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regulating coordinated cardiovascular and respiratory function
during stress and arousal

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Abstract

Actual or potentially threatening stimuli in the external environment (i.e. psychological stressors) trigger highly coordinated defensive behavioral responses that are accompanied by appropriate autonomic and respiratory changes. As discussed in this review, several brain regions and pathways have major roles in subserving the cardiovascular and respiratory responses to threatening stimuli, which may vary from relatively mild acute arousing stimuli to more prolonged life-threatening stimuli. One key region is the dorsomedial hypothalamus, which receives inputs from the cortex, amygdala and other forebrain regions and which is critical for generating autonomic, respiratory and neuroendocrine responses to psychological stressors. Recent studies suggest that the dorsomedial hypothalamus also receives an input from the dorsolateral column in the midbrain periaqueductal gray, which is another key region involved in the integration of stress-evoked cardiorespiratory responses. In addition, it has recently been shown that neurons in the midbrain colliculi can generate highly synchronized autonomic, respiratory and somatomotor responses to visual, auditory and somatosensory inputs. These collicular neurons may be part of a subcortical defense system that also includes the basal ganglia, and which is well adapted to responding to threats that require an immediate stereotyped response that does not involve the cortex. The basal ganglia/colliculi system is phylogenetically ancient, while the defense system that includes the dorsomedial hypothalamus and cortex evolved at a later time, and appears to be better adapted to generating appropriate responses to more sustained threatening stimuli that involve cognitive appraisal.

Key words: defensive behavior, sympathetic activity, respiratory activity, hypothalamus, midbrain, sympathetic premotor nuclei
List of abbreviations used in the text:

Amy  Amygdaloid complex
BAT  Brown adipose tissue
BNST  Bed nucleus of the stria terminalis
CVLM  Caudal ventrolateral medulla
dIPAG  Dorsolateral perifornical area
DMH  Dorsomedial hypothalamus
dmPAG  Dorsomedial perifornical area
IC  Inferior colliculus
IPAG  Lateral perifornical area
mPFC  Medial prefrontal cortex
NTS  Nucleus tractus solitarius
PAG  Periaqueductal gray
PeF  Perifornical area
PVN  Paraventricular nucleus
RPG  Respiratory pattern generator
RVLM  Rostral ventrolateral medulla
RVMM  Rostral ventromedial medulla
SC  Superior colliculus
spSNA  Splanchnic sympathetic activity
viPAG  Ventrolateral perifornical area
An animal’s survival is dependent upon being able to respond appropriately to stressors, which can be categorized into two different groups: interoceptive (also called physical) stressors, and exteroceptive (also called psychological) stressors (44, 133). Physical stressors are those that directly threaten homeostasis, such as hypoxia, hemorrhage or infection, while psychological stressors can be defined as actual or perceived threats in the external environment. Psychological stressors can be further subdivided into conditioned stressors (i.e. ones that are normally innocuous but which are perceived as threatening because of previous experiences) or unconditioned stressors (i.e. ones that are intrinsically threatening, such as the sight, sound or odor of a predator). Physical and psychological stressors activate different populations of neurons in the brain (44, 63, 102). Similarly, there is evidence that the autonomic responses to conditioned and unconditioned psychological stressors are also mediated by different pathways in the brain (62). In this review, which is based on my 2013 Carl Ludwig Distinguished Lecture to the American Physiological Society, I shall consider the brain mechanisms that coordinate the physiological responses to unconditioned psychological stressors, which may range in intensity from mild alerting stimuli to extreme stimuli that may be life-threatening. Such stressors evoke a wide range of physiological responses including autonomic, hormonal and behavioral responses. I shall focus particularly on the brain mechanisms that generate coordinated cardiovascular and respiratory changes that support behavioral responses to psychological stress and arousal.

Pattern of cardiovascular and respiratory responses to stress and arousal

In animals, brief alerting stimuli such as an unexpected noise or light will evoke immediate autonomic and respiratory responses, characterized by strong cutaneous vasoconstriction and respiratory activation (17,18, 84, 109, 161). In
contrast, such non-noxious brief stimuli evoke only small and transient increases in arterial pressure and little or no changes in blood flow to the mesenteric, renal and hindlimb vascular beds (109, 162), indicating that the sympathetic outflow to the cutaneous vascular bed is rather selectively activated by mild alerting stimuli. Consistent with this, alerting stimuli in humans reliably increase cutaneous sympathetic activity (156). Brief alerting stimuli also evoke variable and often biphasic changes in heart rate (1, 8, 33, 161), due to the fact that there is co-activation of cardiac sympathetic and vagal parasympathetic activity (1, 8, 33, 123). Casto et al (32) suggested that the initial response to a novel alerting stimuli is an orienting reflex, which may lead to what has been termed a “defense reaction” or “visceral alerting reaction” (74) if the stimulus is prolonged or more threatening. Consistent with this view, Yu & Blessing (161) proposed that the cutaneous vasoconstriction that is initially evoked is associated with cerebral vasodilation, such that cerebral blood flow is increased at a time when the salience of the stimulus needs to be assessed. Regardless of the physiological significance of the initial orienting reflex, however, it has long been known that more prolonged threatening stimuli typically elicit a more complex autonomic response, which is characterized by an increase in arterial blood pressure, cardiac output and heart rate, together with marked vasoconstriction in renal and mesenteric vascular beds and usually also vasodilation in skeletal muscle beds, in both humans (6, 14, 27, 34, 60, 68, 75, 95, 99, 119, 157) (Fig. 1) and animals (24, 65, 106, 155). These cardiovascular changes are accompanied by a marked increase in total noradrenaline spillover in humans, indicative of an overall increase in sympathetic activity (158).

In humans direct measurements of the activity of sympathetic nerves innervating blood vessels in arm and leg skeletal muscle shows variable changes, including increases, decreases or no change, during psychological stress (mental
Thus the vasodilation in skeletal muscle and consequent increase in skeletal muscle blood flow evoked by psychological stress is due to non-neurogenic factors, which are believed to be primarily an increase in the level of circulating adrenaline as well as the effects of endothelial nitric oxide (99). Consistent with observations in humans, the activity of lumbar sympathetic nerves (which supply mainly skeletal muscle) is little altered during psychological stress in rats, whereas renal sympathetic nerve activity is greatly increased under these conditions (160). In summary, studies in both humans and animals indicate that psychological stress evokes a differentiated pattern of sympathetic responses, with marked increases in the activity of sympathetic nerves supplying the heart, skin, renal and mesenteric beds and adrenal medulla, but with highly variable effects on skeletal muscle sympathetic activity.

It is often assumed that the increase in arterial pressure during psychological stress is associated with inhibition of the baroreceptor reflex, based on early studies showing that electrical stimulation of sites within the classic hypothalamic “defense area” inhibits the depressor response normally evoked by baroreceptor stimulation (38, 108). It is now clear, however, that during naturally evoked psychological stress the baroreflex control of sympathetic vasomotor activity is not inhibited but instead is reset, such that both the arterial pressure and sympathetic vasomotor activity is regulated over a higher operating range, without any decrease in reflex gain, both in normotensive and hypertensive animals (20, 85).

Increases in respiratory rate are evoked by either brief alerting stimuli or more prolonged psychological stressors in both humans and animals (16-18, 84, 145). During more prolonged stress, the ventilation is also increased as demonstrated by a decreased end-tidal CO₂ (145). Hyperventilation is also a characteristic feature of panic disorder in humans (136), which is an extreme type of psychological stress.
Brain regions activated during acute psychological stress

Many previous studies have identified brain regions that are activated during unconditioned psychological stress, as indicated by c-Fos expression (e.g. 44-46, 64, 101, 121, 138, 139, 151)(Fig. 2). Many of these regions also play an important role in sympathetic and/or respiratory regulation, such as the paraventricular nucleus (PVN), dorsomedial hypothalamus (DMH), perifornical area (PeF), midbrain periaqueductal gray (PAG), parabrachial region, nucleus tractus solitarius (NTS) and the ventrolateral medulla. Such studies, however, do not clearly identify the particular neurons and pathways that mediate stress-evoked increases in arterial pressure, sympathetic activity and respiratory activity. Furthermore, c-Fos expression occurs only after sustained stimulation of neurons and so this method cannot be used to identify cell populations activated by brief alerting stimuli. Nevertheless, even though many questions remain unanswered, recent studies have provided much new information concerning the central pathways and mechanisms that generate coordinated sympathetic and respiratory responses during psychological stress and arousal.

In this review I shall propose that there are essentially two different systems in the brain that generate defensive behavioural responses associated with appropriate autonomic and respiratory changes. One of these defense systems includes the DMH and dorsolateral PAG as key components, and is dependent upon inputs to these regions from the amygdala, cortex and other forebrain regions. The second system is subcortical, and includes the basal ganglia and midbrain colliculi. This system is phylogenetically ancient, and is well adapted to responding to threats that require an immediate stereotyped response that does not involve the cortex. Before discussing the mechanisms by which these two defense systems generate
autonomic and respiratory responses to external threatening stimuli, however, I shall first consider which sympathetic premotor neurons in the brain drive increased sympathetic activity during psychological stress.

**Premotor nuclei driving stress-evoked sympathetic activity**

Sympathetic premotor neurons within the rostral ventrolateral medulla (RVLM), many of which are adrenaline-synthesizing neurons of the C1 group, are known to have a critical role in the tonic and reflex control of sympathetic vasomotor activity and blood pressure (41, 70). It is often assumed that these neurons would be strongly activated and thus drive increased sympathetic vasomotor activity in response to psychological stress. In a recent study from our laboratory (64), however, we found that air puff stress, an unconditioned psychological stressor of moderate intensity (138, 139, 149) that elicits significant increases in arterial pressure, resulted in only a small increase in c-Fos expression in neurons within the RVLM sympathetic premotor region (Figs. 2, 3A), and virtually no c-Fos expression in C1 cells (Fig. 3C). In response to baroreceptor unloading, however, which reflexly activates RVLM sympathetic premotor neurons (41, 70), there was a large increase in c-Fos expression in the RVLM, including C1 cells (Figs. 3C, 3D). Similarly, Dayas et al (44) found that another psychological stressor, noise stress, evoked c-Fos expression in only 4% of C1 cells, although there was substantial c-Fos expression in other brain regions that are known to be activated by psychological stress, including the PVN, medial amygdala, and A1 cell group in the caudal ventrolateral medulla.

In contrast, air puff stress, but not baroreceptor unloading, evoked a large increase in c-Fos expression in the region medial to the RVLM that has been termed the rostral ventromedial medulla (RVMM) (39, 144) (Figs. 2, 3). Similar to our results, Carrive and Gorissen (26) found that another psychological stressor
(conditioned fear) also evokes c-Fos expression in the RVMM but not in the RVLM. The RVMM, like the RVLM, contains sympathetic premotor neurons, some of which synthesize serotonin (144), but their precise function in cardiovascular regulation is not known. It is interesting to note, however, that Cox and Brody showed over 20 years ago that inhibition of neurons in the RVMM, but not the RVLM, greatly reduced responses evoked by stimulation of the hypothalamus (39). It is therefore possible that sympathetic premotor neurons in the RVMM (including serotonin neurons) are one of the main drivers of stress-evoked sympathetic hyperactivity (Fig. 4).

A study using retrograde transport of viral tracers showed that the neurons that regulate the sympathetic outflow to the kidney are located within the RVMM of the rat at a level just caudal to the facial nucleus (134), consistent within the fact that psychological stress increases renal sympathetic activity (85, 160). The more rostral part of the RVMM, at the level of the facial nucleus, contains premotor neurons that regulate the sympathetic outflow to the stellate ganglion (82) which contains sympathetic postganglionic neurons innervating sweat glands (2). Consistent with this, glutamate microinjections into this rostral part of the RVMM evokes sweating in the paw of the cat, but has no effect on arterial pressure (135). Sweating in the paw of the cat or rat may be analogous to sweating in the human hand, which is typically evoked by psychological stress (100). Furthermore, a brain imaging study in humans has shown that psychogenic sweating is associated with activation of a region in the rostral medulla that is homologous to the rostral RVMM region described above (56). Thus, taking all of these observations together, I suggest that psychological stress activates different groups of sympathetic premotor neurons in the RVMM that, as first suggested by Shafton and McAllen (135), are topographically organized such that premotor neurons regulating the renal and other vasomotor
outflows are located more caudally, while those regulating the sudomotor outflow are located more rostrally.

As mentioned above, the baroreceptor-sympathetic reflex is reset during psychological stress. Thus, although RVLM sympathetic premotor neurons may not be primary drivers of stress-evoked increases in sympathetic activity, they would still play a major indirect role by regulating sympathetic activity and arterial pressure within an increased operating range (Fig. 4).

The raphe pallidus in the midline medulla also contains sympathetic premotor neurons, particularly those that regulate the sympathetic outflow to the heart, cutaneous vasculature and brown adipose tissue (BAT) (112). Neurons within the raphe pallidus mediate stress-evoked tachycardia (54), cutaneous vasoconstriction (120) and activation of the sympathetic outflow to BAT, leading to thermogenesis and an increased body temperature (86) (Fig. 4). Many of the sympathetic premotor neurons within the raphe pallidus contain serotonin and also express inhibitory 5-HT1A receptors (72). In conscious rabbits activation of 5-HT1A receptors in the raphe pallidus greatly reduces the tachycardia evoked by psychological stress (116). Similarly, in anesthetized rats activation of 5-HT1A receptors in the raphe pallidus reduces leptin-evoked increases in BAT thermogenesis (111). Because activation of 5-HT1A autoreceptors inhibits serotonergic neurons, these observations suggest that the tachycardia and BAT thermogenesis in response to psychological as well as physical stressors are mediated to a substantial degree by serotonergic neurons within the raphe pallidus.

It is well known that neurons in the hypothalamic PVN regulate neuroendocrine responses to psychological stress (9)(Fig. 4). The PVN also contains sympathetic premotor neurons as well as neurons that project indirectly to the spinal sympathetic outflow via the RVLM (9), but a study in conscious rats showed that inhibition of the
PVN had no effect on the pressor response and tachycardia evoked by air jet stress, although it blocked the stress-evoked neuroendocrine response (142). Therefore, sympathoexcitatory neurons in the PVN are not a critical component of the central pathways mediating sympathetic responses to unconditioned psychological stress, although such neurons are activated by physical stressors such as an immune challenge or hypovolemia (3, 19). At the same time, PVN sympathoexcitatory neurons may also contribute to sympathetic responses evoked by unconditioned psychological stress, because it has been shown that approximately 10% of spinally-projecting PVN neurons are activated by a conditioned stressor (conditioned fear) (26). In addition, a small proportion (~7%) of PVN neurons projecting to the NTS are activated by air puff stress in rats (64). Therefore PVN neurons projecting to the NTS, and/or those projecting to the RVLM and spinal cord, may also contribute to resetting of the baroreceptor-sympathetic reflex during psychological stress (Fig. 4).

**Role of DMH and PeF in integrating stress-evoked responses**

Several observations clearly demonstrate the critical importance of the DMH in mediating stress-evoked cardiovascular and respiratory responses. In landmark studies, DiMicco and co-workers first demonstrated that inhibition of neurons within the DMH greatly reduced the pressor response and tachycardia evoked by air jet stress, whereas, as mentioned above, inhibition of neurons in the PVN had no effect (142, 143). This finding was later confirmed by others (101, 110). Similarly, inhibition of the DMH almost completely abolishes the neuroendocrine and respiratory responses to psychological stressors (17, 53, 142), while blockade of NMDA receptors in the DMH reduces the increases in arterial pressure, heart rate and respiratory rate evoked by lactate infusion in a rat model of panic disorder (83). In addition, activation of neurons in the DMH evokes a pattern of autonomic and respiratory effects, including a resetting
of the baroreceptor reflex, that are very similar to naturally evoked stress responses (23, 52, 53, 59, 76, 103, 104, 146). Finally, psychological stress evokes a marked increase in c-Fos expression in the DMH (64, 101, 132, 138) (Fig. 2).

It is well known that central respiratory activity can influence sympathetic activity, via connections from respiratory neurons to sympathetic premotor neurons within the brain stem (57). In response to disinhibition of DMH neurons, however, McDowall et al. (103) found that there was no consistent change in the amplitude of the respiratory-related variations in renal sympathetic nerve activity, despite the marked increase in the amplitude and frequency of phrenic nerve activity bursts that occur under those conditions. This indicates that the marked increase in sympathetic vasomotor activity in response to disinhibition of the DMH is not simply a consequence of the increased central respiratory drive. Similarly, Tanaka and McAllen (146) found that there was little correlation between increases in cutaneous sympathetic activity and phrenic nerve activity in response to activation of different sites within the DMH. It is therefore likely that the increased sympathetic and respiratory activities that are evoked by activation of DMH are mediated by separate independent descending pathways (Fig. 4).

Output pathways from the DMH mediating stress-evoked cardiovascular and respiratory responses. Since there is no direct projection from the DMH to the spinal cord (147), DMH neurons can only generate autonomic and respiratory responses via premotor supraspinal nuclei. There are direct descending projections from neurons in the DMH to the raphe pallidus in the midline medulla (86, 122, 132, 147, 150), and there is substantial evidence that these projections mediate the stress-induced tachycardia, cutaneous vasoconstriction and activation of the sympathetic outflow to BAT (86, 132,
150), which as discussed above is dependent upon activation of the raphe pallidus (Fig. 4).

Apart from the pathway to the raphe pallidus, however, descending direct projections from the DMH to the lower brainstem are relatively sparse (122, 147). Therefore the questions remains: what are the output pathways from the DMH mediating stress-evoked increases in sympathetic vasomotor activity and respiration? The DMH projects very heavily to other hypothalamic regions, including the PVN and PeF (147), both of which project to the spinal sympathetic outflow directly or via premotor nuclei in the medulla. As discussed above, there is little evidence that PVN neurons makes a major contribution to the increased sympathetic vasomotor activity evoked by unconditioned psychological stress, although PVN neurons projecting to the NTS may contribute to the stress-evoked baroreflex resetting. On the other hand, several lines of evidence suggest that the PeF may be a major source of sympathetic vasomotor drive during arousal or psychological stress. First, unlike the PVN (67), there is a strong direct connection from the PeF to the RVMM (122), which as discussed above may be a major driver of stress-evoked sympathetic activation. Second, there is also a projection from the PeF to the spinal cord, and the results of a combined c-Fos/retrograde labeling study suggests that the PeF-spinal pathway is one of the main sources of increased sympathetic activity during the conditioned fear response (26). In addition, there is a projection from the PeF to the NTS which, like the projection from PVN to NTS, may contribute to stress-evoked baroreflex resetting (Fig. 4)(64).

There are also significant projections from PeF neurons to brainstem regions that contain respiratory neurons, including the dorsomedial medulla and Kölliker-Fuse nucleus (125, 163), and disinhibition of PeF neurons can powerfully increase respiratory activity (80). It is therefore possible that DMH neurons activated by psychological stress
may increase respiratory activity via connections with Pef neurons that project to brainstem respiratory neurons.

Inputs to the DMH mediating stress-evoked cardiovascular and respiratory responses. The majority of inputs to the DMH arise from other hypothalamic nuclei. In fact, virtually every major hypothalamic nucleus provides an input to the DMH (148). In addition, there are major inputs from the bed nucleus of the stria terminalis (BNST), amygdaloid nuclei and medial prefrontal cortex (35, 78, 148, 153). In contrast, direct inputs from brainstem nuclei are sparse, except for a substantial input from the parabrachial nucleus (148). Given the fact that the DMH subserves many different functions, including ingestive behavior, thermoregulation and circadian rhythms in addition to stress responses (12, 54, 148), and the fact that the inputs to the DMH arise from many different sources, it is difficult to identify specifically the inputs that generate stress-evoked cardiovascular and respiratory responses.

Nevertheless, some information is available. The classic study by Klüver and Bucy (91) first demonstrated that lesions of the temporal lobes (including the amygdaloid complex) abolished fear responses normally elicited by threatening stimuli. Later, Blanchard and Blanchard (15) showed that this was due specifically to lesions of the amygdaloid complex. More recently, it has been shown that inhibition of neurons in the amygdaloid nuclei by microinjection of muscimol greatly reduces both the cutaneous vasoconstrictor and respiratory responses evoked by brief alerting stimuli such as noise or light (18, 109, 162). Furthermore, lesions of the amygdaloid complex also reduce the hindlimb vasodilation and mesenteric vasoconstriction that are evoked by a more prolonged threatening stimulus (65). Psychological stress activates neurons in the medial and basolateral amygdala (44, 64), both of which project to the DMH both directly and indirectly via the BNST (35, 93, 148). In addition, disinhibition of both the
basolateral amygdala and BNST evokes increases in arterial pressure and respiratory activity, similar to those evoked by psychological stress (165), and these effects appear to be mediated by excitatory amino acid receptors in the DMH (137). Taken together, these observations indicate that neurons in the medial and basolateral amygdala that are activated by threatening stimuli activate neurons in the DMH via both direct and indirect pathways (Fig. 4).

There is also a major input to the DMH from the infralimbic cortex in the medial prefrontal cortex (78, 153), which is believed to have a role in regulating autonomic functions associated with stress (40, 117). Activation of neurons in the infralimbic cortex reduces the pressor and tachycardic response evoked by psychological stress (115), while inhibition of the ventral part of the medial prefrontal cortex that includes the infralimbic cortex results in increased c-Fos expression in the DMH and also the medial amygdala (101). These findings suggest that the input from the infralimbic cortex acts to reduce stress-evoked autonomic (and possibly also respiratory) activation, via an inhibitory action on DMH neurons or on neurons in the medial amygdala which, as discussed above, provide an excitatory input to the DMH.

In summary, the DMH is a critical region for integrating autonomic and respiratory responses to psychological stress and arousal. The DMH is immediately medial to the PeF, however, and it is not clear whether the neurons that are critical for the expression of these responses are essentially confined within the DMH or whether they extend into the PeF. Although microinjections of muscimol centered on the DMH greatly reduce stress-evoked cardiovascular and respiratory responses (see above), the effects of muscimol microinjections centered on the adjacent PeF have not been performed. It has been shown, however, that neurotoxic lesions centered on the PeF largely abolish the cardiovascular response associated with conditioned fear (62). As discussed above, there are extensive interconnections between the DMH and PeF (147, 148), and it is
therefore possible that DMH neurons may regulate autonomic and respiratory activity via projections to PeF neurons. The precise functional relationship between DMH and PeF neurons in regulating stress-evoked responses is an important question that will need to be addressed in future studies.

*The role of orexin neurons in regulating stress-evoked cardiovascular and respiratory responses.* Neurons containing orexin (also known as hypocretin) are located within the DMH, PeF and lateral hypothalamus (125). It is believed that orexin neurons located in the PeF and DMH are primarily involved in arousal and stress, whereas those located more laterally in the lateral hypothalamus are primarily involved in reward processing (71). In orexin knockout mice, the cardiovascular and respiratory responses evoked by disinhibition of the DMH/PeF are greatly reduced compared to responses in wildtype mice (87, 164). Similarly, systemic administration of the dual orexin receptor antagonist Almorexant reduces the cardiovascular responses associated with arousal and psychological stress but not those associated with cold stress, a physical stressor (63). In addition, the increases in arterial pressure, heart rate, renal sympathetic nerve activity and respiratory rate evoked by disinhibition of neurons in the PeF and DMH is reduced by about 50% after systemic administration of Almorexant (80). Consistent with these observations, arousal and psychological stress activates orexin neurons in the PeF and DMH, but not those in the lateral hypothalamus (63).

Orexin neurons in the PeF and DMH project to multiple targets in the brain stem, including regions that have an autonomic and/or respiratory function, such as the midbrain PAG, parabrachial and Kölliker-Fuse nuclei, NTS, RVLM and RVMM (125, 131). It might be suggested, therefore, that orexin neurons in the DMH and PeF projecting to autonomic and respiratory centers in the brainstem generate the
cardiorespiratory responses to psychological stress. Double-labeling studies indicate, however, that orexin neurons constitute only a minority of the projection neurons from the PeF and DMH. For example, only approximately 20% of PeF neurons projecting to the NTS and RVMM contain orexin (150, 166). Although it is possible that 20% of projection neurons from the DMH and PeF could be sufficient to mediate stress-evoked cardiovascular and respiratory responses, other evidence indicates that orexin neurons primarily act to modulate responses mediated by non-orexinergic neurons, rather than being the prime mediators of these responses. In particular, as reviewed by Kuwaki and Zhang (96), the firing rate of orexin neurons is altered greatly during a variety of behaviors, including the different phases of sleep, exercise, and psychological stress, leading to the conclusion that their principal function is state-dependent modulation of physiological responses. In support of the view that orexinergic inputs act to amplify the actions of other inputs, it has been shown that the excitatory actions of orexin on BAT sympathoexcitatory neurons in the RVMM are dependent on the presence of ongoing activity in those neurons (150), while a study at the cellular level has shown that orexin can act presynaptically to modulate the release of glutamate from nerve terminals (58).

**Role of midbrain PAG in integrating stress-evoked responses**

There is a very extensive literature that has clearly documented the role of the midbrain PAG in the production of coordinated somatomotor and autonomic responses to a wide variety of stressors, both physical and psychological (for reviews see 4, 5, 25, 88, 154). The PAG consists of four longitudinal columns referred to as the dorsomedial (dmPAG), dorsolateral (dlPAG), lateral (IPAG) and ventrolateral (vIPAG) subdivisions, which differ with respect to their functional properties, anatomical connections and chemical properties (4, 5, 25, 42, 88, 154). The
findings of functional and anatomical studies have led to the proposal, first put forward by Bandler and co-workers (4,5), that the dlPAG has a major role in generating the behavioral and autonomic changes associated with defensive responses evoked by unconditioned threatening psychological stimuli. The evidence for this hypothesis has been discussed in detail in previous reviews (see refs. 4 or 42) but can be briefly summarised as follows: (1) the dlPAG is the only PAG subregion that receives inputs related to visual, auditory and olfactory signals (10, 113, 118)(Fig. 5); (2) auditory, visual or olfactory threatening stimuli (e.g. ultrasound application, the sight or odor of a cat) that are known to trigger defensive behavior in rats also evoke strong c-Fos expression in the dlPAG but much less expression in other PAG regions (21, 50, 89, 113); (3) lesions of the dorsal PAG that include the dlPAG reduce the behavioral and cardiovascular response to the sight or odor of a cat (51); and (4) activation of neurons in the dlPAG, but not other PAG subregions, evoke increases in sympathetic activity and respiratory rate that closely resemble responses naturally evoked by psychological stressors (79). In contrast to the dlPAG, the lPAG receives afferent inputs arising from somatic receptors, and generates increases in sympathetic activity in response to physical stressors (e.g. cutaneous pain) via direct descending projections to the medulla (42, 88)(Fig. 5).

**Functional relationship between the PAG and DMH.** If both the dlPAG and DMH have important roles in integrating the cardiovascular and respiratory responses to psychological stressors, then what is the relationship between these key regions? It has previously been suggested that the dlPAG is a component of the descending pathway mediating cardiovascular responses evoked by activation of DMH neurons (43), but a recent study has shown that inhibition of neurons in the dlPAG has no effect on cardiovascular and respiratory responses evoked from the
DMH (77). In contrast, however, the increases in arterial pressure, heart rate, renal sympathetic nerve activity and respiratory activity evoked by stimulation of dIPAG neurons are virtually abolished by inhibition of neurons in the DMH in both conscious and anesthetized rats (48, 77). There are two possible explanations for this observation. The simplest explanation is that there is an ascending pathway from the dIPAG that activates DMH neurons that in turn generate the cardiovascular and respiratory responses, as illustrated in Figs. 4 and 8. There is only a minor direct projection from the dIPAG to the DMH (148), but there are much stronger projections to the cuneiform nucleus (11, 127) and superior lateral parabrachial nucleus (94), both of which project to the DMH and other hypothalamic nuclei (13, 61, 97) (Fig. 5).

The alternative possibility, as suggested previously (42), is that there are separate descending pathways from the DMH and dIPAG that converge on cardiovascular and respiratory neurons in the lower brainstem. In that case, it is conceivable that cardiovascular and respiratory responses would only be evoked by activation of the dIPAG if the descending pathway from the DMH were also active. Although there are no direct descending projections from the dIPAG to the lower brainstem (42), there is as mentioned above a projection from the dIPAG to the cuneiform nucleus, which has descending projections to cardiovascular and respiratory nuclei in the lower brainstem and is believed to be involved in stress-evoked cardiovascular responses (92, 152) (Fig. 5). Future studies will be required, however, to determine the precise functional relationship between the DMH and dIPAG in generating stress-evoked response.

Role of midbrain colliculi in integrating stress-evoked responses

The superior colliculus (SC) receives inputs arising from visual, auditory, and somatosensory stimuli, and can generate appropriate highly coordinated orienting, defensive, or escape behavioral responses to stimuli that require immediate action
Similarly, the inferior colliculus (IC), which receives convergent signals from brainstem auditory nuclei, can also generate appropriate behavioral responses to auditory signals, via projections to the SC (30). In a recent study in anesthetized rats, my colleagues and I found that natural auditory, visual and somatosensory stimuli could evoke highly synchronized increases in sympathetic, respiratory and somatomotor activity, but only after disinhibition (by microinjection of the GABA receptor antagonists picrotoxin or bicuculline) of certain sites within the SC or IC (114) (Fig. 6). Although the evoked responses were highly synchronized, the onsets of the evoked increases in respiratory and somatomotor activity slightly preceded the onset of the evoked increases in sympathetic activity (114)(Figs. 6B,C). These differences in onset times are consistent with the hypothesis that the sympathetic, respiratory and somatomotor responses are driven by a common population of “command neurons” within the colliculi, as shown in Fig. 6D, because the conduction velocity of unmyelinated sympathetic postganglionic axons is much slower than that of myelinated phrenic and sciatic motor pathways (81, 126)(Fig. 6D). The time differences in onset of the evoked increases in sympathetic, respiratory and somatomotor activity (Figs. 6B,C) are consistent with the hypothesis that all of these responses are activated simultaneously from a common population of “command neurons” within the colliculi (Fig. 6D). Many neurons within the deep layers of the SC receive convergent visual, auditory and somatosensory inputs, and also have descending projections to the brain stem (107). Such multisensory neurons could therefore conceivably act as command neurons driving synchronized changes in sympathetic, respiratory and somatomotor output in response to multisensory inputs. The projection targets of the putative collicular command neurons are unknown. It is proposed, as depicted in Fig. 6D, that such neurons provide collateral inputs to premotor sympathetic, respiratory and somatomotor neurons in the brain stem.
Alternatively, in regard to the synchronized sympathetic and respiratory responses, it could be argued that such neurons project to respiratory neurons in the brain stem that in turn provide an input to sympathetic premotor neurons. As mentioned above, central respiratory activity can modulate sympathetic activity, via connections from respiratory neurons in the lower brain stem to sympathetic premotor neurons (57). Such coupling occurs under resting conditions and is exaggerated during hypoxia when both respiratory activity and sympathetic activity is increased (49). The pattern of such modulation, however, is distinctly different from that observed following activation of collicular neurons that drive synchronized increases in sympathetic and respiratory activity. In particular, under both resting conditions and hypoxia, splanchnic sympathetic activity (spSNA) is increased during the expiratory phase (49). In contrast, as illustrated in Fig. 6C, in response to auditory, visual or somatosensory stimulation following disinhibition of certain sites in the colliculi, the increase in spSNA occurs primarily during the inspiratory phase of respiration (i.e. when phrenic nerve activity is increased) (114). It therefore seems unlikely that the observed sympathetic-respiratory synchronization is simply a consequence of sympathetic premotor neurons receiving a modulatory input from central respiratory neurons that in turn are activated by inputs from the colliculi.

In summary, these observations are consistent with the hypothesis that there are neurons within the SC or IC that are capable of generating immediate behavioral responses to external stimuli, supported by cardiovascular and respiratory changes that are appropriate for the particular behavior. Such neurons are normally inhibited by tonic GABAergic inputs, which arise from the substantia nigra pars reticulata (31, 37). The substantia nigra pars reticulata itself receives inhibitory inputs from the striatum that in turn receives inputs from the deep layers of the SC relayed via the thalamus as well as from the cortex and amygdala (31, 69, 105)(Fig. 7). The
The functional relationship between the dIPAG and the basal ganglia-colliculi system is not known, although as pointed out in a previous review (42), they share certain anatomical and functional properties. In particular, both the dIPAG and deep layers of the SC receive inputs from several nuclei in the cortex, hypothalamus and medulla (10, 22, 90, 159). In addition, the pattern of sympathetic and respiratory changes evoked by stimulation of neurons in the dIPAG is similar to that evoked from the deep layers of the SC (79, 80). Furthermore, the dIPAG (but not other PAG subregions) receives a direct input from the deep layers of the SC (129). At the same time, there are significant differences in the connections of the dIPAG and...
deep layers of SC; for example, there is a dense projection from the medial prefrontal
cortex to the dlPAG but not to the adjacent SC (4), and a descending projection to
the pontomedullary reticular formation from the deep layers of SC but not from the
dlPAG (42, 128). Furthermore, cardiovascular and respiratory responses evoked
from the dlPAG, but not those evoked from the SC, are dependent on the activation
of the DMH (48, 77, 114). Thus, although the dlPAG and deep layers of the SC have
some features in common, they appear to be essentially distinct defense systems.

Summary and conclusions

It is clear that several different brain regions and pathways play major roles in
subserving the cardiovascular and respiratory responses to threatening stimuli. As
discussed above, these stimuli may vary from relatively mild acute alerting stimuli to
more prolonged life-threatening stimuli. It is likely that in the course of vertebrate
evolution different defense systems have developed that subserve different functions.
The basal ganglia and colliculi are phylogenetically ancient and highly conserved
(140, 141), while the defense system that includes the DMH and cortex evolved at a
later time, and may subserve more complex behaviors. The basal ganglia-colliculi
system is well adapted to responding to threats that require an immediate but highly
stereotyped action that does not require cognitive appraisal of the stimulus. In
particular, it is able to elicit different types of coordinated behaviors (e.g. orienting,
pursuit, escape) depending on the pattern of inputs (visual, auditory and/or
somatosensory) that trigger the behavior (47, 105). These behaviors are
accompanied by appropriate autonomic and respiratory responses (47). In contrast
to the basal ganglia-colliculi defense system, the hypothalamic defense system
appears to be better adapted to generating appropriate responses to more sustained
threatening stimuli, that presumably involve cognitive appraisal and/or recollection of memories, via inputs arising from the cortex and/or amygdaloid complex.

A common feature of these different defense systems is that they all produce highly coordinated behavioral, cardiovascular and respiratory responses. Even though much is still unknown about the precise brain circuits that subserve these coordinated responses, it is possible to put forward a working hypothesis of these circuits, as shown in Fig. 8. It is proposed that the basal ganglia/corticostriatal system evokes immediate defensive responses to alerting stimuli, while the DMH is a critical component of a system that includes the cortex, amygdala and midbrain PAG. Threatening stimuli (i.e. the sight, sound or odor of a predator) activate neurons in the DMH/PeF via inputs from the cortex, amygdala and/or PAG, while output neurons in the DMH/PeF convey descending signals to premotor nuclei in the pons and medulla that generate appropriate cardiovascular and respiratory responses. These premotor nuclei have not been definitively identified, except for the midline medullary raphe nuclei that mediates the stress-evoked increased activity of the sympathetic outflow to the heart, cutaneous blood vessels and BAT. In regard to stress-evoked sympathetically mediated visceral vasoconstriction, it is proposed that sympathetic premotor neurons in the RVMM are one of the main drivers of this activity (Fig. 4), while the sympathetic premotor neurons in the RVLM play a critical role in subserving the baroreflex control of sympathetic vasomotor activity, which is reset to a higher operating range via descending inputs to the NTS (Fig. 4). The output neurons in the DMH/PeF include orexin neurons, whose principal role may be to amplify increases in sympathetic and respiratory activity via their projections to sympathetic and respiratory premotor nuclei in the pons and medulla (96).

The general scheme shown in Fig. 8 is highly simplified and incomplete, and is very likely to be greatly modified and added to as new knowledge is gained from
future studies. Many important questions remain unanswered. For example, locomotion is associated with coordinated cardiovascular and respiratory changes, but the extent to which brain pathways regulating exercise-induced cardiorespiratory responses overlap with those regulating stress-evoked cardiorespiratory responses is not known. Another major general issue concerns the role of noradrenergic neurons in the locus coeruleus and serotonergic neurons in the dorsal raphe, both of which are activated during stress and arousal and have widespread projections to many parts of the brain, including key nuclei involved in cardiovascular and respiratory regulation (7, 98, 124). Furthermore, the nucleus incertus in the pons and lateral habenula in the midbrain have recently been shown to contain neurons that are strongly activated by stress and which also project very widely throughout the brain (130, 73). There is a large body of evidence indicating that all of these neuronal systems can modify stress-evoked responses (98, 124, 130) but their precise role in stress-evoked cardiorespiratory responses remain unclear and will need to be defined in future studies.
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DISCLOSURES

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**LEGENDS**

*Figure 1.* Changes (mean ± SE) in mean arterial pressure (MAP), heart rate (HR) and conductance in the renal, mesenteric and forearm vascular beds during a 5-min period of mental stress (mental arithmetic) in normal subjects lying supine, compared to the baseline (B) values measured before the onset of the mental stress. The values during the recovery (R) period are also shown. Modified from Figs. 1-4 in the study by Kuipers et al. (95), with permission.

*Figure 2.* Upper panel, sagittal section of the rat brain showing location of different brain regions that were examined for c-Fos expression following a period air puff stress in rats. Lower panel, histograms showing numbers of c-Fos-positive cells per section (mean ± SEM) in the different brain regions under control conditions (n = 4) and during air puff stress (n = 5). Abbreviations: BLA, basolateral amygdala; c, commissural; CeA, central nucleus of the amygdala; CVLM, caudal ventrolateral medulla; dl, dorsolateral; dm, dorsomedial; DMH, dorsomedial hypothalamus; KF, Kölliker-Fuse nucleus; l, lateral; LC, locus coeruleus; LH, lateral hypothalamus; m, medial; MeA, medial amygdala; PAG, periaqueductal gray; PeF, perifornical area; PVN, paraventricular nucleus; RPa, raphe pallidus; RVLM, rostral ventrolateral medulla; RVMM, rostral ventromedial medulla; vl, ventrolateral. **P < 0.01, *P < 0.05 vs. control. Modified from Fig. 2 in the study by Furlong et al. (64), with permission.
Figure 3. Data from the study by Furlong et al. (57), showing the distribution of c-Fos-positive cells in the rostral ventral medulla following air puff stress (A, combined data from three rats), and following hypotension (B, example from one rat). The numbers above each section indicate the level caudal (−) to the bregma (mm). The RVLM and RVMM were defined as the regions ventral to the nucleus ambiguus (NA), separated by a vertical line placed 0.2 mm medial to the medial edge of the NA and extending from approximately 12.0 to 13.2 mm caudal to bregma. The RVMM region included the ventral part of the gigantocellular reticular nucleus (GiV) and medial parts of the lateral paragigantocellular nucleus (LPG). (C,D) Examples of cells labelled for TH (brown-stained cytoplasm) immunoreactivity and c-Fos (black-stained nuclei) within the RVLM following air puff stress and hypotension, respectively. Note the lack of double-labeled c-Fos/TH cells following air puff stress (C) in contrast to the large number of double-labeled cells (indicated by asterisks) following hypotension (D). Abbreviations: 12N, hypoglossal nucleus; Sp5, spinal trigeminal nucleus. For other abbreviations see Fig. 2.

From Fig. 3 in the study by Furlong et al. (64), with permission.

Figure 4. Upper panel, sagittal section of the rat brain showing location of different brain regions that referred to in the diagram below. Lower panel, schematic diagram showing input and output connections of the DMH/PeF that subserve the sympathetic, respiratory and neuroendocrine responses to psychological stress. The unbroken lines indicate known connections (which may be monosynaptic or polysynaptic), while the dashed lines indicate postulated connections that have not yet been defined. Abbreviations: ACTH, adrenocorticotropic hormone; AVP, arginine vasopressin; mPFC, medial prefrontal cortex. For other abbreviations see Fig. 2.
Figure 5. Schematic diagram showing major inputs to the dIPAG and the proposed output pathways subserving the co-ordinated changes in sympathetic vasomotor and respiratory activity regulated by the dIPAG. The lines with arrows indicate projections, that include both direct (monosynaptic) or indirect (polysynaptic) projections. In contrast to the dIPAG, the IPAG is activated primarily by physical stressors, and regulates sympathetic and respiratory activity via direct descending projections to medullary nuclei. Abbreviations: CnF, Cuneiform nucleus; PBsl, superior lateral parabrachial nucleus. For other abbreviations see Fig. 2. Modified from Fig. 5 in the study by Dampney et al. (42), with permission.

Figure 6. Cardiovascular, respiratory, and somatomotor responses evoked by different stimuli after microinjection of picrotoxin into the midbrain colliculi. A: example in which auditory (clap), visual (light) and somatosensory (pinch) stimuli all evoked responses in splanchnic nerve activity (SpSNA), phrenic nerve activity (PNA), and sciatic nerve activity (ScNA) after, but not before, picrotoxin (GABA receptor antagonist) microinjection. B: Cycle-triggered average of 10 responses evoked by single claps following picrotoxin microinjection into one site in the colliculi, where the trigger was the onset of the evoked SpSNA burst. C: portion of the averaged response (indicated by shaded portion in B), showing that the onsets of the bursts of PNA and ScNA occurred slightly before the onset of the burst of SpSNA. The time of onset in each case is indicated by the vertical dashed line. These differences in onset time are consistent with activation of sympathetic, respiratory and somatomotor outputs by a single population of collicular neurons, as shown schematically in (D), given the relatively slow conduction velocity of sympathetic efferent pathways compared with that of phrenic and sciatic motor pathways (114). From Figs. 2 and 3 in the study by Müller-Ribeiro et al. (114), with permission.
Figure 7. Schematic diagram showing the subcortical loop involving the superior colliculus (SC) and basal ganglia. The deep layers of the SC receive topographically organised visual, auditory and somatosensory inputs, while superficial layers receive only visual inputs. There are multiple parallel projections from the deep layers of the SC to the striatum, relayed via intralaminar nuclei in the thalamus. The striatum also receives inputs from the cortex, and may act as an “action selector”, comparing inputs from the SC with those from the cortex, so that the most appropriate action can be selected for a particular set of inputs. This is done by disinhibition of the particular output neurons in the SC that regulate the appropriate coordinated somatomotor/autonomic response, by inhibiting neurons in the substantia nigra pars reticulata which in turn are inhibitory to specific neurons within the deep layers of SC. The red lines indicate an excitatory projection, while the blue lines indicate an inhibitory projection. Modified from Fig. 2 in McHaffie et al. (105), with permission.

Figure 8. Schematic diagram showing a general hypothesis of the systems subserving cardiovascular, respiratory and behavioral responses to psychological stressors or alerting stimuli. It is proposed that the pathways subserving the DMH/cortical system are essentially separate to those of the basal ganglia-colliculi system, although it is possible that both activate the same premotor nuclei in the pons and medulla. Also shown in the diagram are inputs to the IPAG arising from somatosensory inputs. For further details of the connections of the DMH, the dlPAG and the basal ganglia/colliculi see Figs. 4, 5 and 7, respectively.
ΔMAP (mmHg) vs. Mental stress (min)

ΔHR (bpm) vs. Mental stress (min)

ΔRenal conductance (% baseline) vs. Mental stress (min)

ΔMesenteric conductance (% baseline) vs. Mental stress (min)

ΔForearm conductance (% baseline) vs. Mental stress (min)
Fos-positive cells/section

Forebrain/midbrain

PVN, CeA, MeA, BLA, DMH, PeF, LH, dmPAG, dpAG, IPAG, vIPAG

Pons/medulla

LC, KF, PB, A5, NTSm, NTSc, NTSI, RPa, RVLM, RVMM, CVLM

control

stress
**A** Air puff stress

-12.0

**B** Hypotension

**C** -12.0

**D**
Acute psychological stressor via mPFC & amygdala

PVN

DMH/PeF

Baroreflex resetting

ACTH, AVP secretion

NTS

CVLM

RVLM

mPFC

PAG

Amy

RP

RVMM

PVN

DMH/PeF

Sympathetic outflow

Premotor nucleus (RVMM?)

Raphe pallidus

Central respiratory nuclei

Phrenic nucleus

Baroreceptor inputs

Visceral vasoconstriction

Heart rate

Cutaneous vasoconstriction

BAT activity

Respiratory activity

Baroreceptor inputs

Visceral vasoconstriction

Heart rate

Cutaneous vasoconstriction

BAT activity

Respiratory activity
Psychological stressors

Auditory
- Auditory cortex

Visual
- Visual cortex

Olfactory
- Amygdala

Perceived emotional stressor
- Medial PFC

Physical stressors

Somatic receptors
- Spinal cord & brainstem

Sympathetic activity

Respiratory activity
Averaged integrated ScNA (units)

Before picrotoxin

After picrotoxin

Arterial pressure (mmHg)

SpSNA (units)
PNA (units)

Integrated SpSNA (units)

Integrated PNA (units)

Integrated ScNA (units)

clap

5 s

light on

pinch

Averaged integrated SpSNA (units)

Averaged integrated PNA (units)

Averaged integrated ScNA (units)

Cycle-triggered averaging

Command neuron (colliculus)

Premotor nuclei (pons/medulla)

Motor nuclei (spinal cord)

Sympathetic activity

Phrenic nerve activity

Sciatic nerve activity
Visual, auditory & somatosensory inputs

Thalamus
- Rostral intralaminar n.
- Caudal intralaminar n.

Striatum

Substantia nigra pars reticulata

Inputs from cortex & amygdala

Superior colliculus
- Superficial layers
- Deep layers

Somatomotor & autonomic outputs

Coordinated behavioral & autonomic response
Psychological stressor (e.g. sight/sound/odor of predator, or perceived threat)

via cortex and hypothalamus

dIPAG

via spinal cord and brainstem

Somatosensory stressor (e.g. painful stimulus)

via cortex and hypothalamus

DMH

Alerting stimulus (sight, sound, touch)

Basal ganglia/colliculi system

Cardiovascular response

Behavioral response

Respiratory response

Pons/medulla

dIPAG

IPAG

Basal ganglia/colliculi system