Central mechanisms regulating coordinated cardiovascular and respiratory function during stress and arousal

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Submitted 12 February 2015; accepted in final form 28 May 2015

Dampney RA. Central mechanisms regulating coordinated cardiovascular and respiratory function during stress and arousal. Am J Physiol Regul Integr Comp Physiol 309: R429–R443, 2015. First published June 3, 2015; doi:10.1152/ajpregu.00051.2015.—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. In contrast, 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.

defensive behavior; sympathetic activity; respiratory activity; hypothalamus; midbrain; sympathetic premotor nuclei

AN ANIMAL’S SURVIVAL is dependent on 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, whereas 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 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 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 nonnoxious 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 coactivation 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 and Blessing (161) proposed that the cutaneous vasoconstrictive that is initially evoked is associated with cerebral vasodilatation, 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 norepinephrine 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 arithmetic) (27–29, 55, 75, 95). Thus the vasodilation in skeletal muscle and consequent increase in skeletal muscle blood flow evoked by psychological stress is due to nonneurogenic factors, which are believed to be primarily an increase in the level of circulating epinephrine 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 propose that there are essentially two different systems in the brain that generate defensive behavioral 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 on 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 will 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 epinephrine-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 and 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 (Fig. 3, B and D). 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 and 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 (39) 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. 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 with 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

Fig. 3. Data from the study by Furlong et al. (64) 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 ~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 and D: examples of cells labeled for tyrosine hydroxylase (TH), a marker of C1 cells (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 *) following hypotension (D). 12N, hypoglossal nucleus; Sp5, spinal trigeminal nucleus. For other abbreviations see Fig. 2. From Fig. 3 in Furlong et al. (64).
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 neuroendoctrine 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 ~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 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 (142, 143) 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. 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), whereas 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, which 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 neurons 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 on 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 brain stem are relatively sparse (122, 147). Therefore, the question 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 make 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 brain stem 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 brain stem 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). In contrast, direct inputs from brain stem 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 Klüber 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 vasaconstrictor 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 Role of DMH and PeF in Integrating Stress-Evoked Responses), 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.

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 with responses in wild-type 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 are 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 brain stem 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 ~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 nonorexinergic 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. Supporting 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 sympathetic excitatory neurons in the RVMM are dependent on the presence of ongoing activity in those neurons (150), whereas 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 Refs. 4, 5, 25, 88, 154). The PAG consists of four longitudinal columns referred to as the dorsomedial (dmPAG), dorsolateral (dlPAG), lateral (lPAG), and ventrolateral (vl-PAG) 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

![Fig. 5. Schematic diagram showing major inputs to the dlPAG and the proposed output pathways subserving the coordinated changes in sympathetic vasoactivity and respiratory activity regulated by the dlPAG. The lines with arrows indicate projections, which include both direct (monosynaptic) or indirect (polysynaptic) projections. In contrast to the dlPAG, the IPAG is activated primarily by physical stressors and regulates sympathetic and respiratory activity via direct descending projections to medullary nuclei. CnF, cuneiform nucleus; PBsl, superior lateral parabrachial nucleus. For other abbreviations see Fig. 2. From Dampney et al. (Fig. 5, 42).](http://ajpregu.physiology.org/doi/abs/10.1152/ajpregu.00051.2015)
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 summarized 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 IPAG 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 dlPAG 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 dlPAG 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 dlPAG 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 dlPAG that converge on cardiovascular and respiratory neurons in the lower brain stem. In that case, it is conceivable that cardiovascular and respiratory responses would only be evoked by activation of the dlPAG if the descending pathway from the DMH were also active. Although there are no direct descending projections from the dlPAG to the lower brain stem (42), there is, as mentioned above, a projection from the dlPAG to the cuneiform nucleus, which has descending projections to cardiovascular and respiratory nuclei in the lower brain stem 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 dlPAG 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 (36, 47, 66). Similarly, the inferior colliculus (IC), which receives convergent signals from brain stem 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 (114) 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 (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) (Fig. 6, B and 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 unmynelinated sympathetic postganglionic axons is much slower than that of myelinated phrenic and sciatic motor pathways (81, 126) (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.

Fig. 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 somatomotom 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 Müller-Ribeiro et al. (Figs. 2 and 3, 114).
**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 light on pinch**

**Cycle-triggered averaging**

- **Averaged integrated SpSNA (units)**
- **Averaged integrated PNA (units)**
- **Averaged integrated ScNA (units)**

**Command neuron (colliculus)**

- **Premotor nuclei (pons/medulla)**
- **Motor nuclei (spinal cord)**

**Sympathetic activity**

**Phrenic nerve activity**

**Sciatic nerve activity**
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, with 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. 6, 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 summary, these observations support 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 substantia nigra pars reticulata and striatum are components of the basal ganglia, and it has been proposed that this subcortical loop involving the basal ganglia and SC allows selection of an appropriate response to competing inputs, including those that arise from the cortex (105). The inputs to the SC from visual, auditory, and somatosensory inputs, as well as all the connections within this subcortical loop, are highly topographically organized, so that the pattern of inputs to the striatum are highly specific for a particular stimulus (47, 105). Similarly, the inputs to the striatum from the cortex are also highly topographically organized (105). Thus it has been suggested (105) that the striatum 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, by withdrawing inhibition from the particular output neurons in the SC that regulate the appropriate coordinated somatomotor/autonomic response. For example, visual or auditory inputs signaling the presence of a predator may via this loop result in disinhibition of collicular neurons that trigger escape behavior accompanied by appropriate cardiovascular and respiratory activation, while other alternative behavioral responses continue to be inhibited.

The functional relationship between the dlPAG 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 dlPAG and deep layers of the SC receive inputs from several nuclei in the cortex (105). The inputs to the SC from visual, auditory, and somatosensory inputs, as well as all the connections within this subcortical loop, are highly topographically organized, so that the pattern of inputs to the striatum are highly specific for a particular stimulus (47, 105). Similarly, the inputs to the striatum from the cortex are also highly topographically organized (105). Thus it has been suggested (105) that the striatum 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, by withdrawing inhibition from the particular output neurons in the SC that regulate the appropriate coordinated somatomotor/autonomic response. For example, visual or auditory inputs signaling the presence of a predator may via this loop result in disinhibition of collicular neurons that trigger escape behavior accompanied by appropriate cardiovascular and respiratory activation, while other alternative behavioral responses continue to be inhibited.

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Perspectives and Significance

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 earlier, 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), whereas the defense system that includes the DMH and cortex evolved at a later time. 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-colliculi subcortical 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, whereas 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 mediate the stress-evoked increased activity of the sympathetic outflow to the heart, cutaneous blood vessels, and BAT. With regard to stress-evoked sympathetically mediated visceral vasconstriction, it is proposed that sympathetic premotor neurons in the RVMM are one of the main drivers of this activity (Fig. 4), whereas 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 (73, 130). 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.

ACKNOWLEDGEMENTS

I am very grateful to the following colleagues (in alphabetical order) who worked with me at the University of Sydney and at Macquarie University on our studies that are discussed in this review: Marco Fontes, Teri Furlong, Ann Goodchild, Jouji Horüchi, Kameron Iigaya, Suzanne Killinger, Lachlan McDowall, Simon McMullan, Flavia Müller-Ribeiro, and Jaime Polson. I also have benefitted from many discussions with other colleagues, particularly Robin McAllen, Pascal Cariwre, and Bill Blessing. I thank Howard Schultz and the Neural Control and Regulation Section of the American Physiological Society for giving me the privilege of presenting the Carl Ludwig Lecture.

GRANTS

Studies described in this review that were performed in my laboratory were supported by the National Health and Medical Research Council of Australia, the Australian Research Council, and the National Heart Foundation of Australia.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS

Author contributions: R.A.D. drafted manuscript; R.A.D. edited and revised manuscript; R.A.D. approved final version of manuscript.

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