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REVIEW ARTICLE

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Modulation of forebrain function by nucleus incertus and relaxin-3/RXFP3 signaling

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Summary

The nucleus incertus (NI) in the pontine tegmentum sends ascending projections to the midbrain, hypothalamus, amygdala, basal forebrain, hippocampus, and prefrontal cortex, and has a postulated role in modulating several forebrain functions. A substantial population of GABAergic NI neurons expresses the neuropeptide, relaxin-3, which acts via the $G_{i/o}$-protein-coupled receptor, RXFP3, present throughout the forebrain target regions. Broad and specific manipulations of these systems by activation or inhibition of the NI or modulating RXFP3 signaling have revealed key insights into the likely influence of the NI/relaxin-3/RXFP3 system on modalities including arousal, feeding, stress responses, anxiety and addiction, and attention and memory. This range of actions corresponds to a likely impact of NI/(relaxin-3) projections on multiple integrated circuits, but makes it difficult to draw conclusions about a generalized function for this network. This review will focus on the key physiological process of oscillatory theta rhythm and the neural circuits that promote it during behavioral activation, highlighting the ability of NI and relaxin-3/RXFP3 signaling systems to modulate these circuits. A better understanding of these mechanisms may provide a way to therapeutically adjust malfunction of forebrain activity present in several pathological conditions.

KEYWORDS
arousal, brainstem, feeding, GABA, hippocampus, septum, social interaction, theta rhythm

1 | INTRODUCTION

Primary functions of the mammalian brain are to perceive the features and details of the environment, give emotional value to the cues and contexts, and store or retrieve information that can be useful in new scenarios. As a result of these processes, motor or visceral responses can be performed following determined stereotypes using particular sets of forebrain interconnections. These processes are, in turn, modulated by ascending, subcortical neural projections that enhance or reduce activities in different regions of the forebrain depending on the particular circumstances needed, levels of arousal, the time within the circadian rhythm or the level of metabolic demand. Cholinergic, dopaminergic, histaminergic, serotoninergic, adrenergic, and noradrenergic projections to the forebrain have established roles in these modulatory actions.[1-4] Indeed, agonists and antagonists, and allosteric or enzymatic modulators affecting these transmitter systems and their multiple receptors are the basis for the primary

treatments of most neurological and mental illnesses.[5] However, these systems are quite complex in nature, as these monoamine transmitters are coreleased with other transmitters including neuropeptides.[6,7] Some of these transmitters may act via non-synaptic mechanisms, such as extra-synaptic receptors or volume transmission.[8,9] Notably, some long, ascending projections from the brainstem use GABA as a primary (fast) transmitter, which is coexpressed with a number of different transmitters/modulators. One such GABA network arises from the *nucleus incertus* (NI) in the pontine tegmentum and these large GABA neurons coexpress neuropeptides including relaxin-3, cholecystokinin, and neuromedin-B[10] (Figure 1A-D). Unlike the monoamine transmitter systems that have been extensively characterized experimentally, the NI GABA/peptide system is still relatively uncharacterized. This review is focused on basic mechanisms at the cellular level (eg, ERK phosphorylation) and the system level (eg, theta rhythm) that are controlled by the NI GABA/relaxin-3 system, and that may be common to most functions modulated by this system.

2 | NUCLEUS INCERTUS

The term *nucleus incertus* (Latin for "uncertain nucleus") was first introduced by George Streeter[11] to describe a group of neurons in the human brain located in the midline of the floor of the 4th ventricle at the level of the genu of the facial nerve. Thereafter, in 2001, a precise anatomical study[12] revealed a widespread neural network originating in the NI that was distributed throughout various midbrain and forebrain areas. This NI network was subsequently confirmed and its description extended.[13]

Originally, it was proposed that the NI system functions via a strong triangular circuit involving the NI, median raphe and interpeduncular nuclei, with ascending connections from these nodes producing a general activation of forebrain circuits including the hippocampus (Figure 2A).[11] We further proposed that the NI is engaged in bidirectional communication between the septohippocampal system and the NI, as analysis revealed that the NI successively connects regions that, in turn, project to the next node, so that the hippocampus receives the cumulative connections of all nuclei/areas within the pathway. Successive connections in this major pathway involve the median raphe, interpeduncular nucleus, supramammillary nucleus, medial septum, and the hippocampus (Figure 2B).[12] We have additionally observed that the medial septum is a source of descending projections to the NI.[13]

These early studies were motivated by the observation that NI neurons in the rat brain express a high level of corticotropin-releasing factor receptor-1 (CRF_1) mRNA,[14,15] which suggested the NI may mediate behavioral responses to stressful conditions.

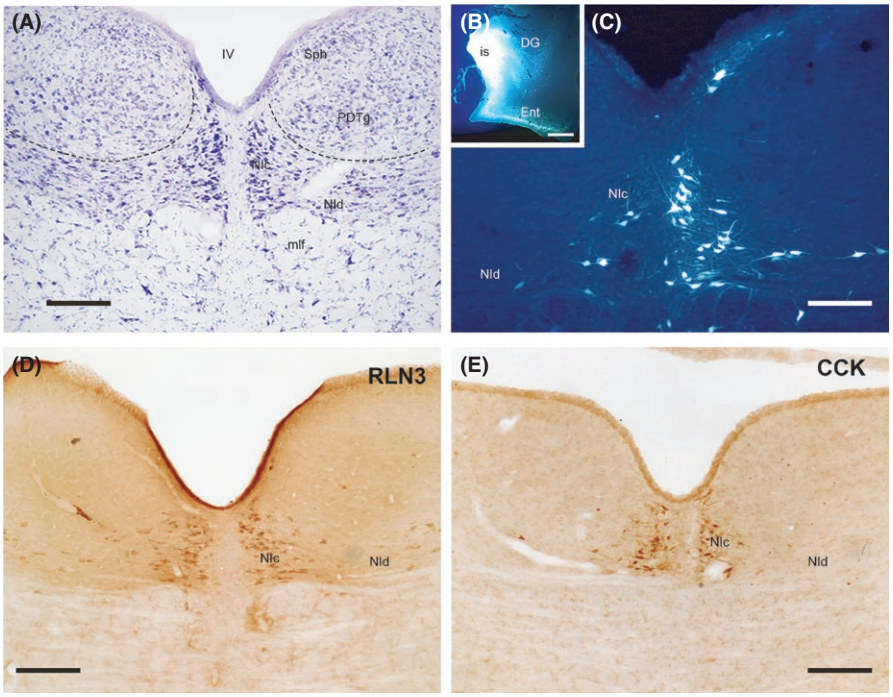

FIGURE 1 Morphological features of the rat nucleus incertus (NI). A, A Giemsa-stained section of the pontine tegmentum at the level of the floor of the 4th ventricle (IV). The NI (outlined) is composed of the pars compacta in the midline (NIc) and the pars dissipata (NId), just dorsal to the medial longitudinal fasciculus (mlf), with the posterodorsal tegmentum (PDTg) and the sphenoid nucleus (Sph) dorsolateral to the NI at this level. B, An injection site (is) for the retrograde tracer, fluorogold (FG) in the dentate gyrus of the caudo-ventral hippocampus, displaying some retrograde labeling in the entorhinal cortex (Ent). C, Retrograde labeling in the ipsilateral NI resulting from the injection in (B), with some retrograde labeling in the contralateral NI. D, Immunohistochemical detection of relaxin-3 (RLN3) in the NI. E, Immunohistochemical detection of cholecystokinin (CCK) in the NIc. Calibration bars in A, C, D and E, 200 μm, and B, 500 μm

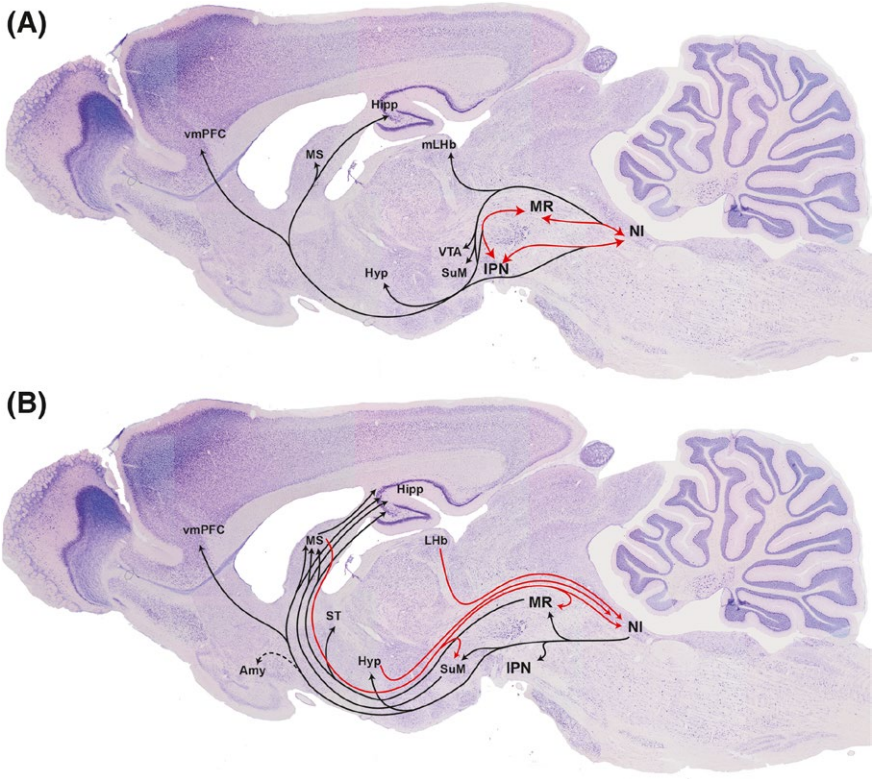

FIGURE 2 Two schematic models of NI connectivity. A, Based on their anatomical mapping studies, Goto et al[11] proposed a strong neural circuit between the median raphe (MR), the interpeduncular nucleus (IPN) and the NI and proposed that from this circuit, ascending projections could reach the ventral tegmental area (VTA), supramammillary nucleus (SuM), hypothalamus (Hyp), medial septum (MS), hippocampus (Hip), and the medial prefrontal cortex (mPFC). B, In subsequent studies, Olucha-Bordonau et al[12] proposed a series of overlapping ascending projections from the NI to the hippocampus, in which each progressive step recapitulated previous projections on route to the hippocampus. This scheme also incorporated the bed nucleus of the stria terminalis (ST) and the amygdala (Amy). Descending projections from the septum, hypothalamus, and lateral habenula (LHb) are also illustrated, which constitute a bidirectional pathway[13]

3 | THE RELAXIN-3/RXFP3 NEUROPEPTIDE/G-PROTEIN-COUPLED RECEPTOR SIGNALING SYSTEM

Concurrent with the mapping of NI anatomical connections, the neuropeptide, relaxin-3, was identified in human, mouse, and rat and its expression in different tissues was reported.[16,17] Using in situ hybridization and immunohistochemistry, the peptide was found to be highly expressed in an area in the ventromedial pontine tegmentum that corresponded to the NI.[10,17,18] In addition to the large NI population, smaller populations of relaxin-3 neurons were observed in the central periaqueductal gray, lateral part of the substantia nigra, and pontine raphe nucleus.[10,18-20] In NI neurons, relaxin-3 is coexpressed with the major inhibitory transmitter, GABA, and the distribution pattern of relaxin-3 fibers is similar among rodents and in primate brain.[10,18,19] Similar anatomical characteristics of the NI, relaxin-3 neurons, and their projections in the *Macaca fascicularis* have also been described.[20]

Analysis of different taxa revealed relaxin-3 as the ancestral form of the relaxin family peptides in vertebrates.[21] The native peptide is synthetized from the preprorelaxin-3 gene, and mRNA is translated to a peptide which is cleaved into 3 peptides (A, B and C), with peptides A and B linked by 2 disulfide bonds. The B-peptide contains the characteristic sequence "RXXXRXX(I/V)" which is key to the interaction with the peptide's receptor.[16] Phylogenetic evidence revealed that the relaxin-3 gene emerged before the divergence tree of vertebrates and the ancestral form of the relaxin family is a relaxin-3-like peptide. In zebrafish, mRNA expression is diffuse in early development until 72 days postpharyngula stage and then abundant transcripts appear in the periaqueductal gray and the brainstem, in a structure identified as the NI equivalent in *Danio nerio*.[22]

Shortly after the discovery of relaxin-3, a type 1 G-protein-coupled receptor, GPCR135 (renamed "RXFP3"),[23] was identified as its cognate receptor. Relaxin-3 binds to RXFP3 with high affinity (K_d 300 pmol/L) and RXFP3 activation produces inhibition of cAMP, normally associated with $G_{i/o}$-protein coupling.[24,25] Studies in Chinese hamster ovary (CHO-K1) cells transfected with RXFP3 revealed that receptor activation also resulted in ERK1/2 phosphorylation.[26]

Chemical modifications of the relaxin-3 structure have produced specific RXFP3 agonists and antagonists,[27-29] which have provided several functional insights into relaxin-3/RXFP3 biology. Further insights have also been obtained by studying the phenotype of

mice lacking the relaxin-3 or RXFP3 gene and peptide/protein.[30-32] Studies employing direct activation or inactivation of the NI and its neuronal inputs and targets have also provided information about the likely roles of relaxin-3/RXFP3 systems. Thus, considerable data implicates the relaxin-3/RXFP3 system in arousal and motivated feeding and drug-seeking behavior, stress responses and anxiety, and attention and memory.[33-35] Several investigations have examined the neurophysiological events that are driven by the NI/relaxin-3 networks, with consistent effects observed on the septohippocampal system and hippocampal theta rhythm, and on expression of immediate early genes and transcription factors in target nuclei,[36,37] but further studies are required of these processes that underlie the complex behavioral actions observed.

4 | SYSTEM AND CELLULAR PROCESSES AFFECTED BY THE NI-RELAXIN-3/RXFP3 SYSTEM

The pattern of forebrain connections of the NI was consistent with the ascending brainstem control of the hippocampus either directly or through the septal area,[12,38] and this observation led to a series of experiments that confirmed the role of the NI/relaxin-3/RXFP3 system in control of hippocampal theta rhythm. Stimulation of the NI in urethane-anesthetized rats induced hippocampal theta and lesion of the NI eliminated the hippocampal theta activity produced by electrical stimulation of the *nucleus reticularis pontis oralis* (RPO).[39] Similar effects were observed after manipulations of the relaxin-3/RXFP3 system. Notably, injection of an RXFP3 agonist into the medial septum induced hippocampal theta rhythm and affected exploration and spatial working memory in the spontaneous alternation test.[40] Septal infusion of an RXFP3 antagonist impaired RPO stimulation-induced theta.[40] Furthermore, there was a correlation between the phase of theta oscillations in the hippocampus and in NI,[36] and there is a causal interdependence between hippocampal theta rhythm and NI firing, whereby different types of neurons drive the flow of the causality either from the NI to the hippocampus or vice versa.[41,42]

Nucleus incertus neurons express high levels of CRF_1 mRNA,[15] suggesting a role for the nucleus in relaying stress response signals to telencephalic circuits. In a recent study, we determined that the NI is composed of different populations of neurons, but all RLN3 neurons express CRF_1 and intracerebroventricular (icv) infusion of CRF-activated relaxin-3 neurons.[36] Furthermore, a recent study reported that CRF infusion into the NI impaired long-term potentiation in the medial prefrontal cortex produced by hippocampal stimulation,[43] suggesting the hippocampus-prefrontal cortex (and amygdala) circuit is modulated by relaxin-3-containing GABA afferents.

A major target of NI projections is the medial septum,[38] which in turn sends projections to the hippocampus and amygdala and to a lesser extent the cerebral cortex. The pathways arising from the NI and extending across the telencephalic "emotional" centers appear, for the most part, to parallel the monoaminergic systems. These data lead us to consider the ascending NI-relaxin-3/RXFP3 system as an important arousal network that represents a putative target of the pathology associated with mental illnesses and/or a therapeutic target for drug development.[33,44,45]

There has been less research on the consequences of intrinsic neuronal mechanisms driven by RXFP3 activation. Ex vivo studies reported that RXFP3 activation induced ERK phosphorylation and inhibition of cAMP synthesis.[24,26] In vivo studies observed neuronal ERK phosphorylation in the septum following icv injection of an RXFP3 agonist,[37] but more recent studies suggest this effect within cholinergic neurons was indirect via RXFP3 activation of presynaptic circuits or local GABA interneurons.[46] Patch-clamp studies in the intergeniculate thalamic nucleus and the paraventricular hypothalamic nucleus indicated that the primary direct effect of RXFP3 activation is inhibition of receptor-expressing neurons.[47,48]

5 | ROLES OF THE NI-RELAXIN-3/RXFP3 SYSTEM

5.1 | Feeding behavior

Manipulating the relaxin-3/RXFP3 system consistently alters feeding behavior in rats. Direct hypothalamic or icv infusion of relaxin-3 resulted in an increase in cumulative food intake in satiated rats 1-4 hours postinjection.[49] Similar effects were observed when RXFP3-specific agonists were administered, which were attenuated by prior infusion of an RXFP3 antagonist.[50] The NI is activated by stressors that may alter energy balance and food intake,[51,52] and sex differences in relaxin-3/RXFP3 signaling related to feeding behavior have been described.[52] Chronic stress and repeated food restriction increased body weight gain in female, but not male Wistar rats, which was associated with significantly increased relaxin-3 mRNA levels in the NI of female, but not male rats, compared to controls.[52] The gender-specific effect of relaxin-3 is further highlighted by the fact that this neuropeptide has been shown to produce a significant increase in plasma corticosterone and the expression of CRF and c-*fos* mRNAs in the parvocellular paraventricular hypothalamic nucleus (PVN) in male, but not female rats. Subsequently, female rats displayed greater sensitivity and a stronger food intake increase in response to relaxin-3 treatment.[53] In a model of stress-induced binge eating, female rats were prone to develop binge-like eating and displayed increased relaxin-3 mRNA in the NI compared to binge eating-resistant rats.[54]

Modulation of relaxin-3/RXFP3 signaling also alters feeding behavior in mice, under some conditions.[34] It has also been observed in mice that icv injection of RXFP3 antagonists affects behaviors related to food intake. These effects include reduced food anticipatory activity before meal time during food restriction, consumption of highly palatable food, consumption of regular chow during the initial dark phase, and consumption of regular chow after mild food deprivation.[55] Specific effects on salt appetite have also been reported in mice, whereby icv injection of an RXFP3 antagonist reduced the volume of salt consumed in a dose-dependent manner, when offered to sodium-depleted mice, relative to vehicle-treated controls; and this effect was not observed in *Rxfp3* gene knockout mice.[56]

5.2 | Stress and anxiety

The putative role of the relaxin-3/RXFP3 system in stress and anxiety behavior was first highlighted by the strong expression of CRF_1 mRNA in the rat NI.[14,15] It was subsequently observed that icv CRF infusion activated relaxin-3 positive neurons in the rat NI[18] and stressful stimuli-modified relaxin-3 expression in the NI.[57] Repeated forced swim stress increased relaxin-3 heteronuclear and mRNA levels, 1-2 hours after the second swim, and these effects were attenuated by prior injection of the CRF_1 antagonist, antalarmin.[57]

Central (icv) injection of RXFP3 agonist was found to induce an anxiolytic effect in the elevated plus maze and light/dark box tests in rats.[58] However, the anxiolytic effect of RXFP3 activation appears to depend on the basal emotional condition prior to testing. In non-stressed, naive mice, icv infusion of RXFP3 agonist did not alter anxiety levels, which was significantly reduced after treatment with an anxiogenic benzodiazepine,[59] suggesting that endogenous relaxin-3/RXFP3 signaling may not strongly participate in regulating basal levels of anxiety. In this regard, one strain of relaxin-3 gene knockout mice was reported to display mild anxiety-like behavior, although the phenotype was not consistently observed.[60] In another strain, *Rxfp3* gene knockout mice displayed reduced activity in the voluntary home-cage running wheels and decreased anxiety-like traits in the elevated plus maze.[31] The NI has also been proposed to contribute to the anxiogenic effects of high doses of buspirone, a clinically used anxiolytic drug,[61] as an anxiogenic effect was observed after direct injection of buspirone into the NI and this effect was abolished by prior NI lesion.[61]

5.3 | Alcohol-seeking

Relapse represents one of the most difficult clinical problems in treating patients with alcohol-use disorders. In a rat model of alcohol use and alcohol-seeking, icv administration of RXFP3 antagonist has been shown to decrease self-administration of alcohol in a dose-related manner, and attenuate cue- and stress-induced reinstatement following extinction.[62] No effect of the antagonist injections was observed on sucrose-seeking or general food intake behavior, indicating specific regulation of alcohol intake and relapse-like behavior.[62] Furthermore, there was a positive correlation between relaxin-3 mRNA levels in the NI and levels of alcohol intake in alcohol-preferring rats.[63] Addiction and relapse are strongly driven by stress and anxiety, and injection of a relaxin-3 antagonist into the central amygdala has been reported to attenuate the relapse to alcohol induced by the anxiogenic drug, yohimbine.[64] Reinstatement of alcohol-seeking was also disrupted by intra-NI infusion of a CRF_1, but not CRF_2, receptor antagonist.[65] Similarly, bilateral NI infusion of an orexin-2 (OX2), but not OX1, receptor antagonist decreased reinstatement of alcohol-seeking,[66] which is consistent with the presence of an orexin innervation of OX2-positive neurons in the rat NI.[67]

The mesolimbic system, which is a pivotal element in neural reward mechanisms, is not strongly or directly linked to NI circuits, but other regions, such as the hippocampus and the amygdala, are capable of modulating components of the mesolimbic system involved in reward and addictive processes. For example, mice receiving chronic morphine treatment display a strong increase in CA1 theta rhythm.[68] Moreover, theta burst stimulation of the hippocampal ventral subiculum, which projects to the nucleus accumbens, resulted in a blunted reinstatement of cocaine consumption.[69] It has also been found that intermittent alcohol self-administration is sufficient to reduce theta synchrony in the hippocampus, amygdala and prefrontal cortex.[70] Thus, it is likely that signaling pathways downstream of RXFP3 activation are involved in alcohol-seeking and relapse, and synchronization in the theta band, a process driven by the NI-relaxin-3 projection to the septum and hippocampus.

5.4 | Arousal

Strong anatomical data suggest the NI and the associated relaxin-3/RXFP3 system could be involved in adapting cognitive and emotional functions to circadian activity. The NI strongly projects to the median preoptic and ventrolateral preoptic nuclei, lateral hypothalamic area, medial division of the lateral habenula, dorsal raphe, and interpeduncular nuclei.[11,12] All these nuclei have been associated with shifts between different sleep states including wake, slow wave sleep (SWS), and rapid eye movement (REM) sleep.[71-73] In addition, NI neurons express receptors for transmitters/neuropeptides involved in these processes, including orexin receptors OX1 and OX2[67,74,75] and melanin-concentrating hormone (MCH) receptors.[76] In this regard, the firing of NI neurons can be activated by orexin-A[77] and inhibited by MCH (Blasiak A, Ma S, Gundlach AL, unpublished data). However, there is currently no direct indication of the role of NI relaxin-3/RXFP3 signaling in shifting sleep/wake states and circadian rhythmicity.[77] Nonetheless, icv infusion of the *Rxfp3* agonist, R3/I5, increased arousal-related locomotor activity and exploration, and increased wakefulness;[78] and relaxin-3 and *Rxfp3* gene knockout mice display circadian hypoactivity manifested by reduced running wheel activity and increased inactivity and sleep duration during the dark, active phase.[30,31]

All these data are consistent with a role for the NI and relaxin-3/RXFP3 systems in promoting active behavior. Notably, ipsilateral microstimulation of the NI in rats produced increased locomotion and rotation behavior,[79] and icv infusion of a relaxin-3 agonist produced increased locomotor activity of rats in a T-maze, although this was accompanied by a disruption in natural spontaneous alternation.[37] In recent studies, sustained chemogenetic activation of NI neurons in rats produced long-lasting locomotor activity, associated with increased vigilance behavior (head-scanning) following fear conditioning, effects paralleled by increased cortical electroencephalograph (EEG) desynchronization, and increased theta rhythm activity.[80]

5.5 | Learning and memory

Given the direct connections of NI-relaxin-3 neurons with structures directly involved in memory processes, the effects of interference with RXFP3 signaling on spatial memory have been investigated. Infusion of RXFP3 antagonist into the septal area resulted in disruption of spatial working memory in the spontaneous

alternation test,[40] while icv infusion of an agonist induced impairment of short-term memory in the T-maze.[37] While these results seem contradictory, these paradigms measure different aspects of memory (spatial working memory and short-term spatial memory), and the mode of RXFP3 modulation was different in each test.

The effects of interference with NI activity on spatial memory has also been analyzed using transient lidocaine inactivation of NI neurons,[81,82] which impaired long-term spatial memory in the Morris water maze[81] and impaired retention in a passive avoidance learning task.[82] In the latter study, it was proposed that the increased excitability promoted by the NI may facilitate processing along the trisynaptic pathway. Using a direct approach, Cre-dependent deletion of RXFP3 in receptor-positive hilar neurons of the dentate gyrus in mice, impaired spatial alternation assessed in a Y-maze.[83]

Furthermore, oscillatory hippocampal theta activity can be driven by a specific type of parvalbumin-positive, GABAergic septal neuron, named as Teevra cells, which selectively innervate CA3 interneurons and pyramidal cell assemblies, to modulate phase-firing during hippocampal theta activity.[84] The fact that RXFP3 is broadly expressed in septal GABAergic neurons,[46] some of which are likely to be parvalbumin-positive, further highlights the likely role of relaxin-3 transmission in driving hippocampal theta rhythm. In addition, optogenetic induction of hippocampal theta oscillations by rhythmic activation of glutamatergic septal neurons is associated with initiation of locomotion within hundreds of milliseconds of stimulus onset.[85] The association of theta rhythm activation and locomotor induction may underlie the well-characterized effects of NI and relaxin-3 neuron activity in promoting movement.[37,79,80]

5.6 | Affective behavior

The amygdala receives relaxin-3-positive projections from the NI.[12,86] The medial amygdala nuclei and medial nuclei of the bed nucleus of the stria terminalis are among the areas containing the densest NI neuron and relaxin-3 fibers, although fibers are also dispersed

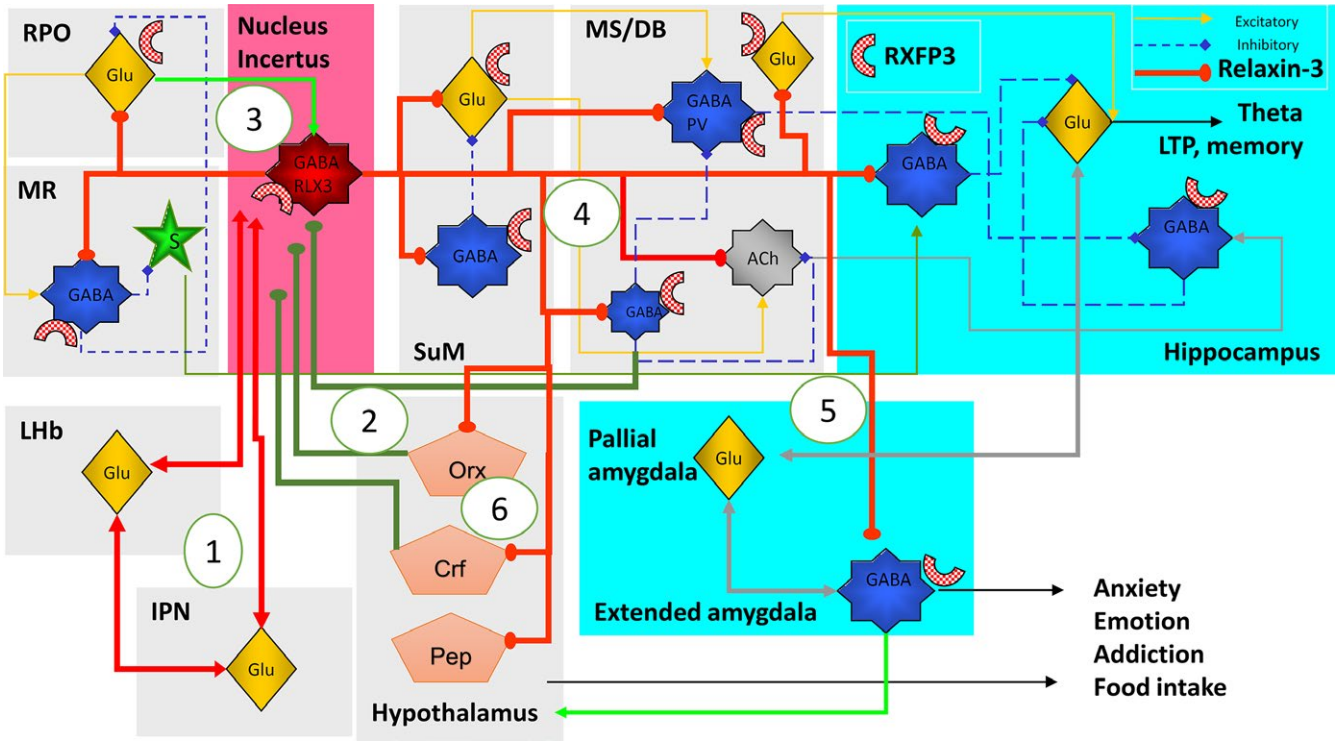

FIGURE 3 Organization of the connections of the NI and its specific targets. A loop in the brainstem exists where the median raphe induces theta desynchronization, and the reticularis pontis oralis (RPO) is the source for tonic firing during somatosensory stimulation and may relay on the NI. The NI can integrate stress-related information to activate arousal, emotion, and learning and memory mechanisms in a set of ascending connections. At the next level, the supramammillary nucleus (SuM) modulates NI firing to regulate rhythmic output to the septum, which is a primary regulator of rhythmic input to the hippocampus. Several neural circuit loops can be anatomically defined. (1) The central processing loop is composed of the lateral habenula (LHb), the interpeduncular nuclei (IPN) and the NI. (2) The NI also receives hypothalamic projections from corticotropin-releasing factor (CRF) and orexin (Orx) peptidergic projections and also from GABAergic projections arising from the septal area. (3) The NI receives projections from the RPO which is responsible for driving theta from somatosensory stimulation. (4) The NI then sends ascending connections to the median raphe (MR), supramammillary nucleus (SuM), medial septum/diagonal band (MS/DB), and hippocampus, which largely underlie subcortically-driven theta rhythm. (5) NI projections also innervate the extended amygdala, which is important for emotionally-driven behaviors, including addictive behaviors and anxiety. (6) Some of these behaviors, such as feeding, could be modulated by NI projections to hypothalamic nuclei and interactions with other peptidergic systems (Pep)

in other amygdaloid nuclei, in close contact with some calcium-binding protein-positive neurons.[86] These data suggest a role of the system in affective (emotion)-related behaviors. Selective lesion of the NI with saporin-CRF has been found to prolong freezing in a fear conditioning paradigm[87] and electrolytic lesions of NI impair normal extinction[88] in rats. In contrast, as mentioned, excitatory DREADD activation of the NI in rats resulted in increased head-scanning and vigilant behaviors during conditioned fear recall.[80]

6 | A UNIFIED ROLE FOR THE NI-RELAXIN-3/RXFP3 SYSTEM?

As discussed, existing data suggest the NI neural network and its relaxin-3/RXFP3 signaling component alter a wide variety of somewhat independent behaviors. A major driver of NI function in the rat is CRF/CRF_1 signaling, which presumably arises from the hypothalamus[36] and potentially from local CRF neurons,[89] in addition to orexin inputs activating OX2 receptors in the NI.[66,67] Therefore, under stressful conditions, a widespread neural reaction can be driven from a broad network of GABA/peptidergic connections involving the brainstem, hypothalamus, amygdala, septum, hippocampus, and prefrontal cortex. At the molecular level, the consequences of RXFP3 activation are inhibition of cAMP synthesis and possibly phosphorylation of ERK. At the behavioral level, activation of the NI promotes locomotor activity.[37,79,80] Another consequence of NI activation is the occurrence of theta oscillations in the hippocampus[39,40] (Figure 3). These synchronized oscillations may be maintained by bidirectional connections between the hippocampus and the NI,[13] which provide phase locking between the brainstem and hippocampal circuits.[36,41] Theta synchronization also occurs between the prefrontal cortex, hippocampus, and amygdala, during fear acquisition and retrieval processes.[90] Thus, the NI and relaxin-3/RXFP3 signaling may work to induce intracellular metabolic cascades in their target areas, including cAMP inhibition and ERK phosphorylation, which enable mutual and somehow reciprocal neural interconnections, resulting in oscillatory synchrony in several areas such as the hippocampus. As a result, arousal mechanisms are potentiated and memory processes are modulated, which subserve a variety of functions, including spatial learning, anxiety processes, feeding behavior, and alcohol-seeking. Further knowledge of how this system modulates these processes may offer a new therapeutic perspective on treating malfunctions associated with neuropathology and psychiatric disorders.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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