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Anatomic and Functional Topography of the Dorsal Raphe Nucleus

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ABSTRACT: Serotonergic systems play an important and generalized role in regulation of sleep–wake states and behavioral arousal. Recent *in vivo* electrophysiologic recording studies in animals suggest that several different subtypes of serotonergic neurons with unique behavioral correlates exist within the brainstem raphe nuclei, raising the possibility that topographically organized subpopulations of serotonergic neurons may have unique behavioral or physiologic correlates and unique functional properties. We have shown that the stress-related and anxiogenic neuropeptide corticotropin-releasing factor can stimulate the *in vitro* neuronal firing rates of topographically organized subpopulations of serotonergic neurons within the dorsal raphe nucleus (DR). These findings are consistent with a wealth of behavioral studies suggesting that serotonergic systems within the DR are involved in the modulation of ongoing anxiety-related behavior and in behavioral sensitization, a process whereby anxiety- and fear-related behavioral responses are sensitized for a period of up to 24 to 48 h. The dorsomedial subdivision of the DR, particularly its middle and caudal aspects, has attracted considerable attention as a region that may play a critical role in the regulation of acute and chronic anxiety states. Future studies aimed at characterization of the molecular and cellular properties of topographically organized subpopulations of serotonergic neurons are likely to lead to major advances in our understanding of the role of serotonergic systems in stress-related physiology and behavior.

KEYWORDS: fear; anxiety; anatomy; stress; serotonin; 5-hydroxytryptamine; 5-HT; topographic

INTRODUCTION

Serotonergic systems within the brainstem raphe complex are topographically organized with respect to their anatomic and functional properties. This anatomic and functional specificity raises the possibility that topographically organized subpopulations of serotonergic neurons may have unique stress-related functional properties. Although there are clear differences between the anatomic and functional properties of the dorsal raphe nucleus (DR) and the median raphe nucleus (MnR),¹ in this review we focus on the anatomic and functional topographic organization within the

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DR, a brainstem raphe nucleus that contains an extensive group of serotonergic neurons (estimated to number 235,000 in the human brain²) projecting in a topographically organized manner to forebrain and brainstem targets. Recent technical advances have demonstrated that although the DR has been defined as a single nucleus by cytologic criteria, it comprises multiple subdivisions that differ with respect to the characteristics of individual serotonergic neurons found within them. Topographically organized subpopulations of serotonergic neurons within the DR differ in their morphologic features, membrane and cellular properties, afferent regulation, and efferent projection patterns. In this review, we evaluate whether or not these differences among subpopulations of serotonergic neurons have important implications for neural mechanisms underlying serotonergic modulation of stress-related physiology and behavior. In addition, we provide atlases of the rat and mouse DR and a proposed nomenclature that may prove valuable for future studies exploring the fine anatomic and functional topography of this nucleus.

SEROTONERGIC NEURONS IN DIFFERENT SUBDIVISIONS OF THE DR HAVE UNIQUE MORPHOLOGIC AND CELLULAR FEATURES

Serotonergic neurons in the rat DR appear in topographically organized groups of small round cells, medium-sized fusiform and bipolar cells, large fusiform cells, and very large multipolar cells.³ Similarly, serotonergic neurons in the human DR appear in topographically organized groups of round, ovoid, fusiform, and multipolar cells.² Although we cannot predict functional properties of serotonergic cells based on morphologic features, the fact that serotonergic neurons with different morphologic features are topographically organized within different subdivisions of the DR suggests a potential link between structure and function. Recently developed and recently optimized technical approaches, such as whole-cell patch-clamp electrophysiologic studies in visualized brain slices, should allow us to begin to investigate structure–function relationships.

In addition to regional differences in the morphology of topographically organized subpopulations of serotonergic neurons, immunohistochemical co-localization studies have demonstrated that topographically organized subpopulations of serotonergic neurons co-express different neurotransmitters, neuropeptides, or other proteins. For example, recent studies have identified a subpopulation of serotonergic neurons within the dorsomedial subdivision of the DR (~–8.00 mm bregma) that also expresses corticotropin-releasing factor (CRF).⁴ This same study revealed, consistent with previous studies,⁵ that this region of the DR projects to the central nucleus of the amygdala (CeA), a stress-related forebrain structure that is part of a distributed central autonomic control system. These observations, along with the high expression of neurokinin-1 immunoreactivity on neuronal fibers within the dorsomedial DR, suggest that this subdivision may play a role in serotonergic modulation of stress-related physiology and behavior in rats and in stress-related neuropsychiatric disorders in humans.

EFFERENT PROJECTIONS ARISING FROM THE DR ARE TOPOGRAPHICALLY ORGANIZED

The midbrain raphe nuclei, including the DR and the MnR, are the main sources of serotonergic innervation of forebrain stress circuits.¹ The most consistent finding

in anatomic studies is that serotonergic neurons within the DR are organized topographically in the rostrocaudal plane. That is, serotonergic neurons located more rostrally project selectively to specific forebrain sites (e.g., the caudate putamen, substantia nigra, and virtually all neocortical regions), whereas serotonergic neurons located more caudally project to other forebrain sites (e.g., the septum, hippocampus, and entorhinal cortex^{3,6-8}). Serotonergic neurons that project to the caudate putamen do not project to the hippocampus, and vice versa.⁹ These simple anatomic observations suggest that topographically organized subpopulations of serotonergic neurons have unique functional properties associated with modulation of specific forebrain systems.

SEROTONERGIC NEURONS GIVE RISE TO COLLATERAL PROJECTIONS TO FUNCTIONALLY RELATED TARGETS

The use of multiple retrograde tracers in a single animal has led to the proposal that not only are serotonergic neurons topographically organized within the mid-brain and pontine raphe nuclei, but that individual serotonergic neurons give rise to collateral (branched) projections to functionally related targets, such as those involved in stress responses.¹ These collateral serotonergic projections could allow temporally coordinated modulation of specific neural circuits, such as those implicated in central autonomic control, anxiety, and conditioned fear, which include the CeA and the paraventricular nucleus (PVN) of the hypothalamus.¹ Collateral projections from serotonergic neurons innervating the CeA and PVN are located primarily in medial regions of the middle DR and in bilateral clusters of cells near the cerebral aqueduct more caudally.^{5,10} These double retrograde-tracing studies and others¹⁰⁻¹³ provide clear evidence for an anatomic topography within the DR and suggest that individual serotonergic neurons give rise to functionally related target structures, providing an anatomic basis for serotonergic modulation of distributed functional systems within the brain.

AFFERENT PROJECTIONS INNERVATING THE DR ARE TOPOGRAPHICALLY ORGANIZED

Different subdivisions of the DR receive afferent input from different sources. Retrograde and anterograde studies provide evidence for specific afferents to the ventral and dorsal parts of the middle (~-8.00 mm bregma) DR, the rostral DR (~-7.30 mm bregma), and the ventrolateral DR.^{14,15} A recent immunohistochemical study in rats found that CRF-ir fibers in the DR are organized along a rostral-to-caudal gradient and that CRF-ir fibers innervating different subdivisions of the DR have different proportions of excitatory versus inhibitory synaptic specializations, with the majority of synapses between CRF-ir terminals and dendrites in the dorsolateral DR being excitatory and the majority of those in the ventromedial region being inhibitory.¹⁶ Although the origin of the full complement of CRF-ir fibers innervating the DR is uncertain, a proportion of CRF-ir afferents arises from the CeA, suggesting that they may play an important role in differential modulation of topographically organized subpopulations of serotonergic neurons under stress-related conditions.¹⁷

FUNCTIONAL TOPOGRAPHIC ORGANIZATION OF THE DR

Behavioral Correlates of Neuronal Activity

Some of the strongest evidence for topographic organization of subpopulations of serotonergic neurons comes from studies combining electrophysiologic data with behavioral observations. Single-unit extracellular recording studies in cats suggested the existence of two different types of serotonergic neurons, designated type I and type II serotonergic neurons, based on behavioral correlates. Type I serotonergic neurons are active during waking arousal but show decreased activity during stages of quiet waking (QW) and slow-wave sleep (SWS) and cease firing during paradoxical sleep (PS) or rapid eye movement sleep. Type II serotonergic neurons are highly topographically organized and are found only in small numbers in bilateral columns of cells at the caudal interface of the dorsal and median raphe nuclei (reminiscent of the interfascicular dorsal raphe nucleus in the rat brain). Type II serotonergic neurons, like type I neurons, are active during waking arousal and QW but remain active during SWS and PS.¹⁸ Due to the overwhelming preponderance of type I serotonergic neurons, it is likely that most conclusions drawn from single-unit electrophysiology studies are drawn from random sampling of this population of cells.

A recent study sampling a large number of presumed serotonergic neurons within the DR using single-unit recording methods has classified them into six different subtypes based on their electroencephalographic and behavioral correlates in cats.¹⁹ Serotonergic neurons were classified within two large categories: (1) typical serotonergic neurons (e.g., similar to type I serotonergic neurons described previously) and (2) atypical serotonergic neurons. Among the typical and atypical serotonergic neurons, subpopulations were identified that may correspond to type I and type II serotonergic neurons described previously (TABLE 1). According to Sakai *et al.*,¹⁹ type I-A and type I-B serotonergic neurons are evenly distributed in the DR, discharge regularly at a high rate during active waking and at progressively slower rates during SWS, and cease firing during SWS or PS (type I-A) or only during PS (type I-B); atypical type I-C serotonergic neurons are unevenly distributed in the DR (located predominantly within the ventral DR, similar to the atypical type II serotonergic neurons) and discharge regularly at a high rate during active waking but have sustained activity during SWS and PS. Other atypical serotonergic neurons (types II-A, II-B, and II-C) have unique topographic distributions within the DR. Type II-A neurons are located predominantly within the dorsomedial DR and show reductions in firing during QW and SWS; unlike typical serotonergic neurons, the level of α_1 -adrenergic receptor activation does not seem to be a major determinant of neuronal activity during SWS and PS. This study of a large sample of serotonergic neurons within the DR points toward a greater degree of functional heterogeneity of serotonergic neurons within the DR than had previously been suspected.

Cellular Properties and Afferent Control Mechanisms

Consistent with findings that the DR consists of multiple topographically organized subpopulations of serotonergic neurons with unique behavioral correlates, studies investigating the responses of serotonergic neurons to specific afferent signals (neurotransmitters, neuropeptides) have demonstrated that responses are dependent on the topographic location of the recordings.

TABLE 1. Typical and atypical serotonergic cells of the DR in freely moving cats (as described by Rasmussen *et al.*¹⁸ and Sakai and Crochet¹⁹)

Type	Firing Pattern	Distribution	Behavioral Correlates	% Cessation Blocked By
Typical groups				
I-A (similar to Type I serotonergic neurons in Rasmussen <i>et al.</i> ¹⁸)	Clock-like	Evenly distributed	Cease firing during SWS with PGO waves and PS	40 Histamine or phenylephrine
I-B (similar to Type I serotonergic neurons in Rasmussen <i>et al.</i> ¹⁸)	Clock-like	Evenly distributed	Cease firing during SWS only	27 Histamine or phenylephrine
Atypical groups				
I-C (similar to Type II serotonergic neurons in Rasmussen <i>et al.</i> ¹⁸)	Clock-like	Unevenly distributed (ventral DR)	Sustained activity during SWS and PS	6 Histamine or phenylephrine
II-A	Nonclock-like	Unevenly distributed (dorsomedial DR)	Reduction in firing during QW and SWS. Ceased firing before the occurrence of PGO waves and remained silent during SWS with PGO waves and PS	8 Phenylephrine has no effect; histamine blocks cessation of discharge during sleep
II-B	Nonclock-like	Unevenly distributed (rostral dorsal portion of DR, near the oculomotor n.)	Highest rate of discharge during SWS with suppression of activity during waking and PS; strongly activated during feeding or grooming	12 Phenylephrine
II-C	Nonclock-like	Unevenly distributed (ventral DR near mlf and nucleus annularis)	Sustained activity during PS	8 Histamine

ABBREVIATIONS: DR, dorsal raphe nucleus; SWS, slow-wave sleep; PGO, ponto-geniculo-occipital; PS, paradoxical sleep; QW, quiet waking.

Electrophysiologic studies suggest that intracerebroventricular injection of CRF at low doses inhibits the firing rates of DR 5-HT neurons *in vivo* in a topographically dependent manner; the majority of the cells tested (60.7%) in the ventromedial DR (−7.80 mm bregma) were inhibited by CRF, whereas responses of cells in the dorsomedial and lateral wings (ventrolateral DR) were more variable.^{20,21} *In vitro* experiments in our laboratory have found a greater proportion of CRF-responsive neurons in the ventral midline of the caudal DR compared with the dorsal region of the DR at the same rostrocaudal level (−8.00 to −8.40 mm bregma).²² Recent studies using visualized whole-cell patch-clamp electrophysiologic techniques reveal that serotonergic neurons within the dorsomedial and ventromedial DR differ with respect to active and passive membrane characteristics,²³ further supporting distinctions among cellular properties and afferent control mechanisms of topographically organized serotonergic neurons within this nucleus.

Heterogeneity of 5-HT neuronal firing is also demonstrated in responses to compounds related to circadian fluctuations in behavioral arousal. The cessation of serotonergic neuronal firing in serotonergic neurons of the DR observed during PS is not the result of GABA-mediated inhibition²⁴ but is rather the result of lack of facilitation resulting from the local release of norepinephrine and histamine.¹⁹ Different subtypes of serotonergic DR neurons respond differentially to microdialysis application of phenylephrine (an α_1 -adrenergic agonist) and histamine. Typical I-A and I-B neurons' normal quiescence during PS is blocked by phenylephrine or histamine, whereas the atypical II-A and II-C neurons' low firing rates are unaffected by phenylephrine but are totally (II-A) or partially (II-C) blocked by histamine (leading to substantial tonic activity during sleep).¹⁹ These results strongly suggest additional heterogeneity in the behavioral correlates and afferent control mechanisms of topographically organized subpopulations of serotonergic neurons within the DR.

Behavioral Studies

Anatomic and functional heterogeneity of the DR is nowhere more evident than in the modulation of stress-related neural circuits and stress responses. Behavioral studies involving site-specific injections of CRF into subregions of the DR support the hypothesis that the DR has a functional topographic organization in the rostrocaudal plane. A series of studies has demonstrated that increased serotonergic activity arising from the DR plays a critical role in the development of learned helplessness, including increases in behavioral measures of anxiety and fear measured 24 to 48 h after exposure to inescapable stress.^{25–27} Further studies have shown that these behavioral changes are dependent on CRF actions within the DR. Microinjection of the nonselective CRF receptor antagonist D-Phe-CRF_(12–41) into specific sites in the caudal DR (particularly at ~−8.30 mm bregma) blocks behavioral changes induced by inescapable stress.²⁸ Microinjections of CRF into the caudal DR (but not the rostral DR) are sufficient to induce learned helplessness 24 h later in the absence of any exposure to inescapable stress,²⁸ consistent with the hypothesis that CRF actions on serotonergic systems arising from the caudal portion of the DR mediate these behavioral changes. This is consistent with the finding that exposure to inescapable stress increases the expression of the protein product of the immediate-early gene *c-fos* within the middle and caudal portions of the DR but not the rostral portion. This

evidence points toward an essential role for the caudal DR in modulating the behavioral consequences of inescapable stress.

IMMEDIATE-EARLY GENE EXPRESSION STUDIES

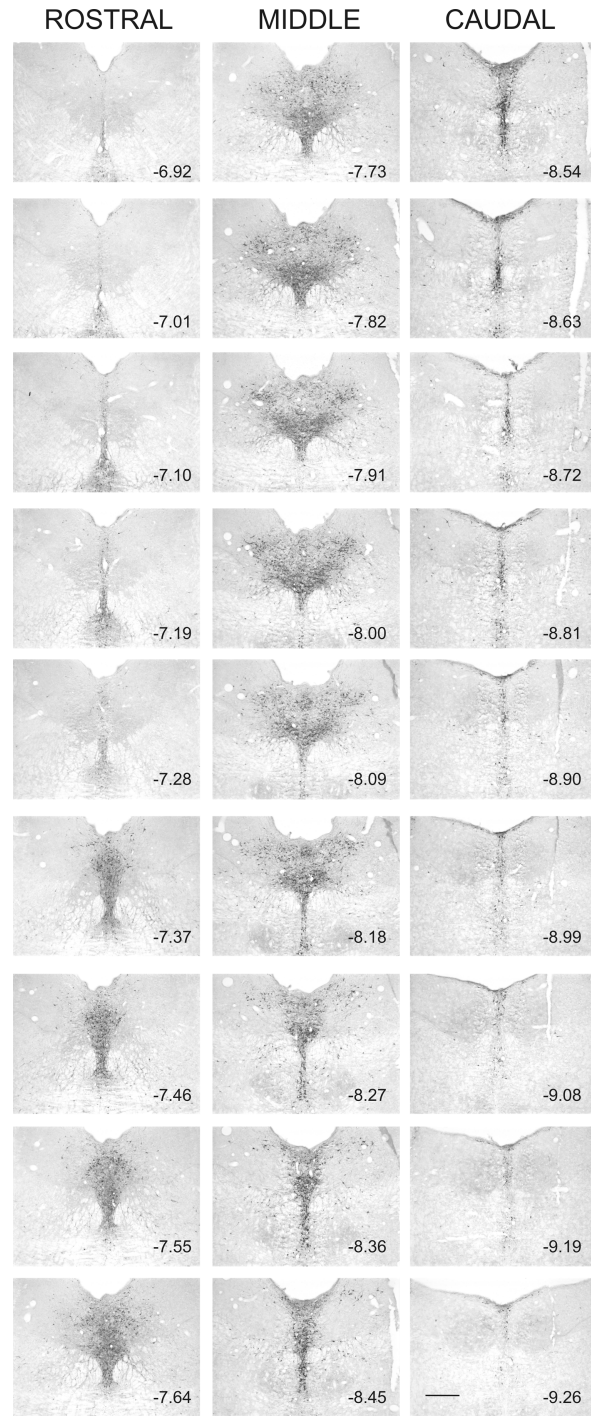
There is a large body of data concerning the effects of stressful stimuli on the expression of immediate-early genes within the DR.²⁹ Investigation of the effects of stress-related stimuli on the expression of the protein products of immediate-early genes such as *c-fos* is an effective means of evaluating the effects of a temporally defined stimulus on large populations of cells, with resolution of functional responses at the single-cell level. Paradigms associated with increased anxiety or conditioned fear,^{30–41} opiate withdrawal,^{42,43} and intracerebroventricular infusion of CRF or similar anxiogenic neuropeptides^{44,45} are effective at increasing immediate-early gene expression within caudal regions of the DR. When these data are taken together with those indicating increased serotonergic neurotransmission in the DR in response to stressful stimuli,^{46,47} especially when the stressor is intense, uncontrollable, or unpredictable,^{48–51} they suggest a mechanism whereby anxiogenic or fear-inducing stimuli increase serotonergic neurotransmission via actions on subsets of serotonergic neurons within the caudal regions of the DR that give rise to a meso-limbocortical serotonergic innervation of the forebrain.

THE NEED FOR DEFINITIVE NEUROANATOMIC MAPS

With the goal of developing better treatments for human neuropsychiatric disorders in mind, it is essential that rostrocaudal levels of the DR and other raphe nuclei be carefully defined. A survey of the literature shows widely variable definitions, with some groups' definitions of "caudal" regions^{20,21} being almost as far forward as others' definitions of "rostral" ones²². Therefore, we propose consensus definitions based on standard stereotaxic atlases of the rat⁵² and mouse⁵³ brain and our own atlases of rat (FIG. 1) and mouse brain (FIG. 2) immunostaining with tryptophan hydroxylase.

According to these atlases, the "rostral" DR (regions associated with the caudal linear nucleus, ventrally) comprises anatomic levels from -6.92 to -7.64 mm bregma in the rat brain (-4.12 to -4.48 mm bregma in the mouse); the "middle" DR includes anatomic levels from -7.73 to -8.45 mm bregma in the rat brain (-4.54 to -4.90 mm bregma in the mouse), and "caudal" DR includes anatomic levels from -8.54 to -9.26 mm in the rat brain (-4.96 to -5.32 mm bregma in the mouse). We hope that these maps will prove useful in making meaningful comparisons among different studies of anatomic and functional subsets of serotonergic neurons in rodent brain.

FIGURE 1. Anatomic atlas of tryptophan hydroxylase (TrpOH) immunohistochemical (ir) staining in the rat DR. Sections ($30\ \mu\text{m}$) were obtained from an adult male Wistar rat, and every third section was immunostained with a sheep anti-TrpOH antibody. Bregma levels (mm) are indicated in each photograph. Scale bar represents $50\ \mu\text{m}$. Anatomic levels assigned based on comparisons with a rat stereotaxic atlas.⁵²



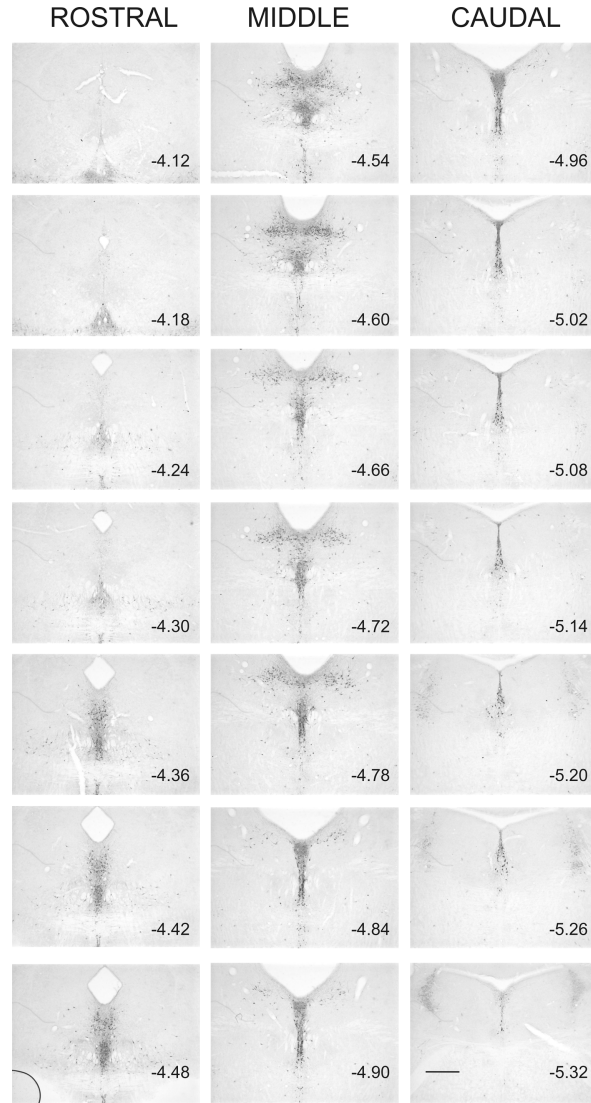


FIGURE 2. Anatomic atlas of TrpOH-ir staining in the mouse DR. Sections (30 μm) were obtained from an adult male Balb/c mouse, and every second section was immunostained with a sheep anti-TrpOH antibody. Bregma levels (mm) are indicated in each photograph. Scale bar represents 50 μm. Anatomic levels assigned based on comparisons with a mouse stereotaxic atlas.⁵³

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