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Functional topography of serotonergic systems supports the Deakin/Graeff hypothesis of anxiety and affective disorders

Evan D Paul and Christopher A Lowry

Abstract

Over 20 years ago, Deakin and Graeff hypothesized about the role of different serotonergic pathways in controlling the behavioral and physiologic responses to aversive stimuli, and how compromise of these pathways could lead to specific symptoms of anxiety and affective disorders. A growing body of evidence suggests these serotonergic pathways arise from topographically organized subpopulations of serotonergic neurons located in the dorsal and median raphe nuclei. We argue that serotonergic neurons in the dorsal/caudal parts of the dorsal raphe nucleus project to forebrain limbic regions involved in stress/conflict anxiety-related processes, which may be relevant for anxiety and affective disorders. Serotonergic neurons in the "lateral wings" of the dorsal raphe nucleus provide inhibitory control over structures controlling fight-or-flight responses. Dysfunction of this pathway could be relevant for panic disorder. Finally, serotonergic neurons in the median raphe nucleus, and the developmentally and functionally-related interfascicular part of the dorsal raphe nucleus, give rise to forebrain limbic projections that are involved in tolerance and coping with aversive stimuli, which could be important for affective disorders like depression. Elucidating the mechanisms through which stress activates these topographically and functionally distinct serotonergic pathways, and how dysfunction of these pathways leads to symptoms of neuropsychiatric disorders, may lead to the development of novel approaches to both the prevention and treatment of anxiety and affective disorders.

Keywords

Anxiety, depression, functional topography, panic disorder, raphe nucleus, serotonergic pathways, serotonin, stress

Introduction

Serotonergic systems are implicated in coordinating the appropriate behavioral and physiologic responses to aversive stimuli, and dysfunction of these serotonin systems is postulated to contribute to the etiology and pathophysiology of stress-related psychiatric disorders, including anxiety and affective disorders. Even though this view is generally accepted, there are controversies surrounding the particular role of 5-hydroxytryptamine (5-HT) in controlling defensive responses to aversive stimuli and the role of 5-HT in anxiety (such as whether 5-HT is anxiogenic or anxiolytic) and affective disorders (Griebel, 1995; Millan, 2003; Soubrie, 1986).

Early investigations into the role of 5-HT in defense and anxiety used conflict models of anxiety, in which the animal is trained to perform an operant response for a reward (e.g. pushing a lever for a food pellet), and this behavior is in turn suppressed through a response-contingent shock (Geller and Seifter, 1960). These conflict models found that interventions resulting in reduced serotonergic neurotransmission increase punished responding, an effect indicative of an anxiogenic role for 5-HT (Geller and Blum, 1970; Graeff and Schoenfeld, 1970; Robichaud and Sledge, 1969; Wise et al., 1972). Paradoxically, in other animal models of aversive behavior, such as electrical stimulation of the dorsal periaqueductal gray (DPAG), 5-HT has an anti-aversive (i.e. anti-panic) effect. Stimulation of the DPAG in animals rapidly elicits fight-or-flight behaviors and sympathetic responses, resulting in tachycardia, hypertension and hyperventilation (Keay and Bandler, 2001); a set of behavioral and physiological responses that is strikingly similar to the reaction provoked by an immediate threat, such as exposure to a predator (Blanchard et al., 1986). Studies using this model of DPAG-stimulated aversion reveal that pharmacological treatments that reduce serotonergic neurotransmission facilitate escape-responding behavior (Kiser and Lebovitz, 1975; Kiser et al., 1978b; Schenberg and Graeff, 1978). In other words, reducing 5-HT neurotransmission increases operant responding in order to shut down DPAG stimulation, an effect that suggests 5-HT inhibits DPAG stimulation-induced aversion. Similarly, interventions that increase serotonergic neurotransmission attenuate lever-pressing to escape DPAG stimulation (Kiser et al., 1978a), again suggesting that 5-HT decreases the aversiveness of DPAG stimulation. Taken together with other evidence investigating the role of 5-HT in DPAG stimulation-induced aversion (for comprehensive reviews and references, see Del-Ben and Graeff, 2009; Graeff, 2004), these data illustrate that facilitation of serotonergic neurotransmission has anti-aversive-like effects in the DPAG, which is interpreted as an anti-panic-like effect of 5-HT.

Department of Integrative Physiology and Center for Neuroscience, University of Colorado Boulder, Boulder, USA

Corresponding author:
Evan D Paul, Department of Integrative Physiology and Center for Neuroscience, University of Colorado Boulder, Boulder, CO 80309-0354, USA.
Email: evan.paul@colorado.edu

Table 1. Serotonin receptor subtypes, their locations and affinities for serotonin.

Receptor	Type	Location	Affinity (pk_i)[a]	References
5-HT_{1A}	GPCR ($G_{i/o}$)	presynaptic/ postsynaptic	9.1–9.7	Kalipatnapu et al., 2004; Newman-Tancredi et al., 1992; Newman-Tancredi et al., 1997; Newman-Tancredi et al., 1998a; Newman-Tancredi et al., 1998b; Newman-Tancredi et al., 1999.
5-HT_{1B}	GPCR ($G_{i/o}$)	presynaptic/ postsynaptic	7.4–9.0	Davidson et al., 1997; Newman-Tancredi et al., 1999; Newman-Tancredi et al., 2000; Selkirk et al., 1998; Watson et al., 1996; Weinshank et al. et al., 1992
5-HT_{2A}	GPCR ($G_{q/11}$)	postsynaptic	6.0–8.4	Almaula et al., 1996; Branchek et al., 1990; Johnson et al., 1994; Knight et al., 2004; May et al., 2003; Sleight et al., 1996
5-HT_{2C}	GPCR ($G_{q/11}$)	postsynaptic	6.8–8.6	Egan et al., 2000; Fitzgerald et al., 1999; Kimura et al., 2004; Knight et al., 2004; May et al., 2003

[a]Affinities are for human serotonin receptors and were obtained from the IUPHAR database (Sharman et al., 2013). See the IUPHAR website (http://www.iuphar-db.org/index.jsp) for further information about all serotonin receptor subtypes.
5-HT: 5-hydroxytryptamine; GPCR: G-protein coupled receptor; IUPHAR: International Union of Basic and Clinical Pharmacology

The Deakin/Graeff hypothesis

To reconcile the anxiogenic effect of 5-HT in conflict tasks with the panicolytic effect of 5-HT in DPAG models of aversion/anxiety, Deakin and Graeff (1991) proposed that different types of aversive stimuli (e.g. unconditioned versus conditioned aversive stimuli; and acute versus chronic stress) activate different 5-HT pathways that send unique patterns of efferents to specific forebrain and brainstem structures, in order to coordinate an adaptive behavioral and physiologic response, and that dysfunction of these neural circuits results in distinct pathological entities. Serotonin is thought to mediate its effects through activation of a myriad of postsynaptic 5-HT receptors, including: 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 receptors. Serotonin receptors consist of at least 14 different subtypes that predominantly belong to the family of G-protein coupled receptors (GPCR), except for the 5-HT_3 receptors, which are ligand-gated ion channels. Further adding to this complexity, 5-HT receptors are located presynaptically or postsynaptically, or both; they can undergo extensive post-translational modifications through RNA editing (e.g. 5-HT_{2C} receptors) and alternate splicing, they have different affinities for 5-HT, they can form homo- and heterodimers, and they can be modulated by a number of accessory proteins (for a review of serotonin receptor subtypes, see Artigas, 2013; Hannon and Hoyer, 2008; Hoyer et al., 1994; Hoyer et al., 2002; Millan et al., 2008; Werry et al., 2008). In this review, we will limit the discussion to the 5-HT receptors discussed by Deakin and Graeff (Table 1); however, it is likely that a complex interplay between postsynaptic 5-HT receptors is necessary to coordinate an adaptive stress response. The Deakin/Graeff hypothesis proposes three distinct serotonergic systems controlling behavioral responses to aversive stimuli. It was proposed that the first serotonergic pathway, consisting of the dorsal raphe periventricular tract, restrains fight-or-flight behavior in response to either (a) stimulation of the DPAG or (b) exposure to acute unconditioned aversive stimuli (e.g. predator exposure, pain or aversive interoceptive stress), resulting in freezing/quiescence. The inhibitory action of 5-HT in the DPAG is thought to be mediated by stimulation of 5-HT_{1A} and/or 5-HT_{2A} receptors (described below). It was hypothesized that dysfunction of this serotonergic pathway results in unrestrained bouts of sympathetic and behavioral arousal reminiscent of panic disorder (PD). It was proposed that a second pathway (consisting of the dorsal raphe forebrain bundle tract innervating structures such as the amygdala, hippocampus and prefrontal cortex) is recruited by exposure to acute conditioned aversive stimuli, with the goal of facilitating risk assessment and avoidance behaviors in order to direct the organism away from potential or distal danger. In this pathway, serotonin was thought to mediate its effects via binding to 5-$HT_{2A/2C}$ and 5-HT_3 receptors. It was hypothesized that abnormalities in this serotonergic circuit relate to anxiety disorders like Generalized Anxiety Disorder (GAD). Finally, it was proposed that a third serotonergic pathway, consisting of the median raphe forebrain bundle tract and projecting to the septo-hippocampal system, is activated by chronic unconditioned and conditioned stimuli, promoting resilience or tolerance to chronic stress through activation of postsynaptic 5-HT_{1A} receptors in the hippocampus. It was hypothesized that dysfunction of this serotonergic pathway, and consequently failure to adapt to chronic stress, is relevant to depression. The experimental evidence that led to the Deakin/Graeff hypothesis and the evidence generated since their proposal have been reviewed extensively (the following are excellent reviews: Deakin and Graeff, 1991; Graeff, 1990; Graeff, 1991; Graeff, 2004; Graeff et al., 1996).

Here, we describe studies from our laboratory and others, published over the previous 20 years, that are relevant to the Deakin/Graeff hypothesis. These studies are largely confirmatory of the Deakin/Graeff hypothesis and suggest that topographically-organized subpopulations of serotonergic neurons in the dorsal (DR) and median (MnR) raphe nuclei are differentially activated by a number of stressful stimuli, have unique patterns of afferents/efferents, and are implicated in the specific symptoms associated with stress-related neuropsychiatric disorders. More specifically, we hypothesize that serotonergic neurons in (a) the dorsal part of the DR (DRD)/caudal part of the DR (DRC), (b) the ventrolateral part of the DR (DRVL)/ventrolateral periaqueductal gray (VLPAG), and (c) the interfascicular part of the DR (DRI)/MnR constitute the serotonergic subpopulations that give rise to the pathways originally described by the Deakin/Graeff hypothesis (Deakin and Graeff, 1991; Graeff et al., 1996).

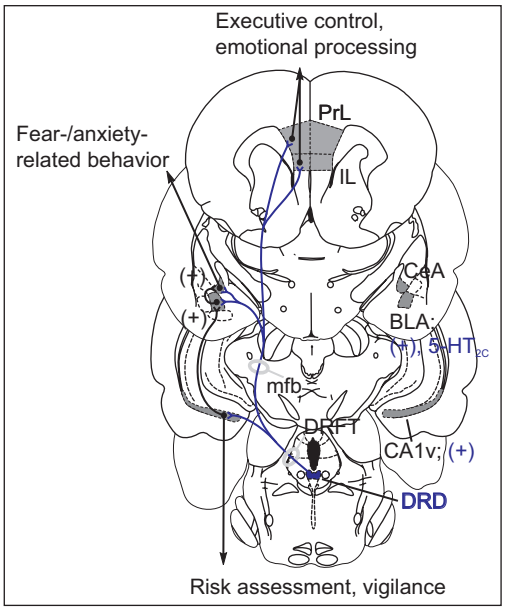

Figure 1. Schematic diagram depicts the serotonergic projections of the dorsal part of the DRD, which constitutes the "anxiety" pathway originally proposed by Deakin and Graeff. Serotonergic neurons in the DRD give rise to axons traveling through the DRFT to innervate forebrain limbic structures involved in controlling anxiety-related behavior, like the BLA, CeA and BnST (not shown). The BLA also projects to the CeA and BnST (not shown), two structures integral for fear and anxiety responses, respectively. Dysfunction of this pathway is thought to relate to symptoms of GAD. Coronal section templates reproduced with from Paxinos G and Watson C (1998) *The Rat Brain in Stereotaxic Coordinates*, 4th Edition. San Diego: Academic Press: 1998 with permission from Elsevier.

BLA: basolateral amygdaloid nucleus; BnST: bed nucleus of the stria terminalis; CA1v: field CA1 of ventral hippocampus; CeA: central nucleus of the amygdala; DRD: dorsal raphe nucleus, dorsal part; DRFT, dorsal raphe forebrain bundle tract; GAD: Generalized Anxiety Disorder; IL: infralimbic cortex; mfb: medial forebrain bundle; PrL: prelimbic cortex; (+): excitation; (–): inhibition.

Different methodological approaches have been used in previous studies to investigate the functional topography of serotonergic neurons in specific subregions of the midbrain raphe complex. The first, and most extensively used, approach is immunohistochemical staining of tryptophan hydroxylase (TPH) or serotonin (to identify serotonergic neurons) and immediate-early gene proteins (e.g. c-Fos, to identify biochemically-activated neurons) following acute stress-related challenges. A second approach is in situ hybridization histochemistry, with high-resolution analysis of expression of serotonergic genes throughout the midbrain raphe complex, such as *tph2*, encoding TPH 2; *slc6a4*, encoding the high affinity, sodium-dependent serotonin transporter; and *htr1a*, encoding the 5-HT_{1A} receptor. This approach has the advantage of identifying both developmental and other long-term influences, and acute stimulus-induced changes in gene expression in subregions of the midbrain raphe complex. A third approach involves microdissections of subregions of the midbrain raphe complex, followed by measurement of in vivo TPH activity, or measurement of tissue concentrations of serotonin and the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA). Importantly, these three approaches provide convergent evidence for the functional properties of topographically-organized subpopulations of serotonergic neurons, and the evidence strongly supports the original tripartite model of serotonergic systems in anxiety and affective disorders, originally proposed by Deakin and Graeff.

Hodology and functional topography of DRD/DRC serotonergic neurons: Role in conflict anxiety and affective disorders

Serotonergic neurons in the DRD/DRC project to forebrain structures involved in emotional control and anxiety-related behavior

Consistent with Deakin and Graeff's pathway involved in anxiety, serotonergic neurons of the DRD/DRC send projections through the dorsal raphe forebrain bundle tract to innervate forebrain structures that are involved in emotional control and anxiety-related behavior, including the basolateral nucleus of the amygdala (BLA) (Hale et al., 2008a; Ottersen, 1981), central nucleus of the amygdala (CeA) (Commons et al., 2003), bed nucleus of the stria terminalis (BnST) (Petit et al., 1995) and the ventral medial prefrontal cortex (vmPFC) (Van Bockstaele et al., 1993). Serotonergic neurons of the DRD also send collateral projections to functionally-related structures involved in mediating fear and conflict anxiety-like behavior, including the BLA and ventral hippocampus (Imai et al., 1986), the CeA and vestibular nuclei (Halberstadt and Balaban, 2006), and the hippocampus and entorhinal cortex (Kohler and Steinbusch, 1982). Furthermore, many of these forebrain targets in turn project back to the DRD/DRC subregions (Peyron et al., 1998). These functional neuroanatomical studies support the notion that DRD/DRC serotonergic neurons are part of a widespread, interconnected stress- and anxiety-related circuit (Figure 1) (For a full list of DRD/DRC efferents and afferents, see the following reviews: Hale and Lowry, 2011; Hale et al., 2012; Lowry, 2002; Lowry et al., 2008b).

Anxiety- and stress-related stimuli activate DRD/DRC serotonergic neurons

Anxiogenic drugs activate DRD/DRC serotonergic neurons. According to Deakin and Graeff (1991), a 5-HT pathway innervating forebrain limbic structures is activated by acute aversive conditioned stimuli, in order to guide the organism away from potential danger. Based on neuroanatomical tracing and functional neuroanatomical studies, serotonergic neurons of the DRD and the functionally related DRC are part of a stress- and anxiety-related circuit. These serotonergic neurons are selectively activated by a number of anxiety-related and aversive stimuli and likely constitute the subpopulation of serotonergic neurons originally discussed by Deakin/Graeff. Serotonergic neurons in the DRD and DRC are selectively activated after intraperitoneal (i.p.) administration of diverse anxiogenic drugs, including caffeine (an adenosine receptor antagonist), m-chlorophenyl piperazine (mCPP; a 5-$HT_{2A/2C}$ receptor agonist), and N-methyl-beta-carboline-3-carboxamide (FG-7142; a partial inverse agonist at the benzodiazepine allosteric site on the γ-aminobutyric acid A ($GABA_A$) receptor) (Abrams et al., 2005).

Stress- and anxiety-related neuropeptides activate DRD/DRC serotonergic neurons. DRD/DRC serotonergic neurons are selectively activated by the stress- and anxiety-related neuropeptide urocortin 2 (Ucn 2), when administered either through the intracerebroventricular (i.c.v.) route (Hale et al., 2010b; Staub et al., 2005) or directly into the mid-rostrocaudal and caudal DR (Amat et al., 2004). Likewise, chronic infusion of Ucn 2 or ovine corticotropin-releasing factor (CRF), adjacent to the rostral DR, both result in a nearly 30% reduction in *slc6a4* mRNA expression selectively in the DRD subregion, confirming studies above demonstrating that the DRD is a CRF/Ucn-sensitive subpopulation of serotonergic neurons. The same study reports that chronic administration of Ucn 2 or ovine CRF increases the ratio of the expression of *tph2* mRNA to the expression of *slc6a4* mRNA within the core of the DRD, relative to the ventromedial DR. These neuropeptide-induced alterations of DRD serotonergic gene expression are associated with increases in anxiety-like behavior (Clark et al., 2007).

Diverse stress-related stimuli activate DRD/DRC serotonergic neurons. Serotonergic neurons in the DRD/DRC are potently activated by a number of diverse stressors. Uncontrollable stress, relative to controllable stress, activates serotonergic neurons in the mid-rostrocaudal to caudal DR, as measured by increases in extracellular 5-HT and c-Fos expression in 5-HT neurons (Amat et al., 2005). Exposure to unpredictable noise stress, but not sham noise stress, increases in vivo TPH activity selectively, within the DRC (Evans et al., 2009). Likewise, chronic oral corticosterone administration abolishes the diurnal rhythm of *tph2* gene expression within the DRD/DRC region (and in the ventral part of the DR (DRV); see supplementary Figure S2) and increases anxiety- and depressive-like behavior (Donner et al., 2012b). In the elevated T-maze, which is a behavioral test used to simultaneously measure anxiety- and panic-/escape-like behavior, inhibitory avoidance (i.e. conflict anxiety-like behavior) increases c-Fos immunoreactivity in serotonergic neurons of the DRD/DRC (and DRI/MnR, see below), whereas one-way escape, a measure of panic-like behavior, does not (Spiacci et al., 2012). Other more ethologically-relevant stressors such as social defeat also result in activation of DRD serotonergic neurons (Gardner et al., 2005).

Serotonergic neurons in the DRD/DRC facilitate conflict-anxiety-like behavior through projections to forebrain structures

Serotonergic projections from DRD/DRC 5-HT neurons to the basolateral amygdaloid nucleus. Consistent with the evidence showing that DRD 5-HT neurons are activated by anxiogenic drugs and anxiety-related neuropeptides, exposure of rats to an open-field arena, which is mildly stressful and anxiogenic, increases the expression of the neuronal activation marker c-Fos in DRD 5-HT neurons, including a subpopulation of 5-HT neurons that project to the BLA (Hale et al., 2008a), a critical structure for processing anxiety- and fear-related stimuli (Davis et al., 1994; Hale et al., 2006; Ottersen, 1981). Administration of Ucn 2 into the mid-rostrocaudal and caudal DR also increases extracellular 5-HT within the BLA (Amat et al., 2004). A study by Hale and colleagues (2010a) reports that administration of the same anxiogenic drugs (except mCPP) discussed above increases neuronal activity in a subset of parvalbumin (PV)-expressing GABAergic interneurons that also co-express the 5-HT_{2A} receptor; the increases in activation of PV-GABA interneurons is positively correlated with the increases in activation of serotonergic neurons within the mid-rostrocaudal and caudal DR, as well as the increases in anxiety-like behavior observed by Abrams et al. (2005; Hale et al., 2010a). These PV-expressing GABAergic neurons are one of at least four subgroups of local inhibitory GABAergic interneurons that can be distinguished by the presence of calcium-binding proteins and neuropeptide content (McDonald and Mascagni, 2001). The function of PV-expressing GABAergic interneurons may be to terminate anxiety-related responses in the BLA (Hale et al., 2010a).

These anxiogenic drugs also activate non-PV-expressing neurons (presumably glutamatergic) that may facilitate anxiety (Hale et al., 2010a). Prior exposure to uncontrollable stress leads to an exaggerated elevation of extracellular 5-HT in the BLA during a social exploration task and it reduces social exploration, an anxiogenic effect that is attenuated by microinjection of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) into the mid-rostrocaudal and caudal DR, or by microinjection of the 5-HT_{2C} receptor antagonist SB 242,084 into the BLA (Christianson et al., 2010). Overall, these studies support Deakin and Graeff's view that stress- and anxiety-related stimuli facilitate anxiety-like behavior through activation of serotonin neurons (located in the DRD/DRC) that project to the amygdala.

Serotonergic projections from DRD/DRC 5-HT neurons to the hippocampal formation. In addition to the BLA, serotonergic neurons of the DRD/DRC may facilitate anxiety-like behavior through projections to the hippocampal formation (Hale and Lowry, 2011; Imai et al., 1986; Kohler and Steinbusch, 1982). Exposure to an open-field arena increases c-Fos expression in a subpopulation of neurons in the CA1 of the ventral hippocampus, subiculum and entorhinal cortex that project to the BLA (Hale et al., 2008b). The former structure is intimately involved in anxiety-like behaviors (Bannerman et al., 2004), while the latter two structures are thought to be important for cognitive conflict resolution and response-oriented conflict resolution, respectively, and form important components in Gray's "behavioral inhibition system" (BIS), which functions to assess risk in conflict anxiety situations (Gray, 1982; McNaughton and Corr, 2004). In addition to a role of DRD/DRC serotonergic neurons in behavioral inhibition in order to avoid aversive outcomes, a study recently found that serotonergic activity in the midrostrocaudal DR (i.e. DRD and DRV) is necessary for inhibiting behavioral activity to wait for delayed rewards (Miyazaki et al., 2012). These data support the hypothesis that DRD/DRC serotonergic neurons facilitate anxiety-like behavior (i.e. risk assessment behaviors) through projections to a distributed system of interconnected structures in the hippocampal formation that also project to the BLA.

Serotonergic projections from DRD/DRC 5-HT neurons to the ventromedial prefrontal cortex. DRD/DRC 5-HT neurons may control anxiety-related behavior through projections to the vmPFC, including the infralimbic (IL) and prelimbic (PrL)

cortices; which both integrate information about aversive stimuli, in order to coordinate the proper emotional response through projections to the amygdala (Sotres-Bayon and Quirk, 2010; Sylvester et al., 2012; Varela et al., 2012). Administration of the anxiogenic drug FG-7142 (i.p.) increases serotonin metabolism in the PrL (Evans et al., 2006; Singewald and Sharp, 2000). Microinjection of the anxiety-related neuropeptide CRF into the mid-rostrocaudal to caudal DR (an area encompassing the DRD, DRV and DRVL/VLPAG) increases extracellular 5-HT in the medial prefrontal cortex, a measure that correlates with the cessation of freezing (Forster et al., 2006). Activation of the ventral medial prefrontal cortex blocks the behavioral consequences of uncontrollable stress, as well as the stress-induced activation of serotonergic neurons in the mid-rostrocaudal to caudal DR (Amat et al., 2008). Likewise, controllable stress activates DR-projecting PrL neurons, suggesting that the prefrontal cortex modulates the stress response through connections with the DR (Baratta et al., 2009). Taken together, there is strong evidence suggesting that serotonergic neurons in the DRD/DRC are potently activated by a multitude of stress- and anxiety-related stimuli, and that these neurons control anxiety-like behavior through projections to forebrain structures that are critical in processing the emotional salience of aversive stimuli and coordinating the appropriate behavioral and physiologic response.

Stress-induced activation of DRD/DRC serotonergic neurons by CRF afferents

Stress- and anxiety-related stimuli activate DRD/DRC serotonergic systems, as well as a distributed interconnected network of brain regions (Singewald and Sharp, 2000; Singewald et al., 2003) in addition to the ones described above, offering multiple sites for serotonin to modulate anxiety states and, in turn, many candidate brain structures that can modulate serotonergic activity in response to stress. One mechanism through which such diverse stressors can alter DRD/DRC neurotransmission, is through stress-induced alterations in CRF afferent input from forebrain structures. In addition to Ucn 2, microinjection of CRF into the mid-rostrocaudal DR, but not the rostral DR, mimics the behavioral deficits observed following uncontrollable stress (Hammack et al., 2002). The behavioral consequences of uncontrollable stress are reversed by microinjection of antisauvagine-30, a CRF_2 receptor antagonist, into the mid-rostrocaudal DR (Hammack et al., 2003). Likewise, antagonism of CRF receptors with d-Phe-CRF (which displays 2–10 times greater affinity for the $CRFR_2$ receptor than the $CRFR_1$ receptor) in the mid-rostrocaudal to caudal DR reduces social isolation stress-induced increases in anxiety-like behavior (Lukkes et al., 2009). Chronic stimulation of CRF_2 receptors, by overexpressing the high affinity CRF_2 receptor agonist urocortin 3, results in elevated basal anxiety, as well as blunted responses to stress and differential 5-HT metabolism in forebrain projection regions of the DRD/DRC, including the BLA (Neufeld-Cohen et al., 2012). CRF-enhanced acoustic startle increases c-Fos expression in serotonergic neurons in the caudal DR that project to the IL cortex, raising the possibility that stress-induced activation of the caudal DR by CRF may control anxiety-related behavior through increased 5-HT release in the frontal cortex (Meloni et al., 2008). A recent study by Sink et al. (2012) may shed light on the origin of endogenous CRF input to the DR, as overexpression of CRF within the BnST, a region that innervates the DRD/DRC region (Peyron et al., 1998), alters fear conditioning and selectively decreases CRF_2 receptor mRNA expression within the DRD/DRC. The DRD/DRC projects back to the BnST (Petit et al., 1995) and serotonin controls anxiety-related behavior mediated by the BnST through complex actions of 5-HT on multiple cell types, differentially expressing multiple 5-HT receptor subtypes (e.g. 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2c}, 5-HT_7 receptors), resulting in 5-HT having both anxiogenic and anxiolytic effects (Guo et al., 2009; Hammack et al., 2007; Hammack et al., 2009; Hazra et al., 2012). Moreover, unpredictable shock stress, which produces long-lasting increases in anxiety-like behavior, differentially alters 5-HT receptor mRNA in a cell-type specific manner, underlining the complexity of BnST-5-HT interactions (Hazra et al., 2012). An important direction of future research is to elucidate the complex effects of serotonin in the BnST on anxiety-related behavior and stress-related disease.

Serotonin autoreceptors control DRD/DRC serotonergic activity: Implications for stress- and anxiety-related behavior

Serotonergic neurons contain 5-HT_{1A} and 5-HT_{1B} autoreceptors located on the cell body and terminal, respectively, providing important negative feedback control that may be relevant to stress-induced changes in serotonergic neurotransmission. A recent study by McDevitt and colleagues (2011) reveals that overexpression or stimulation of inhibitory 5-HT_{1B} autoreceptors in the DRC reduces the expression of conditioned fear, as well as depressive-like behavior, consistent with the hypothesis that DRD/DRC serotonergic neurons facilitate conditioned fear responses. Since the 5-HT_{1B} receptor is located pre-synaptically on the DRC terminals, this treatment presumably results in altered 5-HT release in DRC projection regions like the amygdala, a region involved in mediating conditioned fear responses. On the other hand, uncontrollable stress, relative to controllable stress, compromises 5-HT_{1A} receptor-mediated autoinhibition of DRD neuronal activity and this impairment closely follows the behavioral consequences of stress, including exaggerated fear conditioning (Rozeske et al., 2011). Highlighting the importance of the 5-HT_{1A} autoregulatory receptor, a recent study reports that a 5-HT_{1A} receptor polymorphism associates with comorbid depression and generalized anxiety disorder (Molina et al., 2011). These alterations in $5\text{-HT}_{1A/1B}$ autoinhibitory mechanisms in the DRD/DRC region may influence fear- and anxiety-related behavior through changes in serotonin release in forebrain regions such as the BLA. Consistent with the Deakin/Graeff hypothesis, these studies suggest aversive stimuli alter autoregulatory mechanisms within DRD/DRC serotonergic neurons that may be relevant for 5-HT release in forebrain targets and anxiety-like behavior.

Serotonergic markers in the DRD/DRC are altered in depressed patients

Studies of post-mortem human brain tissue in select populations of depressed patients reveal subregion-specific alterations in

neuronal markers of serotonergic neurotransmission within the DRD/DRC. For example, depressed suicide victims have increases in TPH protein in the mid-rostrocaudal DR (Underwood et al., 1999b). Another study confirmed the increases in TPH protein, albeit with the effects being more predominant in the rostral DR (Boldrini et al., 2005). Likewise, depressed suicides have increases in *tph2* mRNA in the DR, which is mainly driven by a robust increase in *tph2* mRNA in the DRD (Bach-Mizrachi et al., 2006). In addition, the increase in *tph2* mRNA and TPH protein observed in depressed suicide victims is reflected in higher cellular transcriptional capacity of the *tph2* gene, especially in serotonergic neurons within the mid-rostrocaudal to caudal DR (Bach-Mizrachi et al., 2008). A study in alcohol-dependent depressed suicide victims revealed increases in TPH immunoreactivity that are restricted to the DRD subregion (Bonkale et al., 2006). These studies suggest that increases in *tph2* mRNA and TPH protein expression in the DRD and DRC are evident in select populations of depressed suicide patients. Overall, the functional topography of DRD serotonergic neurons supports the hypotheses that these neurons are an important component of a stress- and anxiety-related network via their projections to forebrain structures, and that dysfunction of serotonergic neurotransmission in this circuit is implicated in the pathophysiology of anxiety and affective disorders (Commons et al., 2003; Deakin and Graeff, 1991; Graeff et al., 1996; Lowry et al., 2008b).

Neuromodulation of DRD serotonergic neurons: Implications for anxiety and affective disorders

Serotonergic neurons in the DRD co-express corticotropin-releasing factor

In addition to 5-HT, the DRD contains a multitude of other neurotransmitters and neuropeptides that are important for modulating serotonergic neurotransmission (Lowry et al., 2008a; Michelsen et al., 2007; Valentino and Commons, 2005; Vasudeva et al., 2011). The DRD contains a dense cluster of corticotropin-releasing factor (CRF)-containing neurons and almost all of them (96%) co-localize with 5-HT (Commons et al., 2003); these CRF co-expressing 5-HT neurons also innervate CRF neurons in the lateral portion of the central nucleus of the amygdala, suggesting that CRF has a unique modulatory role for 5-HT activity both at the level of the midbrain raphe nuclei as well as in terminal regions that mediate anxiety- and fear-related responses (Commons et al., 2003; LeDoux et al., 1988). This highlights a role for intrinsic CRF systems in the DRD (in addition to CRF afferents) in fine-tuning serotonergic activity (Valentino and Commons, 2005).

The DRD contains vesicular glutamate transporter 3-expressing neurons

The DRD, specifically the shell of the DRD, contains a subpopulation of vesicular glutamate transporter 3 (VGLUT3)-expressing, non-serotonergic neurons with distinct projections (Hioki et al., 2010). Moreover, genetic deletion of VGLUT3 increases anxiety-like behavior, decreases 5-HT_{1A} receptor-mediated autoinhibition and alters 5-HT neurotransmission in forebrain limbic regions (Amilhon et al., 2010). Further research on the role of VGLUT-expressing serotonergic and non-serotonergic neurons in modulating 5-HT neurotransmission and anxiety-like behavior is warranted.

The DRD contains glutamatergic fibers immunoreactive for the neurokinin 1 receptor: Role of substance P

The DRD also contains a dense plexus of glutamatergic fibers that are immunoreactive for the neurokinin-1 (NK1) receptor; these processes encapsulate the CRF co-expressing 5-HT neurons (Commons and Valentino, 2002; Commons et al., 2003; Valentino et al., 2003), which may explain why application of substance P, an endogenous agonist of the NK-1 receptor, into the DR produces excitation in the DRD, whereas inhibition of 5-HT neurons is observed in other subregions of the DR (Valentino et al., 2003). This latter inhibitory effect may be mediated by substance P-induced activation of DRD serotonergic neurons and subsequent release of 5-HT in adjacent DR subregions, which would be expected to inhibit the other 5-HT neurons via 5-HT_{1A} receptor-mediated autoinhibition (Valentino and Commons, 2005). Considering the involvement of the substance P/NK1 receptor system and CRF in stress- and anxiety-related behavior (Binder and Nemeroff, 2010; Ebner and Singewald, 2006; Ebner et al., 2008; Nemeroff, 1996), and clinical evidence revealing the therapeutic potential for NK1 receptor and CRF receptor antagonists in treating anxiety and affective disorders (Adell, 2004; Chatzaki et al., 2006; Koob and Zorrilla, 2012; Kramer, 2000; Kramer et al., 1998; Kramer et al., 2004; Rupniak and Kramer, 1999; Zorrilla and Koob, 2010; Zoumakis and Chrousos, 2010), an important objective for future research will be to delineate the contributions of these neuromodulatory neuropeptidergic and glutamatergic systems in controlling the activity of serotonergic neurons in adaptive and disease states.

Hodology and functional topography of DRVL/VLPAG serotonergic neurons: Role in sympathomotor inhibition and panic disorder

Serotonergic neurons in the DRVL/VLPAG project to brainstem structures involved in fight-or-flight responses

The Deakin/Graeff hypothesis suggests that a second serotonergic system arising from the DR inhibits the innate defensive responses generated by the DPAG in response to exposure to an acute unconditioned stimulus, innate defensive responses that resemble the strong autonomic and behavioral arousal associated with PD. Serotonergic neurons of the DRVL/VLPAG (also called the "lateral wings" of the DR), predominately project to brainstem structures involved in controlling autonomic activity and fight-or-flight responses to aversive stimuli. DRVL/VLPAG serotonergic neurons give rise to axons that likely travel through the DR periventricular tract to innervate the DPAG (Beitz, 1982; Stezhka and Lovick, 1997). The 5-HT neurons of the DRVL/VLPAG provide virtually all the serotonergic input from the DR to the C1

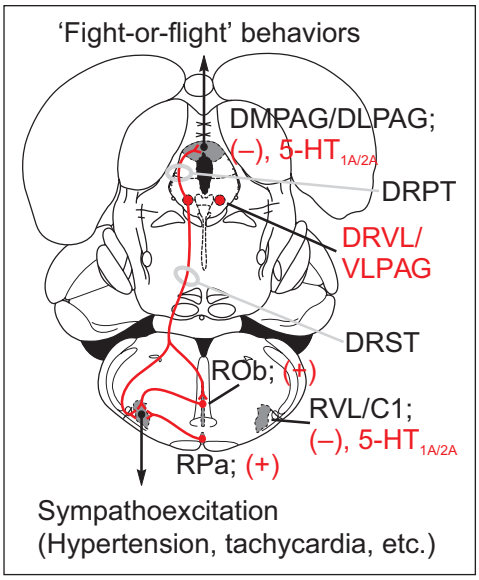

Figure 2. Schematic diagram shows the serotonergic pathways of the DRVL and the adjacent VLPAG, which corresponds to the "panic" circuit proposed by the Deakin and Graeff hypothesis. The DRVL/VLPAG serotonergic neurons send projections through the DRPT and DRST, to innervate the dorsal periaqueductal gray (DMPAG/DLPAG) and the RVL/C1, respectively, and inhibit structures involved in eliciting the autonomic and behavioral components of the fight-or-flight response. Dysfunction of this pathway is thought to relate to symptoms of panic disorder. Coronal section templates reproduced with permission from Paxinos G and Watson C (1998) *The Rat Brain in Stereotaxic Coordinates*, 4th Edition. San Diego: Academic Press: 1998 with permission from Elsevier.

DRVL: dorsal raphe nucleus, ventrolateral part; VLPAG: ventrolateral periaqueductal gray; DMPAG: dorsomedial periaqueductal gray; DLPAG: dorsolateral periaqueductal gray; DRPT: dorsal raphe periventricular tract; DRST: dorsal raphe spinal tract; Rob: raphe obscurus nucleus; RPa: raphe pallidus nucleus; RVL/C1: rostroventrolateral medulla/C1 adrenaline cells; (+): excitation; (–): inhibition.

adrenaline neurons of the rostroventrolateral medulla (C1/RVL). Indeed, these serotonergic neurons appear to be part of a sympathomotor command center and send multisynaptic projections to both the hindlimb skeletal muscle and the adrenal gland, controlling somatomotor and sympathetic responses to stress (Kerman et al., 2006). The hodology of DRVL/VLPAG 5-HT neurons suggests these neurons constitute the original serotonergic pathway innervating the DPAG, proposed by Deakin and Graeff (Figure 2). For a full list of DRVL/VLPAG efferents and afferents, see the following reviews (Hale and Lowry, 2011; Hale et al., 2012; Johnson et al., 2004; Lowry, 2002).

Stress-induced activation of DRVL/VLPAG 5-HT neurons: Inhibition of fight-or-flight and sympathetic arousal

Functional neuroanatomical studies of DRVL/VLPAG serotonergic neurons, combined with the hodology described above, strongly support a role for this subregion in controlling the behavioral and physiological responses to aversive stimuli, including acute unconditioned aversive stimuli, as originally suggested by the Deakin/Graeff hypothesis. For example, exposure to acute social defeat in adulthood elevates expression of *tph2* and *slc6a4* mRNA selectively within the DRVL/VLPAG, but only in rats previously exposed to maternal separation (Gardner et al., 2009a; Gardner et al., 2009b). Maternally-separated rats also display a more passive-submissive coping style, characterized by increases in anxiety- and fear-like behaviors during social defeat in adulthood (Gardner et al., 2005). Consistent with these development by environment interactions, immune challenge of mice with lipopolysaccharide (LPS) early in life increases the expression of *tph2* and *slc6a4* mRNA and decreases *htr1a* mRNA selectively, within the DRVL/VLPAG and DRV (Sidor et al., 2010), which all have the net effect of increasing DRVL/VLPAG 5-HT activity. Peripheral immune activation with LPS activates DRVL/VLPAG (and DRI) 5-HT neurons and also decreases behavioral arousal (e.g. seen by locomotion or grooming), which is consistent with the hypothesis that DRVL/VLPAG 5-HT neurons inhibit sympathetic arousal (Graeff et al., 1996; Hollis et al., 2006; Keay and Bandler, 2001). DRVL/VLPAG and DRI serotonergic neurons are co-activated by exposure to warm ambient temperature (Hale et al., 2011) and cold water swim stress (Kelly et al., 2011), suggesting these 5-HT neurons may be important for thermoregulatory mechanisms.

A convergent line of evidence shows that diverse inescapable stressors (e.g. deep muscular pain, cutaneous pain and visceral pain) increase the expression of the neuronal activity marker c-Fos in the VLPAG longitudinal column, which includes 5-HT neurons in the lateral wings. These stressors all produce passive coping responses, characterized by behavioral quiescence, hyporeactivity, hypotension, bradycardia and opioid-mediated analgesia (Keay and Bandler, 2001). This, together with the functional role of the DPAG, has led to the suggestion that the VLPAG (and the adjacent DRVL) and DPAG have opposing roles in mediating behavioral coping strategies to aversive stimuli, with the former eliciting passive (reactive) coping strategies and the latter promoting active coping strategies, characterized by fight-or-flight responses, hypertension, tachycardia, hindlimb vasodilation and non-opioid analgesia (Johnson et al., 2004; Keay and Bandler, 2001). This notion is consistent with Deakin and Graeff's hypothesis that the DPAG produces active defensive responses in the presence of a proximal threat, like a predator, and that 5-HT normally restrains these innate fight-or-flight responses (Deakin and Graeff, 1991; Graeff et al., 1996).

It is unclear how DRVL/VLPAG serotonergic neurons are activated by such diverse stressors that encompass multiple sensory modalities, but one potential mechanism may be through the unique afferent input to this subregion. DRVL/VLPAG serotonergic neurons receive afferents from medullary structures involved in autonomic control, including the parabrachial nucleus, nucleus of the solitary tract, and viscerosensory areas of the glossopharangeal and vagal nerves; and from forebrain limbic structures involved in controlling defensive responses, such as the CeA and BnST (for a full list of afferents and references, see Hale and Lowry, 2011; Hale et al., 2012). Serotonergic neurons in the lateral wings have unique electrophysiological properties that make them more excitable than serotonergic neurons located in other DR subregions, suggesting that inherent properties of DRVL/VLPAG 5-HT neurons make them more likely to become activated by stressful stimuli (Crawford et al., 2010). Further research is needed, but the unique properties of DRVL/VLPAG 5-HT neurons, which potentially make them more susceptible to activation by stress, and their afferent input from structures involved in autonomic control, support the hypothesis that this subset

of serotonergic neurons inhibits fight-or-flight responses and facilitates a passive (reactive) coping style in response to aversive stimuli, including acute unconditioned aversive stimuli, as predicted by the Deakin/Graeff hypothesis.

DRVL/VLPAG 5-HT neurons inhibit fight-or-flight responses and sympathetic outflow through projections to brainstem structures

Serotonergic projections from DRVL/VLPAG neurons to the DPAG. Serotonergic neurons in the DRVL/VLPAG are believed to be a critical node in a "defense circuit" and can restrain fight-or-flight responses generated by the DPAG. Chemical or electrical stimulation of the DPAG elicits a behavioral and autonomic response characterized by fight-or-flight behaviors, hypertension and tachycardia, which closely mimics the rodent's response to a natural predator (Bandler and Depaulis, 1988; Bandler et al., 2000; Canteras, 2002; Keay and Bandler, 2001). Chemical stimulation of the DRVL/VLPAG region (as well as the DRD/DRV) elevates extracellular 5-HT 14-fold in the DPAG in addition to blocking one-way escape in the elevated T-maze, indicative of an antipanic-like effect of DRVL/VLPAG 5-HT (Viana et al., 1997). Likewise, electrical stimulation of the DR (especially the lateral wing region) is also effective at reducing operant response to escape DPAG stimulation (Kiser et al., 1980). The panicolytic effect (i.e. impaired escape) of DR stimulation can be blocked by local microinjection of 5-HT_{1A} or 5-HT_{2A} receptor antagonists into the DPAG (Pobbe and Zangrossi, 2005). Similarly, microinjections of 5-HT_{1A} and 5-HT_{2A} receptor agonists into the DPAG impair escape from an unconditioned stressor like a predator (Pobbe et al., 2011). The inhibitory 5-HT_{1A} receptor is likely expressed by DPAG output neurons and/or excitatory amino acid "*on* cells," which activate DPAG output neurons, whereas the excitatory 5-HT_{2A} receptor appears to be located on GABAergic "*off* cells," which inhibit DPAG output neurons (Brandao et al., 2008). Recent evidence suggests that local endogenous opioids in the DPAG may interact with serotonergic systems to produce panicolytic effects. For example, application of 5-HT or fluoxetine (SSRI) into the DPAG impairs escape in the elevated T-maze, indicative of a panicolytic effect; however, this effect is attenuated by prior intra-DPAG microinjection of naloxone, an opioid inverse agonist that is a competitive antagonist at multiple opioid receptors (Graeff, 2012; Roncon et al., 2012). Altogether, these data support the Deakin/Graeff hypothesis that 5-HT, likely arising from serotonergic neurons located in the DRVL/VLPAG, produces anti-panic behavioral effects in the DPAG.

Serotonergic projections from DRVL/VLPAG neurons to the C1/RVL region. The 5-HT neurons of the DRVL/VLPAG send dense projections to the C1/RVL, suggesting an important role for DRVL/VLPAG serotonergic neurons in controlling the cardiovascular responses to emotionally salient events (Bago et al., 2002; Underwood et al., 1999a). Lesions of the DR cause drastic reductions in 5-HT and 5-HIAA (a major metabolite of 5-HT) concentrations and 5-HT transporter binding within the C1/RVL region (Underwood et al., 1999a). The C1/RVL region contains an abundance of 5-HT_{1A} receptor-immunoreactive catecholaminergic and non-catecholaminergic neurons that project to the intermediolateral cell column (Helke et al., 1997). Microinjection of 8-OH-DPAT into the RVL results in inhibition of sympathetic renal and hindlimb skeletal muscle nerve activity (Bago et al., 1999). Likewise, sympathoinhibition elicited by electrical stimulation of the VLPAG is blocked by intra-RVL microinjection of the 5-HT_{1A} receptor antagonist, WAY-100635 (Bago and Dean, 2001). These data support the notion of a serotonergic pathway, predominantly arising from the DRVL/VLPAG to the C1/RVL, that is involved in inhibiting motor and visceral sympathetic outflow.

Altered DRVL/VLPAG serotonergic function in rodent models of panic

There is evidence in rodent models of panic that compromise of the DRVL/VLPAG 5-HT system leads to robust sympathetic and behavioral arousal, in the presence of stimuli that normally do not warrant such a response, that are strikingly similar to the symptoms of a panic attack. Shekhar et al. (1996) report that chronic disinhibition of the dorsomedial hypothalamus produces an anxiety-like phenotype as well as susceptibility to panic-like responses induced by exposure to sodium lactate or elevated carbon dioxide (5%), both of which can elicit panic attacks in patients with panic disorder, but have no effect in healthy volunteers (Gorman et al., 1994; Liebowitz et al., 1986; Pitts and McClure, 1967). Exposure to intravenous (i.v.) sodium lactate increases c-Fos expression in DRVL/VLPAG serotonergic neurons, but only in control rats that do not show panic-like behavioral and physiological responses. On the other hand, sodium lactate fails to activate DRVL/VLPAG serotonergic neurons in panic-prone rats and these rats display panic-like responses (Johnson et al., 2008). These data suggest that DRVL/VLPAG 5-HT neurons normally suppress sympathetic activation, following exposure to innocuous stimuli. Consistent with this idea, priming of the BLA by chronic microinjection of the stress- and anxiety-related neuropeptide Ucn 1 increases baseline anxiety and sensitivity to panicogenic agents (Rainnie et al., 2004; Sajdyk et al., 1999) and also elevates *tph2* mRNA within the DRVL/VLPAG (Donner et al., 2012a). Taken altogether, these data suggest that DRVL/VLPAG 5-HT neurons normally inhibit sympathetic responses to seemingly innocuous stimuli, possibly through projections to the DPAG and C1/RVL, but dysregulation of this system leads to unrestrained behavioral (i.e. fight-or-flight) and sympathetic (i.e. increased heart rate, blood pressure, etc.) responses that may be relevant for the pathophysiology of panic disorder.

Hodology and functional topography of DRI/MnR serotonergic neurons: Role in coping with stress and depression

Serotonergic neurons in the DRI/MnR project to forebrain structures implicated in coping, resilience and tolerance to stress

The final 5-HT pathway discussed by Deakin and Graeff is hypothesized to originate in the MnR and through its projections through the median raphe forebrain bundle tract to the hippocampus promotes tolerance or adaptation to chronic stress. Serotonergic neurons of the DRI/MnR send dense projections through this fiber tract to innervate the septohippocampal system, including the dorsal hippocampus and medial septum/diagonal

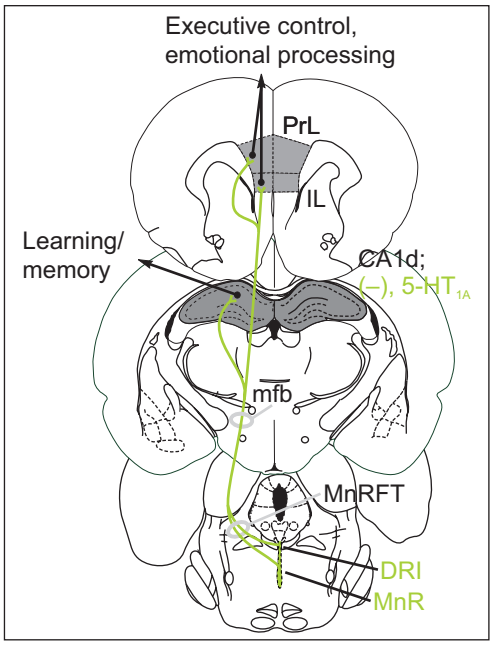

Figure 3. Serotonergic neurons of the interfascicular part of the dorsal raphe nucleus and the developmentally and anatomically related median raphe nucleus (DRI/MnR) constitute Deakin and Graeff's "depression" pathway and project via the MnRFT, to forebrain limbic structures that are involved in controlling anxiety- and depressive-like behavior, and function to suppress hippocampal theta and to promote resilience to aversive stimuli. Dysfunction of this pathway is thought to relate to symptoms of depression. Coronal section templates reproduced with permission from Paxinos G and Watson C (1998) *The Rat Brain in Stereotaxic Coordinates*, 4th Edition. San Diego: Academic Press: 1998 with permission from Elsevier.
CA1d: field CA1 of dorsal hippocampus; DRI: dorsal raphe nucleus, interfascicular part; IL: infralimbic cortex; MnR: median raphe nucleus; MnRFT: median raphe forebrain tract; mfb: medial forebrain bundle; PrL: prelimbic cortex; (+): excitation; (–): inhibition.

band of Broca region (MS/DBB), two structures that are critical for theta rhythm generation (Crooks et al., 2012; Petsche and Stumpf, 1962; Petsche et al., 1962); a significant number of these 5-HT neurons send collateral projections to both structures (Acsady et al., 1996; Kohler et al., 1982; McKenna and Vertes, 2001). The DRI also sends projections to the IL and PL cortices of the vmPFC (Van Bockstaele et al., 1993), which, in the case of the PL, is known to promote resilience to stress when activated pharmacologically or by behavioral control over the stressor (Amat et al., 2008; Amat et al., 2005; Baratta et al., 2009). The anatomical projections of DRI/MnR 5-HT neurons are consistent with Deakin and Graeff's predicted pathway that innervates forebrain structures like the hippocampus, in order to promote resilience to stress (Figure 3). For a full list of DRI/MnR efferents and afferents, see the following reviews (Hale and Lowry, 2011; Hale et al., 2012).

Serotonergic neurons in the DRI and MnR share a common embryonic origin

In addition to their hodological similarities, there is strong evidence that DRI serotonergic neurons and serotonergic neurons in the dorsal part of the MnR share a common embryonic origin. For example, at gestational day 15 there are two groups of 5-HT-immunoreactive neurons that give rise to the DR and MnR, which later (gestational day 17) separate, with one group forming the DRI and MnR and the other group forming the other DR subregions (Azmitia and Gannon, 1986; Jacobs and Azmitia, 1992). Consistent with this, serotonergic neurons of the DRI and dorsal MnR appear to share a common genetic lineage, as both populations of serotonergic neurons are derived from rhombomere 1, whereas 5-HT neurons in the ventral MnR and more caudal raphe nuclei develop from rhombomeres 2–7 (Jensen et al., 2008). These developmental similarities, together with the hodological and functional similarities of the DRI and MnR serotonergic neurons are consistent with the hypothesis that these neurons promote coping and resilience to stress through projections to forebrain limbic structures (Graeff et al., 1996; Hale and Lowry, 2011; Lowry, 2002).

DRI/MnR serotonergic neurons promote coping, resilience or tolerance to stress

Stress-induced alcohol reinstatement: Role of MnR serotonergic neurons. Evidence supporting a role for MnR 5-HT neurons in coping with stress comes from studies showing the importance of the MnR in stress-induced reinstatement of alcohol administration. For example, inactivation of MnR serotonergic neurons by intra-MnR administration of 8-OH-DPAT, muscimol (a $GABA_A$ receptor agonist), or CRF causes alcohol relapse, an effect that mimics the effects of stress- (foot shock-) induced reinstatement of alcohol administration (Le et al., 2002; Le et al., 2008). Antagonism of MnR CRF receptors blocks the effects of stress on alcohol relapse and also prevents the stress-induced increases in *c-fos* mRNA expression in the CeA (Funk et al., 2003; Le et al., 2002). These studies highlight a complex interplay between CRF, $GABA_A$ receptors and MnR serotonergic neurons on stress-induced alcohol reinstatement, while supporting a role for the MnR in tolerance to stress.

The MnR promotes resilience to the physiological and immunological consequences of stress. Another line of evidence supporting a role for MnR serotonergic neurons in stress resilience comes from the observation that lesions of the MnR enhance sensitivity to stress (e.g. food deprivation, brain surgery and restraint stress) as measured by increases in stress-induced gastrointestinal ulcer formation (Graeff et al., 1996; Hoshino and Sugizaki, 1986), whereas lesions of the DR have no effect on stress-induced ulcer formation following food deprivation (Hoshino and Sugizaki, 1986). Also, MnR lesions, compared to sham lesions, result in blunted splenic immune responses to the mitogen, concanavalin A, suggesting these cultured rat splenic cells are more sensitive to surgical stress (Graeff et al., 1996). These data support a role for the MnR in promoting tolerance to the physiological and immunological consequences of stress.

Serotonergic neurons of the MnR and anxiety-like behavior. Serotonergic neurons in the MnR are also involved in the behavioral effects of anxiety-provoking tasks, possibly through altered 5-HT activity in forebrain targets. For example, selective

neurotoxic lesions of 5-HT neurons in the MnR result in increased inhibitory avoidance (an anxiety-like behavior) in the elevated T-maze, but have no effect on escape (a panic-like behavior) (Andrade et al., 2004). Consistent with this, inhibitory avoidance in the elevated T-maze increases c-Fos expression in 5-HT neurons within the DRI/MnR (as well as the anxiety-related DRD/DRC subregions), but not in serotonergic neurons in the panic-related DRVL/VLPAG (Spiacci Jr et al., 2012). Noradrenergic modulation of MnR serotonergic neurons alters anxiety-like behavior in the elevated plus-maze (Mansur et al., 2010; Mansur et al., 2011), which may be mediated by altered 5-HT release in forebrain structures such as the hippocampus and amygdala (Adell and Artigas, 1999; Adell et al., 2002; Mansur et al., 2011). Together, these data support the notion that activation of MnR 5-HT neurons promotes adaptation to stress, possibly through altered 5-HT release in forebrain targets.

Activation of DRI serotonergic neurons has antidepressant-like effects. Like the MnR, serotonergic neurons in the DRI are implicated in cognition, control of emotional behavior and antidepressant-like effects. We have found that DRI as well as DRVL/VLPAG (Hale et al., 2012) serotonergic neurons are activated by peripheral immune stimulation with LPS (Hollis et al., 2006) and a heat-killed preparation of the nonpathogenic, saprophytic bacteria *Mycobacterium vaccae* (Lowry et al., 2007). The latter effect is associated with increased 5-HT metabolism in DRI projection regions like the infralimbic and prelimbic cortices, as well as antidepressant-like behavioral effects in the forced swim test (Lowry et al., 2007; Porrino and Goldman-Rakic, 1982).

Serotonergic modulation of hippocampal theta rhythm

Theta rhythms and aversive learning and memory: Implications for anxiety and affective disorders

Theta rhythms are characteristic wave patterns in the electroencephalogram (EEG) that are generated during waking exploratory behavior, as well as REM sleep (Bland, 1986; Buzsaki, 2002; Grosmark et al., 2012; Sharman et al., 2013; Vanderwolf, 1969). Evidence suggests hippocampal theta rhythms are important for a number of learning and memory processes, including spatial memory, sensory gating and long-term potentiation (Bland, 1986; Vertes, 2005). In addition to mnemonic functions, theta rhythms (e.g. type 2 theta) are generated by conditioned aversive stimuli and unconditioned aversive stimuli, including predator exposure and foot shock (Graeff et al., 1980; Hsiao et al., 2012; Sainsbury et al., 1987).

More recently, theta rhythms have garnered attention in the context of anxiety and affective disorders. McNaughton and colleagues (2007) reported that all classes of effective anxiolytics, including anxiolytics that act on serotonergic systems (e.g. 5-HT_{1A} receptor agonists and SSRIs), suppress the theta rhythm. Indeed, the antiepileptic, phenytoin, has anxiolytic effects in the elevated plus-maze, while also suppressing theta, offering *prima facie* evidence for the predictive validity of the theta suppression model (Yeung et al., 2012). Somatostatin administered i.c.v. has anxiolytic- and antidepressant-like effects that are associated with reductions in hippocampal theta elicited by simulation of the reticular formation (Engin et al., 2008). Likewise, a sub-anesthetic dose of ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist with rapid-acting antidepressant effects in healthy volunteers, leads to reductions in prefrontal theta cordance, a quantitative EEG measure that correlates well with cortical perfusion measured by positron emission tomography (Engin et al., 2009; Horacek et al., 2010). Conflict tasks that provoke anxiety in humans evoke a marked increase in right frontal cortex theta rhythm, which correlates with behavioral indices of anxiety such as neuroticism and avoidance (Neo and McNaughton, 2011); anxiolytics block this conflict-induced frontal theta, leading the authors to suggest this type of theta could be used as a human anxiety-specific biomarker (McNaughton et al., 2012). Theta oscillations in structures such as the prefrontal cortex can be entrained by hippocampal theta and this is thought to be critical for the integration of information across disparate neocortical networks (Sirota et al., 2008). Indeed, a recent study revealed that theta coupling between the hippocampus, lateral amygdala and medial prefrontal cortex occurs during retrieval of conditioned fear memory and recall of extinction memory; however, theta coupling in this network decreased during extinction learning (Lesting et al., 2011). These studies support a functional role of theta rhythms in processing fear- and anxiety-related memories, which may be relevant for stress-related human disease.

Serotonergic neurons in the MnR suppress hippocampal theta

There is evidence that serotonergic systems arising from the MnR suppress hippocampal theta, in part through projections to the septum. As mentioned earlier, it is notable that serotonergic neurons of the DRI/MnR densely innervate the hippocampus and medial septum/diagonal band of Broca region (MS/DBB), two structures that are critical for theta rhythm generation (Crooks et al., 2012; Petsche and Stumpf, 1962; Petsche et al., 1962), and a significant number of these serotonergic neurons send collateral projections to both structures (Acsady et al., 1996; Kohler et al., 1982; McKenna and Vertes, 2001). Stimulation of MnR serotonergic neurons desynchronizes hippocampal theta (Jackson et al., 2008; Nitz and McNaughton, 1999; Vertes, 1981), whereas lesions of the MnR or inactivation of the MnR using 8-OH-DPAT results in persistent theta activation (Maru et al., 1979; Vertes et al., 1994).

Serotonergic neurons in the MnR likely desynchronize hippocampal theta through excitation of a subpopulation of GABAergic neurons and/or inhibition of cholinergic (and possibly glutamatergic) pacemaker neurons in the MS/DBB that all contribute to the generation of hippocampal theta (Buzsaki, 2002; Crooks et al., 2012; Leranth and Vertes, 1999; Sharman et al., 2013; Vertes and Kocsis, 1997). In addition, the MnR (similar to the DR) has reciprocal connections with the supramammillary area, a region known to control the frequency of hippocampal theta rhythm probably through connections with structures implicated in modulating hippocampal theta (for review, see Pan and McNaughton, 2004). The mechanisms for how stress alters MnR serotonergic activity and hippocampal theta are beginning to be elucidated (Figure 4) (Hsiao et al., 2012). There is a paucity of information on the role of DRI serotonergic neurons in controlling theta rhythms (possibly due to the small size of this subregion); therefore, future studies should address this knowledge gap. The

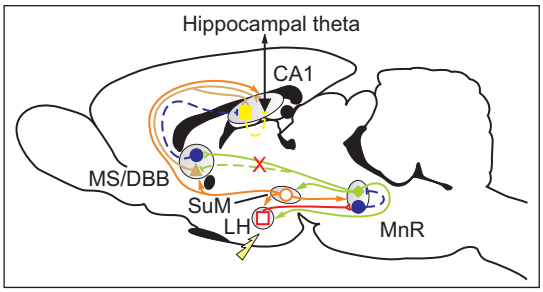

Figure 4. Schematic diagram of a sagittal section of the rat brain illustrating the effects of stress on serotonergic activity in the MnR and on hippocampal theta rhythms. Hippocampal theta rhythms are generated through a complex interaction of excitatory and inhibitory inputs onto the soma and dendrites of hippocampal CA1 pyramidal neurons (inverted triangle), likely coming from GABAergic interneurons (closed circle) and cholinergic pacemaker cells (triangle) in the MS/DBB, hippocampal basket cell interneurons (closed square) and the entorhinal cortex (not shown: Buzsaki, 2002). Serotonergic neurons of the MnR (diamond) normally desynchronize hippocampal theta rhythms through projections to the MS/DBB. Here, serotonin can activate GABAergic neurons (closed circle) or inhibit cholinergic pacemaker neurons (triangle), with the net effect being inhibition of hippocampal theta. Stress (e.g. foot shock) activates hypocretin/orexin neurons (open square) located in the PeF/LH that project to and activate GABAergic interneurons (closed circle) in the MnR (Hsiao et al., 2012). These GABAergic interneurons suppress MnR serotonergic activity, removing the desynchronizing serotonergic input to the MS/DBB and resulting in stress-induced hippocampal theta. The MnR and DR project back to the PeF/LH and, at least in regards to the DR, some projections directly innervate hypocretin/orexin neurons (Yoshida et al., 2006). The MnR also has reciprocal connections with the SuM (open circle), a structure known to control theta frequency (Pan and McNaughton, 2004). Solid lines ending in inverted arrowheads represent excitatory pathways, dashed lines ending in rectangles represent inhibitory pathways, solid curved lines ending in arrowheads represent projections with complex or uncertain biological effects and solid straight lines ending in arrowheads represent functional systems output. Sagittal section template reproduced with permission from Paxinos G and Watson C (1998) *The Rat Brain in Stereotaxic Coordinates*, 4th Edition. San Diego: Academic Press: 1998 with permission from Elsevier.
CA1: field CA1 of dorsal hippocampus; DR: dorsal raphe nucleus; GABA: gamma-aminobutyric acid; MnR: median raphe nucleus; MS/DBB: medial septum/diagonal band of Broca region; PeF/LH: perifornical/lateral hypothalamus; SuM: supramammillary area

observations that serotonergic neurons of the MnR (and possibly DRI) desynchronize hippocampal theta, including stress-induced theta, are consistent with the Deakin/Graeff hypothesis that the MnR serotonergic-hippocampal pathway promotes resilience or tolerance to chronic stress, possibly in part through desynchronizing hippocampal theta rhythms, and that the dysfunction of this pathway leads to stress-related anxiety and affective disorders (Deakin and Graeff, 1991; Graeff et al., 1996).

Implications of the functional topography of serotonergic systems

The functional topography of serotonergic systems raises a number of interesting questions. For example, are these different pathways activated independently? Do they interact? What are the advantages to having a coordinated behavioral response that increases fear, but reduces panic? Evidence suggests that different serotonergic pathways are activated independently. For example, a number of studies have now shown that when the midline DRD/DRC serotonergic systems (implicated in facilitation of conflict anxiety) are active, DRVL/VLPAG and DRI serotonergic systems (implicated in inhibition of panic-like responses and stress resilience, respectively) are not (Abrams et al., 2005; Gardner et al., 2005; Hale et al., 2010b; Hale et al., 2012; Staub et al., 2006). Conversely, when DRVL/VLPAG and DRI serotonergic systems are active, midline DRD/DRC serotonergic systems are not (Commons, 2008; Hale et al., 2012; Hollis et al., 2006; Lowry et al., 2007). It is likely that functional subsets of serotonergic systems interact, perhaps in reciprocal inhibition in the case of competing behavioral strategies (Jasinska et al., 2012). A clear advantage to having a coordinated behavioral response that increases fear, but reduces panic, is evident in the case of contextual fear. In contexts where a predator has been observed previously, but where the presence or absence (and location) of a predator is uncertain, increased contextual fear (freezing behavior) and inhibition of a flight/escaping behavior is clearly adaptive in order to avoid detection by, or direct confrontation with, a potential predator (Blanchard and Blanchard, 1988; Blanchard and Blanchard, 1989; Graeff, 2011; Graeff and Zangrossi, 2010; McNaughton and Corr, 2004).

Conclusions

Over 20 years have passed since Deakin and Graeff hypothesized that different 5-HT pathways respond to diverse types of aversive stimuli and that dysregulation of these pathways contributes to the pathophysiology of anxiety and affective disorders. Here we present convergent lines of evidence supporting the involvement of topographically organized subpopulations of serotonergic neurons comprising the original 5-HT pathways. Although Deakin and Graeff argue that specific aversive (e.g. unconditioned versus conditioned stress and acute versus chronic stress) stimuli selectively activate distinct 5-HT pathways, it seems numerous characteristics interact to determine which 5-HT pathway is activated, including factors like the proximity of the threat, the subjective perception of threat, the escapability from/controllability of the stressor, the sensory modality involved, and whether the aversive stimulus is interoceptive or exteroceptive; however, the data overwhelmingly support the idea that specific 5-HT pathways are activated by diverse aversive stimuli and function to coordinate the appropriate behavioral and physiologic response in order to avoid, escape, remove or cope with the threat. Overall, experimental evidence strongly supports the core of the Deakin/Graeff hypothesis: that different 5-HT pathways are intimately involved in controlling stress-induced anxiety- and depressive-like behavioral and physiologic responses, and that dysfunction of these pathways can lead to anxiety and affective disorders. In this review, we've extended the original hypothesis to identify the location of topographically-organized subpopulations of serotonergic neurons involved. Future research should elucidate the mechanisms of how specific stressors activate these topographically-organized serotonergic pathways and how they mediate changes in behavior and physiology.

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Conflict of interest

ED Paul declares that there is no conflict of interest. CA Lowry reports the following activities for the previous 2 years: consultant for Enlight Biosciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Mental Health (NIMH) or the National Institutes of Health (NIH).

References

Abrams JK, Johnson PL, Hay-Schmidt A, Mikkelsen JD, Shekhar A and Lowry CA (2005) Serotonergic systems associated with arousal and vigilance behaviors following administration of anxiogenic drugs. *Neuroscience* 133: 983–997.

Acsady L, Arabadzisz D, Katona I and Freund TF (1996) Topographic distribution of dorsal and median raphe neurons with hippocampal, septal and dual projection. *Acta Biol Hung* 47: 9–19.

Adell A (2004) Antidepressant properties of substance P antagonists: Relationship to monoaminergic mechanisms? *Curr Drug Targets CNS Neurol Disord* 3: 113–121.

Adell A and Artigas F (1999) Regulation of the release of 5-hydroxytryptamine in the median raphe nucleus of the rat by catecholaminergic afferents. *Eur J Neurosci* 11: 2305–2311.

Adell A, Celada P, Abellan MT and Artigas F (2002) Origin and functional role of the extracellular serotonin in the midbrain raphe nuclei. *Brain Res Rev* 39: 154–180.

Almaula N, Ebersole BJ, Zhang D, Weinstein H and Sealfon SC (1996) Mapping the binding site pocket of the serotonin 5-hydroxytryptamine 2A receptor. Ser3.36(159) provides a second interaction site for the protonated amine of serotonin but not of lysergic acid diethylamide or bufotenin. *J Biol Chem* 271: 14672–14675.

Amat J, Baratta MV, Paul E, Bland ST, Watkins LR and Maier SF (2005) Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. *Nat Neurosci* 8: 365–371.

Amat J, Paul E, Watkins LR and Maier SF (2008) Activation of the ventral medial prefrontal cortex during an uncontrollable stressor reproduces both the immediate and long-term protective effects of behavioral control. *Neuroscience* 154: 1178–1186.

Amat J, Tamblyn JP, Paul ED, Bland ST, Amat P, Foster AC, et al. (2004) Microinjection of urocortin 2 into the dorsal raphe nucleus activates serotonergic neurons and increases extracellular serotonin in the basolateral amygdala. *Neuroscience* 129: 509–519.

Amilhon B, Lepicard E, Renoir T, Mongeau R, Popa D, Poirel O, et al. (2010) VGLUT3 (Vesicular Glutamate Transporter Type 3) contribution to the regulation of serotonergic transmission and anxiety. *J Neurosci* 30: 2198–2210.

Andrade TG, Macedo CE, Zangrossi H, Jr and Graeff FG (2004) Anxiolytic-like effects of median raphe nucleus lesion in the elevated T-maze. *Behav Brain Res* 153: 55–60.

Artigas F (2013) Future directions for serotonin and antidepressants. *ACS Chem Neurosci* 4: 5–8.

Azmitia EC and Gannon PJ (1986) Anatomy of the serotonergic system in the primate and sub-primate brain. *Adv Neurol* 43: 407–468.

Bach-Mizrachi H, Underwood MD, Kassir SA, Bakalian MJ, Sibille E, Tamir H, et al. (2006) Neuronal tryptophan hydroxylase mRNA expression in the human dorsal and median raphe nuclei: Major depression and suicide. *Neuropsychopharmacol* 31: 814–824.

Bach-Mizrachi H, Underwood MD, Tin A, Ellis SP, Mann JJ and Arango V (2008) Elevated expression of tryptophan hydroxylase-2 mRNA at the neuronal level in the dorsal and median raphe nuclei of depressed suicides. *Mol Psychiatry* 13: 507–513.

Bago M and Dean C (2001) Sympathoinhibition from ventrolateral periaqueductal gray mediated by 5-HT(1A) receptors in the RVLM. *Am J Physiol Regul Integr Comp Physiol* 280: R976–984.

Bago M, Marson L and Dean C (2002) Serotonergic projections to the rostroventrolateral medulla from midbrain and raphe nuclei. *Brain Res* 945: 249–258.

Bago M, Sprtel BM and Dean C (1999) Modulation of sympathetic nerve activity by microinjection of the 5-HT1A receptor agonist 8-OH-DPAT into the rostroventrolateral medulla. *J Auton Nerv Syst* 76: 127–134.

Bandler R and Depaulis A (1988) Elicitation of intraspecific defence reactions in the rat from midbrain periaqueductal grey by microinjection of kainic acid, without neurotoxic effects. *Neurosci Lett* 88: 291–296.

Bandler R, Keay KA, Floyd N and Price J (2000) Central circuits mediating patterned autonomic activity during active vs. passive emotional coping. *Brain Res Bull* 53: 95–104.

Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T, et al. (2004) Regional dissociations within the hippocampus–memory and anxiety. *Neurosci Biobehav Rev* 28: 273–283.

Baratta MV, Zarza CM, Gomez DM, Campeau S, Watkins LR and Maier SF (2009) Selective activation of dorsal raphe nucleus-projecting neurons in the ventral medial prefrontal cortex by controllable stress. *Eur J Neurosci* 30: 1111–1116.

Beitz AJ (1982) The organization of afferent projections to the midbrain periaqueductal gray of the rat. *Neuroscience* 7: 133–159.

Binder EB and Nemeroff CB (2010) The CRF system, stress, depression and anxiety-insights from human genetic studies. *Mol Psychiatry* 15: 574–588.

Blanchard DC and Blanchard RJ (1988) Ethoexperimental approaches to the biology of emotion. *Annu Rev Psychol* 39: 43–68.

Blanchard RJ and Blanchard DC (1989) Attack and defense in rodents as ethoexperimental models for the study of emotion. *Prog Neuropsychopharmacol Biol Psychiatry* 13:S3–14.

Blanchard RJ, Flannelly KJ and Blanchard DC (1986) Defensive behavior of laboratory and wild *Rattus norvegicus*. *J Comp Psychol* 100: 101–107.

Bland BH (1986) The physiology and pharmacology of hippocampal formation theta rhythms. *Prog Neurobiol* 26: 1–54.

Boldrini M, Underwood MD, Mann JJ and Arango V (2005) More tryptophan hydroxylase in the brainstem dorsal raphe nucleus in depressed suicides. *Brain Res* 1041: 19–28.

Bonkale WL, Turecki G and Austin MC (2006) Increased tryptophan hydroxylase immunoreactivity in the dorsal raphe nucleus of alcohol-dependent, depressed suicide subjects is restricted to the dorsal subnucleus. *Synapse* 60: 81–85.

Branchek T, Adham N, Macchi M, Kao HT and Hartig PR (1990) [3H]-DOB(4-bromo-2,5-dimethoxyphenylisopropylamine) and [3H] ketanserin label two affinity states of the cloned human 5-hydroxytryptamine 2 receptor. *Mol Pharmacol* 38: 604–609.

Brandao ML, Zanoveli JM, Ruiz-Martinez RC, Oliveira LC and Landeira-Fernandez J (2008) Different patterns of freezing behavior organized in the periaqueductal gray of rats: Association with different types of anxiety. *Behav Brain Res* 188: 1–13.

Buzsaki G (2002) Theta oscillations in the hippocampus. *Neuron* 33: 325–340.

Canteras NS (2002) The medial hypothalamic defensive system: Hodological organization and functional implications. *Pharmacol Biochem Behav* 71: 481–491.

Chatzaki E, Minas V, Zoumakis E and Makrigiannakis A (2006) CRF receptor antagonists: Utility in research and clinical practice. *Curr Med Chem* 13: 2751–2760.

Christianson JP, Ragole T, Amat J, Greenwood BN, Strong PV, Paul ED, et al. (2010) 5-hydroxytryptamine 2C receptors in the basolateral amygdala are involved in the expression of anxiety after uncontrollable traumatic stress. *Biol Psychiatry* 67: 339–345.

Clark MS, McDevitt RA, Hoplight BJ and Neumaier JF (2007) Chronic low dose ovine corticotropin releasing factor or urocortin II into the rostral dorsal raphe alters exploratory behavior and serotonergic gene expression in specific subregions of the dorsal raphe. *Neuroscience* 146: 1888–1905.

Commons KG (2008) Evidence for topographically organized endogenous 5-HT-1A receptor-dependent feedback inhibition of the ascending serotonin system. *Eur J Neurosci* 27: 2611–2618.

Commons KG and Valentino RJ (2002) Cellular basis for the effects of substance P in the periaqueductal gray and dorsal raphe nucleus. *J Comp Neurol* 447: 82–97.

Commons KG, Connolley KR and Valentino RJ (2003) A neurochemically distinct dorsal raphe-limbic circuit with a potential role in affective disorders. *Neuropsychopharmacol* 28: 206–215.

Crawford LK, Craige CP and Beck SG (2010) Increased intrinsic excitability of lateral wing serotonin neurons of the dorsal raphe: A mechanism for selective activation in stress circuits. *J Neurophysiol* 103: 2652–2663.

Crooks R, Jackson J and Bland BH (2012) Dissociable pathways facilitate theta and non-theta states in the median raphe-septohippocampal circuit. *Hippocampus* 22: 1567–1576.

Davidson C, Ho M, Price GW, Jones BJ and Stamford JA (1997) (+)-WAY 100135, a partial agonist, at native and recombinant 5-HT1B/1D receptors. *Br J Pharmacol* 121: 737–742.

Davis M, Rainnie D and Cassell M (1994) Neurotransmission in the rat amygdala related to fear and anxiety. *Trends Neurosci* 17: 208–214.

Deakin JFW and Graeff FG (1991) 5-HT and mechanisms of defence. *J Psychopharmacol* 5: 305–315.

Del-Ben CM and Graeff FG (2009) Panic disorder: Is the PAG involved? *Neural Plast* 2009: 108–135.

Donner NC, Johnson PL, Fitz SD, Kellen KE, Shekhar A and Lowry CA (2012a) Elevated tph2 mRNA expression in a rat model of chronic anxiety. *Depress Anxiety* 29: 307–319.

Donner NC, Montoya CD, Lukkes JL and Lowry CA (2012b) Chronic non-invasive corticosterone administration abolishes the diurnal pattern of tph2 expression. *Psychoneuroendocrinol* 37: 645–661.

Ebner K, Muigg P, Singewald G and Singewald N (2008) Substance P in stress and anxiety: NK-1 receptor antagonism interacts with key brain areas of the stress circuitry. *Ann NY Acad Sci* 1144: 61–73.

Ebner K and Singewald N (2006) The role of substance P in stress and anxiety responses. *Amino Acids* 31: 251–272.

Egan C, Grinde E, Dupre A, Roth BL, Hake M, Teitler M, et al. (2000) Agonist high and low affinity state ratios predict drug intrinsic activity and a revised ternary complex mechanism at serotonin 5-HT(2A) and 5-HT(2C) receptors. *Synapse* 35: 144–150.

Engin E, Stellbrink J, Treit D and Dickson CT (2008) Anxiolytic and antidepressant effects of intracerebroventricularly administered somatostatin: Behavioral and neurophysiological evidence. *Neuroscience* 157: 666–676.

Engin E, Treit D and Dickson CT (2009) Anxiolytic- and antidepressant-like properties of ketamine in behavioral and neurophysiological animal models. *Neuroscience* 161: 359–369.

Evans AK, Abrams JK, Bouwknecht JA, Knight DM, Shekhar A and Lowry CA (2006) The anxiogenic drug FG-7142 increases serotonin metabolism in the rat medial prefrontal cortex. *Pharmacol Biochem Behav* 84: 266–274.

Evans AK, Heerkens JL and Lowry CA (2009) Acoustic stimulation in vivo and corticotropin-releasing factor in vitro increase tryptophan hydroxylase activity in the rat caudal dorsal raphe nucleus. *Neurosci Lett* 455: 36–41.

Fitzgerald LW, Conklin DS, Krause CM, Marshall AP, Patterson JP, Tran DP, et al. (1999) High-affinity agonist binding correlates with efficacy (intrinsic activity) at the human serotonin 5-HT2A and 5-HT2C receptors: Evidence favoring the ternary complex and two-state models of agonist action. *J Neurochem* 72: 2127–2134.

Forster GL, Feng N, Watt MJ, Korzan WJ, Mouw NJ, Summers CH, et al. (2006) Corticotropin-releasing factor in the dorsal raphe elicits temporally distinct serotonergic responses in the limbic system in relation to fear behavior. *Neuroscience* 141: 1047–1055.

Funk D, Li Z, Shaham Y and Le AD (2003) Effect of blockade of corticotropin-releasing factor receptors in the median raphe nucleus on stress-induced c-fos mRNA in the rat brain. *Neuroscience* 122: 1–4.

Gardner KL, Hale MW, Lightman SL, Plotsky PM and Lowry CA (2009a) Adverse early life experience and social stress during adulthood interact to increase serotonin transporter mRNA expression. *Brain Res* 1305: 47–63.

Gardner KL, Hale MW, Oldfield S, Lightman SL, Plotsky PM and Lowry CA (2009b) Adverse experience during early life and adulthood interact to elevate tph2 mRNA expression in serotonergic neurons within the dorsal raphe nucleus. *Neuroscience* 163: 991–1001.

Gardner KL, Thrivikraman KV, Lightman SL, Plotsky PM and Lowry CA (2005) Early life experience alters behavior during social defeat: Focus on serotonergic systems. *Neuroscience* 136: 181–191.

Geller I and Blum K (1970) The effects of 5-HTP on para-chlorophenylalanine (p-CPA) attenuation of "conflict" behavior. *Eur J Pharmacol* 9: 319–324.

Geller I and Seifter J (1960) The effects of meprobamate, barbiturates, d-amphetamine and promazine on experimentally induced conflict in the rat. *Psychopharmacologia* 1: 482–492.

Gorman JM, Papp LA, Coplan JD, Martinez JM, Lennon S, Goetz RR, et al. (1994) Anxiogenic effects of CO2 and hyperventilation in patients with panic disorder. *Am J Psychiatry* 151: 547–553.

Graeff FG (1990) Brain defense systems and anxiety. In: Roth M, Burrows GD and Noyes R (eds) *Handbook of Anxiety*. Amsterdam: Elsevier, pp. 307–354.

Graeff FG (1991) Neurotransmitters in the dorsal periaqueductal gray and animal models of panic anxiety. In: Briley M and File SE (eds) *New Concepts in Anxiety*. London: MacMillan Press, pp 288–312.

Graeff FG (2004) Serotonin, the periaqueductal gray and panic. *Neurosci Biobehav Rev* 28: 239–259.

Graeff FG (2011) Defense-related emotions in humans. *Psychology Neurosci* 4: 183–189.

Graeff FG (2012) New perspective on the pathophysiology of panic: Merging serotonin and opioids in the periaqueductal gray. *Braz J Med Biol Res* 45: 366–375.

Graeff FG and Schoenfeld RI (1970) Tryptaminergic mechanisms in punished and nonpunished behavior. *J Pharmacol Exp Ther* 173: 277–283.

Graeff FG and Zangrossi H Jr (2010) The dual role of serotonin in defense and the mode of action of antidepressants on generalized anxiety and panic disorders. *Cent Nerv Syst Agents Med Chem* 10: 207–217.

Graeff FG, Guimaraes FS, De Andrade TG and Deakin JF (1996) Role of 5-HT in stress, anxiety, and depression. *Pharmacol Biochem Behav* 54: 129–141.

Graeff FG, Quintero S and Gray JA (1980) Median raphe stimulation, hippocampal theta rhythm and threat-induced behavioral inhibition. *Physiol Behav* 25: 253–261.

Gray JA (1982) *The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septo-hippocampal System*. Oxford: Clarendon Press.

Griebel G (1995) 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: More than 30 years of research. *Pharmacol Ther* 65: 319–395.

Grosmark AD, Mizuseki K, Pastalkova E, Diba K and Buzsaki G (2012) REM sleep reorganizes hippocampal excitability. *Neuron* 75: 1001–1007.

Guo JD, Hammack SE, Hazra R, Levita L and Rainnie DG (2009) Bidirectional modulation of bed nucleus of stria terminalis neurons by

5-HT: Molecular expression and functional properties of excitatory 5-HT receptor subtypes. *Neuroscience* 164: 1776–1793.

Halberstadt AL and Balaban CD (2006) Serotonergic and nonserotonergic neurons in the dorsal raphe nucleus send collateralized projections to both the vestibular nuclei and the central amygdaloid nucleus. *Neuroscience* 140: 1067–1077.

Hale MW and Lowry CA (2011) Functional topography of midbrain and pontine serotonergic systems: Implications for synaptic regulation of serotonergic circuits. *Psychopharmacol (Berl)* 213: 243–264.

Hale MW, Bouwknecht JA, Spiga F, Shekhar A and Lowry CA (2006) Exposure to high- and low-light conditions in an open-field test of anxiety increases c-Fos expression in specific subdivisions of the rat basolateral amygdaloid complex. *Brain Res Bull* 71: 174–182.

Hale MW, Dady KF, Evans AK and Lowry CA (2011) Evidence for in vivo thermosensitivity of serotonergic neurons in the rat dorsal raphe nucleus and raphe pallidus nucleus implicated in thermoregulatory cooling. *Exp Neurol* 227: 264–278.

Hale MW, Hay-Schmidt A, Mikkelsen JD, Poulsen B, Bouwknecht JA, Evans AK, et al. (2008a) Exposure to an open-field arena increases c-Fos expression in a subpopulation of neurons in the dorsal raphe nucleus, including neurons projecting to the basolateral amygdaloid complex. *Neuroscience* 157: 733–748.

Hale MW, Hay-Schmidt A, Mikkelsen JD, Poulsen B, Shekhar A and Lowry CA (2008b) Exposure to an open-field arena increases c-Fos expression in a distributed anxiety-related system projecting to the basolateral amygdaloid complex. *Neuroscience* 155: 659–672.

Hale MW, Johnson PL, Westerman AM, Abrams JK, Shekhar A and Lowry CA (2010a) Multiple anxiogenic drugs recruit a parvalbumin-containing subpopulation of GABAergic interneurons in the basolateral amygdala. *Prog Neuropsychopharmacol Biol Psychiatry* 34: 1285–1293.

Hale MW, Shekhar A and Lowry CA (2012) Stress-related serotonergic systems: Implications for symptomatology of anxiety and affective disorders. *Cell Mol Neurobiol* 32: 695–708.

Hale MW, Stamper CE, Staub DR and Lowry CA (2010b) Urocortin 2 increases c-Fos expression in serotonergic neurons projecting to the ventricular/periventricular system. *Exp Neurol* 224: 271–281.

Hammack SE, Guo JD, Hazra R, Dabrowska J, Myers KM and Rainnie DG (2009) The response of neurons in the bed nucleus of the stria terminalis to serotonin: Implications for anxiety. *Prog Neuropsychopharmacol Biol Psychiatry* 33: 1309–1320.

Hammack SE, Mania I and Rainnie DG (2007) Differential expression of intrinsic membrane currents in defined cell types of the anterolateral bed nucleus of the stria terminalis. *J Neurophysiol* 98: 638–656.

Hammack SE, Richey KJ, Schmid MJ, LoPresti ML, Watkins LR and Maier SF (2002) The role of corticotropin-releasing hormone in the dorsal raphe nucleus in mediating the behavioral consequences of uncontrollable stress. *J Neurosci* 22: 1020–1026.

Hammack SE, Schmid MJ, LoPresti ML, Der-Avakian A, Pellymounter MA, Foster AC, et al. (2003) Corticotropin releasing hormone type 2 receptors in the dorsal raphe nucleus mediate the behavioral consequences of uncontrollable stress. *J Neurosci* 23: 1019–1025.

Hannon J and Hoyer D (2008) Molecular biology of 5-HT receptors. *Behav Brain Res* 195: 198–213.

Hazra R, Guo JD, Dabrowska J and Rainnie DG (2012) Differential distribution of serotonin receptor subtypes in BNST(ALG) neurons: Modulation by unpredictable shock stress. *Neuroscience* 225: 9–21.

Helke CJ, Capuano S, Tran N and Zhuo H (1997) Immunocytochemical studies of the 5-HT(1A) receptor in ventral medullary neurons that project to the intermediolateral cell column and contain serotonin or tyrosine hydroxylase immunoreactivity. *J Comp Neurol* 379: 261–270.

Hioki H, Nakamura H, Ma YF, Konno M, Hayakawa T, Nakamura KC, et al. (2010) Vesicular glutamate transporter 3-expressing nonserotonergic projection neurons constitute a subregion in the rat midbrain raphe nuclei. *J Comp Neurol* 518: 668–686.

Hollis JH, Evans AK, Bruce KP, Lightman SL and Lowry CA (2006) Lipopolysaccharide has indomethacin-sensitive actions on Fos expression in topographically organized subpopulations of serotonergic neurons. *Brain Behav Immun* 20: 569–577.

Horacek J, Brunovsky M, Novak T, Tislerova B, Palenicek T, Bubenikova-Valesova V, et al. (2010) Subanesthetic dose of ketamine decreases prefrontal theta cordance in healthy volunteers: Implications for antidepressant effect. *Psychol Med* 40: 1443–1451.

Hoshino K and Sugizaki M (1986) Ulcerogenic effect of the lesion of the median raphe nucleus in fasted rats. *Braz J Med Biol Res* 19: 123–130.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, et al. (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* 46: 157–203.

Hoyer D, Hannon JP and Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* 71: 533–554.

Hsiao YT, Jou SB, Yi PL and Chang FC (2012) Activation of GABAergic pathway by hypocretin in the median raphe nucleus (MRN) mediates stress-induced theta rhythm in rats. *Behav Brain Res* 233: 224–231.

Imai H, Steindler DA and Kitai ST (1986) The organization of divergent axonal projections from the midbrain raphe nuclei in the rat. *J Comp Neurol* 243: 363–380.

Jackson J, Dickson CT and Bland BH (2008) Median raphe stimulation disrupts hippocampal theta via rapid inhibition and state-dependent phase reset of theta-related neural circuitry. *J Neurophysiol* 99: 3009–3026.

Jacobs BL and Azmitia EC (1992) Structure and function of the brain serotonin system. *Physiol Rev* 72: 165–229.

Jasinska AJ, Lowry CA and Burmeister M (2012) Serotonin transporter gene, stress and raphe-raphe interactions: A molecular mechanism of depression. *Trends Neurosci* 35: 395–402.

Jensen P, Farago AF, Awatramani RB, Scott MM, Deneris ES and Dymecki SM (2008) Redefining the serotonergic system by genetic lineage. *Nat Neurosci* 11: 417–419.

Johnson MP, Loncharich RJ, Baez M and Nelson DL (1994) Species variations in transmembrane region V of the 5-hydroxytryptamine type 2A receptor alter the structure-activity relationship of certain ergolines and tryptamines. *Mol Pharmacol* 45: 277–286.

Johnson PL, Lightman SL and Lowry CA (2004) A functional subset of serotonergic neurons in the rat ventrolateral periaqueductal gray implicated in the inhibition of sympathoexcitation and panic. *Ann NY Acad Sci* 1018: 58–64.

Johnson PL, Lowry CA, Truitt W and Shekhar A. (2008) Disruption of GABAergic tone in the dorsomedial hypothalamus attenuates responses in a subset of serotonergic neurons in the dorsal raphe nucleus following lactate-induced panic. *J Psychopharmacol* 22: 642–645.

Kalipatnapu S, Pucadyil TJ, Harikumar KG and Chattopadhyay A (2004) Ligand binding characteristics of the human serotonin1A receptor heterologously expressed in CHO cells. *Biosci Rep* 24: 101–115.

Keay KA and Bandler R (2001) Parallel circuits mediating distinct emotional coping reactions to different types of stress. *Neurosci Biobehav Rev* 25: 669–678.

Kelly KJ, Donner NC, Hale MW and Lowry CA (2011) Swim stress activates serotonergic and nonserotonergic neurons in specific subdivisions of the rat dorsal raphe nucleus in a temperature-dependent manner. *Neuroscience* 197: 251–268.

Kerman IA, Shabrang C, Taylor L, Akil H and Watson SJ (2006) Relationship of presympathetic-premotor neurons to the serotonergic transmitter system in the rat brainstem. *J Comp Neurol* 499: 882–896.

Kimura Y, Hatanaka K, Naitou Y, Maeno K, Shimada I, Koakutsu A, et al. (2004) Pharmacological profile of YM348, a novel, potent and orally active 5-HT2C receptor agonist. *Eur J Pharmacol* 483: 37–43.

Kiser RS and Lebovitz RM (1975) Monoaminergic mechanisms in aversive brain stimulation. *Physiol Behav* 15: 47–53.

Kiser RS, Brown CA, Sanghera MK and German DC (1980) Dorsal raphe nucleus stimulation reduces centrally-elicited fearlike behavior. *Brain Res* 191: 265–272.

Kiser RS, German DC and Lebovitz RM (1978a) Serotonergic reduction of dorsal central gray area stimulation-produced aversion. *Pharmacol Biochem Behav* 9: 27–31.

Kiser RS, Lebovitz RM and German DC (1978b) Anatomic and pharmacologic differences between two types of aversive midbrain stimulation. *Brain Res* 155: 331–342.

Knight AR, Misra A, Quirk K, Benwell K, Revell D, Kennett G, et al. (2004) Pharmacological characterisation of the agonist radioligand binding site of 5-HT(2A), 5-HT(2B) and 5-HT(2C) receptors. *Naunyn Schmiedebergs Arch Pharmacol* 370: 114–123.

Kohler C and Steinbusch H (1982) Identification of serotonin and non-serotonin-containing neurons of the mid-brain raphe projecting to the entorhinal area and the hippocampal formation. A combined immunohistochemical and fluorescent retrograde tracing study in the rat brain. *Neuroscience* 7: 951–975.

Kohler C, Chan-Palay V and Steinbusch H (1982) The distribution and origin of serotonin-containing fibers in the septal area: A combined immunohistochemical and fluorescent retrograde tracing study in the rat. *J Comp Neurol* 209: 91–111.

Koob GF and Zorrilla EP (2012) Update on corticotropin-releasing factor pharmacotherapy for psychiatric disorders: A revisionist view. *Neuropsychopharmacol* 37: 308–309.

Kramer MS (2000) Update on Substance P (NK-1 receptor) antagonists in clinical trials for depression. *Neuropeptides* 34: 255.

Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, et al. (1998) Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science* 281: 1640–1645.

Kramer MS, Winokur A, Kelsey J, Preskorn SH, Rothschild AJ, Snavely D, et al. (2004) Demonstration of the efficacy and safety of a novel substance P (NK1) receptor antagonist in major depression. *Neuropsychopharmacol* 29: 385–392.

Le AD, Funk D, Harding S, Juzytsch W, Li Z and Fletcher PJ (2008) Intra-median raphe nucleus (MRN) infusions of muscimol, a GABA-A receptor agonist, reinstate alcohol seeking in rats: Role of impulsivity and reward. *Psychopharmacol (Berl)* 195: 605–615.

Le AD, Harding S, Juzytsch W, Fletcher PJ and Shaham Y (2002) The role of corticotropin-releasing factor in the median raphe nucleus in relapse to alcohol. *J Neurosci* 22: 7844–7849.

LeDoux JE, Iwata J, Cicchetti P and Reis DJ (1988) Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *J Neurosci* 8: 2517–2529.

Leranth C and Vertes RP (1999) Median raphe serotonergic innervation of medial septum/diagonal band of broca (MSDB) parvalbumin-containing neurons: Possible involvement of the MSDB in the desynchronization of the hippocampal EEG. *J Comp Neurol* 410: 586–598.

Lesting J, Narayanan RT, Kluge C, Sangha S, Seidenbecher T and Pape HC (2011) Patterns of coupled theta activity in amygdala-hippocampal-prefrontal cortical circuits during fear extinction. *PLoS One* 6(6): e21714. doi:10.1371/journal.pone.0021714.

Liebowitz MR, Gorman JM, Fyer A, Dillon D, Levitt M and Klein DF (1986) Possible mechanisms for lactate's induction of panic. *Am J Psychiatry* 143: 495–502.

Lowry CA (2002) Functional subsets of serotonergic neurons: Implications for control of the hypothalamic-pituitary-adrenal axis. *J Neuroendocrinol* 14: 911–923.

Lowry CA, Evans AK, Gasser PJ, Hale MW, Staub DR and Shekhar A (2008a) Topographical organization and chemoarchitecture of the dorsal raphe nucleus and the median raphe nucleus. In: Monti JM, Pandi-Perumal BL, Jacobs BL and Nutt DL (eds) *Serotonin and Sleep: Molecular, Functional and Clinical Aspects*. Basel: Birkhauser, pp. 25–68.

Lowry CA, Hale MW, Evans AK, Heerkens J, Staub DR, Gasser PJ, et al. (2008b) Serotonergic systems, anxiety, and affective disorder: Focus on the dorsomedial part of the dorsal raphe nucleus. *Ann NY Acad Sci* 1148: 86–94.

Lowry CA, Hollis JH, De VA, Pan B, Brunet LR, Hunt JR, et al. (2007) Identification of an immune-responsive mesolimbocortical serotonergic system: Potential role in regulation of emotional behavior. *Neuroscience* 146: 756–772.

Lukkes J, Vuong S, Scholl J, Oliver H and Forster G (2009) Corticotropin-releasing factor receptor antagonism within the dorsal raphe nucleus reduces social anxiety-like behavior after early-life social isolation. *J Neurosci* 29: 9955–9960.

McDevitt RA, Hiroi R, Mackenzie SM, Robin NC, Cohn A, Kim JJ, et al. (2011) Serotonin 1B autoreceptors originating in the caudal dorsal raphe nucleus reduce expression of fear and depression-like behavior. *Biol Psychiatry* 69: 780–787.

McDonald AJ and Mascagni F (2001) Colocalization of calcium-binding proteins and GABA in neurons of the rat basolateral amygdala. *Neuroscience* 105: 681–693.

McKenna JT and Vertes RP (2001) Collateral projections from the median raphe nucleus to the medial septum and hippocampus. *Brain Res Bull* 54: 619–630.

McNaughton N and Corr PJ (2004) A two-dimensional neuropsychology of defense: Fear/anxiety and defensive distance. *Neurosci Biobehav Rev* 28: 285–305.

McNaughton N, Kocsis B and Hajos M. (2007) Elicited hippocampal theta rhythm: A screen for anxiolytic and procognitive drugs through changes in hippocampal function? *Behav Pharmacol* 18: 329–346.

McNaughton N, Swart C, Neo P, Bates V and Glue P (2012) Anti-anxiety drugs reduce conflict-specific "theta." A possible human anxiety-specific biomarker. *J Affect Disord* 148: 104–111.

Mansur SS, Terenzi MG, Neto JM, Faria MS and Paschoalini MA (2010) Changes in food intake and anxiety-like behaviors after clonidine injected into the median raphe nucleus. *Behav Brain Res* 212: 71–77.

Mansur SS, Terenzi MG, Neto JM, Faria MS and Paschoalini MA (2011) Phenylephrine into the median raphe nucleus evokes an anxiolytic-like effect in free-feeding rats but does not alter food intake in free-feeding rats. *Behav Brain Res* 217: 209–214.

Maru E, Takahashi LK and Iwahara S (1979) Effects of median raphe nucleus lesions on hippocampal EEG in the freely moving rat. *Brain Res* 163: 223–234.

May JA, Chen HH, Rusinko A, Lynch VM, Sharif NA and McLaughlin MA (2003) A novel and selective 5-HT2 receptor agonist with ocular hypotensive activity: (S)-(+)-1-(2-aminopropyl)-8,9-dihydropyrano[3,2-e] indole. *J Med Chem* 46: 4188–4195.

Meloni EG, Reedy CL, Cohen BM and Carlezon WA Jr (2008) Activation of raphe efferents to the medial prefrontal cortex by corticotropin-releasing factor: Correlation with anxiety-like behavior. *Biol Psychiatry* 63: 832–839.

Michelsen KA, Schmitz C and Steinbusch HW (2007) The dorsal raphe nucleus - from silver staining to a role in depression. *Brain Res Rev* 55: 329–342.

Millan MJ (2003) The neurobiology and control of anxious states. *Prog Neurobiol* 70: 83–244.

Millan MJ, Marin P, Bockaert J and Mannoury CC (2008) Signaling at G-protein-coupled serotonin receptors: Recent advances and future research directions. *Trends Pharmacol Sci* 29: 454–464.

Miyazaki KW, Miyazaki K and Doya K (2012) Activation of dorsal raphe serotonin neurons is necessary for waiting for delayed rewards. *J Neurosci* 32: 10451–10457.

Molina E, Cervilla J, Rivera M, Torres F, Bellon JA, Moreno B, et al. (2011) Polymorphic variation at the serotonin 1-A receptor gene is associated with comorbid depression and generalized anxiety. *Psychiatr Genet* 21: 195–201.

Nemeroff CB (1996) The corticotropin-releasing factor (CRF) hypothesis of depression: New findings and new directions. *Mol Psychiatry* 1: 336–342.

Neo PS and McNaughton N (2011) Frontal theta power linked to neuroticism and avoidance. *Cogn Affect Behav Neurosci* 11: 396–403.

Neufeld-Cohen A, Kelly PA, Paul ED, Carter RN, Skinner E, Olverman HJ, et al. (2012) Chronic activation of corticotropin-releasing factor type 2 receptors reveals a key role for 5-HT1A receptor responsiveness in mediating behavioral and serotonergic responses to stressful challenge. *Biol Psychiatry* 72: 437–447.

Newman-Tancredi A, Audinot V, Moreira C, Verriele L and Millan MJ (2000) Inverse agonism and constitutive activity as functional correlates of serotonin h5-HT(1B) receptor/G-protein stoichiometry. *Mol Pharmacol* 58: 1042–1049.

Newman-Tancredi A, Conte C, Chaput C, Verriele L, Udinot-Bouchez V, Lochon S, et al. (1997) Agonist activity of antimigraine drugs at recombinant human 5-HT1A receptors: Potential implications for prophylactic and acute therapy. *Naunyn Schmiedebergs Arch Pharmacol* 355: 682–688.

Newman-Tancredi A, Cussac D, Audinot V and Millan MJ (1999) Actions of roxindole at recombinant human dopamine D2, D3 and D4 and serotonin 5-HT1A, 5-HT1B and 5-HT1D receptors. *Naunyn Schmiedebergs Arch Pharmacol* 359: 447–453.

Newman-Tancredi A, Gavaudan S, Conte C, Chaput C, Touzard M, Verriele L, et al. (1998a) Agonist and antagonist actions of antipsychotic agents at 5-HT1A receptors: A [35S]GTPgammaS binding study. *Eur J Pharmacol* 355: 245–256.

Newman-Tancredi A, Verriele L, Chaput C and Millan MJ (1998b) Labelling of recombinant human and native rat serotonin 5-HT1A receptors by a novel, selective radioligand, [3H]-S 15535: Definition of its binding profile using agonists, antagonists and inverse agonists. *Naunyn Schmiedebergs Arch Pharmacol* 357: 205–217.

Newman-Tancredi A, Wootton R and Strange PG (1992) High-level stable expression of recombinant 5-HT1A 5-hydroxytryptamine receptors in Chinese hamster ovary cells. *Biochem J* 285: 933–938.

Nitz DA and McNaughton BL (1999) Hippocampal EEG and unit activity responses to modulation of serotonergic median raphe neurons in the freely behaving rat. *Learn Mem* 6: 153–167.

Ottersen OP (1981) Afferent connections of the amygdaloid complex of the rat with some observations in the cat. III. Afferents from the lower brain stem. *J Comp Neurol* 202: 335–356.

Pan WX and McNaughton N (2004) The supramammillary area: Its organization, functions and relationship to the hippocampus. *Prog Neurobiol* 74: 127–166.

Paxinos G and Watson C (1998) *The Rat Brain in Stereotaxic Coordinates*, 4th Edition. San Diego: Academic Press.

Petit J-M, Luppi P-H, Peyron C, Rampon C and Jouvet M (1995) VIP-like immunoreactive projections from the dorsal raphe and caudal linear nuclei to the bed nucleus of the stria terminalis demonstrated by a double immunohistochemical method in the rat. *Neurosci Lett* 193: 77–80.

Petsche H and Stumpf C (1962) [The origin of theta-rhythm in the rabbit hippocampus]. *Wien Klin Wochenschr* 74: 696–700.

Petsche H, Stumpf C and Gogolak G (1962) The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus. I. The control of hippocampus arousal activity by the septum cells. *Electroencephalogr Clin Neurophysiol* 14: 202–211.

Peyron C, Petit J-M, Rampon C, Jouvet M and Luppi P-H (1998) Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods. *Neuroscience* 82: 443–468.

Pitts FN Jr and McClure JN Jr. (1967) Lactate metabolism in anxiety neurosis. *N Engl J Med* 277: 1329–1336.

Pobbe RL and Zangrossi H Jr (2005) 5-HT(1A) and 5-HT(2A) receptors in the rat dorsal periaqueductal gray mediate the antipanic-like effect induced by the stimulation of serotonergic neurons in the dorsal raphe nucleus. *Psychopharmacol (Berl)* 183: 314–321.

Pobbe RL, Zangrossi H Jr, Blanchard DC and Blanchard RJ (2011) Involvement of dorsal raphe nucleus and dorsal periaqueductal gray 5-HT receptors in the modulation of mouse defensive behaviors. *Eur Neuropsychopharmacol* 21: 306–315.

Porrino LJ and Goldman-Rakic PS (1982) Brainstem innervation of prefrontal and anterior cingulate cortex in the *rhesus* monkey revealed by retrograde transport of HRP. *J Comp Neurol* 205: 63–76.

Rainnie DG, Bergeron R, Sajdyk TJ, Patil M, Gehlert DR and Shekhar A (2004) Corticotrophin releasing factor-induced synaptic plasticity in the amygdala translates stress into emotional disorders. *J Neurosci* 24: 3471–3479.

Robichaud RC and Sledge KL (1969) The effects of p-chlorophenylalanine on experimentally induced conflict in the rat. *Life Sci* 8: 965–969.

Roncon CM, Biesdorf C, Santana RG, Zangrossi H Jr, Graeff FG and Audi EA (2012) The panicolytic-like effect of fluoxetine in the elevated T-maze is mediated by serotonin-induced activation of endogenous opioids in the dorsal periaqueductal grey. *J Psychopharmacol* 26: 525–531.

Rozeske RR, Evans AK, Frank MG, Watkins LR, Lowry CA and Maier SF (2011) Uncontrollable, but not controllable, stress desensitizes 5-HT1A receptors in the dorsal raphe nucleus. *J Neurosci* 31: 14107–14115.

Rupniak NM and Kramer MS (1999) Discovery of the antidepressant and anti-emetic efficacy of substance P receptor (NK1) antagonists. *Trends Pharmacol Sci* 20: 485–490.

Sainsbury RS, Heynen A and Montoya CP (1987) Behavioral correlates of hippocampal type 2 theta in the rat. *Physiol Behav* 39: 513–519.

Sajdyk TJ, Schober DA, Gehlert DR and Shekhar A (1999) Role of corticotropin-releasing factor and urocortin within the basolateral amygdala of rats in anxiety and panic responses. *Behav Brain Res* 100: 207–215.

Schenberg LC and Graeff FG (1978) Role of the periaqueductal gray substance in the antianxiety action of benzodiazepines. *Pharmacol Biochem Behav* 9: 287–295.

Selkirk JV, Scott C, Ho M, Burton MJ, Watson J, Gaster LM, et al. (1998) SB-224289 - A novel selective (human) 5-HT1B receptor antagonist with negative intrinsic activity. *Br J Pharmacol* 125: 202–208.

Sharman JL, Benson HE, Pawson AJ, Lukito V, Mpamhanga CP, Bombail V, et al. (2013) IUPHAR-DB: Updated database content and new features. *Nucleic Acids Research* 41: D1083–1088.

Shekhar A, Keim SR, Simon JR and McBride WJ (1996) Dorsomedial hypothalamic GABA dysfunction produces physiological arousal following sodium lactate infusions. *Pharmacol Biochem Behav* 55: 249–256.

Sidor MM, Amath A, MacQueen G and Foster JA (2010) A developmental characterization of mesolimbocortical serotonergic gene expression changes following early immune challenge. *Neuroscience* 171: 734–746.

Singewald N and Sharp T (2000) Neuroanatomical targets of anxiogenic drugs in the hindbrain as revealed by Fos immunocytochemistry. *Neuroscience* 98: 759–770.

Singewald N, Salchner P and Sharp T (2003) Induction of c-Fos expression in specific areas of the fear circuitry in rat forebrain by anxiogenic drugs. *Biol Psychiatry* 53: 275–283.

Sink KS, Walker DL, Freeman SM, Flandreau EI, Ressler KJ and Davis M (2012) Effects of continuously enhanced corticotropin releasing factor expression within the bed nucleus of the stria terminalis on conditioned and unconditioned anxiety. *Mol Psychiatry* 18: 308–319.

Sirota A, Montgomery S, Fujisawa S, Isomura Y, Zugaro M and Buzsaki G (2008) Entrainment of neocortical neurons and gamma oscillations by the hippocampal theta rhythm. *Neuron* 60: 683–697.

Sleight AJ, Stam NJ, Mutel V and Vanderheyden PM (1996) Radiolabelling of the human 5-HT2A receptor with an agonist, a partial agonist and an antagonist: Effects on apparent agonist affinities. *Biochem Pharmacol* 51: 71–76.

Sotres-Bayon F and Quirk GJ (2010) Prefrontal control of fear: More than just extinction. *Curr Opin Neurobiol* 20: 231–235.

Soubrie P (1986) Reconciling the role of central serotonin neurons in human and animal behavior. *Behav Brain Sci* 9: 319–364.

Spiacci A Jr, Coimbra NC and Zangrossi H Jr (2012) Differential involvement of dorsal raphe subnuclei in the regulation of anxiety- and panic-related defensive behaviors. *Neuroscience* 227: 350–360.

Staub DR, Evans AK and Lowry CA (2006) Evidence supporting a role for corticotropin-releasing factor type 2 (CRF2) receptors in the regulation of subpopulations of serotonergic neurons. *Brain Res* 1070: 77–89.

Staub DR, Spiga F and Lowry CA (2005) Urocortin 2 increases c-Fos expression in topographically organized subpopulations of serotonergic neurons in the rat dorsal raphe nucleus. *Brain Res* 1044: 176–189.

Stezhka VV and Lovick TA (1997) Projections from dorsal raphe nucleus to the periaqueductal grey matter: Studies in slices of rat midbrain maintained in vitro. *Neurosci Lett* 230: 57–60.

Sylvester CM, Corbetta M, Raichle ME, Rodebaugh TL, Schlaggar BL, Sheline YI, et al. (2012) Functional network dysfunction in anxiety and anxiety disorders. *Trends Neurosci* 35: 527–535.

Underwood MD, Arango V, Bakalian MJ, Ruggiero DA and Mann JJ (1999a) Dorsal raphe nucleus serotonergic neurons innervate the rostral ventrolateral medulla in rat. *Brain Res* 824: 45–55.

Underwood MD, Khaibulina AA, Ellis SP, Moran A, Rice PM, Mann JJ, et al. (1999b) Morphometry of the dorsal raphe nucleus serotonergic neurons in suicide victims. *Biol Psychiatry* 46: 473–483.

Valentino RJ and Commons KG (2005) Peptides that fine-tune the serotonin system. *Neuropeptides* 39: 1–8.

Valentino RJ, Bey V, Pernar L and Commons KG (2003) Substance P acts through local circuits within the rat dorsal raphe nucleus to alter serotonergic neuronal activity. *J Neurosci* 23: 7155–7159.

Van Bockstaele EJ, Biswas A and Pickel VM (1993) Topography of serotonin neurons in the dorsal raphe nucleus that send axon collaterals to the rat prefrontal cortex and nucleus accumbens. *Brain Res* 624: 188–198.

Vanderwolf CH (1969) Hippocampal electrical activity and voluntary movement in the rat. *Electroencephalogr Clin Neurophysiol* 26: 407–418.

Varela JA, Wang J, Christianson JP, Maier SF and Cooper DC (2012) Control over stress, but not stress per se increases prefrontal cortical pyramidal neuron excitability. *J Neurosci* 32: 12848–12853.

Vasudeva RK, Lin RC, Simpson KL and Waterhouse BD (2011) Functional organization of the dorsal raphe efferent system with special consideration of nitrergic cell groups. *J Chem Neuroanat* 41: 281–293.

Vertes RP (1981) An analysis of ascending brain stem systems involved in hippocampal synchronization and desynchronization. *J Neurophysiol* 46: 1140–1159.

Vertes RP (2005) Hippocampal theta rhythm: A tag for short-term memory. *Hippocampus* 15:923–935.

Vertes RP and Kocsis B (1997) Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. *Neuroscience* 81: 893–926.

Vertes RP, Kinney GG, Kocsis B and Fortin WJ (1994) Pharmacological suppression of the median raphe nucleus with serotonin 1A agonists, 8-OH-DPAT and buspirone, produces hippocampal theta rhythm in the rat. *Neuroscience* 60: 441–451.

Viana MB, Graeff FG and Loschmann PA (1997) Kainate microinjection into the dorsal raphe nucleus induces 5-HT release in the amygdala and periaqueductal gray. *Pharmacol Biochem Behav* 58: 167–172.

Watson JM, Burton MJ, Price GW, Jones BJ and Middlemiss DN (1996) GR127935 acts as a partial agonist at recombinant human 5-HT1D alpha and 5-HT1D beta receptors. *Eur J Pharmacol* 314: 365–372.

Weinshank RL, Zgombick JM, Macchi MJ, Branchek TA and Hartig PR (1992) Human serotonin 1D receptor is encoded by a subfamily of two distinct genes: 5-HT1D alpha and 5-HT1D beta. *Proc Natl Acad Sci USA* 89: 3630–3634.

Werry TD, Loiacono R, Sexton PM and Christopoulos A (2008) RNA editing of the serotonin 5HT2C receptor and its effects on cell signalling, pharmacology and brain function. *Pharmacol Ther* 119: 7–23.

Wise CD, Berger BD and Stein L (1972) Benzodiazepines: Anxiety-reducing activity by reduction of serotonin turnover in the brain. *Science* 177: 180–183.

Yeung M, Treit D and Dickson CT (2012) A critical test of the hippocampal theta model of anxiolytic drug action. *Neuropharmacol* 62: 155–160.

Yoshida K, McCormack S, Espana RA, Crocker A and Scammell TE (2006) Afferents to the orexin neurons of the rat brain. *J Comp Neurol* 494: 845–861.

Zorrilla EP and Koob GF (2010) Progress in corticotropin-releasing factor-1 antagonist development. *Drug Discov Today* 15: 371–383.

Zoumakis E and Chrousos GP (2010) Corticotropin-releasing hormone receptor antagonists: An update. *Endocr Dev* 17: 36–43.