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The Visible Burrow System: A View From Across the Hall

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Abstract

The visible burrow system (VBS) is an ethologically relevant social stress model that creates a distinct dominance hierarchy in rats. Randall Sakai's laboratory performed an impressive series of studies documenting the very different impact of VBS exposure on the brain and behavior of dominants (DOM) and subordinates (SUBs). Hierarchy formation causes pronounced changes in metabolism in SUBs relative to both DOMs and unstressed controls, resulting in marked weight loss and metabolic imbalance. Stress testing revealed multiple phenotypes in the VBS, including DOMs, stress-responsive SUBs and stress-non-responsive SUBs. Stress-responsive SUBs have adrenal hypertrophy and elevated baseline corticosterone, consistent with prolonged HPA axis activation; however, peak acute stress responses are not sensitized. In contrast, stress non-responsive individuals do not mount a response to an acute stress, suggesting HPA axis hypofunction. In brain, SUBs exhibit a pattern of gene regulation consistent with impaired stress inhibition (e.g., hippocampal adrenocorticosteroid receptor down-regulation and dendritic retraction) and drive of stress pathways (e.g., increased locus coeruleus tyrosine hydroxylase expression). The non-responsive phenotype is distinguished by down-regulation of paraventricular nucleus corticotropin releasing hormone expression and enhanced neuropeptide Y expression in amygdala. The brain 'signature' created by VBS hierarchy formation differed substantially from that of another well-studied chronic stress model (chronic variable stress). Thus, the impact of VBS is mediated by neurocircuit mechanisms at least in part distinct that of other chronic stress modalities, and suggests that the nature of the stressor may be an essential consideration in development of treatment strategies for stress-related diseases.

Introduction

Stress is a pervasive biological problem that contributes to a variety of disease states in all species. Physiological responses to stress are adaptive in nature, causing redistribution of resources to meet a real or anticipated challenge. However, chronic or severe stress can prolong or exaggerate physiological (as well as psychological) reactions, a process that if left unchecked can lead to morbidity and mortality. Importantly, whether or not stress is

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‘maladaptive’ depends on both the intensity of the adverse event(s) and the individual’s ability to cope [1]. The latter component is evident in the heterogeneity of stress responses across individuals, and lies at the heart of Randall Sakai’s groundbreaking work using the Visible Burrow System (VBS).

A variety of animal models are used to study the impact of chronic stress on physiology and behavior. The vast majority of these rely on manipulations with limited ethological relevance, involving exposure of animals to stressors or situations that are not part of the animals’ ecological niche. For example, the chronic variable stress (CVS) protocol I use involves exposing rats to cold, swimming, restraint, altered housing density, hypoxia, etc. While we use it to limit predictability and habituation (and it results in a reliable and reproducible spectrum of stress endpoints), the majority of the manipulations in the CVS regimen involve physical interactions with conditions and apparatus that do not mimic stimuli found in the rat’s ecological niche.

The VBS relies on development of dominance to drive individual stress phenotypes in male rats (see [2]). In this model, male rats are housed with females in a large burrow apparatus, consisting of a large open area and two side chambers separated by tunnels (a full description is available in [cite article in this issue]). The presence of females is a key feature, as it drives development of the hierarchy. Importantly, the animals are placed in the burrow and left largely undisturbed for a prolonged period of time (usually two weeks), during which the animals sort themselves into dominants and subordinates. The dominants typically spend the most time in the large open area, and will pursue and bite subordinates, generally driving them into the side chambers. Interactions are videotaped to help identify dominants and subordinates, using behavioral characteristics as well as wounding (location and number) as indicators of social position. Dominants exhibit more aggressive behaviors than subordinates (biting, chasing, pinning). Subordinates tend to have wounds in the tail and body (chase wounds), dominants in the head and face area (defense wounds) [2, 3]. The ‘self-organizing’ aspect of the hierarchy is its ethological strength, as it is generated by the animals themselves and not the experimenter.

The VBS Stress ‘Phenotype’

Exposure to the VBS produces differential stress phenotypes in the dominants and subordinates. Generally, both dominants and subordinates lose significant weight upon initial placement in the burrow and maintain a lower body weight throughout the VBS exposure [3]. In virtually all experiments performed in the VBS (Table 1), subordinates lose significantly more weight than dominants. Indeed, this invariant feature of the dominance hierarchy fueled Randall’s extensive work focusing on social stress and metabolism, much of which was performed during his time at Cincinnati (e.g., see [3–8]).

The VBS dominance hierarchy typically produces alterations in HPA axis function. In about two thirds of burrow experiments measuring HPA endpoints, baseline corticosterone (typically taken after removal from the VBS) was increased relative to controls and/or dominants (see Table 1 for references). In a subset of experiments corticosterone was also elevated in dominants, but typically to a level below that of subordinates. Elevated

corticosterone is accompanied by reduced levels of corticotropin binding globulin in subordinates [9], predictive of an even more pronounced increase in free corticosterone. Adrenal weight was disproportionately increased in subordinates in about 63% of experiments, consistent with enhanced long-term drive of the HPA axis. Moreover, in 40% of experiments, thymic atrophy was observed, consistent with long-term glucocorticoid hypersecretion.

It is not entirely surprising that enhanced HPA axis drive is a somewhat variant feature of the VBS. There are several reasons why this may occur. First, corticosterone is secreted in a pulsatile manner [10], which may introduce noise and obscure detection of differences, particularly when baseline secretory activity is assessed during the circadian nadir (as was characteristic for VBS studies). Notably, reproducing baseline increases in corticosterone secretion can also be an issue using other chronic stress models (including CVS: contrast [11] and [12]). Second, the VBS model necessitates removing the animals from the burrow to sample blood. Thus, the values attained will reflect the timing and treatment during removal, which may vary between experiments. Third, assessment of baseline values ignores corticosterone ‘spikes’ linked to agonistic interactions in the subordinates, which creates a cumulative glucocorticoid burden that is not detectable in the isolated ‘baseline’ situation. Finally, elevated basal corticosterone was observed in 80% of studies performed in Hawaii, and <50% of studies performed at Cincinnati.

Differences in the mechanics of the VBS protocol may account for differences in the apparent ‘magnitude’ of the stress phenotype, which appears more pronounced in the Hawaii studies. There are several factors that may contribute to these differences. One of the authors (KLT) had the opportunity to run studies at both Hawaii and UC, and notes several differences in the background of animals used for VBS studies. First, the source of Long-Evans rats differed between sites: rats from the Hawaii studies were derived from a long-standing colony maintained at the University of Hawaii, whereas rats included in the UC studies were non-littermates purchased from commercial vendors (Harlan, Charles River). Genetic isolation is sufficient to produce behavioral differences that may affect stress outcomes (see [13]). Indeed, the Long-Evans rats from the Hawaii colony appeared to be more aggressive, as often subordinates needed an occasional separation from the colony due to excessive wounding. Second, in the Hawaii burrows food was available only in the large chamber, whereas food was available in all three chambers in the UC apparatus. The need to enter the dominant’s ‘domain’ to eat may have engendered more agonistic interactions. Finally, Harlan and Charles River wean rats into pens of about 20, permitting some degree of socialization, whereas rats were weaned to cages of 2–3 in Hawaii. Increased early socialization may limit the overall ‘impact’ of the VBS experience in the UC colonies.

The 4 (or 5) male:2 female colony reliably yields one dominant (hereafter designed as DOM) and 3–4 subordinate (SUB) males. However, it is also clear that not all subordinates are equal. Some VBS colonies can produce subordinates that have severely blunted 60 minute corticosterone responses, to the point where there is no significant difference between baseline, peak and recovery time points (i.e., they are stress ‘non-responsive’ subordinates (NRS))(e.g., see [3, 14, 15]). Typically only 1 of 3–4 subordinates emerged as a non-responder for any given colony. Corticosterone responses to restraint typically do not

differ between stress-responsive subordinates (SRS) and DOM or controls [3, 14, 15], suggesting that the stress non-responders alone had an HPA axis stress response phenotype following subordination.

Recent work from Melhorn (this issue) has further clarified the issue of heterogeneity within subordinate subpopulations [16]. On the basis of wound burden and location, her study identifies a more severe subordinate stress group (referred to as Omegas) that have markedly potentiated weight loss relative to DOM and other subordinates (SRS), including rather marked loss of lean body mass. Again, there is typically one Omega rat per colony. Importantly, identified Omegas are also stress hyporesponsive, having a negligible corticosterone response to restraint challenge. These data suggest the hierarchical structure extends beyond a single dominant and submissive phenotype, and that the Omega individuals may be the most severely affected of all groups [16].

It is important to consider the significance of the NRS phenotype. Work exploring stressor severity in mice suggests that severe social stress can produce a marked adrenal insufficiency [17]. This is of course a maladaptive and potentially life-threatening condition. In addition, the loss of lean body mass seen in the Omega subordinates [16] represents a severe metabolic challenge, undeniably maladaptive. The nature and severity of the Omega phenotype recalls the failure phase of Selye's General Adaptation Syndrome, wherein the system is pushed beyond the ability to adapt effectively [18]. While significant mortality is not observed in the Omega subordinates, it is highly possible that continuing exposure beyond the typical period may result in further debilitation and may eventually be life-endangering.

In many of Randall's VBS studies at Cincinnati, clear stress non-responsive individuals were not identified. This raises the question as to whether the non-responsive subordinate is an invariant component of the VBS hierarchies. However, it is important to consider that prior to the most recent work [16], the non-responsive 'call' was made on the basis of HPA axis stress profiles, rather than wounding or body weight. Given the recent data on wounding and body weight, it is possible that the existence of the OMEGA individuals may have been underestimated by reliance on corticosterone profiles as the primary identifier. In this respect, it would be interesting to retrospectively mine prior studies.

Randall's group spent a considerable amount of time understanding how the dynamics of the colony social structure determined the stress phenotypes. Switching from 4 male:2 female to all male completely blocked formation of dominance hierarchy. Indeed, in the all-male colonies that majority of social behavior was 'rough and tumble' play, with little in the way of aggression or biting. As was the case for DOM in the standard colonies, all animals lost a modest amount of weight, likely due to the additional activity afforded by the physical as well as social environment [7].

In contrast, switching to a 2 male:4 female colony resulted in a more pronounced stress phenotype in the subordinate males [3]. Unlike the 4 male:2 female colonies, the females also showed aggression toward the subordinate male, perhaps contributing to the stress

phenotype. The cause of the severe weight loss seen in this VBS configuration is unclear, but may be related to differences in the behavior of the females in the female biased condition.

Impact of VBS exposure on the brain

Randall was keenly interested in understanding how social status affects brain structures responsible for stress regulation, as a means toward identification of potential mechanisms underlying the very different stress phenotypes seen in DOMs vs. SUBs. In line with strong evidence for the hippocampus in stress signaling, his group spent considerable effort studying the impact of VBS exposure on hippocampal endpoints (Table 2). Expression of both glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) was decreased in subfield CA1 of the hippocampus in both SRS and NRS subordinates relative to controls [19]. Expression of GR in CA1 (or whole hippocampus) is decreased in other chronic stress models (e.g., water immersion-restraint [20], swim in combination with restraint [21]), providing a cross-validation that VBS subordination is read by the CNS as a stressor. Both GR and MR genes are subject to regulation by exogenous glucocorticoids [22], and it is generally thought that down-regulation by stress is a cumulative effect of elevated glucocorticoids (which is supported in the case of VBS and other stress models by adrenal hypertrophy and/or thymic atrophy). Loss of hippocampal GR is associated with HPA axis hyper-reactivity following stress, due to impaired negative feedback [23]. However, subordinates in the VBS do not show any consistent evidence of increased stress reactivity, and indeed in the case of non-responders, show hypo-reactivity. These data may reflect compensation for decreased hippocampal GR signaling in other regions. Alternatively, it is possible that the degree of GR (and MR) down-regulation (generally quite small and limited to CA1) is not sufficient to generate a change in functional protein. Indeed, GR and MR binding do not appear to be affected in SUBs [2].

Subordinates (subtype not specified) also exhibit decreased GAP43 expression in subfield CA1 [19]. This gene is linked to neurite growth [24], and may be related to known effects of chronic stress on dendritic complexity. Indeed, morphological analysis indicates decreased apical dendrite branching in CA3 [25]. However, it is important to note that decreased branching was also observed in DOM animals, and indeed, only DOM animals show decreased apical dendrite length [25]. Thus, the morphological consequences may be driven by the VBS experience itself, independent of social status.

Hippocampal serotonin and GABA are thought to play key roles in integration of hippocampal information processing (as well as mood). Receptor binding studies indicate decreased 5HT1A binding in DOM and SRS groups, and decreased serotonin transporter binding in DOM and SUB groups [15], suggesting reduced serotonin-mediated inhibition and reuptake, respectively, and thereby enhanced serotonin signaling capacity. However, it is important to note that reduced 5HT1A binding was not observed in the NRS group [15], differentiating this group from both DOM and SRS animals and implying intact serotonergic inhibition of hippocampal output neurons. Elevated expression of glutamic acid decarboxylase 67 (GAD67) mRNA is observed in all hippocampal subfields, as well as the medial prefrontal cortex of SUB animals [26]. GAD67 is an enzyme responsible for conversion of glutamate to GABA in neurons, and contributes to generation of a readily

releasable (rather than stored) pool of GABA at terminals [27]. Enhanced GAD67 mRNA would be predicted to promote inhibition of hippocampal and prefrontal output in the context of stress. Since these regions are implicated in trans-synaptic inhibition of stress responses [28], enhanced inhibition would be expected to enhance physiological and behavioral responses to stress, consistent with the observed changes in behavior and adrenal weight in subordinates.

Corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) are key neuropeptides involved in hormonal and behavioral responses to stress [29]. HPA axis responses to stress are mediated primarily by hypophysiotrophic CRH and CRH/AVP neurons in the paraventricular nucleus (PVN) [29], making this a logical place for assessment of changes in central drive as a result of VBS exposure. Cellular expression of CRH mRNA in the PVN was increased in DOM and SRS rats relative to non-responsive animals but not controls [14], suggesting that the VBS regimen did not produce a pronounced central drive of CRH expression. In contrast, expression of CRH mRNA in NRS rats was equivalent to that of control in one experiment and lower than control in another. Decreased CRH expression is consistent with the lack of HPA axis drive in the NRS group [14]. It is not known whether this change is due to active suppression of CRH gene expression, presumably following a history of elevated glucocorticoids (or an alternative mechanism), or by a phenotypic failure of the NRS group to engage CRH expression as a consequence of stress. Exposure to VBS did not enhance PVN AVP mRNA in any group [14].

Both CRH and AVP are involved in processing of emotional information. Anxiety and fear behaviors are believed to involve CRH neurons in the central amygdaloid nucleus (CeA) and bed nucleus of the stria terminalis (BST) (see [30]). Expression of CRH mRNA is upregulated in the CeA of both SRS and NRS rats, and in the oval (but not fusiform) nucleus of the BST (BSTov) of SUB rats [14, 31]. The CeA and BSTov have a close anatomical linkage, and it has been proposed that CRH acts in both regions to enhance physiological and behavioral responses to stress [32]. Moreover, CRH expression is increased by glucocorticoid exposure in both regions [33, 34]. Thus, it is possible that long-term activation of these regions may underlie behavioral and physiological sequelae of subordination.

The medial amygdala (MeA) plays an important role in social behavior and aggression [35]. Gene expression data indicate that AVP mRNA is increased, and NPY mRNA decreased in the MeA of SRS, NRS and Omega groups [14, 16]. AVP plays a role in social behavior and aggression [36], and loss of AVP may be associated with submissive behaviors in subordinates. Notably, MeA AVP is positively regulated by testosterone [37, 38], which is reduced in SUBs, suggesting a connection between the loss of gonadal steroids and submission. In general, NPY is thought to inhibit CRH neurons in the amygdala [39], which may further reduce MeA-mediated aggressive behavior.

The locus coeruleus (LC) also plays a general role in stress and arousal [40, 41] via widespread noradrenergic projections to limbic forebrain structures and autonomic relays. Expression of tyrosine hydroxylase (mRNA and protein), the rate-limiting enzyme for

norepinephrine (NE) synthesis, is increased in the LC of SRS and NRS rats [42]. Expression of the co-localized neuropeptide galanin is also increased in SUB rats [43]. The data support increased synthesis of NE and galanin, which may enhance LC-mediated excitation in stress-regulatory targets.

It is important to note that for most endpoints, SRS, NRS and Omega groups show similar gene expression changes. One notable exception to this rule is NPY mRNA expression in the basolateral amygdala (BLA) and CeA, which are only up-regulated in the Omega group. Given that both regions are critically involved in emotional regulation, and that NPY is generally inhibitory to amygdalar neurons [39] [44], these data raise the intriguing possibility that dysregulation of amygdala output underlies the aggressive phenotype observed in the Omega submissive, perhaps due to a failure to mount appropriate inhibitory responses.

Overall, the numerous studies, performed in different experimental iterations and in different labs, provide a rather consistent picture of a stress-activated brain in subordinate groups. In general, changes restricted to DOMs are more minor in extent, limited to decreased GAD67 expression in PVN projecting neurons of the peri-PVN zone and intrafascicular BST [26] and enhanced expression of orexin receptors in the prefrontal cortex [45]. However, while the weight of evidence supports a wide-reaching alteration in brain stress circuits, it is important to consider the limitations of these data. The vast majority of the evidence comes from in situ hybridization studies, which measure mRNA expression but do not provide a test of function. In addition, many of the mRNA changes are relatively small, in many cases <25%. Moreover, while it is clear that stress pathways are engaged, it is difficult to pinpoint whether expression changes drive the social status, or result from establishment of the social hierarchy or the stress associated with hierarchy formation. Answering these questions will require intervention studies to address the role of anatomical and/or molecular targets in the VBS phenotypes.

The VBS vs. Chronic Variable Stress

It is important to consider whether the VBS subordinate ‘phenotype’ presents an example of a generalized reaction to chronic stress, or has general and regimen-specific features, or represents its own distinct spectrum of responses to chronic drive. To begin to address this issue, included in Tables 2 and 3 are columns summarizing data from taken from our group, focusing on changes in stress regulatory pathways following the CVS regimen commonly used in my lab. This procedure involves exposure of rats to a series of unpredictable stressors twice per day over a prolonged period (minimum of one week, usually 14 days) (see [11]). We use this regimen to provide a random (to the rat) exposure of stressors at unpredictable times, limiting the ability of the animal to anticipate stress exposure and obviating potential habituation to stressors as a result of repeated exposure. This regimen produces a fairly reliable spectrum of HPA axis changes, including reduced weight gain, adrenal hypertrophy, thymic atrophy, and baseline elevations in circulating corticosterone in the AM (see [11]). All of these changes can also be observed in VBS studies, suggesting that physiological endpoints are a common feature of chronic stress exposure and not characteristic of either CVS or social stress. As was the case for VBS, the body weight

phenotype is observed in all experiments, and the other endpoints in most (but not all) studies.

Changes in brain tell another story. Inspection of Table 2 shows a quite different pattern across circuits assessed in both CVS and the VBS. (It is notable that many of the in situ hybridization studies were performed in the same lab environment (my group and Randall's), using the same protocols). It is particularly noteworthy that no alterations in gene expression were observed in the CeA or BST following CVS [46], in contrast to changes seen in the VBS [14, 31]. While the list of commonly assessed genes is short, the data suggest that in aggregate, VBS exposure results in more prominent engagement of key amygdala nuclei linked to social behavior, aggression and anxiety/fear. In contrast, the CVS paradigm causes increased expression of GAD65 mRNA in a number of PVN projecting regions, including the anterior subdivisions of the BST, dorsomedial hypothalamic nucleus, medial preoptic nucleus, and peri-PVN region [47], areas linked to trans-synaptic inhibition of the PVN. These results are more consistent with CVS actions on subcortical structures relaying limbic information to the PVN, and imply a different locus of control.

Up-regulation of LC transcripts and TH protein are observed in VBS subordinates but not following CVS, suggested selective recruitment of LC neurons in the former. However, it is important to note that CVS causes increased TH mRNA and protein expression in the nucleus of the solitary tract [48], which projects to the CRH-containing division of the PVN [49] as well as PVN-projecting regions in the hypothalamus and anterior BST. To our knowledge NTS TH expression has not been assessed in VBS studies, and thus it is not clear whether TH up-regulation is specific to LC or common across other noradrenergic cell groups. Nonetheless, the differences between the stress regimens point toward differential engagement of noradrenergic neurons by unpredictable vs. social adversity.

Common features to both VBS and CVS include up-regulation of GAD67 mRNA expression in hippocampus and prefrontal cortex [26, 47]. This implies that both stressors may promote inhibition of stress regulatory structures, which may participate in upstream control of glucocorticoid negative feedback.

It should be noted that the full HPA axis phenotype differs between CVS and VBS. While both regimens can produce elevated basal levels of corticosterone, the CVS regimen is associated with a marked sensitization or facilitation of responses to new stressors (e.g., in [46]), whereas acute responses to (novel) restraint challenge do not differ amongst control, DOM and SRS groups in any VBS study (Table 1). In addition, both CRH and AVP mRNA expression are induced in the PVN of rats following CVS, but VBS only results in increased CRH mRNA (in both DOM and SRS), and that only relative to the NRS group [11, 14]. It is well known that AVP synergizes with CRH to promote ACTH release at the corticotrope and elevated AVP would be predictive of greater central drive of the HPA axis in CVS vs. VBS. Overall, the data would appear to point toward CVS being a more 'severe' stress regimen (from an HPA axis perspective) than VBS, at least for standard subordinates.

Perspective

The first author considers himself fortunate to have been able to interact with Randall for some 15 years, the last 10 with our offices across the hall from each other. As a consequence of our frequent interactions and participation in our student's dissertation committees, I grew to appreciate his keen insight on the problem of social stress and its relation to his true scientific 'love', ingestive behavior. He opened my mind to the value of considering stress in a social setting, and his work, in combination with my own, highlighted the heterogeneous impact of stress on the brain. The marked differences between central actions of social subordination and chronic unpredictable stress indicate that the conditions create in essence a different 'brain'. Understanding the nature of the circuitries involved will be important in determining how social adversity (e.g., 'bullying') and situation stress (e.g., economic pressures) may drive stress-related pathologies via distinct mechanisms.

There is still considerable work to be done on understanding the impact of VBS exposure on brain and behavior. For example, it is probably naïve to assume that the social stress environment has minimal effect on the DOM, based on the measures we currently have in our pocket. The pressures of 'maintaining the alpha position' may well play out via different stress effector system, e.g., sympathoadrenomedullary drive. In addition, it would be of immense interest to understand the impact of the VBS on females, who in fact are involved in generating the hierarchy and are 'witnesses' to the impact of aggression on both DOMs and SUBs. Finally, the NRS/Omega individuals are of keen interest for understanding factors responsible for stress coping strategies. The passing of Randall, as well as his mentor Bob Blanchard, undoubtedly slows progress on these important questions. Nonetheless, Bob and Randall's pivotal work on the model has influenced new interest in social stress models, and I anticipate that work in this area will be an important component of stress neurobiology research moving forward.

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Table 1

Summary of Visible Burrow System Stress Endpoints

Study	Elevated CORT	Peak CORT	Thymus	Adrenal	Body weight	Where?	M/F ratio
Albeck et al (1997) exp 1	↑DOM, NRS, SRS	↓NRS				Hawaii	5:2
Albeck et al (1997) exp 2	No	↓NRS				Hawaii	5:2
Blanchard et al (1995) exp 1	↑DOM, SUB		No	↑SUB	SUB<DOM<CON	Hawaii	4:2
Blanchard et al (1995) exp 2	↑SUB		↓DOM, SUB	No	SUB<DOM<CON	Hawaii	4:2
Chao et al (1993)	↑NRS, SRS	↓NRS				Hawaii	
Choi et al (2006)	↑SUB		No	↑SUB	SUB<DOM<CON	UC	4:2
Davis et al (2009)	↑SUB				SUB<DOM<CON	UC	4:2
Duncan et al (2006)	No				↓SUB	UC	4:2
Hardy et al (2002)	↑SUB, DOM	↑SUB				Hawaii	4:2
Lucas et al (2004)	No	↓NRS				Hawaii	4:2
McKittrick et al (1995)	↑SRS	↓NRS		NRS>DOM, CON		Hawaii	5:2
McKittrick et al (2000)	↑SUB, DOM		SUB<DOM<CON	SUB>DOM>CON	SUB<DOM<CON	Hawaii	4:2/5:2
Melhorn et al (2010)	↑SUB			↑SUB	SUB<DOM<CON	UC	4:2
Monder et al (1994)	↑SUB			SUB,DOM>CON		Hawaii	4:2
Nguyen et al (2007)	No				SUB<DOM<CON	UC	4:2
Melhorn et al (2017)	No	↓OM			OM<hSUB<DOM<CON	UC	4:2
Smeltzer et al (2012)	No				SUB<DOM<CON	UC	4:2
Tamashiro et al (1997)	↑SUB				SUB<DOM<CON	UC	4:2
Tamashiro et al (2004)	↑SRS	↓NRS	↓SUB	No	SRS,NRS<DOM<CON	UC	4:2
Watanabe et al (1995) exp 1	↑SRS	↓NRS			SRS,NRS <DOM<CON	Hawaii	4:2
Watanabe et al (1995) exp 2	↑DOM	↓NRS				Hawaii	4:2

↑ = increased expression relative to control; ↓ = decreased expression to control; - = no change from control; n.d. = not determined

DOM = dominant, SUB = subordinate (subgrouping not specified or identified); NRS = non-responsive subordinate; SRS = stress responsive subordinate; OM = Omega grouping (Melhorn et al, [16]).

Table 2

Impact of Visible Burrow System and Chronic Variable Stress on Hippocampal Gene Expression, Receptor Binding and Morphology

	Dom	Sub	NRS	CVS
Gene Expression				
Glucocorticoid Receptor	-	↓ ^I (CA1)	↓(CA1)	↓(CA1,DG),- ^I
Mineralocorticoid Receptor	-	↓ ^I (CA1)	↓(CA1)	↓(CA1,CA3,DG),- ^I
GAP43	-	↓(CA1)	n.d.	n.d.
Proenkephalin	-	-	n.d.	n.d.
GAD65	-	-	n.d.	-
GAD67	-	↑(CA1,CA3,DG)	n.d.	↑(CA3,DG)
BDNF	-	-	-	-
Receptor Autoradiography				
5HT1A	↓(CA1, CA3)	↓(CA1,CA3,DG)	-	n.d.
5HTT	↓(CA3)	↓(CA3)	n.d.	n.d.
Receptor Binding				
Glucocorticoid Receptor	-	-	-	n.d.
Mineralocorticoid Receptor	-	-	-	n.d.
Morphology (CA3)				
Pyramidal Cell Branching: basal	-	-	-	n.d.
Pyramidal Cell Branching: apical	↓	↓	-	n.d.
Dendritic Length: basal	-	-	-	n.d.
Dendritic Length: apical	↓	-	-	n.d.

Data compiled from refs [2, 11, 12, 15, 19, 25, 26, 55]

↑ = increased expression relative to controls; ↓ = decreased expression relative to controls; -= no change from control; n.d. = not determined

^I = small decrease (~20%) observed in [11], but not replicated in [12].

Table 3

Impact of Visible Burrow System and Chronic Variable Stress on Extra-Hippocampal Gene/Protein Expression

	Dom	Sub	NRS	CVS
Gene Expression				
Medial Prefrontal Cortex				
GAD67	-	↑	-	↑
Orexin-1 Receptor	↑	-	-	n.d.
Orexin-2 Receptor	-	-	-	n.d.
Amygdala: Central n.				
CRH	-	↑	↑	-
GAD65	-	-	n.d.	-
GAD67	-	-	n.d.	-
NPY	-	-	↑	-
Amygdala: Medial n.				
AVP		↓	↓	n.d.
GAD65	-	-	n.d.	-
GAD67	-	-	n.d.	-
NPY	-	↑	↑	-
Amygdala: Basolateral n.				
NPY	-	-	↑	-
Bed Nucleus of the Stria Terminalis				
CRH (Oval)	-	↑	n.d.	-
GAD67 (Oval)	-	-	n.d.	-
BDNF (Oval)	-	↑	n.d.	-
CRH (Fusiform)	-	-	-	-
GAD65 (Principle)	-	-	n.d.	-
GAD67 (Principle)	-	-	n.d.	-
GAD65 (Intrafascicular)	↓	↓	n.d.	- /
GAD67 (Intrafascicular)	↓	-	n.d.	-
GAD65 (Anterior)	-	-	-	↑
GAD67 (Anterior)	-	-	-	↑
Hypothalamus: PVN				
CRH	-	- , ↑ /	↓	↑
AVP	-	-	-	↑
Hypothalamus: periPVN				
GAD65	n.d.	n.d.	n.d.	↑
GAD67	↓	-	n.d.	-
Hypothalamus: Arcuate n.				-

	Dom	Sub	NRS	CVS
NPY	↑	↑	n.d.	↑
POMC	n.d.	n.d.	n.d.	↓
Hypothalamus: Dorsomedial n.				
GAD65	n.d.	n.d.	n.d.	↑
GAD67	-	↑ ²	n.d.	-
NPY	-	-	n.d.	n.d.
Hypothalamus: Preoptic Area				
GAD65 (medial preoptic n)	-	-	n.d.	↑
Locus Coeruleus				
Galanin	-	↑	n.d.	
Tyrosine Hydroxylase (mRNA)	-	↑	↑	-
Tyrosine Hydroxylase (protein)	-	↑	↑	-

Data from references [11, 14, 16, 26, 31, 42, 43, 45, 47, 52, 53, 56–58]

↑ = increased expression; ↓ = decreased expression; - = no change from control; n.d. = not determined

¹ = # detectable CRH neurons increased relative to controls; no change in cellular expression level relative to controls [14].

² = increased relative to dominant only.