Disinhibition of the midbrain colliculi unmasks coordinated autonomic, respiratory and somatomotor responses to auditory and visual stimuli

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Abstract

The midbrain superior and inferior colliculi have critical roles in generating coordinated orienting or defensive behavioral responses to environmental stimuli, and it has been proposed that neurons within the colliculi can also generate appropriate cardiovascular and respiratory responses to support such behavioral responses. We have previously shown that activation of neurons within a circumscribed region in the deep layers of the superior colliculus and in the central and external nuclei of the inferior colliculus can evoke a response characterized by intense and highly synchronized bursts of renal sympathetic nerve activity and phrenic nerve activity. In this study we tested the hypothesis that, under conditions where collicular neurons are disinhibited, co-ordinated cardiovascular, somatomotor and respiratory responses can be evoked by natural environmental stimuli. In response to natural auditory, visual or somatosensory stimuli, powerful synchronized increases in sympathetic, respiratory and somatomotor activity were generated following blockade of GABA\(_A\) receptors in a specific region in the midbrain colliculi of anesthetized rats, but not under control conditions. Such responses still occurred after removal of most of the forebrain, including the amygdala and hypothalamus, indicating that the essential pathways mediating these coordinated responses were located within the brainstem. The temporal relationships between the different outputs suggest that they are driven by a common population of “command neurons” within the colliculi.

Key words: command neuron, defensive behavior, arterial pressure, sympathetic activity, phrenic nerve activity
INTRODUCTION

Detection of danger generates rapid and integrated behavioral and physiological responses that facilitate an animal’s survival. The superior colliculus (SC) has a critical role in generating highly co-ordinated orienting, defensive or escape behavioral responses to visual, auditory and/or somatosensory stimuli that require immediate action (6,8,10). Similarly, the inferior colliculus (IC), which receives convergent signals from auditory nuclei in the brainstem, also has a critical role in mediating appropriate behavioral responses to auditory signals, via projections to the SC (3). Microinjection of the GABA<sub>A</sub> receptor antagonist picrotoxin into the colliculi of conscious animals can evoke defensive or escape responses to otherwise innocuous stimuli (2,4,8) indicating that tonic GABAergic input to the region normally inhibits such responses.

It would be expected that immediate escape or defensive behaviors are supported by appropriate cardiovascular and respiratory responses. Stimulation of neurons in the SC can evoke increases in blood pressure, heart rate and respiratory activity (23,24), and recently we reported that activation of neurons within a specific region within the colliculi evokes highly synchronized increases in renal sympathetic nerve activity, blood pressure, and respiratory activity in anesthetized rats (17). This observation raises the possibility that such synchronized autonomic and respiratory effects are part of a more generalized response that includes increases in somatomotor activity, such as might occur during escape or defensive reactions generated by activation of neurons within this collicular region. If so, it would be predicted that natural alerting stimuli (i.e. visual, auditory and/or somatosensory stimuli) could trigger a co-ordinated somatomotor, autonomic and respiratory response under conditions where the tonic GABAergic inhibition of collicular neurons that generate such responses is removed. In this study we tested the hypothesis that blockade of GABAergic input to the collicular region identified in our previous study (17) would permit natural
alerting stimuli to activate command neurons that simultaneously drive cardiorespiratory and somatomotor outputs in anesthetized rats.

MATERIALS AND METHODS

General Procedures. Experiments were performed on a total of 29 adult male Sprague Dawley (SD) rats (330-600g) supplied by the Animal Resources Centre (Perth, Australia). All experimental procedures were approved by the Animal Ethics Committee of Macquarie University and carried out in accordance with the Australian code of practice for the care and use of animals for scientific purposes. Anesthesia was induced by intraperitoneal (i.p.) injection of 10% urethane (1.3g/kg) and maintained at surgical depth throughout experiments, with supplementary urethane added when required. An adequate level of anesthesia before neuromuscular blockade was assessed by the absence of a corneal reflex and changes in breathing and blood pressure in response to strong hind paw pinch, and after neuromuscular blockade by the absence of heart rate and blood pressure responses to paw pinch. The right femoral artery and vein were cannulated for arterial pressure (AP) measurement and drug administration, respectively. A tracheostomy was performed to ensure clear airways and to permit artificial ventilation. All rats were bilaterally vagotomized, paralyzed (pancuronium bromide 2 mg ml⁻¹, induction: 0.4 ml i.v.; maintenance: 0.2 ml h⁻¹ i.v.; Astra Pharmaceuticals, Australia) and artificially ventilated with room air plus additional O₂ at a rate that maintained end-tidal CO₂ levels within the range 3.5-4.5%. Arterial blood gases were measured once the preparation was complete and once or twice during the experiment to ensure appropriate ventilation. Core temperature was measured with a rectal probe and maintained at 37°C with a homoeothermic blanket. The left greater splanchnic nerve was exposed using a retroperitoneal incision and the left phrenic nerve via a dorsolateral approach as previously described (32,37). The sciatic nerve was exposed at mid-thigh from a dorsolateral approach,
the biceps femoris muscle was separated from the vastus lateralis muscle and the nerve was then dissected. All nerves were carefully isolated from surrounding tissues, cut distally, placed on bipolar electrodes and immersed in mineral oil. Signals from splanchnic, phrenic and sciatic nerves were amplified, filtered (0.1 – 3 kHz bandpass), and sampled (2 kHz) with a Power 1401plus and Spike 2 software (Cambridge Electronic Design, UK). The mean arterial pressure (MAP) and heart rate (HR) signals were derived from the pulsatile arterial pressure signal and recorded on a computer using Spike 2 software. The head was placed in a stereotaxic frame with the incisor bar fixed 3.3 mm below the interaural line. Blunt ear bars were used, and care was taken to ensure that the tympanic membrane was not punctured. A small area of the dorsal surface of the brain was exposed to allow insertion of micropipettes into the SC and IC.

**Decerebrate preparation.** A pre-collicular decerebration was performed in 4 rats anesthetized with urethane. Briefly, two round holes were drilled in the skull on both sides to allow access to the brain. The dura was then incised and a transection above the colliculi was made using a fine forceps. A suction system was then used to remove the brain tissue above the level of the transection. The internal carotid arteries were not ligated. During suction of the tissue, mean arterial pressure was lowered by pentobarbitone infusion (13 mg kg⁻¹, 5 ml i.v. at a rate of 1 ml min⁻¹) to reduce bleeding.

**Drug microinjection.** Microinjections of the GABA<sub>A</sub> receptor antagonist picrotoxin (50 pmol in 50 nl, Sigma-Aldrich Inc.) or bicuculline methiodide (50 pmol in 50 nl, Tocris) were made into sites in the SC and IC using a glass micropipette held vertically in a micromanipulator. We have previously shown that a 50 pmol dose of bicuculline was effective in evoking cardiorespiratory responses from restricted sites within the colliculi (17). The same dose (50 pmol) of picrotoxin was selected based on previous studies showing that equal or similar doses of bicuculline or picrotoxin evoked responses of similar magnitude when injected into the brain or spinal cord (12, 45). In all cases the injectate was 10 mM PBS
adjusted to pH 7.4, and also contained rhodamine beads (0.5% Fluospheres) for later
histological verification of microinjection sites. At the end of each experiment, the rat was
euthanized with an overdose of potassium chloride (KCl, 3 M, 1 ml i.v.). The brain was
removed and after fixation in 4% paraformaldehyde solution, coronal brain sections (100 μm)
were cut on a vibrating microtome and mounted onto gelatinized glass slides. Injection sites
were determined using a fluorescence microscope and mapped using the atlas of Paxinos and
Watson (38) as a reference. In experiments where decerebration was performed, horizontal or
sagittal brain sections (100 μm) were cut and stained using cresyl violet to determine the level
of decerebration.

Experimental Procedures. Natural stimuli were tested in all experiments before and
after microinjection of picrotoxin or bicuculline into sites in the superior or inferior colliculi.
Three different stimulus modalities were used: auditory, visual and somatosensory. The
auditory stimulus consisted of a hand clap made approximately 30 cm from the animal. In a
separate test, a wide band microphone (B&K 4138 1/8 inch microphone 20 Hz to 160 kHz)
and measuring amplifier (B&K 2610) was used to record the sound generated by typical claps
as used in the experiment, made at the same distance (30 cm) from the microphone. The
output of the recorded signal was digitized at 96 kHz (Edirol UA25EX) and spectral analysis
performed using Matlab (The Mathworks – V7.11). Assuming that the ear bars resulted in an
attenuation of 40 dB (which is greater than the attenuation produced by industrial ear plugs),
the amplitude of the sound after attenuation was 30-40 dB greater than the audiometric
threshold for the rat over the frequency range 8 kHz to 30 kHz, which corresponds to the
frequency range at which rats hear best (14). The visual stimulus consisted of a bright light
with a duration of 3 s directed to both eyes from a distance of approximately 20 cm, while the
somatosensory stimulus was a hard pinch, with a duration of approximately 2 s, applied by
forceps to a hind paw.
For picrotoxin microinjections into the colliculi, the tip of the micropipette was positioned stereotaxically at rostrocaudal levels ranging from 7.1 to 8.6 mm caudal to bregma in tracks located 1.0 to 2.5 mm lateral to the midline and depth varying from 4.0 to 6.0 mm ventral to bregma, according to the atlas of Paxinos and Watson (38). In each track injections were made into sites 0.5 mm apart, starting at the most dorsal site. Once the most ventral site in each track was reached, the micropipette was placed in a new track that was 0.5 mm lateral, medial, rostral or caudal to the previous track. Injections were applied by pressure, and the volume of each injection measured by movement of the meniscus against a grid corresponding to 20 nl volumes monitored using an operating microscope. The number of injection sites in each experiment varied between 2 and 14. The sites into which microinjections were made included the region in which, as mapped previously, microinjection of bicuculline evoked synchronized bursts of renal SNA and PNA (17). If a natural stimulus evoked a response following picrotoxin microinjection, there was a waiting period of at least 30 min before the next picrotoxin microinjection. In experiments in which decerebration was performed, the procedure was the same except that (i) bicuculline was microinjected instead of picrotoxin, (ii) only auditory stimuli were applied, (iii) the procedure of bicuculline microinjection followed by application of auditory stimuli was performed before and after decerebration and (iv) PNA was not recorded in 3 of the 4 experiments.

Data analysis. Natural stimuli evoked synchronized bursts of splanchnic nerve activity (SpSNA), phrenic nerve activity (PNA) and sciatic nerve activity (ScNA) after GABA-receptor blockade at many sites in the colliculi. At some of these sites, the stimulus was repeated several times so that cycle-triggered averaging could be used to determine the time relationships between the bursts of SpSNA, PNA and ScNA and the associated changes in MAP evoked by an auditory, visual or somatosensory stimulus. For cycle-triggered averaging the recorded digitized SpSNA, PNA and ScNA signals were first rectified and then integrated (time constant 20 ms). For each stimulus, the onset of the evoked SpSNA burst was
first identified and then samples of the signals for all four simultaneously recorded variables (integrated SpSNA, integrated PNA, integrated ScNA and MAP) were taken over the period from 2.5 s before to 2.5 s after this trigger point (200 samples/s) (e.g. Fig. 3B). This process was done for 4-19 stimuli and the average of these sweeps was calculated for all four variables. The time-point of the onset of increases in these averaged integrated SpSNA, PNA, and ScNA signals was then identified, in each case, as the point where (i) the value of averaged signal increased, relative to the immediately preceding value, by an amount that was greater than twice the standard deviation of the preceding 10 values, and (ii) the averaged signal continued to increase thereafter towards the peak value.

Cycle-triggered averaging was also used to compare the magnitudes of the increases in MAP and of the bursts of SpSNA and PNA (expressed relative to their baseline levels of activity) evoked by the auditory, visual and somatosensory stimuli. For this purpose, at each site where cycle-triggered averaging was performed, the maximum increases in the averaged MAP, integrated SpSNA and integrated PNA variables, relative to their respective pre-stimulus baseline levels, were measured. The pre-stimulus baseline level was, in each case, the mean value of the averaged variable over the time period from 2.5 to 0.5 s before the stimulus. The magnitude of the integrated ScNA burst amplitude could not be measured in this way, however, because there was no detectable baseline ScNA activity above the noise level.

A paired $t$-test was used to determine if the time differences between the onsets of the increases in (i) SpSNA and PNA, and (ii) SpSNA and ScNA evoked by auditory or visual stimuli were significantly different from zero. An unpaired $t$-test was then used to determine whether these time differences were significantly different from each other and also whether they were significantly different when responses were evoked by a visual stimulus as compared to those evoked by an auditory stimulus. An unpaired $t$-test was also used to determine if the magnitudes of the evoked increases in MAP, SpSNA or PNA were
significantly different when evoked by the different stimuli. P-values of < 0.05 were taken as statistically significant.

RESULTS

Cardiovascular and respiratory responses to auditory, visual and somatosensory stimuli, following microinjection into the colliculi. Under baseline conditions, before injection of picrotoxin into sites in the colliculi, no changes in SpSNA, PNA, or ScNA were evoked by auditory (clap: Fig. 1A), visual (light flash) or somatosensory (pinch) stimuli in urethane-anesthetized, vagotomized, paralyzed and artificially ventilated rats. However, following microinjection of picrotoxin (50 pmol/ 50 nl) at 57 out of 124 sites in the midbrain colliculi one or more of these stimuli triggered immediate and coordinated activation of SpSNA and PNA, followed by increases in arterial pressure (Fig. 1A,B). At most (37 out of 57) of these positive sites the picrotoxin microinjection itself had no effect on the baseline levels of SpSNA, PNA or ScNA, and variable effects at the remaining 20 sites (either a transient response or a more sustained modest response). At 7 positive sites, after picrotoxin microinjection synchronized bursts of SpSNA and PNA also occurred spontaneously, in addition to the stimulus-evoked synchronized bursts (Fig. 2A).

After picrotoxin injection the auditory stimuli evoked a response at 96% of the positive sites, while the visual and somatosensory stimuli evoked responses at 46 and 40%, respectively, of the positive sites at which they were tested. The somatosensory stimulus (pinch) was applied to the hind paw on one side only, but responses to this stimulus were obtained after picrotoxin microinjections into sites in either the ipsilateral or contralateral colliculi. All 3 types of stimuli evoked responses in 9 of the 35 positive sites in which all three were tested (e.g. Fig. 1A). At all 57 positive sites, increases in both SpSNA and PNA were always evoked. The sympathetic and respiratory responses to alerting stimuli were also
accompanied by a somatomotor response (increase in ScNA) at 30 of the 42 positive sites in which ScNA was also measured (Fig. 1A,B).

**Magnitudes of the evoked responses.** At sites at which cycle-triggered averaging was performed, the magnitudes of the averaged responses evoked by clap, light or pinch were determined (Fig. 3). As shown in Fig. 4, the three different stimuli evoked increases in the averaged MAP and in the amplitudes of the SpNA and PNA bursts that were of similar magnitude for all stimuli. Changes in heart rate were also evoked, but these were very small (< 20 beats/min), probably because the rats were vagotomized. A pairwise comparison (clap vs light, clap vs pinch, and light vs. pinch) revealed no significant difference in the magnitudes of the evoked increases in MAP, or in the averaged SpSNA and PNA burst amplitudes ($P > 0.5$ in all cases).

**Time relationships of the evoked responses.** The most striking feature of the responses to natural sensory stimuli was the tight synchronization of the recorded sympathetic, respiratory and somatomotor responses (Fig. 3). Using cycle-triggered averaging, the onsets of the PNA and ScNA responses to the auditory stimuli consistently were found to precede the onset of the SpSNA response (Fig. 3C) by 26.0 ± 2.7 and 41.7 ± 2.7 ms, respectively (Fig. 5). Similarly, the onsets of the PNA and ScNA responses to the visual stimuli preceded the onset of the SpSNA response by 30.0 ± 4.2 and 48.3 ± 4.4 ms, respectively (Fig. 5). These time differences were in all cases significantly different from zero, for both the visual and auditory evoked responses ($P < 0.001$). Furthermore, the time difference between the onsets of the ScNA and SpSNA responses was greater than that between the onsets of the PNA and SpSNA responses, when either auditory or visual stimuli were applied ($P<0.01$ and $P<0.05$, respectively, Fig. 5). Thus, following either an auditory or visual stimulus, the ScNA response occurred first followed by the PNA response and then the SpSNA response. The latencies of the SpSNA responses relative to the PNA or ScNA responses, however, were not
significantly different when the effects of auditory and visual stimuli were compared ($P > 0.3$

in both cases, Fig. 5).

*Location of positive sites.* The centers of the 57 positive sites were located

predominantly within the deep layers of the SC and the external and central nuclei of the IC,
with a few sites in the adjacent lateral periaqueductal gray and precuneiform nucleus (Fig. 6).
The midbrain region identified previously as a region capable of evoking synchronized
sympathetic/respiratory responses (17) contained virtually all the positive response sites
identified in the present study.

*Effects of decerebration.* To determine whether forebrain regions were essential for the
expression of cardiovascular, respiratory and somatomotor responses to alerting stimuli, we
examined the effects of decerebration ($n=4$). In all these experiments, we first confirmed that,
before decerebration, responses to auditory stimuli could be evoked after disinhibition of a
site in the SC or IC. Following removal of most of the forebrain (Fig. 7C), including the
prefrontal cortex, amygdala, auditory cortex and dorsomedial, perifornical and lateral
hypothalamus, auditory stimuli still evoked bursts of SpSNA after (but not before) collicular
disinhibition in all four experiments. In one of these experiments PNA was also recorded,
and in this case the stimuli also evoked bursts of PNA that were highly synchronized with the
bursts of SpSNA (e.g. Figs. 7A,B). As previously reported (17), bursts of SpSNA and PNA
also occurred spontaneously after bicuculline injection (Fig. 2B), in addition to the stimulus-
evoked synchronized bursts.

**DISCUSSION**

The main finding of the present study is that disinhibition of neurons within parts of the
SC and IC permits the production of highly synchronized sympathetic, respiratory and
somatomotor responses to different sensory stimuli. Such responses are suppressed under
baseline conditions in anesthetized rats. In the following Discussion, we shall consider (i) whether these responses are driven by a common population of command neurons in the colliculi, (ii) the central circuitry that subserves these responses, and (iii) the physiological significance of our findings. First, however, we will consider some methodological issues.

**Methodological considerations.** The auditory stimulus was a handclap, made approximately 30 cm from the animal. The rat was placed in atraumatic ear bars, such that the tympanic membrane was not punctured. The presence of ear bars would have reduced the amplitude of the auditory signal, but as described in the Methods measurements of the intensity and power spectra of handclaps indicated that the amplitude of the sound would have exceeded the auditory threshold, even allowing for the attenuating effect of the ear bars. Furthermore, the fact that responses were evoked by the auditory stimuli demonstrates that the signal was not eliminated. We did not systematically examine the relationship between the magnitude of the auditory stimulus and the magnitude of the evoked responses, although in some experiments softer and louder handclaps were made. In those cases, there was no discernible effect of stimulus strength on the magnitude of the evoked responses, suggesting that the responses were “all-or-nothing” rather than graded. Similarly, visual or somatosensory stimuli either evoked a clear response or no response at all.

The sciatic nerve contains sympathetic postganglionic fibers as well as somatomotor fibers (43), but two lines of evidence indicate that the recorded evoked responses in this nerve represent increased somatomotor activity. First, the onset of the evoked ScNA responses to auditory and visual stimuli preceded the onset of the SpSNA responses by 41.7 ± 2.7 and 48.3 ± 4.4 ms, respectively (Fig. 5), consistent with a response mediated by fast-conducting myelinated somatomotor fibers rather than slow-conducting unmyelinated sympathetic postganglionic fibers. Second, unlike SpSNA, there was no detectable resting level of ScNA, which would be expected if sympathetic activity were being recorded. Although we cannot rule out the possibility that increased sympathetic activity may have contributed to the ScNA
response, we believe that this response predominantly reflects increased somatomotor nerve activity.

The auditory and visual stimuli were bilateral, but the somatosensory stimulus was applied to the hind paw on one side only. Responses to this stimulus, however, were obtained after picrotoxin microinjections into sites in either the ipsilateral or contralateral colliculi, showing that unilateral disinhibition of collicular neurons was capable of unmasking responses to somatosensory inputs originating from either the ipsilateral or contralateral side. Consistent with this, anatomical studies have shown that somatosensory inputs project bilaterally to the superior colliculus (50), and that there are also extensive interconnections between the right and left sides of the superior colliculi (20,21).

Following picrotoxin microinjection into 7 sites (out of a total of 124 sites), synchronized bursts of SpSNA and PNA also occurred spontaneously (i.e. in the absence of auditory or other stimuli). In contrast, in a previous study from our laboratory (17), such spontaneous synchronized bursts of SpSNA and PNA were evoked following microinjection of bicuculline methiodide at 55 out of a total of 129 sites. The fact that spontaneous synchronized bursts of SpSNA and PNA occurred more frequently following bicuculline injection as compared to picrotoxin injection may be due to differences in the pharmacological properties of the two drugs. Apart from blocking GABA\textsubscript{A} receptors, bicuculline methiodide also blocks small-conductance calcium-activated potassium channels (28), which can potentiate N-methyl-D-aspartate-dependent burst firing in neurons (22). In contrast, picrotoxin does not have this effect (28). Therefore under resting conditions bicuculline methiodide may be more effective than picrotoxin in generating bursts of activity in collicular neurons that in turn generate synchronized bursts of SpSNA and PNA. Consistent with our observations, highly synchronized bursts of neural activity are reliably evoked by application of bicuculline methiodide to brain slices containing the thalamus, whereas these occur much less frequently following application of picrotoxin in the same
preparation (28). Similarly, previous studies have also shown that injection of bicuculline
into other regions, such as the hypothalamic paraventricular nucleus or the spinal cord, can
evoke low-frequency bursts of sympathetic activity (12,25,26).

Although they have some different pharmacological properties, a common property of
picrotoxin and bicuculline is that they both block GABA<sub>A</sub> receptors (28). Our results
therefore show that in anesthetized rats blockade of GABA<sub>A</sub> receptors within the deep layers
of the SC or external and central nuclei of the IC allows the expression of stimulus-evoked
synchronized sympathetic, respiratory and somatomotor responses that are normally
suppressed under control conditions. Further studies will be required, however, to determine
whether the GABA<sub>A</sub> receptors that suppress the evoked responses are synaptic or
extrasynaptic, since picrotoxin and bicuculline block both types of receptors (44).

Collicular neurons driving the sympathetic, respiratory and somatomotor responses.

Two lines of evidence suggest that the sympathetic and respiratory components of the evoked
responses are driven by a common population of command neurons in the colliculi. First, in
all cases where a response was evoked, there were marked increases in both SpSNA and
PNA. Second, the increases in SpSNA and PNA were highly synchronized, such that the
onset of the auditory or light-triggered SpSNA bursts lagged the onset of the PNA bursts (by
25-30 ms). It seems unlikely that such a time lag could be explained by a difference in the
number of central synapses in the descending pathways mediating the increases in SpSNA
and PNA, because the transmission time for synapses in the brain stem is typically < 1 ms
(39). This time lag is, however, consistent with activation of respiratory and sympathetic
outputs by a single population of collicular neurons, given the relatively slow conduction
velocity of sympathetic efferent pathways compared to that of phrenic motor pathways
(19,40). At the same time, we cannot rule out the possibility that the sympathetic and
respiratory responses are driven by separate populations of collicular neurons that are
simultaneously activated by auditory, visual or somatosensory inputs.
At 30 of the 42 positive sites at which ScNA was recorded in addition to SpSNA and PNA, stimulus-evoked increases in SpSNA and PNA were also accompanied by an increase in ScNA, the onset of which also preceded the onset of the auditory or light-triggered SpSNA bursts. Again, this difference in onset time can be explained by the fact that sciatic motor nerves have a much higher conduction velocity than sympathetic efferent pathways (19,48).

The inability to detect ScNA responses in some cases could reflect technical difficulties associated with detecting motor activity in the sciatic nerve, or differential sensitivity of motor compared to phrenic/sympathetic outputs under anesthesia. Alternatively, some collicular neurons driving cardiovascular/respiratory responses may not influence somatomotor activity. Nevertheless, the data are consistent with the hypothesis that a population of command neurons in the colliculi can generate synchronized sympathetic, respiratory and somatomotor responses to multimodal sensory inputs, even under surgical anesthesia. In this regard, it is interesting to note that the intermediate and deep layers of the SC contain multisensory neurons that respond to visual, auditory and somatosensory stimuli (15,16,27,31,33,34). In particular, Meredith and Stein (34) demonstrated a convergence of visual, auditory and somatosensory inputs on to many individual cells in the deep layers of the SC. Further studies are required, however, to determine whether multisensory neurons in the SC can simultaneously drive sympathetic, respiratory and somatomotor responses, or whether these responses are driven by separate populations of collicular neurons.

Central pathways mediating the reflex responses. The essential circuitry subserving the coordinated sympathetic, respiratory and motor responses (at least to auditory stimuli) must be contained within the brainstem and spinal cord, as responses were present after removal of virtually the entire forebrain. This is consistent with previous reports that behavioral responses, when triggered by visual, somatosensory or auditory stimuli signaling a threat, are dependent upon relays in the SC and (for auditory stimuli) the IC, but not the thalamus or cortex (3,5).
Similarly, our finding that responses to auditory stimuli were evoked in decerebrate rats following bicuculline microinjection into the colliculi also indicates that the tonic GABAergic inhibition that blocks the responses to natural stimuli in anesthetized rats must also arise from neurons within the brainstem or spinal cord. These neurons may be local interneurons (35), or may project to the SC or IC from other nuclei such as the substantia nigra. There is a direct GABAergic pathway from the substantia nigra pars reticulata (SNpr) to the colliculi (4,41), and this pathway may therefore tonically inhibit command neurons that generate coordinated behavioral and autonomic responses to natural stimuli. It is very interesting to note that defensive behavioral responses evoked by electrical or chemical stimulation of the SC or IC in conscious rats are enhanced following blockade of neurons in the SNpr, whereas activation of such neurons in the SNpr has the opposite effect (4,7,36).

Output pathways from the SC have been studied primarily with respect to the role of the SC in somatomotor functions associated with orienting and escape behavior (8,10). There are direct descending projections to the pontomedullary reticular nuclei that control somatomotor activity (42), but there is little anatomical information concerning possible connections with brainstem nuclei that control sympathetic or respiratory function, such as the rostral ventrolateral medulla or the dorsal and ventral respiratory groups. Similarly, the possible pathways by which neurons in the IC could regulate sympathetic or respiratory function are also unknown. There is a strong projection from the IC to the SC, however (11), and it is possible that this projection may target neurons within the SC that regulate sympathetic and respiratory activity.

Perspectives

LeDoux (29) suggested that a subcortical pathway that includes the lateral amygdala mediates rapid subconscious responses to threatening stimuli. In the present study we
observed no significant effect of removal of the amygdala on responses to auditory stimuli unmasked by collicular disinhibition. It therefore seems likely that the colliculi may mediate an even more rapid and primitive response. The SC and IC are the mammalian homologues of the optic tectum and torus semicircularis, respectively, both of which evolved very early and are highly conserved in vertebrates (1,47). As in mammals (31,33,34), many neurons in the optic tectum of reptiles and fish are multisensory and the optic tectum in these species also has a critical role in generating defensive responses, triggered by signs of a predator (46). Thus, it is possible that the synchronized responses generated by the colliculi are part of a phylogenetically ancient and highly conserved defense system (47) that generates immediate somatomotor and supportive autonomic responses in reaction to the perception of danger. For example, it has been shown in humans that a sudden noise or unexpected somatosensory stimulus commonly evokes both a sudden inspiration and increase in skin sympathetic activity (13).

The ability of the auditory system to perceive danger, or an unexpected stimulus in the environment, depends upon what has been termed stimulus-specific adaptation, such that there is neuronal adaptation to repeated sounds while maintaining responsiveness to uncommon sounds (18). Neurons with such a capability were first identified in the auditory cortex (49) but more recently have also been identified in the IC (9,30). Neurons that display stimulus-specific adaptation have also been identified in the optic tectum of the barn owl, and so this may be a general characteristic of some neurons in the SC as well as in the IC. Such neurons in the IC and SC would therefore be good candidates as neurons that generate immediate co-ordinated physiological responses to novel or threatening stimuli.

Finally, consistent with our observations, the threshold for evoking avoidance and escape behavior in conscious rats is reduced by SC disinhibition (8). These findings, together with the data presented here, support our proposal that command neurons within the SC and IC, controlled by a GABAergic input, generate appropriate co-ordinated increases in
autonomic, respiratory and somatomotor activity as part of a generalized behavioral response to natural stimuli that require immediate action.
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DISCLOSURES

None of the authors have any conflicts of interest.

AUTHOR CONTRIBUTIONS

F.C.F.M.-R contributed to data collection, analysis and interpretation of data, and revision of the manuscript. R.A.L.D. and A.K.G. contributed to the conception and design of the experiments, and drafting and revision of the manuscript. S.M. contributed to the analysis and interpretation of data, and revision of the manuscript. M.A.P.F. contributed to the conception and design of the experiments, and revision of the manuscript. All authors approved the final version of the manuscript.
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Figure 1. Cardiovascular, respiratory and somatomotor responses evoked by different stimuli after microinjection of picrotoxin into the midbrain colliculi. A, An example where all 3 stimuli evoked responses in SpSNA, PNA and ScNA after, but not before, picrotoxin microinjection. B, An example where only the auditory stimulus (series of claps) evoked responses after picrotoxin microinjection.

Figure 2. Examples of recordings from two different experiments showing spontaneously-occurring synchronized bursts of SpSNA and PNA following microinjection of (A) picrotoxin and (B) bicuculline into the midbrain colliculi.

Figure 3. Cycle-triggered averaging of stimulus-evoked responses. A, Example of bursts of SpSNA, PNA and ScNA evoked by a single clap. B, The average of 10 responses, including the response shown in A, evoked by single claps following picrotoxin microinjection into one site in the colliculi. C, A 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.

Figure 4. Histogram showing the magnitudes (mean ± SEM) of the increase in averaged MAP and the amplitudes of the averaged SpSNA and PNA bursts as determined from cycle-triggered averaging of the responses evoked by auditory (clap), visual (light) or somatosensory (pinch) stimuli. Note that the responses were of very similar magnitude, whether evoked by clap, light or pinch.
*Figure 5.* Histogram showing the time differences (mean ± SE) between the onset of SpSNA bursts and the onsets of PNA bursts or ScNA bursts as determined from cycle-triggered averaging of the responses evoked by auditory (clap) or visual (light) stimuli, following picrotoxin microinjection into different positive sites in the colliculi. The negative values indicate that the onsets of PNA and ScNA bursts preceded the onset of SpSNA bursts.

* P<0.05, ** P<0.01, ns, not significant.

*Figure 6.* Distribution of the centers of sites at which, following a picrotoxin microinjection, a response was evoked by an auditory, visual and/or somatosensory stimulus (positive sites, red-filled circles) and sites at which no response was evoked (negative sites, black-filled circles), mapped on to three coronal sections of the brain at distances (in mm) caudal to bregma. CnF, cuneiform nucleus; dm, dorsomedial; dl, dorsolateral; DpG, deep gray layer of the SC; DpWh, deep white layer of the SC; ECIC, external nucleus of the IC; InG, intermediate gray layer of the SC; l, lateral; PAG, periaqueductal gray; PrCnF, precuneiform nucleus; vl, ventrolateral.

*Figure 7.* Auditory-evoked responses were intact following decerebration. A, Responses evoked by auditory stimuli (series of claps) after microinjection of bicuculline into one positive site after decerebration. B, Cycle-triggered average of 13 SpSNA and PNA auditory-evoked responses for this site, where the trigger point was the onset of the burst of SpSNA. C, Sagittal section of the brain at 1.13 mm lateral to midline showing brain regions (in gray) removed by decerebration in this experiment.
Arterial pressure (mmHg)

SpSNA (units)

PNA (units)

Integrated SpSNA (units)

Integrated PNA (units)

A After picrotoxin

B After bicuculline

5 s
Figure A: Graph showing the cycle-triggered averaging of arterial pressure (mmHg), SpSNA (units), PNA (units), and ScNA (units) over time.

Figure B: Graphs showing the averaged MAP (mmHg), integrated SpSNA (units), integrated PNA (units), and integrated ScNA (units) with a cycle-triggered average highlighted.

Figure C: Graphs showing the averaged integrated SpSNA (units), averaged integrated PNA (units), and averaged integrated ScNA (units) over time with a 100 ms scale.
Averaged ΔMAP (mmHg)

- Black bar: clap (n=25)
- Light grey bar: light (n=9)
- Dark grey bar: pinch (n=3)

Averaged SpSNA burst amplitude (%baseline)

Averaged PNA burst amplitude (%baseline)
Averaged integrated SpSNA (units)

Averaged integrated PNA (units)

Arterial pressure (mmHg)

Integrated SpSNA (units)

Integrated PNA (units)

Cycle-triggered averaging

Cerebral Cortex
Hypothalamus
Midbrain
Pons Medulla
Cerebellum

SC IC

1.0
0.5
0
1.0
0.5
0
0
1.0
0.5
0

Clap clap clap clap clap

5 s

1 s