

Ethology, Neurophysiology, Neuropharmacology, Sex Differences, and Effects of Stress on the Defensive Burying Paradigm: A Review.

Koblanski1  
1University of British Columbia

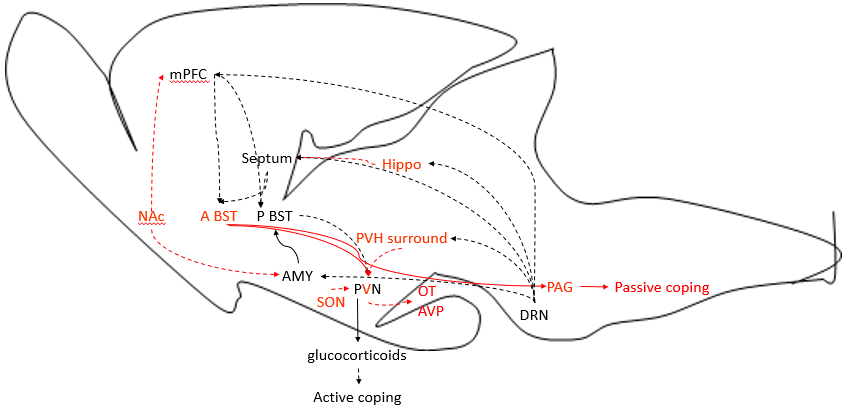
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**Abstract**

he defensive burying paradigm can inform how stressor controllability affects stress adaptation, which has clinical implications with regards to adaptive coping responses following presentation with a stressful situation. Active coping (notably defensive burying) is associated with a controllable stressor, promoting stress adaptation, thus decreases stress hormone levels. In opposition, chronic stress and uncontrollable stressors lead to an increase in passive coping behaviours, with elevated stress hormone levels. Several brain regions have been implicated in active and passive coping, as well as neurotransmitter systems, which can be evaluated via pharmacological manipulation. No sex differences were found in defensive burying, although there were effects of sex hormones within sex.

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# Introduction The defensive burying test explores the rodent neurobiological exhibition of avoidance, fear, and anxiety-related behaviour based on its tendency to use bedding material to bury noxious stimuli. In the wild this is effective to protect against predation/ other rats.1 Shock prods are an example of noxious stimuli2. Although different in function, intensity, and form, rodents may also bury apparently harmless novel objects such as marbles.3 Noxious stimulus exposure causes an increase in stress hormones, heart rate, blood pressure, and body temperature.1,4 Previous literature has operationalized psychological indices by measuring representative behaviours, according to their motor endpoints. All of these behaviours can be quantified by scoring 10-15 minute videos to make an ethogram, using software such as JWatcher.1,5 Behaviours reflecting similar psychological indices can share a CNS basis, which can be modulated by pharmacological sensitivity, sex differences, sex hormones, and stress.

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**Figure 1. Active and passive coping pathways in the rat brain.** Pathways related to active coping behavioural responses are in black, and passive are in red. Solid arrow indicates sufficient confirmation of the pathway in this behavioural paradigm; dashed arrows indicate a likely pathway based on other research. Brain regions only involved in active coping are in black, passive in red, and both in black and red. mPFC = medial prefrontal cortex; A & P BST = anterior & posterior bed nucleus of stria terminalis; AMY = amygdala; SON = supraoptic nucleus; NAc = nucleus accumbens; PVN = paraventricular nucleus of the hypothalamus; PVH = paraventricular hypothalamus; DRN = dorsal raphe nucleus; PAG = periaqueductal grey; Hippo = hippocampus; OT = oxytocin; AVP = arginine vasopressin.

# CNS and pharmacological basis for active coping

Active coping strategies may be mediated at the level of the medial prefrontal cortex (mPFC), septum, medial amygdala (MeA), posterior bed nucleus of stria terminalis (P BST), and dorsal raphe nucleus (DRN) with some effects of serotonin, arginine vasopressin (AVP), and testosterone (T).

Lesions to the mPFC6 and lesions or inhibition of the septum1, led to less defensive burying. The PFC7 and septum8 converge onto the P BST to affect stress hormone release.9

However, subregions in the amygdala that project to the P BST10 (the lateral amygdala (LA) through the posterior bundle via the stria terminalis, the central amygdala (CeA) through the anterior bundle, and the medial amygdala (MeA) through the ansa peduncularis) can affect fear retention, and defensive burying. Lesions to the LA affected fear acquisition, but not expression.11 Lesions to the CeA seemed to be important for fear expression, but did not affect defensive burying on their own.12 However, a positive correlation was found between higher burying, androgen receptor (AR) immunoreactivity, and AVP mRNA in the rostrocaudal extent of the MeA.5 Overall these data suggest a more intricate involvement of the amygdala in fear acquisition and expression.

While Lesions to the P BST also failed to affect burying,1 AR immunoreactivity and AVP mRNA in the P BST (more apparent in the interfascicular and transverse nuclei) was positively correlated to higher burying.5 In addition, AVP expression in the MeA and P BST was found to be dependent on T. Further, there may be a positive correlation between burying and T, as high-bury rats had higher blood T levels in circulation, possibly to sustain AVP levels in the P BST & MeA. Moreover, the principal nucleus of the BST targets the medial parvicellular dorsal (mpd) region of the paraventricular nucleus of the hypothalamus (PVN) to regulate corticotropin releasing hormone (CRH) & AVP.13 This then leads to greater ACTH release from the anterior pituitary gland which leads to higher stress hormone release. Hence, it follows that high-burying animals showed a greater pituitary-adrenal response to acute restraint.5 A greater spike in stress hormones to stress or noxious stimuli may be necessary to enact an active coping response, whereby following a successful response to the stressful stimulus hormone levels can return to normal.1, 4 If however, rats are prevented from burying the noxious stimulus and forced to adopt a passive response, they show higher stress hormone levels, suggesting that burying is more adaptive when compared to passive coping responses.1

Lastly, lesions to the dorsal raphe nucleus (DRN), the site of serotonin synthesis, led to less burying.1 Serotonin could affect amygdala and septum activity, in addition to stimulating the HPA axis by inhibiting GABAnergic neurons in the PVH surround.9 Supporting this role for the DRN, an acute dose of tricyclic antidepressants and an SSRI decreased burying of the noxious stimulus.1 TCAs and SSRIs immediately increase serotonin at the postsynaptic synapse, but soon after cause a reduction in serotonin release due to negative feedback of the presynaptic 5-HT 1A receptor (5-HT 1AR).14 This decrease in presynaptic 5-HT 1AR activity may be responsible for a decrease in burying. Furthermore, 5-HT 1AR agonists also decreased burying. These effects were blocked by 5-HT 1AR antagonists.1 Overall these effects likely occur via the presynaptic 5-HT 1AR located in the DRN, when considering that DRN lesions also lead to decreases in serotonin and active coping.1 Nevertheless, more precise experiments would be required to determine if these drugs act on the pre- or postsynaptic 5-HT 1AR.

In all, these brain regions are involved in active coping, an adaptive coping strategy associated with decreases in glucocorticoid levels.

# CNS and pharmacological basis for passive coping

Passive coping involves the nucleus accumbens (NAc), anterior (A) BST, dorso parvicellular (DP) & posterior magnocellular (PM) neurons of the PVN, supraoptic nucleus (SON), and the hippocampus, with a role of GABA, oxytocin (OT), & AVP.

Activity in the caudal shell of the NAc was associated with passive coping, as inhibition resulted in an increase in defensive burying.15 This makes sense, as the NAc is involved in determining emotional valence and normally inhibits the mPFC and amygdala.16 Increase in defensive burying as a result of NAc caudal shell inhibition has been explained as a motivated response to threat following an assessment of emotional valence.15,16

In the A BST, tracing experiments verified projections from the prelimbic area (PRL) to the A BST neuron population, and from here to HPA effector neurons in the PVN and the central periaqueductal grey (cPAG), the latter of which were involved in passive coping.17 In addition, inhibition and stimulation of this prelimbic–A BST pathway increased and decreased passive coping, respectively, in the defensive burying test, without having any direct effect on burying itself (active coping), suggesting that the A BST is involved in activating a passive coping response.17

In the PVN, a negative correlation was found between OT mRNA levels in the PVN DP region and burying.5 Effects of OT may be mediated by the DP region’s descending influence on sympathetic nervous system activity.5 Burying duration was negatively correlated to AVP and OT mRNA within PVN and SON PM neurons.5 This may be a consequence of coping style rather than anxiety or fear, as AVP levels within these nuclei correlate positively in rats with anxiety in the elevated plus maze (EPM) task.5 Secretion of AVP/ OT in the ventricular system from these PVN subregions could affect other brain regions within this review and may be responsible for long-lasting behavioural changes.5

In the dorsal and ventral hippocampus acetylcholinesterase inhibitors led to defensive buying decreases.18 The number of c-Fos positive cells in CA1 was also positively correlated across animals with burying duration.19 Lack of impact of hippocampal lesions on burying duration, however, suggests that the hippocampus doesn’t act on coping behaviours independently, but rather through its indirect actions on other brain regions.1 That is, hippocampal acetylcholine may activate GABA projections to inhibit the septum.19 Mineralocorticoid receptor antagonists to the hippocampus were also shown to decrease burying,1 suggesting a hippocampal role in turning off the stress response and decreasing burying.9

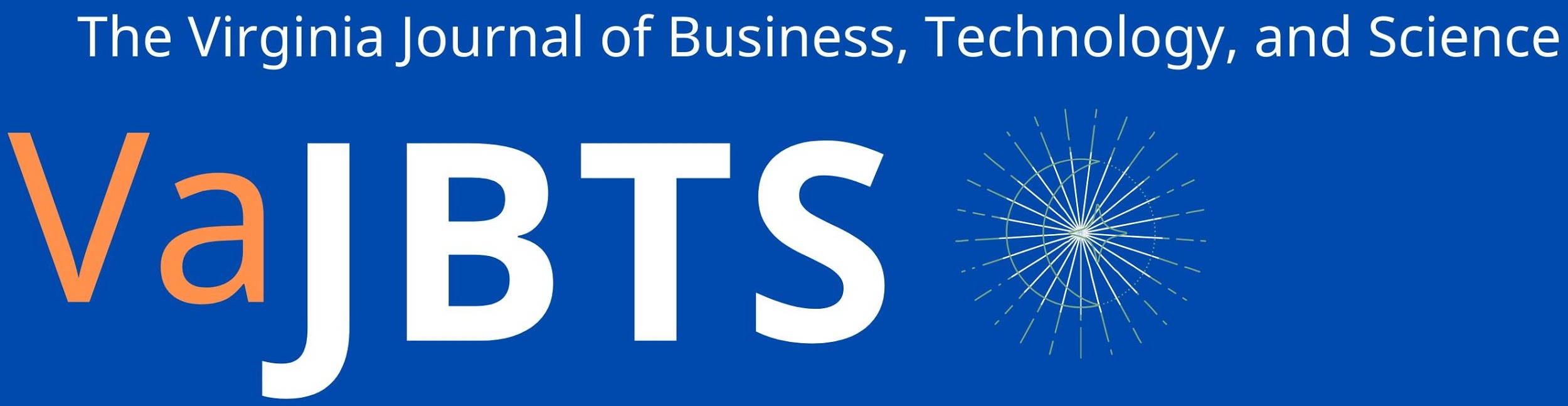
Defensive burying can be pharmacologically suppressed by benzodiazepines, reversed by benzodiazepine/ GABAA-receptor antagonists.1 Additionally, some anxiogenic agents like the GABAA receptor inverse-agonists h-CCE and DMCM enhanced the amount of shock prod burying in rats.1 GABA may also be used by the hippocampus to inhibit other brain regions involved in the active coping response.1 In addition, GABAA receptors are found in the BST and PVN surround where an inverse-agonist would lead to less activation of the GABA receptor, hence reduced inhibition of the BST to PVH pathway to increase burying, and an agonist would do the opposite.20

# Effects of sex hormones and sex

While researchers have not shown sex differences in burying,1 there is little animal research done in females.21 There is an effect of the estrus cycle on burying, possibly related to progesterone (P) levels. Specifically, burying was reduced during late proestrus1 during which there is a peak in P.22 Furthermore there was a decline in burying when ovariectomized females were treated with P, but not with estrogen.23 In addition, while T was positively correlated with burying in males,5 systemic T administration in females reduced burying.1 A similar study has yet to be performed in males. It was concluded that T produced these anxiolytic effects by acting on the GABAA receptor, rather than aromatization into estradiol (E2), as a GABAA antagonist, but not a selective aromatase inhibitor led to reduced burying.24 In all, while no sex differences were found, there were effects of sex hormones on burying within sex.

# Effects of stress

Active coping and burying of a noxious stimulus decreasing stress hormone levels is thought to reflect the concepts of stress controllability and adaptation.1 In contrast, passive coping responses are associated with elevated stress hormone levels and thought to reflect a passive or maladaptive coping strategy. There is a positive correlation between stress controllability and burying.25 Chronic stress on the other hand leads to an increase in passive coping or no change.4 Surprisingly, chronic stress led to an increase in burying when defensive burying tests were performed several days after stress termination.26 In sum, the effects of stress on burying, and how burying can affect stress hormone levels supports the hypothesis that defensive burying is a measure of adaptive vs maladaptive coping strategies.



# Conclusions

While pharmaceuticals have been useful to elucidate the involvement of specific brain regions and neurotransmitter systems, there is a lack of pharmaceutical validation that the defensive burying paradigm measures behaviours that are beyond anxiety and depression. The defensive burying paradigm is not regarded as a test of anxiety and depression and is instead more related to controllability and adaptation as elucidated by controllable, maladaptive, and adaptive stress paradigms.1 Notwithstanding, it is difficult to distinguish between the sedative and anxiolytic effects of benzodiazepines in this paradigm. It is similarly difficult to distinguish between the effects of some 5-HT 1A receptor agonists as causing serotonin syndrome vs decreasing active coping. The EPM test avoids this problem.27 With clinically effective anxiolytics, exploration of the open arms was increased, without increasing exploration in the enclosed arms, indicating that the test reflects anxiety.27 Behaviour in this test is majorly determined by the rat’s unconditioned aversion to open spaces and heights.28 However, the EPM and similar tests of anxiety are limited in their ability to study controllable and adaptive stressors. Therefore, defensive burying could nevertheless contribute to screening for anxiolytic properties of drugs.1

Overall, there has been little animal research done in females,21 and this is an issue as it can negatively impact the health of women.29 For example, treatments that work in men may work differently in women. Additionally, women are more likely to suffer from anxiety and depression than men.29

With these considerations in mind, as this behavioural paradigm reflects stress controllability and adaptation, it could model central components of stress-related psychiatric illness, such as preference for passive, avoidant coping strategies versus adaptive, active coping strategies.1

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