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Regulation of neurological and neuropsychiatric phenotypes by locus coeruleus-derived galanin

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Abstract

Decades of research confirm that noradrenergic locus coeruleus (LC) neurons are essential for arousal, attention, motivation, and stress responses. While most studies on LC transmission focused unsurprisingly on norepinephrine (NE), adrenergic signaling cannot account for all the consequences of LC activation. Galanin coexists with NE in the vast majority of LC neurons, yet the precise function of this neuropeptide has proved to be surprisingly elusive given our solid understanding of the LC system. To elucidate the contribution of galanin to LC physiology, here we briefly summarize the nature of stimuli that drive LC activity from a neuroanatomical perspective. We go on to describe the LC pathways in which galanin most likely exerts its effects on behavior, with a focus on addiction, depression, epilepsy, stress, and Alzheimer's disease. We propose a model in which LC-derived galanin has two distinct functions: as a neuromodulator, primarily acting via the galanin 1 receptor (GAL1), and as a trophic factor, primarily acting via galanin receptor 2 (GAL2). Finally, we discuss how the recent advances in neuropeptide detection, optogenetics and chemical genetics, and galanin receptor pharmacology can be harnessed to identify the roles of LC-derived galanin definitively.

Keywords

locus coeruleus; galanin; norepinephrine; Alzheimer's disease; depression; addiction; epilepsy; stress

Introduction

The brainstem locus coeruleus (LC) is the major noradrenergic nucleus in the brain and is the sole source of norepinephrine (NE) in many parts of the limbic system and forebrain,

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such as the hippocampus and frontal cortex. The LC is implicated in many aspects of physiology and behavior, including attention, arousal, motivation, and stress. Although NE is considered the “primary” neurotransmitter employed by the LC, these neurons also synthesize and release a host of neuropeptides, including galanin, neuropeptide Y, cocaine- and amphetamine-related transcript, and brain-derived neurotrophic factor. In particular, galanin is expressed in most LC neurons in rodents and humans. The purpose of this review is to catalog the putative functions and consequences of LC-derived galanin, integrate them into a comprehensive model, and provide a roadmap for the use of new technologies to define how galanin release from LC neurons impacts brain function and disease.

Overview of LC anatomy

Electrophysiological evidence consistently shows that LC neurons respond to sensory stimulation of all modalities with a high degree of fidelity to the intensity and temporal pattern of the stimulation. The response of LC neurons to sensory stimuli appears to be more sensitive than the brainstem cholinergic, serotonergic, or dopaminergic systems (Koyama et al., 1994; Strecker and Jacobs, 1985), suggesting the LC plays a primary role amongst these systems in directing attentional resources to a dynamic environment. The responsiveness of LC neuronal discharge to novel and conditioned stimuli that represent salient features of the immediate environment is well established in a variety of species under awake, freely behaving conditions (Aston-Jones et al., 1991; Bouret and Sara, 2005; Rasmussen et al., 1986; Sara and Bouret, 2012). An early study of freely behaving cats is particularly informative and demonstrates the wide range and nature of stimuli influencing LC discharge (Rasmussen et al., 1986). These experiments revealed that the treadmill exercise was a potent driver of LC firing, more so than other stimuli, including the presence of rats or other cats. This entrainment to physical activity presumably reflects the high degree of innervation carrying somatosensory, mechanoreceptive, vestibular, and visceral information to the LC, in addition to other sensory inputs.

LC afferents

The LC is remarkably sensitive to a range of general and special sensory input from somatic and visceral sources; however, early tract-tracing studies of LC afferents revealed surprisingly few structures projecting to the LC and identified the nucleus paragigantocellularis (PGi) and nucleus prepositus hypoglossi (NPH) as the major afferent sources (Aston-Jones et al., 1986). Subsequent work expanded the range of systems innervating the LC, although these connections are comparatively minor.

In addition to direct input from the dorsal horn and spinal trigeminal system (Craig, 1992), the nucleus PGi, a structure in the rostral ventrolateral medulla, can account for much of the somatosensory and mechanoreceptor input from the skeletomotor system (Samuels and Szabadi, 2008; Sara and Bouret, 2012). The PGi also carries auditory input via the inferior colliculus (Van Bockstaele et al., 1993). These pathways excite LC neurons primarily via glutamatergic transmission (Aston-Jones et al., 1991; Ennis et al., 1992; Park et al., 2005), though distinct populations of inhibitory, GABAergic neurons arising from the PGi innervate the LC, as well (Sirieix et al., 2012). Sympathetic afferent input to the LC also arises from

the PGI, which itself functions as a sympathoregulatory center. Parasympathetic regulation of the LC occurs via projections from the nucleus tractus solitarius (NTS) (Groves et al., 2005; Van Bockstaele et al., 2001). Though known primarily as a brainstem micturition center, Barrington's nucleus projects to both the LC and a range of sympathetic and parasympathetic targets (Guo et al., 2013; Valentino et al., 1995) and may thus coordinate autonomic input to the LC. GABAergic projections from the NPH constitute a major pathway to the LC that regulates the inhibition of REM sleep induction (Kaur et al., 2001; Sirieix et al., 2012). The NPH also integrates vestibular (Seo et al., 2004) and visual information (Korp et al., 1989) and thus may coordinate kinetic, positional, and visual input to the LC.

A wide range of neural systems involved in motivational, emotional, and cognitive functions impact the LC. Hypothalamic innervation of the LC is substantial and includes projections from the ventrolateral preoptic area (Lee et al., 2005a), tuberomammillary nucleus, and lateral hypothalamus (Espana et al., 2005; Lee et al., 2005b). All of these pathways are implicated in the control of wakefulness and regulation of sleep architecture, but the orexin-containing neurons arising from the lateral hypothalamus are particularly relevant to arousal (Espana et al., 2005). Projections from the preoptic area to the LC may also coordinate noradrenergic activity with thermoregulatory, osmoregulatory, and reproductive functions (Steininger et al., 2001). Extensive innervation of the LC arising from the paraventricular nucleus represents a secondary, indirect output for sympathetic activation originating from the hypothalamus (Luppi et al., 1995; Samuels and Szabadi, 2008).

In addition to the direct sensory and autonomic inputs arising through the circuits outlined above, the LC receives indirect sensory input via excitatory pathways originating in the central nucleus of the amygdala, which contain corticotropin-releasing factor (Charney et al., 1998; Van Bockstaele et al., 1998). Glutamatergic projections arising from the prefrontal cortex (PFC) also innervate the LC (Jodo et al., 1998). These systems thus provide an important path to convey information about stimuli with emotional salience.

Considering the anatomical relationship of the brainstem networks of biogenic amines that regulate arousal, motivation, and stress, the LC system is especially well situated to command these functions. The LC circuitry thus includes extensive, reciprocal connections to cholinergic, dopaminergic, and serotonergic nuclei. The cholinergic lateral dorsal tegmental nucleus (LDT) and pedunculopontine tegmental nucleus (PPT), which are the major sources of the ascending cortical and thalamic projections of the reticular activating system, also project to and activate the LC via nicotinic receptors (Bitner and Nikkel, 2002; Ganesh et al., 2008; Le Novere et al., 1996; Samuels and Szabadi, 2008). Dopaminergic input to the LC arising from the ventral tegmental area (VTA) regulates the activity of LC neurons in a complex fashion, with both excitatory (Deutch et al., 1986; Ornstein et al., 1987) and inhibitory influences reported (Guiard et al., 2008a). By contrast, serotonergic projections from the dorsal raphe to the LC are predominantly inhibitory and mediated via 5-HT_{1A} receptors (Charley et al., 1993; Kim et al., 2004), though more recent evidence suggests this influence may involve polysynaptic rather than direct mechanisms (Pudovkina et al., 2002).

LC efferents: focus on prefrontal cortex, hippocampus, and ventral tegmental area

The widespread and highly collateralized nature of LC efferent projections to the forebrain has been characterized thoroughly in the literature (Sara and Bouret, 2012; Szabadi, 2013). To appreciate the scope of the LC efferent network, it helps to recognize that nearly all the connections described above are reciprocal. Also noteworthy is the relatively high degree to which the LC innervates limbic structures. For the purposes of the present review, the following section will focus on the PFC, hippocampus, and mesolimbic dopamine system because of their prominent roles in stress, addiction, epilepsy, and cognition. As detailed below, regions of LC from which cortical and hippocampal projections arise also tend to express the highest levels of galanin.

The preponderance of anatomical evidence indicates that the LC projects to all of the forebrain isocortex and allocortex, and it accounts entirely for the noradrenergic innervation of these targets (Loughlin et al., 1986; Samuels and Szabadi, 2008). Electrophysiological experiments reveal that the innervation of the PFC is significantly more robust than in other isocortical areas (Chandler et al., 2013; Chandler et al., 2014). The LC projection to the hippocampal formation is similarly robust (Melander et al., 1986b). Noradrenergic transmission in the hippocampus regulates LTD and LTP and induces trophic factor expression in several hippocampal subfields, predominantly via mechanisms involving β -adrenergic receptors (Hansen and Manahan-Vaughan, 2015; Tully and Bolshakov, 2010; Yanpallear et al., 2010). Of the cortical structures to which the LC projects, the olfactory bulb receives by far the most extensive innervation (Shipley et al., 1985), and the major noradrenergic influence on physiology and plasticity in this allocortical structure is well established (Eckmeier and Shea, 2014; Linster et al., 2011). LC pathways thus consistently modulate synaptic plasticity across a range of isocortical and allocortical structures, thereby revealing a prominent role for LC forebrain systems in trophic functions.

LC input to the VTA regulates the firing rate of dopaminergic neurons through α -adrenergic receptors, with both excitatory (Goertz et al., 2015; Grenhoff and Svensson, 1993; Mitrano et al., 2012; Velasquez-Martinez et al., 2012) and inhibitory actions of noradrenergic signaling in the VTA reported (Guiard et al., 2008a; Guiard et al., 2008b; Jimenez-Rivera et al., 2012; Paladini and Williams, 2004; Velasquez-Martinez et al., 2015; White and Wang, 1984). The nature of this regulation is therefore complex and depends on the relative contribution of α_1 , α_2 , and somatodendritic D-2 autoreceptor activation (Guiard et al., 2008a; Weinshenker and Schroeder, 2007).

Galanin coexistence in LC neurons and galanin receptors in prefrontal cortex, hippocampus, and ventral tegmental area

Double-labeling immunocytochemical studies reveal that galanin coexists with tyrosine hydroxylase in approximately 80% of LC neurons in rats, with a distribution concentrated in more dorsal regions (Holets et al., 1988; Melander et al., 1986a). This anatomical distribution is relevant to the function of galanin because neurons of the dorsal LC

predominantly innervate cortex, hippocampus, and hypothalamus (Loughlin et al., 1986; Sara and Bouret, 2012). Galanin expression is also high in the LC of mice (Perez et al., 2001) and humans (Le Maitre et al., 2013).

The LC provides the sole source of galanin-immunoreactive fibers in the cortex and hippocampus, and most of these fibers express dopamine β -hydroxylase (Hokfelt et al., 1998; Xu et al., 1998a). Galaninergic transmission in the cortex and hippocampus may be mediated by either the GAL1 or GAL2 receptor subtype. GAL1 signaling is predominantly inhibitory, with $G_{i/o}$ activation leading to inhibition of adenylate cyclase and the opening of G protein-regulated K^+ channels, whereas GAL2 is much more complex and signals through $G_{i/o}$ and $G_{q/11}$ -type G proteins (Lang et al., 2015), the latter pathway being essential in mediating the trophic actions of galanin (see below). The GAL2 subtype predominates in isocortex, whereas both GAL1 and GAL2 are distributed throughout the hippocampal formation (O'Donnell et al., 1999; Yoshitake et al., 2014). The LC itself expresses high levels of GAL1 and moderate levels of GAL2 (O'Donnell et al., 1999; Yoshitake et al., 2014).

The GAL3 subtype is expressed predominantly in the hypothalamus and brainstem reticular formation in rats (Mennicken et al., 2002). Though the human LC contains high levels of GAL3 (Le Maitre et al., 2013), GAL3 expression in the rodent LC is very low relative to the abundant expression observed in the hypothalamus (Mennicken et al., 2002). Cellular signaling associated with GAL3 is relatively less understood owing to the lack of stable expression of this receptor subtype in commonly used cell lines (Robinson et al., 2013). However, available evidence suggests that signaling through GAL3 is inhibitory and involves interactions with G protein-regulated K^+ channels and inhibition of adenylate cyclase, similar to that of GAL1 (Lang et al., 2015).

The well-established projection from the LC to VTA and the high degree of coexistence of galanin and NE suggest that LC-derived galanin regulates mesocorticolimbic dopamine function (see below). Though galaninergic fibers projecting to the VTA have yet to be definitively characterized, both the VTA and substantia nigra express GAL2 receptors (O'Donnell et al., 1999), and evidence discussed below reveals a role for galanin signaling in the regulation of mesolimbic and mesostriatal dopamine function.

In summary, the anatomy of the LC and the distribution of galanin within LC pathways that mediate stress responses and appetitive motivation support the prediction that galanin function would be tightly linked to drug-seeking and stress-related behaviors. Evidence reviewed below implicates galanin in both phenomena, though many such actions are complex and unanticipated. Conditions that alter galanin gene expression in the LC provide critical insight into the function and nature of LC-derived galanin. Surprisingly, galanin expression in the LC is not consistently altered by stress. Unexpectedly, the literature points more to galanin adaptations to chronic conditions that may alter LC physiology over extended periods, rather than responsiveness to acute stressors per se (Holmes et al., 1995a; Holmes et al., 1995b; Kuteeva et al., 2008b; Sweerts et al., 1999). Our laboratories have shown that various modes of chronic exercise elevate LC galanin expression in a “dose-dependent” manner (Murray et al., 2010). Such regulation is consistent with the role the LC

plays in integrating the range of sensory and visceral activity associated with dynamic interactions with changing environmental stimuli.

Neuromodulatory effects of galanin

The most well-characterized cellular effect of LC-derived galanin is its ability to inhibit LC activity (Xu et al., 2005). Slice electrophysiology experiments indicate that galanin and GAL1-preferring agonists potently hyperpolarize LC neurons, suppress their spontaneous firing rate, and enhance $\alpha 2$ -adrenergic receptor-mediated negative feedback via activation of GAL1 and an increase in K^+ conductance, even when synaptic input is blocked (Ma et al., 2001; Pieribone et al., 1995; Seutin et al., 1989). Immunohistochemical evidence suggests that endogenous galanin is likely released from LC dendrites and soma and acts on GAL1 receptors on LC neurons in an autocrine manner (Pieribone et al., 1995; Vila-Porcile et al., 2009). GAL2 and GAL3 receptors are also expressed in LC neurons and may contribute, although GAL2 agonists suppress LC activity only at very high concentrations (Ma et al., 2001). Furthermore, unlike GAL1, which responds to changes in LC activity and intracellular signaling, the levels of GAL2 and GAL3 receptors appear to be static, further implicating GAL1 as the most important neuromodulatory galanin receptor in LC neurons (Hawes et al., 2005). Analysis of galanin-induced suppression of LC activity in GAL1, GAL2, and GAL3 knockout mice would yield more definitive data. It is possible that galanin also acts as a negative feedback signal at the level of noradrenergic nerve terminals, but this putative action has yet to be explored meticulously.

Galanin released from noradrenergic terminals can also likely modulate the activity of neurons innervated by the LC. As discussed in the anatomy section above, the LC projects to a wide range of brain areas that express galanin receptors, each of which has the potential to transduce a galanin signal. Some prime examples are the dorsal raphe, VTA, basal forebrain, hippocampus, and frontal cortex. From a neuromodulatory standpoint, the main effect of galanin is to suppress the excitability of target cells via GAL1, and many of these nuclei are indeed inhibited by galanin. While these brain regions contain galanin-positive fibers originating from the LC and are inhibited by galanin (Counts et al., 2010; Xu et al., 1998a; Xu et al., 1998b), we still lack conclusive evidence that LC-derived galanin per se is responsible. The best circumstantial evidence to date comes from a study showing that high-frequency stimulation of the LC suppresses dopamine neuron activity in the VTA, an effect that cannot be blocked by adrenergic receptor antagonists and has been attributed to galanin (Grenhoff et al., 1993; Weiss et al., 1998).

Neurotrophic actions of galanin

In addition to its neuromodulatory actions, described above, galanin is a potent trophic and neuroprotective factor throughout the nervous system, and much of its known trophic activity occurs in targets of the LC via activation of GAL2. Galanin promotes neurite outgrowth in hippocampal slices and dorsal root ganglion cultures via activation of GAL2 and a mechanism involving the actin-depolymerizing factor cofilin (Hobson et al., 2008; Hobson et al., 2013). GAL2 signaling is associated with $G_{q/11}$ -mediated inhibition of RhoA GTPase. Inhibition of RhoA subsequently reduces activation of Rho-associated protein

kinase (ROCK) signaling, which dephosphorylates and thereby activates cofilin (Hobson et al., 2008; Hobson et al., 2013). Galanin signaling may also contribute to dendritic spine maintenance through GAL2-mediated inhibition of RhoA. The GAL2 agonist AR-M1896 promotes the aggregation of MAP-2 and β -tubulin in hippocampal neurons (Pirondi et al., 2005a), whereas RhoA signaling contributes to MAP-2/ β -tubulin disaggregation (Koleske, 2013). The aggregation reflects normal microtubule integrity and is vital for delivery of the microtubule-based cargoes necessary to maintain dendritic spines (Koleske, 2013). Consistent with this molecular action, our laboratories have reported that daily administration of galanin protects against acute stress-induced reductions in dendritic spines in layer V prefrontocortical neurons (Sciolino et al., 2015).

GAL2 signaling protects against a variety of physiological insults in vitro, including excitotoxicity and β -amyloid toxicity (Elliott-Hunt et al., 2007; Elliott-Hunt et al., 2011), and this neuroprotection may involve activation of ERK or Akt (Elliott-Hunt et al., 2007). The prominence of galanin-induced neuroprotection in vivo is particularly obvious when examining the response of endogenous galanin systems to neural injury and neurodegeneration. Galanin expression and galanin-mediated plasticity increases in response to a wide range of pathophysiological insults (Counts et al., 2010; Hobson et al., 2008) and is necessary for neural degeneration following injury (Hobson et al., 2008; Holmes et al., 2000). The widely studied changes in galanin associated with Alzheimer's disease offer an instructive example. Upregulation of galanin expression and galaninergic hyperinnervation occur in forebrain structures and LC neurons exhibiting Alzheimer's-related pathology in humans and rodent models (Counts et al., 2010). The fact that galaninergic neurons are relatively spared in late-stage Alzheimer's patients supports a neuroprotective function and reveals a likely source of galaninergic hyperinnervation of the forebrain (Miller et al., 1999). The neuroprotective actions of galanin described above, particularly against β -amyloid toxicity, argue for the theory that galanin overexpression occurs in response to neurodegeneration and serves to slow or reverse this process (Counts et al., 2010; Hobson et al., 2008). On the other hand, the effects of exogenous administration and galanin overexpression on cognitive performance in rodents support the theory that galaninergic hyperinnervation may contribute to some of the cognitive impairments associated with Alzheimer's disease (Crawley, 2008), although it is important to note that these two theories are not mutually exclusive. More specifically, the cognitive impairment could be due to the GAL1-mediated neuromodulatory effects, while the neuroprotective effects of galanin may be mediated by GAL2. While the LC may serve as the primary source of galanin to the cortical and hippocampal structures vulnerable to pathological atrophy, galanin derived from the serotonergic raphe and cholinergic basal forebrain nuclei must also be considered in these trophic and neuromodulatory actions (Counts et al., 2010; Perez et al., 2001; Xu et al., 1998b).

Addiction

Although there has been limited work examining the role of galanin in drug addiction, the information available suggests that the effects of this neuropeptide depend on drug class. In general, galanin protects against the rewarding and aversive withdrawal effects of opiates and psychostimulants, while it facilitates responding for nicotine and ethanol in rodents. The

effects of galanin on opiate responses are the most comprehensively studied. Transgenic overexpression or centrally administered galanin or the synthetic galanin receptor agonist galnon blocks morphine conditioned place preference and withdrawal signs, while galanin knockout mice show enhanced morphine conditioned place preference and exacerbated morphine withdrawal (Hawes et al., 2008; Holmes et al., 2012; Picciotto et al., 2005; Picciotto, 2008; Zachariou et al., 1999; Zachariou et al., 2003). Similarly, galanin knockout mice are more sensitive to cocaine conditioned place preference, which is reversed by galnon (Narasimhaiah et al., 2009). We and others detected no significant effects of increased galanin signaling on cocaine self-administration, but we found that galnon attenuates cocaine-primed reinstatement of cocaine seeking in rats, an operant model of relapse (Brabant et al., 2010; Ogbonmwan et al., 2014).

In contrast, galanin knockout mice have impaired nicotine conditioned place preference and reduced voluntary ethanol consumption, while galanin-overexpressing mice drink more ethanol than their wild-type counterparts (Karatayev et al., 2009; Karatayev et al., 2010; Neugebauer et al., 2011). Notably, not all studies agree on these general findings. For example, another group has reported that galnon administration *decreases* nicotine conditioned place preference (Jackson et al., 2011). Galanin, particularly GAL1, has also been implicated in human drug dependence (Gold et al., 2012; Jackson et al., 2011; Lori et al., 2011), although whether the genetic polymorphisms associated with addiction examined in these studies increase or decrease galanin transmission is unknown. By contrast, a variant in a galanin enhancer that appears to impact galanin expression did not significantly affect cannabis, alcohol, or tobacco use (Richardson et al., 2014).

Several lines of converging evidence suggest that LC-derived galanin is at least partially responsible for some of these phenotypes. First, chronic opiate exposure and withdrawal increases galanin and galanin receptor expression in the LC, and withdrawal-induced LC activity is decreased by galanin (Georgescu et al., 2003; Holmes et al., 2012; McClung et al., 2005; Zachariou et al., 1999; Zachariou et al., 2000). Second, given the suppression of LC firing produced by autocrine release of galanin discussed earlier, one might predict that NE depletion would phenocopy increased galanin transmission; indeed, this is the case in many instances. For example, like transgenic galanin overexpression or galanin receptor agonist administration, selective suppression of NE transmission via knockout of $\alpha 1$ -adrenergic receptors or the NE biosynthetic enzyme dopamine β -hydroxylase, 6-OHDA lesions, or the administration of adrenergic receptor antagonists can attenuate the rewarding effects of morphine and withdrawal symptoms (Drouin et al., 2002; Maldonado, 1997; Mazei-Robison and Nestler, 2012; Olson et al., 2006; Sahraei et al., 2004; Ventura et al., 2005; Weinshenker and Schroeder, 2007; Zarrindast et al., 2002). Similar to manipulations of galanin itself, suppression of NE transmission has no effect for the most part on operant psychostimulant self-administration, but we and others have shown that psychostimulant conditioned place preference and reinstatement are also reduced upon blockade of NE signaling (Leri et al., 2002; Mantsch et al., 2010; Schroeder et al., 2010; Schroeder et al., 2013; Smith and Aston-Jones, 2011; Ventura et al., 2007; Vranjkovic et al., 2014; Wee et al., 2008; Weinshenker and Schroeder, 2007; Zhang and Kosten, 2005; Zhang and Kosten, 2007) (our unpublished data). Both chronic voluntary exercise and galnon block cocaine-primed reinstatement of cocaine seeking, and exercise is associated with increased galanin mRNA specifically in the LC

(Eisenstein and Holmes, 2007; Murray et al., 2010; O'Neal et al., 2001; Ogbonmwan et al., 2015; Sciolino et al., 2012; Sciolino et al., 2015; Van Hoomissen et al., 2004). By contrast, NE depletion and increasing galanin transmission have opposite effects on ethanol and nicotine reward, suggesting that these addiction-related behaviors are mediated by galanin outside of the LC (Forget et al., 2010; Weinshenker et al., 2000). Finally, transgenic mice overexpressing galanin under the control of the noradrenergic-specific dopamine β -hydroxylase promoter, which display a robust increase of galanin in LC neurons, are resistant to morphine withdrawal (Zachariou et al., 2003).

Although the studies described above suggest that LC-derived galanin regulates responses to drugs of abuse, these results should be interpreted with caution. For example, some of the phenotypes associated with blockade of NE transmission probably involve the A2 noradrenergic cell group, rather than the LC (Olson et al., 2006). In addition, not all studies have reported an increase of galanin mRNA in the LC following chronic morphine exposure and withdrawal (Holmes et al., 1995b). Furthermore, the relationship between drug-induced increases of galanin in the LC and subsequent behavioral changes, as well as similarities in the effects of galanin signaling and reduced NE transmission, are evidence of association, not necessarily causation. Although at first blush the attenuation of morphine withdrawal in mice overexpressing galanin in the LC appears to show convincingly that LC-derived galanin is important for this phenotype, it is noteworthy that ectopic overexpression of galanin occurs in several other brain regions in these mice, including the piriform cortex, entorhinal cortex, and subiculum (Crawley et al., 2002; He et al., 2005; Steiner et al., 2001). Combined, these data argue strongly that galanin released from LC neurons modulates some responses to drugs of abuse, but we lack definitive experiments employing manipulations of galanin exclusively in LC neurons.

Both neuromodulatory and trophic actions of galanin probably contribute to its influence on responses to addictive drugs. For example, acute administration of galanin or galnon effectively modulates many of the responses to addictive drugs described above, and GAL1 appears to transduce this signal. On the other hand, GAL2 is the most abundant receptor subtype in the mesocorticolimbic system that controls reward and reinforcement (O'Donnell et al., 1999), and voluntary exercise, which attenuates drug seeking in several different paradigms (Lynch et al., 2013; Ogbonmwan et al., 2015), potently and selectively increases galanin mRNA in the LC in a chronic manner that lends itself to long-term trophic effects (Eisenstein and Holmes, 2007; Murray et al., 2010; O'Neal et al., 2001; Ogbonmwan et al., 2015; Sciolino et al., 2012; Sciolino et al., 2015; Van Hoomissen et al., 2004).

Seizure susceptibility

Galanin has potent endogenous and exogenous anticonvulsant properties. For example, galanin or galnon administration suppresses multiple types of induced seizures, while galanin gene knockout or receptor antagonists exacerbate seizures, and these effects appear to be mediated primarily by GAL1 (Gottsch et al., 2005; Lerner et al., 2008; Mazarati and Lu, 2005; Mitsukawa et al., 2008). The best evidence that LC-derived galanin is anticonvulsant comes from two studies, one employing galanin-overexpressing mice, and the other, exercise. Transgenic mice that overexpress galanin in LC neurons are resistant to

seizures produced by perforant path stimulation, pentylenetetrazol, or kainic acid (Mazarati et al., 2000). However, as noted above, these mice also have ectopic galanin expression in seizure-sensitive brain regions that could contribute to the phenotype. We showed that the anticonvulsant effect of chronic running wheel exercise is abolished by the galanin receptor antagonist M-40 (Reiss et al., 2009). Importantly, exercise upregulates galanin mRNA exclusively in LC neurons (Eisenstein and Holmes, 2007; Murray et al., 2010; O'Neal et al., 2001; Ogbonmwan et al., 2015; Van Hoomissen et al., 2004), strongly suggesting that the galanin in question is coming from the LC. In contrast to the influence of galanin on drug addiction-related behaviors, the protective effects of galanin against seizures would presumably not be autocrine in nature since NE itself is also anticonvulsant (Giorgi et al., 2004; Weinshenker and Szot, 2002) and suppression of LC firing by galanin would be predicted to exacerbate epileptic activity. Instead, galanin released by noradrenergic terminals in the hippocampus probably contributes to the anticonvulsant effect of galanin (Holmes et al., 2015; Reiss et al., 2009). Several clues indicate that neuromodulation by galanin, rather than its trophic effects, underlies its anticonvulsant properties: GAL1 appears to have a greater impact on seizures than GAL2, acute administration of galanin attenuates seizures, and acute administration of galanin receptor antagonist blocks the anticonvulsant effect of galanin and exercise.

Depression

A significant body of preclinical evidence implicates galanin in depressive disorders, but this literature is often contradictory and difficult to ascertain. Human genetic evidence strongly implicates the galanin system in the risk of developing stress-related disorders, such as depression. Juhasz and colleagues identified polymorphisms in genes encoding galanin and its receptors that increase the risk for depression in subjects who have experienced significant stress (Juhasz et al., 2014). Surprisingly, these investigators found that variants in galanin genes are *more* predictive of stress-related depression and anxiety than the canonical 5HTTLPR polymorphism. Several other laboratories report that galanin polymorphisms predict symptom severity in anxiety and depressive disorders in a wide range of different populations (Davidson et al., 2011; Unschuld et al., 2010; Wray et al., 2012). Interpretation of these results is difficult, however, because it is not clear whether the alleles in question increase or decrease galanin transmission. Thus, the human genetic data accumulated so far cannot tell us anything about valence, only that differences in galanin may contribute to depression.

Tests of acute administration of non-selective galanin receptor agonists in preclinical bioassays, such as the forced swim and tail suspension tests in rats and mice, respectively, have resulted in decreased immobility (an “antidepressant-like” pattern) (Bartfai et al., 2004; Lu et al., 2005a), increased immobility (Kuteeva et al., 2008a; Kuteeva et al., 2008b), or no effect (Holmes et al., 2005). Much of this confusion comes from interpreting the results of tests that represent pharmacological “screens” or “bioassays” of acute antidepressant actions in rodents, rather than chronic depression models that meet systematic, validating criteria (Holmes, 2003); potentially opposing effects of GAL1 versus GAL2 activation and site of action also contribute to the confusion.

Although the original “Monoamine Hypothesis of Depression” posited that depression was caused by a deficit in NE (and/or serotonin) levels, a more modern hypothesis based on both clinical and preclinical evidence is that LC hyperactivity causes depression-like behaviors. For example, biochemical findings point to markedly elevated NE in the brains of depressed patients (Ehnavall et al., 2003; Wong et al., 2000), and postmortem studies revealed reduced α_2 inhibitory autoreceptor function in the LC (Ordway et al., 1994; Ordway et al., 2003), indicating diminished negative feedback that would lead directly to elevated LC activity. A convergence of studies, mostly by Dr. Jay Weiss and colleagues, spanning many years and multiple approaches has established LC hyperactivity as a root cause of depression-like behavior in rodents. A strong uncontrollable stressor (tail shock) induced depression-like behavior (reduced psychomotor activity) and heightened burst firing of LC neurons, and the magnitude of the motor activity suppression was highly and significantly correlated with the degree of LC neuron hyper-responsivity in individual animals (Simson and Weiss, 1988). Intra-LC administration of drugs that activate LC neurons produced depressive-like symptoms in rats and mice, whereas drugs that suppress LC activity attenuated these symptoms (Stone et al., 2009; Weiss et al., 1986). An independent line of corroborating evidence comes from the finding that chronic administration of all known clinically effective antidepressant treatments, including multiple classes of antidepressants with different pharmacological actions (e.g. tricyclics, SSRIs, the α_2 -antagonist mirtazapine) and electroconvulsive shock, decreased spontaneous and sensory-evoked burst firing of LC neurons in mature adult rats, while drugs that lacked clinical antidepressant efficacy did not (Grant and Weiss, 2001; West et al., 2009). An “exception that proves the rule” can be seen in young, adolescent rats. There is a “black box” warning on antidepressants because of the risk of increased depression/suicide in human adolescents when they are first treated with antidepressants, and the data indicate that this applies exclusively to selective serotonin reuptake inhibitors (SSRIs). In adolescent rats, SSRIs early in treatment had the effect of increasing, rather than decreasing, LC activity and depression-like behavior. By contrast, tricyclics, which are not associated with increased risk of suicidality in adolescents, decreased LC activity in both adult and adolescent rats (West et al., 2010a; West et al., 2010b).

An important question is how LC hyperactivity might cause behavioral depression. Elevated brain NE, a direct consequence of LC hyperactivity, is unlikely to be responsible for depression-related symptoms because many drugs that increase brain NE transmission lack pro-depressant effects, and in fact have antidepressant properties. One clue that points towards galanin as the mediator of LC hyperactivity-induced depression comes from the finding that low-frequency LC stimulation provides excitatory drive onto dopaminergic neurons in the VTA, whereas burst firing of LC neurons (which occurs prominently when LC neurons are hyperactive) inhibits firing of these cells (Grenhoff et al., 1993). Importantly, the excitatory effects of single-pulse LC stimulation are abolished following blockade of adrenergic receptors, whereas the inhibitory effects of phasic LC stimulation are not (Grenhoff et al., 1993). Given the importance of these mesolimbic dopamine neurons for reward and psychomotor activity, it has been suggested that core symptoms of depression, such as anhedonia and depressed activity, are consistent with and could be caused by inhibition of the VTA (Nestler and Carlezon, 2006). An obvious neuropeptide candidate to

mediate these effects is galanin, which is expressed in LC neurons that project to the VTA (Gundlach et al., 1990; Melander et al., 1986a; Weiss et al., 1998; Xu et al., 1998a) and is a potent inhibitor of dopamine transmission (de Weille et al., 1989; Gopalan et al., 1993; Nordstrom et al., 1987; Ogbonmwan et al., 2014), and its release probably occurs preferentially during high phasic bursting (Bartfai et al., 1988; Consolo et al., 1994; Karhunen et al., 2001; Verhage et al., 1991). Moreover, galanin overexpression is associated with increased immobility in the forced swim test in mice (Kuteeva et al., 2005a; Kuteeva et al., 2005b; Pirondi et al., 2005b). Based on this evidence, Weiss and colleagues have hypothesized that LC hyperactivity produces depression-related behavioral changes by galanin-mediated inhibition affecting the cell bodies of the VTA dopamine neurons that play a critical role in reward and motivation (Weiss et al., 1996; Weiss et al., 1998), not because LC hyperactivity increases NE transmission. This hypothesis is supported by several key results: (1) chronic antidepressant administration was reported to reduce galanin mRNA in the LC by qRT-PCR (Rovin et al., 2012), (2) effective antidepressant treatments (drugs and electroconvulsive shock) increase VTA dopamine neuronal activity, consistent with their decreasing LC neuronal activity and thereby presumably decreasing galanin release (West and Weiss, 2011), and (3) intra-VTA administration of galanin increases immobility in the forced swim test, while blockade of galanin receptors in the VTA reverses stress-induced behavioral depression that is associated with LC hyperactivity and burst firing (Weiss et al., 2005). Although all of these data are consistent with a role for LC-derived galanin in depression, conclusive evidence must await direct measurements of galanin release in the VTA, coupled with behavioral analysis following specific manipulations of LC firing.

These results indicating a pro-depressant effect of LC-derived galanin must be tempered with conflicting evidence suggesting that galanin has potent antidepressant properties, as well. For example, contrary to the findings of the Weiss group (Rovin et al., 2012), others have reported that experimental manipulations that are clinically effective in treating depression, such chronic administration of a variety of antidepressants (Holmes et al., 2006; Lu et al., 2005a), electroconvulsive therapy (Christiansen, 2011), sleep deprivation (Lu et al., 2005a), and exercise (Ogbonmwan et al., 2015; Sciolino et al., 2012), all similarly induce galanin gene expression in the LC. While GAL1-selective agonists increase immobility in the forced swim and tail suspension tests, GAL2 agonists or GAL2 overexpression consistently produce antidepressant-like effects (Kuteeva et al., 2008a; Kuteeva et al., 2008b; Le Maitre et al., 2013; Saar et al., 2013a; Saar et al., 2013b), and the galanin receptor antagonist M40 reverses the antidepressant-like actions of fluoxetine (Lu et al., 2005a). Finally, rapid, antidepressive actions of galanin have also been reported in humans (Murck et al., 1999; Murck et al., 2004).

How can we reconcile these disparate results? The most obvious possibility is that the discrepancies depend on receptor selectivity. Also noteworthy is that antidepressant therapies in humans require chronic treatment for efficacy, many of which increase galanin in the LC (see above). An appealing explanation is that neuromodulatory LC-derived galanin signaling via GAL1 acutely suppresses mesolimbic dopamine function, thereby reducing behavioral activity in a manner reminiscent of some depression symptoms. On the other hand, trophic galanin actions mediated by GAL2 may exert long-lasting antidepressant actions through neural adaptations in mesocorticolimbic targets of the LC. The use of other

tests for depression-like behavior, particularly ones that are only reversed by chronic antidepressant treatment (e.g. novelty-suppressed feeding test, chronic mild stress, or corticolimbic lesion models), will be necessary to test the latter hypothesis. Another potential mechanism that may account for the complexity of galanin-mediated effects could relate to the relative magnitude or patterns of electrophysiological activity in LC-VTA circuits that underlie some depression-like behaviors. Though decreased activity in VTA neurons may produce immobility in the forced swim test, as described above, extremely high firing rates of VTA neurons is associated with a depressive-like phenotype in the social defeat paradigm (Chaudhury et al., 2013; Friedman et al., 2014; Krishnan et al., 2007). Galanin-mediated inhibition of the VTA may therefore produce antidepressant-like effects in this model, either through autoinhibition of LC firing or direct inhibitory influences on the VTA itself. Overall, assessing the role of LC galanin in depression must take into account not only the depression-related behaviors being measured, but also the contribution of a particular LC circuit in those behaviors.

Stress and anxiety

The anatomical capacity for the LC to influence multiple neurotransmitter systems and the direct, neuromodulatory effects of galanin on all such systems support the hypothesis that galanin may potentially modulate stress and addictive behaviors via acute neuromodulatory actions. The literature examining the effects of galanin on stress-related behavior consists mostly of experiments that involved acute administration of galanin receptor ligands and subsequent measures in a variety of standard paradigms to detect anxiolytic-like actions. Most of these investigations tested the prediction that galanin would acutely diminish stress and anxiety-related behaviors and are based on a hypothetical mechanism involving acute reductions in noradrenergic LC activity. Centrally administered galanin or the nonselective agonist galanin indeed exerts anxiolytic actions in rats or mice in the conflict test (Bing et al., 1993) and elevated zero maze (Rajarao et al., 2007), and peripherally administered galanin is reported to produce lasting anxiolytic-like effects on open field exploration in rats (Klenerova et al., 2011). By contrast, centrally administered galanin fails to alter behavior in mice in the elevated plus maze, light-dark exploration, and novel open field tests (Karlsson et al., 2005; Karlsson and Holmes, 2006). Reviewing the literature involving galanin/GAL1/GAL2 transgenic mice, the findings are predominantly negative in most standard anxiety models (Karlsson and Holmes, 2006). Galanin-overexpressing mice do not differ from wild-type controls in baseline behavior in the elevated plus maze, light-dark exploration test, or open field test (Holmes et al., 2002). For the most part, neither GAL1 nor GAL2 knockout influences spontaneous behavior in these models, with the exception of an anxiogenic-like profile in GAL1-knockout mice in the elevated plus maze (Holmes et al., 2003).

As described above, much of the inconsistency concerning a role for galanin in stress responses may be due to the difficulties in interpreting acute effects in bioassays that are optimal for detecting anxiolytic-like activity, rather than paradigms involving prior exposure to stress, which may more closely model anxiety-related disorders. Indeed, a clearer picture emerges in experiments that focus on stress resilience or resistance. These studies consistently reveal that galanin may influence the *longitudinal* impact of stress. Our laboratories have employed a paradigm that involves a single, brief session of footshock that

produces an anxiogenic effect when assessed the following day in the elevated plus maze in rats. Daily icv administration of galanin for 20 days prior to the footshock session blocks the anxiogenic effects observed in the elevated plus maze (Sciolino et al., 2015). This finding reveals that galanin promotes stress resilience and suggests this action may occur through long-term neural adaptations mediated by galanin. Similar to chronic administration of galanin, the same period of voluntary exercise increases galanin expression in the LC and induces resilience to stress. This exercise-induced resilience is reversed by chronic but not acute administration of the galanin receptor antagonist M40 (Sciolino et al., 2015). The results therefore reveal a potential role for LC-derived galanin in promoting stress resilience, potentially via a trophic mechanism.

Several other studies support a role for galanin in stress resilience. Although galanin overexpression does not impact spontaneous behavior in anxiety models, galanin-overexpressing mice are insensitive to the anxiogenic effects of yohimbine in the light-dark exploration test (Holmes et al., 2002). This finding directly supports a neuromodulatory hypothesis in which galanin dampens stress-related behavior by regulating noradrenergic function, though it demonstrates that such a mechanism must be relatively minor since it does not generalize to other anxiety paradigms. In a similar vein, GAL2 knockout had no effect on spontaneous behaviors, such as those assessed by the elevated plus maze, light/dark transition, forced swim, or tail suspension tests (Lu et al., 2008). GAL2 knockout mice did, however, exhibit increased susceptibility to the repeated stress employed in a “learned helplessness”-like paradigm compared to wild-type mice. Unlike the other tests, “learned helplessness” paradigms examine the impact of uncontrollable stress exposure on subsequent responding to repeated stressors. Loss of GAL2 led to a susceptible phenotype in this paradigm, suggesting that galanin signaling through GAL2 is necessary for resilience. In further support of a specific role for GAL2 in resilience, transgenic overexpression of GAL2 in several fronto-cortical areas, including mPFC, was found to decrease immobility in a version of the forced swim test that involved pre-exposure to swim stress on the previous day (Le Maitre et al., 2011). By contrast, GAL2 overexpression had no effect on elevated plus-maze or novel open field exploration in mice not previously exposed to stress. The role of galanin in stress resilience was further demonstrated by the ability of the mixed galanin receptor agonist galnon to prevent the development of an “extreme stress responsive” phenotype in a putative rat model of post-traumatic stress disorder, which measures the impact of an acute stress on the development of subsequent anxiety-like behaviors over time (Kozlovsky et al., 2009).

The behavioral literature thus points to functions for galanin beyond neuromodulatory effects. Galanin, and GAL2 in particular, are evidently involved in protecting against the lasting consequences of an acute stress event by diminishing reactivity to subsequent stressors, rather than by modulating reactivity to the acute stress event itself. This long-term protection points to a role for galanin in the plasticity necessary for resilience.

Summary

The evidence reviewed above reveals the complex actions of LC-derived galanin, which depend on target circuits, receptor subtype, presynaptic or postsynaptic influences, and/or

involvement of NE. Above all, the LC galanin system owes its complexity to the anatomy of the LC itself, and it is thus not surprising that galanin exerts multiple physiological and behavioral effects depending on the particular target innervated by the LC and the receptor subtype involved. Nonetheless, general principles emerge when considering the influence of galanin on physiology and behavior. The model that follows summarizes these principles, specifically as they relate to LC circuitry. By inhibiting activity of the LC itself through somatodendritic autocrine actions, galanin is poised to regulate all noradrenergic functions throughout the brain. GAL1-mediated inhibition of the LC may thus decrease drug seeking and produce anxiolytic and antidepressant effects. It may also lead to cognitive impairment by suppressing attention/arousal mechanisms. Direct inhibition of the LC may also produce a decrease in VTA tone, which could contribute to pro-depressant effects, though dampening hyperexcitability of the VTA may promote resilience to stress and depression-related behaviors. While yet to be definitively established, LC-derived galanin may also regulate VTA electrophysiological activity directly via the abundant GAL2 receptors present in this structure. LC-derived galanin acting via neuromodulatory GAL1-mediated mechanisms appears to have anticonvulsant properties in models of epilepsy and produces cognitive impairment that has been implicated in Alzheimer's disease. By contrast, trophic actions of galanin in the PFC, hippocampus, and LC mediated by GAL2 may contribute to beneficial, long-term adaptations that promote stress resilience, elevate mood, and protect against neurodegeneration.

Future directions

There is substantial evidence pointing to a role for LC-derived galanin in drug addiction, seizure susceptibility, depression, Alzheimer's disease, and stress resilience, although upon close examination, the evidence is all circumstantial. We propose that the following criteria must be met as definitive proof of a functional role for LC-derived galanin: (1) bona fide galanin release must accompany selective LC activity, (2) the effects of LC activity must be blocked by a galanin receptor antagonist or selective depletion of galanin from LC neurons, and (3) selective overexpression of galanin in the LC should mimic the effects of increased LC activity. One reason these criteria have not been met before is that until recently we lacked the necessary tools. Notably, there has been no good way to specifically manipulate LC neurons *in vivo* with the necessary precision or to measure galanin release in the brain. The lack of galanin receptor antagonist specificity has also held the field back. Today, with the development of optogenetics, advances in brain extracellular peptide measurements, and more selective galanin receptor antagonists, the stage is set to rigorously test the neuromodulatory and trophic consequences of galanin release from LC neurons.

The advent of optogenetics, which uses genetically encoded light-sensitive ion channels to selectively activate neurons of a defined brain region and neurotransmitter phenotype within physiological firing parameters, has overcome the limitations of previous techniques (e.g. electrical stimulation, pharmacological activation), transforming the neuroscience and depression fields (Albert, 2014; Nieh et al., 2013; Roeper, 2013; Stuber et al., 2012; Tye et al., 2013). In these studies, a virus containing the light-activated cation channel, channelrhodopsin2 (ChR2), which is driven by a cell type-selective promoter, is injected into a brain region of interest. A fiber optic ferrule or LED is then implanted just dorsal to the

injection site, and a laser delivers light pulses that activate the channel and depolarize the neurons expressing ChR2. Importantly, the laser will only activate neurons that express ChR2, and expression of ChR2 is controlled by the cell type-specific promoter in the virus. Two approaches have been used successfully to drive ChR2 expression selectively in LC neurons. First, Cre-dependent ChR2 viruses can be injected into the LC of transgenic mice or rats expressing Cre recombinase under the control of the tyrosine hydroxylase promoter (Carter et al., 2010; Witten et al., 2011). This approach appears to work well in mice, but despite promising data in the original paper, several labs have failed to observe robust ChR2 expression in the LC of TH-Cre rats (E. Vazey and G. Aston-Jones, personal communication; our unpublished data). To overcome this obstacle, a virus was developed that uses the noradrenergic-specific PRSx8 promoter (Hwang et al., 2001) to drive gene expression in the LC. The initial study describing this method used it to achieve DREADD (Designer Receptors Exclusively Activated by Designer Drugs) expression (Vazey and Aston-Jones, 2014), but it has since been adapted for ChR2-mediated optogenetics (E. Vazey and Gary Aston-Jones, personal communication; our unpublished data). Following implantation of an optic ferrule just dorsal to the viral injection site in the LC, only LC neurons, and not non-LC neurons in the region or fibers of passage/terminals from other brain regions, are activated by laser stimulation. LC neurons fire in two distinct modes: low tonic (typically sustained 0.1–5.0 Hz, but can be as high as 10 Hz) and high phasic (typically 5–15-Hz bursts, with suppression of spontaneous activity in between bursts, but bursts can be as high as 20–25 Hz) (Foote et al., 1983). Although peptide transmission from the LC has been primarily inferred from peripheral nerve data, the release of peptide co-transmitters is thought to be frequency dependent: NE release occurs at both low- and high-frequency discharge, while release of peptide co-transmitters occurs preferentially during high phasic bursting (Bartfai et al., 1988; Consolo et al., 1994; Dutton and Dyball, 1979; Karhunen et al., 2001; Verhage et al., 1991). Moreover, because the activity of the neurons is entrained to the frequency of laser stimulation, we can selectively activate LC neurons in low tonic mode, which preferentially releases NE, or high phasic (bursting) mode, which would be expected to release both NE and galanin, suppress VTA dopamine neuron activity, and induce behavioral depression.

Somatodendritic release of NE from LC neurons and its role in mediating auto-inhibition are well established (Huang et al., 2007; Huang et al., 2012). The overflow of NE in the LC measured by in vivo microdialysis corresponds closely to that occurring in the PFC in response to a variety of manipulations, suggesting that somatodendritic release regulates activity in LC targets (Pudovkina et al., 2001). The co-release of NE and galanin in the pancreas is activity dependent and sensitive to α_2 autoreceptor modulation, suggesting a similar mechanism may exist in the LC (Scheurink et al., 1992). Ultrastructural analyses reveal that such NE release occurs via large, dense-core vesicles, which is characteristic of peptide co-localization (Huang et al., 2007). This finding may thus provide the anatomical evidence for galanin/NE co-release and suggests a mechanism by which galanin may modulate all LC activity. However, the lack of evidence directly confirming activity-dependent release of galanin from LC terminals/varicosities is a major gap in our understanding of LC-derived galanin function. In general, it is very difficult to measure galanin release directly by in vivo microdialysis because of low endogenous extracellular

concentrations, high molecular weight, and poor recovery due to the “sticky” nature of peptides that cause adherence to probe membrane (Shippenberg and Thompson, 2001). A few published studies have reported success with this technique, however. Consolo and coworkers employed in vivo microdialysis to detect galanin release in the ventral hippocampus following local depolarization or stimulation of the cholinergic diagonal band nuclei (Consolo et al., 1994). Though the former manipulation may reflect release from LC projections, the latter only demonstrates a role of cholinergic projections. In vivo microdialysis experiments in the dorsal hippocampus also revealed that 17- β estradiol treatment caused a delayed increase in galanin overflow in anesthetized rats (Hilke et al., 2005), but the source of this release was not determined. Recent advances promise to improve the detection of neuropeptides in brain dialysate. For example, adding antibodies to the perfusion flow can enhance the concentration gradient and probe recovery, and radioimmunoassay, liquid chromatography coupled to mass spectrometry (LC-MS) and capillary liquid chromatography-multistate mass spectrometry (CLC-MS(n)) have facilitated the detection of neuropeptides (Kennedy, 2013; Zhou et al., 2013). We have used enzyme-linked immunosorbent assay (ELISA) successfully to quantify changes in tissue levels of galanin in the brain (Sciolino et al., 2015), and this technique could be adapted for use with dialysate.

By combining optogenetics and new microdialysis techniques, it should be possible now for the first time to directly measure galanin release from noradrenergic terminals in vivo during behavioral testing. It will be critical to test various optogenetic stimulation parameters to confirm that galanin release occurs mainly during high-frequency burst firing of LC neurons. Preliminary studies indicate that high-fidelity activation of LC neurons occurs at ChR2 stimulation frequencies of up to 15 Hz (E. Vazey and G. Aston-Jones, personal communication), which is in the range of the predicted parameters for galanin release (Grenhoff et al., 1993). Newer opsins, such as ChETA (Gunaydin et al., 2010), can sustain higher frequency spike trains, although it has not been tested in LC neurons. The aforementioned DREADDS may also be used to specifically drive the activity of LC neurons (Vazey and Aston-Jones, 2014). DREADDs are derivatives of muscarinic acetylcholine receptors that are engineered to be insensitive to acetylcholine or any other endogenous chemical, but are rapidly activated upon administration of the clozapine derivative, clozapine-N-oxide (CNO), which has no biological activity in the absence of DREADDs (Armbruster et al., 2007; Aston-Jones and Deisseroth, 2013; Wess et al., 2013). Compared to optogenetics, this approach is limited because it cannot precisely control the firing frequency of the neurons: it is more of an all-or-none response and does not appear to be very dose dependent (Vazey and Aston-Jones, 2014). However, because CNO can be administered systemically and a single dose of CNO can modulate neuronal activity for many hours without the need for constant stimulation or connection to a laser (Alexander et al., 2009; Ferguson et al., 2011; Krashes et al., 2011; Rogan and Roth, 2011; Wulff and Arenkiel, 2012), it may be more useful for inducing chronic LC activation and “trophic” galanin release, akin to the ability of chronic voluntary exercise to increase galanin expression in the LC and promote stress resilience. It remains to be seen whether the magnitude of DREADD-mediated LC stimulation is sufficient to induce galanin release.

As mentioned above, the use of DBH-galanin transgenic mice to probe LC-derived galanin function is limited by the ectopic expression of galanin outside the LC. Nevertheless, it should now also be possible to achieve selective galanin overexpression in the LC via intra-LC delivery of PRSx8-galanin virus or Cre-dependent galanin virus in TH-Cre animals. Likewise, these viral vector strategies could be used to reduce LC galanin expression by siRNA or CRISPR. Intra-LC injection of a PRSx8-Cre virus in a “floxed” galanin mouse would presumably achieve galanin ablation in LC neurons, as well. Optogenetic/chemical genetic activation of LC neurons could also be coupled with local infusion of galanin receptor antagonists to establish the contribution of LC-derived galanin to various aspects of neurochemistry and behavior. Finally, optogenetic and chemical genetic tools that suppress, rather than activate, neuronal firing are also available and could be used in attempts to attenuate the effects of presumed LC-derived galanin-mediated processes, such as chronic exercise.

An array of synthetic compounds is currently available to tease apart the relative contributions of GAL1, GAL2, and GAL3 subtypes in the neuromodulatory and trophic functions of galanin. Fragments of the full galanin peptide sequence (29 amino acids in most species, 30 in humans) provide a moderate degree of receptor selectivity. Galanin 2–11 thus exhibits relative selectivity as an agonist for GAL2 and GAL3 (Lang et al., 2007; Webbing et al., 2012). Synthetic peptide agonists, such as M1145 and M617, provide good selectivity and nanomolar affinity for GAL2 and GAL1, respectively (Runesson et al., 2009; Webbing et al., 2012). A series of novel synthetic peptides with high relative affinity for GAL2 were developed recently (e.g. J18), and these compounds exert potent antidepressant-like effects in the forced swim and tail suspension bioassays (Saar et al., 2013a). There are also several nonpeptide agonists available now, the most widely studied being galnon. Like most other nonpeptide agonists, galnon’s affinity for both GAL1 and GAL2 is in the micromolar range and produces off-target effects on other G protein-coupled receptors (Floren et al., 2005; Webbing et al., 2012). Many of the traditional antagonists, such as M35 and M40, are chimeric peptides that bind all receptor subtypes in the nanomolar range (Lang et al., 2007; Webbing et al., 2012). Most of the literature reports antagonist activity of these compounds, although partial agonist activity has been reported, as well (Lu et al., 2005b).

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Highlights

The neuropeptide galanin is expressed in most locus coeruleus (LC) neurons

LC-derived galanin influences neurological and neuropsychiatric phenotypes

Neuromodulatory actions of LC-derived galanin are mediated by GAL1

Neurotrophic actions of LC-derived galanin are mediated by GAL2

New tools and strategies to study LC-derived galanin are proposed

Figure 1.

Hypothetical functions of LC-derived galanin. Shown is a model explaining the differential effects of LC-derived galanin depending on site of action and receptor subtype. In general, GAL1-mediated neuromodulatory effects are responsible for cognitive impairment, anticonvulsant activity, decreased addiction-like behavior, and pro-depressant properties, while GAL2-mediated trophic effects underlie stress resilience, elevated mood, and neuroprotection.