake, while the subordinates showed a continued suppression in both the number and size of meals despite having access to food and water in all areas of the VBS; the diurnal pattern of meal consumption was also shifted among the subordinates perhaps suggesting altered circadian rhythms as well (71, 72).

When animals were removed from the VBS, subordinates were hyperphagic and quickly

regained their body weight, but did so primarily as visceral adipose tissue while dominants regained body weight as both lean and adipose tissue (57, 72). Thus, body composition changed following social stress and this occurrence was hypothesized to be due to the different endocrine conditions that subordinate and dominant animals were in at the end of social housing in the VBS. That is, subordinates had low testosterone and high corticosterone levels and this state would promote adipose tissue deposition. In contrast, since dominant animals have testosterone levels similar to that of controls and corticosterone levels intermediate to that of controls and subordinates, dominants could regain weight as both lean and adipose tissue. Indeed, by treating subordinates with 5 α -dihydrotestosterone (DHT), a non-aromatizable, reduced form of testosterone, weight loss during chronic stress in the VBS was not affected, but DHT promoted weight gain as lean body mass during recovery (55).

While the subordinates had lower levels of insulin and leptin than dominants and controls while in the VBS, they became hyperinsulinemic and hyperleptinemic following the recovery phase (57). The metabolic changes occurring in these animals suggest that chronic subordination may lead to a state that mimics metabolic syndrome in humans, as well as altering patterns of food intake in a way that may contribute to obesity (57, 73). These findings lead to consideration of potential interventions to promote weight gain in subordinates following chronic stress as lean muscle mass rather than adipose tissue. Amylin is a peptide hormone that is co-secreted with insulin from pancreatic β -cells and can act to reduce food intake and decrease weight gain and fat mass while preserving lean mass in obese subjects (74). Administering a low dose of amylin to rats after chronic stress in the VBS prevented hyperphagia in both dominant and subordinate rats for 3 days while having no effect on food intake in controls. Amylin suppressed fat mass gain in dominant and subordinate rats while lean mass was not affected.

Thus, amylin treatment was effective in preventing greater post-stress adiposity in subordinate rats (75). Interventions such as these may be informative in developing useful clinical therapeutics as adverse metabolic conditions, such as obesity, diabetes and cardiovascular disease, are highly co-morbid with stress disorders and related mental illness.

These findings exemplify the important fact that stressors and stress hormones have complex effects on systemic physiology, including adaptive responses that may be facilitated by via acute secretion of glucocorticoids and catecholamines, followed by potentially detrimental changes elicited by chronic stress and persistent high glucocorticoid levels. More broadly, along with the glucocorticoid cascade hypothesis by Robert Sapolsky (14), these findings contributed to the concepts of allostasis and allostatic load, emphasizing the protective as well as damaging effects of stress on the brain and body, operating through the brain (5, 76).

5. Translation to and from the human brain

Using the perspective of allostatic load (77), extension of these findings to the human brain has revealed the enormous range of influences of social factors and health behaviors in hippocampal health and manifestations throughout the brain and body. Studies of the human hippocampus have demonstrated shrinkage of the hippocampus not only in mild cognitive impairment and Alzheimer's disease (78), but also in Type 2 diabetes (79), prolonged major depression (80), Cushing's disease (81) and post-traumatic stress disorder (PTSD) (82). Moreover, in non-disease conditions, such as a 20 year history of chronic stress (83), chronic inflammation (84), lack of physical activity (85) and jet lag (86), smaller hippocampal or temporal lobe volumes have been reported. Regarding the underlying cause of volume reduction, autopsy studies on depression-suicide have indicated loss of glial cells and smaller neuron soma

size (87), which is indicative of a smaller dendritic tree. This is consistent with the findings summarized above from animal models.

With regard to Type 2 diabetes, it should be emphasized that the hippocampus has receptors for, and the ability to take up and respond to insulin, ghrelin, insulin-like growth factor-1 (IGF-1) and leptin; IGF-1 has also been shown to mediate exercise-induced neurogenesis (88). Thus, besides its response to glucocorticoids, the hippocampus is an important target of metabolic hormones that have a variety of adaptive actions in the healthy brain; these functions are likely to be perturbed in metabolic disorders, such as diabetes (88).

The metabolic syndrome, which includes insulin resistance associated with cardiovascular risk factors and obesity, shows a strong relationship to socioeconomic status (SES), (89, 90). Obesity and Type 2 diabetes in low SES teenagers are associated with impaired cognitive impairment, smaller hippocampal volume and altered white matter structure (91). The effects of lower SES extend to other brain regions such as the prefrontal cortex where lower SES is associated with reduced gray matter volume (92), and with obesity, systemic inflammation and altered white matter structure (93, 94). Thus the social environment over the lifecourse plays a powerful role in shaping the brain (77, 95).

But the hippocampus is not the only brain region affected. The young adult human prefrontal cortex reflects the effects of chronic stress by showing impaired cognitive flexibility and reduced functional connectivity that parallels the effects of stress in the young adult rat brain, including the reversibility after the end of the stressful period (96-99). The studies of circadian disruption complement those on the hippocampus/temporal lobe noted above in flight crews suffering from chronic jet lag (86) and raise important questions about how the brain handles shift work, jet lag and chronic sleep deprivation (100). Furthermore, aging in rats is associated

with failure to spontaneously reverse shrinking of medial prefrontal cortical neurons after chronic stress (99) and this harkens back to the glucocorticoid cascade hypothesis (14).

Indeed, when brain circuits remain changed, there are behavioral states and cognitive impairments that also remain and some of these may be maladaptive. Amygdala overactivity is a consequence of exposure to traumatic stressors in a PTSD-like animal model that produces a delayed increase in spine density in basolateral amygdala along with a delayed increase in anxiety-like behavior (101). Amygdala overactivity is also associated with mood disorders (102) and amygdala enlargement is reported in children of chronically depressed mothers (103). Similarly, hippocampal volume reduction in prolonged depression, Type 2 diabetes and Cushing's disease is associated with cognitive and mood impairment (79, 80, 104, 105). These conditions require external intervention that may include use of antidepressants (106), surgery to reduce hypercortisolemia (81), regular physical activity (107) and mindfulness-based stress reduction (108).

6. Closing perspectives

The insight Bob and Caroline Blanchard had in developing and interpreting the findings from the Visible Burrow System made an enormous contribution to the current view that the human brain is very sensitive to the social environment and to agonistic interactions between individuals. Their collaboration with Sakai and McEwen at the Rockefeller extended application of the Visible Burrow System model to demonstrate that it also was a unique and highly relevant neuroethological model with which to study stress and adaptation to stressors. These studies focused on the brain and systemic organ responses to stress and, in turn, described that the brain is also very responsive to changes in systemic physiology.

Finally, we would like to close on a more personal note. Bob Blanchard's scientific contributions to and influence on the field of behavioral neuroscience is clear and examples of this are elegantly discussed in this Special Issue. What may be less often talked about is Bob's commitment to training and providing high caliber biomedical research opportunities to undergraduate students at the University of Hawaii, particularly to underrepresented minority students, the vast majority of whom have gone on to professional careers in biomedical research and medicine. But Bob's influence did not stop there. With the establishment of the collaboration at The Rockefeller University with the McEwen lab and the expansion of use of the Visible Burrow System model at the University of Cincinnati in the Sakai lab, Bob's reach extended from the middle of the Pacific to have national and international impact. Two of the co-authors of this manuscript, McKittrick and Tamashiro, used the Visible Burrow System social stress model in their Ph.D. research projects. Other graduate students and post-doctoral fellows at Rockefeller and Cincinnati also capitalized on the model, continued to build upon the foundation the Blanchards established in Hawaii, and have gone on to successful professional careers of their own, including Drs. David S. Albeck (University of Colorado-Denver), Dennis C. Choi (Emory University), Jon F. Davis (Washington State University), Elizabeth Duncan-Vaidya (Cuyahoga Community College), Eric G. Krause (University of Florida), Susan J. Melhorn (University of Washington), Mary M.N. Nguyen-Choi (Booz-Allen Hamilton), Karen A. Scott (University College Cork, Ireland), Robert L. Spencer (University of Colorado-Boulder), Yoshifumi Watanabe (Yamaguchi University, Japan), and others too numerous to list here. It is clear that Bob Blanchard and his wife Caroline have had, and will continue to have, a significant influence on future generations of scientists and educators far and wide as Bob's legacy lives on. Mahalo nui loa, Bob.

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Figure legends

Figure 1. Diagram of a Visible Burrow System (VBS). The VBS apparatus is constructed primarily from black plexiglas and measures 1 m². There is a large open surface area which is maintained on a 12h:12h light:dark cycle by a light bulb mounted above the area. The open surface area is connected via clear plastic tunnels to one large and one small chamber which have clear plastic lids. The tunnels and chambers are maintained in 24 h darkness to mimic the natural underground burrow systems that rats typically live in. An infrared light source and video camera (not pictured) are mounted directly above each VBS to allow for 24h behavioral observations of all animals in the colony. Food and water are available in the open surface area and in both chambers to provide all animals with adequate access. Microchip identification scanners and associated AccuDiet food intake monitors identify each animal and record the amount and time of each meal.

Figure 2. Corticosterone response to acute novel stressor following 14-day chronic social stress in the VBS. Plasma corticosterone was measured in tail blood collected 3 h after the start of the light cycle (BASAL), after 60-min restraint stress (STRESS), and after 60-min recovery in the home cage (RECOVERY). The stress-nonresponsive subordinate (NRS) rat fails to mount a normal HPA response to acute stress compared to control (CON), dominant (DOM) and stress-responsive subordinate (SRS) rats. * $P < 0.05$ vs. CON, DOM, NRS; # $P < 0.05$ vs. CON, DOM, SRS.

Figure 3. Dominance hierarchies and the CRH (CRF) and HPA system. Consistent with the failure of stress-nonresponsive subordinate (NRS) rats to mount a normal HPA response to acute stress, NRS have reduced hypothalamic CRH mRNA compared to control (CON), dominant (DOM) and stress-responsive subordinate (SRS) rats (A and B). In contrast, CRH mRNA expression in the amygdala of NRS is similar to that of the SRS and elevated compared to that in the DOM and CON (C) (49).

Figure 4. Summary of the neurobiological changes in the brains of subordinate rats following chronic social stress.

Figure 5. Dendritic remodeling in CA3 hippocampal pyramidal neurons of dominant and subordinate rats in VBS. Apical dendrites of CA3 pyramidal neurons of both dominant and subordinate rats are shorter and less branched than controls, with dominant rats showing the shortest length and least branching (47).

Figure 6. Summary of systemic effects of social subordination on components of the HPA axis.

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Table 1. Behavioral, physiological, and endocrine consequences of chronic social stress in rodents.

MEASURE	EFFECT	REFERENCES
BEHAVIORAL		
Offensive or aggressive behavior	↑DOM	Blanchard 1990 (43), 1995(69), Tamashiro 2004 (56)
Defensive or submissive behavior	↑SUB	Blanchard 1990 (43), 1995 (69), Tamashiro 2004 (56)
Reproductive behavior	↓SUB	Blanchard 1995 (69)
Social contact	↓SUB	Blanchard 1989 (49)
PHYSIOLOGICAL		
Adrenal weight	↑DOM, ↑SUB	Blanchard 1995 (69), Tamashiro 2004 (56), McKittrick 1995 (63)
Thymus weight	↓DOM, ↓↓SUB	Blanchard 1995 (69), Tamashiro 2004, (56) McKittrick 1995 (69)
Spleen weight	↑SUB	Blanchard 1995 (69), Tamashiro 2004 (56)
Testes weight	↓SUB	Blanchard 1995 (69), Tamashiro 2004 (56)
METABOLIC		
Body weight	↓SUB	Blanchard 1995 (69), Tamashiro 2004 (56), Melhorn 2010, Nguyen 2007 (55)
Total body adiposity	↓DOM, ↓SUB	Tamashiro 2004 (56), Melhorn 2010 (72), Nguyen 2007 (55), Tamashiro 2007 (57)
Food intake (during stress)	↓SUB	Melhorn 2010 (72)
Food intake (during recovery)	↑SUB	Melhorn 2010 (72)
Visceral:subcutaneous fat	↑SUB	Melhorn 2010 (72), Nguyen 2007 (56), Tamashiro 2007 (57)
Lean body mass	↓SUB	Tamashiro 2004 (56), Melhorn 2010 (72), Nguyen 2007 (55)
Insulin (fasted)	↑SUB	Tamashiro 2007 (57)
Leptin	↓DOM, ↓SUB	Tamashiro 2004 (56)
HPA AXIS		
Baseline corticosterone (CORT)	↑DOM ↑↑SUB	Blanchard 1995 (69), McKittrick 1995 (63), Blanchard 1993, McKittrick 2000 (47)
Corticosterone binding globulin (CBG)	↓DOM, ↓↓SUB	Spencer 1996 (52)
Available splenic glucocorticoid receptors	↓DOM, ↓↓SUB	Spencer 1996 (52)
11β-hydroxysteroid dehydrogenase (11βHSD) oxidative activity	↑DOM	Hardy 2002 (54)
CORT response to acute novel stress	↓Non-responsive SUB	Blanchard 1995 (69), Tamashiro 2004 (56), Albeck 1997 (49)
HPG AXIS		

Testosterone	↑↑DOM, ↓SUB	Blanchard 1995 (69), Tamashiro 2004 (56), Nguyen 2007 (55) , Hardy 2002 (54)
Luteinizing hormone (LH)	↓SUB	Hardy 2002 (54)

Table 2. Neurobiological changes following chronic social stress in rodents.

MEASURE	EFFECT	REFERENCES
MR in CA1 of hippocampus	↓SUB	Chao 1993 (48)
GR in CA1 of hippocampus	↓SUB	Chao 1993 (48)
GAP-43 in CA1 of hippocampus	↓SUB	Chao 1993 (48)
AVP mRNA in medial nucleus of amygdala	↓SUB	Albeck 1997 (49)
AVP mRNA in PVN	No difference	Albeck 1997 (49)
CRH mRNA in central nucleus of amygdala	↑SUB	Albeck 1997 (49)
CRH mRNA in PVN	↑DOM, ↑SRS, ↓NRS	Albeck 1997 (49)
Dopamine system		
Enkephalin mRNA in nucleus accumbens *	↑SUB	Lucas 2004 (65)
Dopamine D2 receptor binding *	↑NRS	Lucas 2004 (65)
Dopamine D1 receptor binding *	No difference	Lucas 2004 (65)
Dopamine transporter binding *	No difference	Lucas 2004 (65)
Serotonin system		
5-HIAA level in limbic areas	↑SUB	Blanchard 1991 (59)
5-HT _{2A} receptor binding in cortex	↑SUB	McKittrick 1995 (63)
5-HT _{1A} receptor binding in hippocampus	↓DOM, ↓SUB	McKittrick 1995 (63)
5-HT _{1A} receptor binding in median raphe	↓SUB	McKittrick 1995 (63)
5-HT transporter binding in hippocampus	↓↓DOM, ↓SUB	McKittrick 2000 (47)
Neuronal morphology		
Dendritic length of apical CA3c pyramidal cells	↓↓DOM, ↓SUB	McKittrick 2000 (47)
Dendritic branch points of apical CA3c pyramidal cells	↓↓DOM, ↓SUB	McKittrick 2000 (47)

* After 3 cycles of VBS stress and recovery

SRS = stress-responsive subordinate

NRS = non-responsive subordinate

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.