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REVIEW ARTICLE

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Behavioural actions of tuberoinfundibular peptide 39 (parathyroid hormone 2)

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Abstract

Tuberoinfundibular peptide of 39 residues (TIP39) acts via its endogenous class B G-protein coupled receptorthe parathyroid hormone 2 receptor (PTH2R). Hence, it is also known as parathyroid hormone 2. The peptide is expressed in the brain by a small number of neurons with a highly restricted distribution, which in turn project to a large number of brain regions that contain PTH2R. This peptide neuromodulator system has been extensively investigated over the past 20 years including its behavioural actions, such as its role in the control of nociception, fear and fear incubation, anxiety and depression-like behaviours, and maternal and social behaviours. It also influences thermoregulation and potentially auditory responses. TIP39 probably exerts direct effect on the neuronal networks controlling these behaviours based on the localization of PTH2R and local TIP39 actions. In addition, TIP39 also affects the secretion of several hypothalamic hormones providing the basis for indirect behavioural actions. Recently developed experimental tools have stimulated further behavioural investigations, and novel results obtained are discussed in this review.

KEYWORDS
anxiety, fear, hypothalamus, neuropeptide, nociception

1 | INTRODUCTION

The parathyroid hormone 2 receptor (PTH2R) was discovered on the basis of its sequence similarity to other class B G-protein coupled receptors.[1] and was named PTH2R because of its significant sequence similarity to the known parathyroid hormone receptor, which is now referred to as PTH1R; and because the human PTH2R is activated by parathyroid hormone.[2] Subsequently, an endogenous ligand of the receptor was identified following its purification from bovine hypothalamus. The new peptide was termed tuberoinfundibular peptide of 39 residues (TIP39) that time.[3] The peptide is now categorized as parathyroid hormone 2. Despite this, however, it is probably not a hormone as its presence in the blood has not been demonstrated. Rather, it is expressed mainly in the brain and has neuromodulatory functions. In addition, expression of TIP39 has been identified in some peripheral organs, such as the kidney, heart, and testis.

2 | PHARMACOLOGY OF TIP39 AND THE PTH2R

Rat (and mouse as these species have the same amino acid sequence for this gene) as well as human TIP39 (different in 4 amino acids) was demonstrated to be an agonist for the rat as well as the human PTH2R.[3] It was shown to increase cAMP as well as intracellular Ca ion levels probably via Gs and Gq proteins, respectively.[4,5] While the known ligands for PTH1R, parathyroid hormone (PTH) and parathyroid hormone related peptide (PTHrP) have very few amino acid residues in common with TIP39, the only known endogenous ligand for PTH2R, their receptors, the PTH1R and PTH2R have about 50% amino acid identity when their sequences are aligned. Therefore, not surprisingly, the three peptides do have a similar three-dimensional structure.[6] This motivated investigation of the relative receptor specificity of the ligands. TIP39 (either human or rat) does not activate the human or rat PTH1R. While PTH and PTHrP have similar potency at PTH1Rs, PTH (rat and human) but not PTHrP (human) activates the human PTH2R. Both rat and human PTH are, however, much less potent at the rat PTH2R. So while PTH does have some ability to activate the PTH2R it is not likely that PTH is a natural ligand for PTH2R. PTH2R expression is greater in the brain than in peripheral tissues based on Northern blot, in situ hybridization histochemistry and immunohistochemistry[1,3,7] while the presence of PTH has not been demonstrated in the brain. Furthermore, in the rat, nanomolar concentrations of PTH do not cause significant activation of PTH2R.[8] Nonetheless, the homologous structures of the receptors and their peptide ligands facilitated experiments in which short sequences or residues were exchanged, allowing functional domains to be identified. Specifically, the amino terminus of the peptide ligand appears to be responsible for activation while the carboxyl region contributes much of the binding energy.[9,10] This information provided the basis for site-directed mutagenesis of TIP39 and the development of a high affinity antagonist of PTH2R by changing four residues of TIP39 and creating a peptide called HYWH-TIP39.[11] This antagonist was employed in several biological experiments described below.

3 | ANATOMICAL DISTRIBUTION

Neuronal somas expressing TIP39 have been identified in just three brain sites in the adult rat and mouse brain based on the mapping of TIP39 mRNA expression and TIP39-immunopositive cell bodies,[12] the periventricular grey of the thalamus (PVG), the posterior intralaminar thalamic nucleus (PIL), and the medial paralemniscal nucleus (MPL) in the lateral pons. Furthermore, no significant expression of TIP39 in other brain sites has been identified. However, the expression level changed in the three major expression sites during ontogeny. At embryonic day 16 during development, the expression of TIP39 was significant in the PIL but had almost completely disappeared from this site during the first postnatal week. In turn, the level of TIP39 increased in the PVG and MPL during postnatal development and started to decrease only around puberty.[13] TIP39 expression levels also correlated with reproductive status as they were increased in the PIL and MPL of mother rats.[14] TIP39-positive fibres have a much wider distribution, which includes limbic, autonomic, hypothalamic, nociceptive, and auditory brain regions.[15] Within the hypothalamus, TIP39 neurons are abundant in different subregions including the part of the preoptic area where oestrogen receptors are located (Figure 1) and hypothalamic regions containing oxytocin (Figure 2). In fact, the distributions of TIP39-positive axon terminals and PTH2R display remarkable similarities, strengthening the argument that TIP39 is the endogenous ligand for PTH2R.[16]

The distribution of the TIP39-PTH2R neuromodulator system is unique as it does not resemble that of any other system.[12] In the PVG, TIP39 neurons are intermingled with the A11 dopaminergic neurons but do not display any colocalization. While A11 dopaminergic neurons have mostly descending projections,[17] TIP39 neurons in this brain regions projects mostly towards the forebrain. Based on previous studies, this brain area may be involved in antinocicieption.[18] In the PIL, TIP39 neurons have an overlapping distribution with galanin-containing fibres, while neurons expressing calcitonin gene-related peptide (CGRP) are located immediately lateral to the TIP39 neurons. While the role of these CGRP neurons is not well known, they project towards the caudate-putamen and the perirhinal cortex.[19] Projections of PIL neurons towards the amygdala were suggested to be involved in auditory fear conditioning.[20] It has been suggested that the PIL can be divided into a medial and a lateral subdivision,[21] the former expressing TIP39, the latter CGRP. The MPL is located immediately lateral to the A7 noradrenergic cell group and immediately medial to the intermediate nucleus of the lateral lemniscus. Because of the vicinity of this auditory relay nucleus, auditory functions of the MPL have been examined.[22] The paralemniscal area could have a role in vocalization.[23]

4 | NEUROCHEMISTRY OF THE TIP39-PTH2R SYSTEM

As suggested by the unique distribution of TIP39-expressing neurons, there were no other neuropeptides identified in these cells. In turn, evidence is available that TIP39 neurons contain glutamate as their major amino acid neurotransmitter. A combination of in situ hybridization histochemistry for vesicular glutamate transporter 2 (VGLUT2) and TIP39 immunolabeling in mother rats suggested that TIP39 neurons in the PIL express VGLUT2 while GABAergic markers were not present in TIP39 neurons.[24] Furthermore, TIP39-containing synapses were identified to be asymmetric suggesting their excitatory nature. In addition, post-embedding electron microscopy demonstrated the presence of glutamate in TIP39-containing presynaptic terminals in the preoptic area.[25]

PTH2R is expressed in a variety of different cell types based on its relatively widespread distribution. Still, the characterization of these cell types is often not sufficient. One reason for that is that the PTH2R immunolabeling labels neuronal processes rather than cell bodies in most brain regions. Technical development of appropriate transgenic animals will solve these issues. The presynaptic localization of the PTH2R promoted the hypothesis that TIP39 may exert its functions in some cases

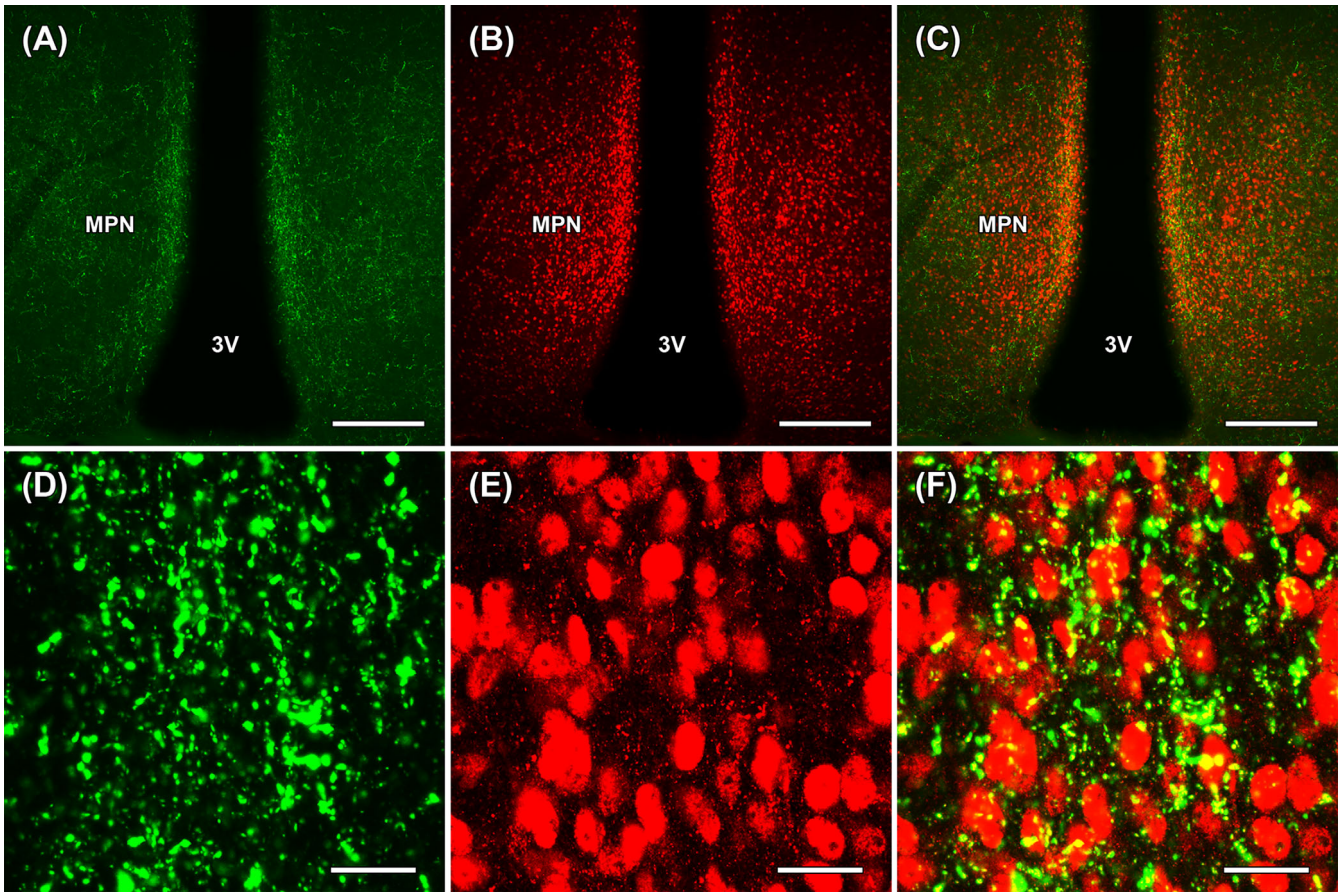

FIGURE 1 Distribution of TIP39-containing nerve fibres in the medial preoptic area. (A–C) TIP39-positive fibres (green) are widely distributed in the medial preoptic area, where ERα-expressing neurons (red) are abundant. The merged image (C) illustrates the overlapping distribution of TIP39 and ERα. (D–F) TIP39-containing fibres surround oestrogen receptor alpha (ERα)-positive medial preoptic neurons. 3 V, third ventricle; MPN, medial preoptic nucleus. Scale bars: 300 μm (A–C), 50 μm (D–F)

on the presynaptic terminal of the target neurons.[26] Another important question is if PTH2R in different neuronal cell types uses Gs to elevate cAMP levels or Gq to elevate calcium ion levels in the intracellular space. While cell culture studies suggested the possibility of both signal transduction pathways, it is not known if both or which one takes place in different neuronal populations expressing the PTH2R.

5 | EXPERIMENTAL TOOLS TO STUDY THE BEHAVIOURAL ACTIONS OF TIP39

The potential roles of TIP39 were evaluated, based on the functions of the brain areas containing TIP39 fibres and PTH2Rs. TIP39 was hypothesized to be involved in sensory processing and the control of hormone release, via which it may indirectly control behaviour. In addition, direct effects of TIP39 on behaviour are plausible and ongoing. The functional studies utilize a growing number of experimental tools. Initially, TIP39 peptide itself was injected into the lateral ventricle or directly to parenchymal sites[27] while pharmacological blockade was performed by injection of an antibody to TIP39 and the PTH2R.[28] Later, nonspecific and specific peptide antagonists were developed.[11] For local delivery of the peptides, viral tools have also been used including a viral vector which encodes a construct in which the PTH2R antagonist HYWH-TIP39 is fused to a signal for cellular secretion, which makes cells in the region where this virus is injected a continual source of the antagonist.[14] In addition, transgenic mice lacking TIP39 and with a null mutation of PTH2R, or expressing Cre recombinase driven by their promoters were also developed.[29] Using these tools and mice, roles for TIP39 have been established in nociception, fear response, anxiety- and depression-like behaviour, auditory responses, and maternal and social behaviours. The involvement of TIP39 in these behaviours is described in more detail in the following sections.

6 | ROLE OF TIP39 IN NOCICEPTION

PTH2 receptors are expressed in many brain regions involved in the processing of nociceptive information. These include regions that are within ascending pathways that convey nociceptive sensory information, as well as within descending pathways to regions involved in modulation of the sensitivity to peripheral stimuli or of responses to nociceptive input. The regions include the spinal cord dorsal horn, PAG,

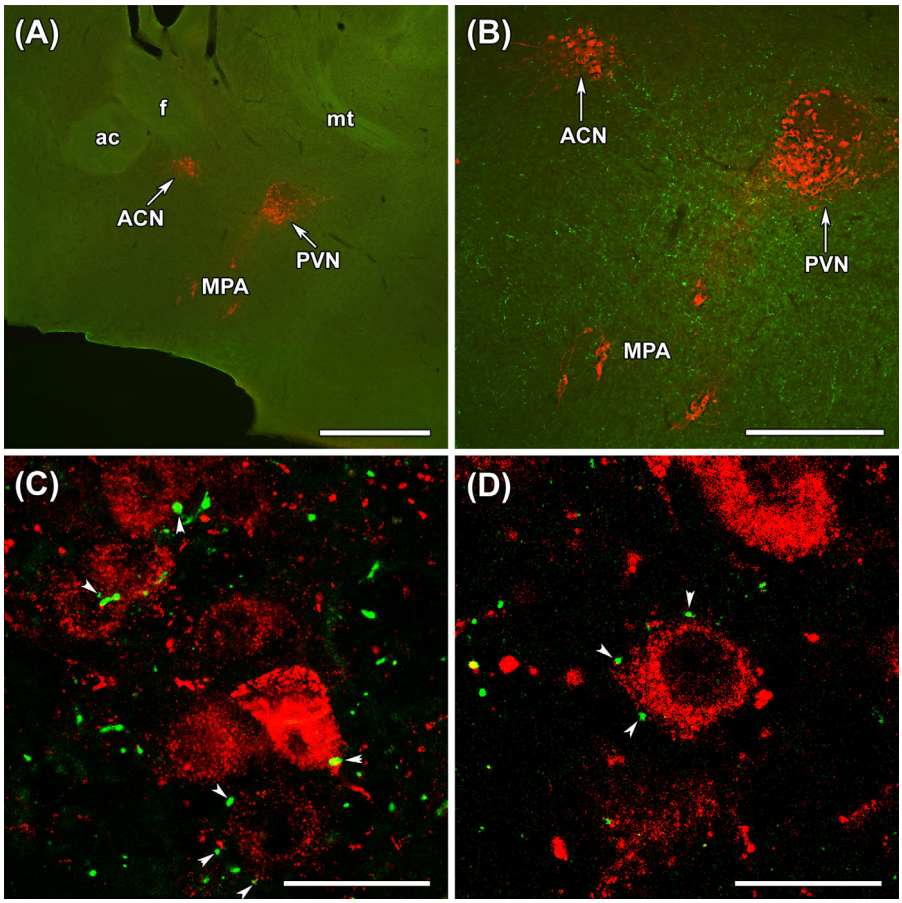

FIGURE 2 Innervation of oxytocin neurons in the hypothalamus by fibre terminals containing TIP39. A sagittal section of the hypothalamus reveals that oxytocin neurons (red) are present in the paraventricular (PVN) and a major accessory nucleus, the anterior commissural (ACN) nucleus, as well as other accessory cell groups in the medial preoptic area (MPA). (B) There are abundant TIP39-positive nerve fibres (green) in these regions. (C–D) TIP39-containing nerve fibre terminals closely appose the cells bodies of the oxytocin-positive neurons (indicated by white arrowheads) in the ACN (C) and the PVN (D). ac, anterior commissure; f, fornix; mt, mamillothalamic tract. Scale bars: 1 mm (A), 500 μm (B), 50 μm (C) and 30 μm (D)

medial and intralaminar thalamic nuclei, several amygdaloid and hypothalamic nuclei, and somatosensory and anterior cingulate cortices. The areas that relay ascending nociceptive information that are enriched in PTH2Rs are considered to be parts of the archispinothalamic and paleospinothalamic tracts, which are considered phylogenetically older ascending pathways and more involved in affective dimensions of pain, in contrast to phylogenetically younger neospinothalamic pathways more involved in sensory-discriminative functions.[30,31] TIP39-containing fibres project to each of these areas of PTH2R expression.

The potential involvement of TIP39-PTH2R signalling in pain processing was evaluated by comparing performance in several standard tests of acute nociceptive sensitivity between control or wild-type mice and mice in which PTH2R signalling was inhibited either by acute administration of a PTH2R antagonist, by null mutation of the PTH2R or by deletion of TIP39.[32] Intracerebroventricular administration of the PTH2R antagonist HYWH-TIP39 increased latency in acute nociceptive withdrawal assays including the tail-flick, and hotplate tests, and in both phases of the formalin test, while intracerebroventricular administration of TIP39 decreased latency in acute nociceptive sensitivity tests. Observations in the mice with constitutive genetic alterations in PTH2R signalling were generally consistent with these observations. The idea that TIP39-PTH2R signalling contributes to physiological modulation of nociceptive function was also evaluated in mice with more long-lasting perturbations.[33] Following peripheral nerve injury, both PTH2R and TIP39 knockout mice developed less tactile and thermal hypersensitivity than controls and returned to baseline sensory thresholds faster. Effects of hindpaw inflammatory injury were similarly decreased in knockout mice. Thus the TIP39-PTH2R system appears to have a role in maintaining the normal sensitivity to nociceptive stimuli and modulating responses to injury.

7 | FUNCTION OF TIP39 MEDIATING FEAR RESPONSES

The TIP39-PTH2R system plays an intricate role in regulating fear memory in rodents. Using Pavlovian fear conditioning, TIP39 knockout mice displayed more freezing during conditioning, cued test, and context text compared to wild-type mice, but displayed normal fear extinction.[34] Furthermore, global deletion of TIP39 signalling in TIP39 knockout and PTH2R knockout male mice enhanced conditioned fear recall at 14 and 28 days, but not 6 days after a single footshock exposure, indicating a fear incubation effect.[35,36] Fear incubation is described as a time-dependent increase in fear responses to fear-conditioned cues in the absence of further stress or cue exposure and a proposed animal model for delayed-onset posttraumatic stress disorder (PTSD). Causes of reported delayed-onset PTSD cases seem to fall into one of two categories, exposure to a one-time traumatic event or chronic traumatic events.[37] Similarly, two different models of fear incubation are commonly used, (1) exposure to a

single inescapable footshock[38] or (2) extended fear conditioning or overtraining[39] and both models demonstrated a conditioned fear response that increases over time. After the experience of a single inescapable footshock, normal TIP39 signalling may lessen the long-term consequences of a single traumatic event, whereas the absence of TIP39 signalling allows for deleterious incubation effects.[36]

The amygdala plays a pivotal role in fear responses.[40] More specifically, aversive events such as predator stress or footshock activates the medial nucleus of the amygdala (MeA).[41] Abundant PTH2R expression and projections from TIP39 neurons in the MeA suggest a potential function of TIP39 signalling in fear regulation.[16] PTH2R knockout mice exhibited reduced c-Fos activation in the MeA following a single footshock and recall testing at 28 days, but had normal activation in the paraventricular nucleus of the hypothalamus and central amygdala compared to WT mice, suggesting the TIP39-PTH2R system in the MeA might play a specific role in fear incubation.[35] Transient inhibition of MeA PTH2R-expressing neurons using designer receptor exclusively activated by designer drugs (DREADD) manipulation before or immediately after the footshock exposure, but not at the time of fear recall, enhanced fear incubation.[35] These findings suggest that TIP39 signalling via MeA PTH2R neurons might influence encoding or consolidation of memory from the fear-associated context. To further reveal the neuronal network responsible for these actions, it will be interesting to establish what cell type of the MeA contain PTH2R.

Contextual conditioned fear not only activates the MeA but also increases plasma concentrations of oxytocin, ACTH, and prolactin.[42] Bilateral lesioning of the MeA significantly impairs these neuroendocrine responses. The TIP39-PTH2R neuromodulatory system exerts an influence in a variety of neuroendocrine systems and functions.[43] Involvement of the TIP39-PTH2R system in the regulation and release of stress hormones, catecholamines, oxytocin, vasopressin, and prolactin may be an additional mechanism that contributes to its effects on fear incubation.

8 | TIP39 HAS ANTI-ANXIOLYTIC AND ANTI-DEPRESSIVE PROPERTIES

In recent years, it has been established that PTH2R plays a role in anxiety- and depression-like behaviours. The first data on the subject dates back to 2004 when acute injection of TIP39 into the lateral cerebral ventricle of male rats had an anxiolytic effect in an elevated plus maze test and an antidepressant-like effect in the forced swim test.[27] Later, it was shown that TIP39 knockout animals demonstrate anxiety-like behaviour, but only if the animals were previously exposed to mild stress.[34] Further, detailed studies demonstrated that after conditioning for fear, TIP39 knockout mice displayed increased anxiety-like and depressive-like behaviour compared to their wild-type littermates.[36] Additional results obtained with PTH2R knockout mice revealed that the lack of the receptor resulted in increased freezing time and rate after electric shock, and increased immobility in the forced swim test. The fact that the TIP39-PTH2R system may play a role not only in the case of electric shock but in other stress loads has been demonstrated by examining mothers. PTH2R knockout mothers also displayed anxiety- and depression-like behaviours compared to wild-type mothers.[44]

9 | MATERNAL BEHAVIOURS

The first indication of a potential effect of the TIP39-PTH2R system was the induction of TIP39 expression in the PIL[45] and MPL areas[46] around parturition and TIP39 levels remain elevated as long as the pups are present suggesting that suckling may activate TIP39 expression.[14] Indeed, suckling induced c-Fos in PIL TIP39 neurons. In turn, if pups were returned to their mothers but a barrier between them prevented suckling, the activation of PIL TIP39 neurons was markedly reduced. Injection of an antagonist of TIP39 into the lateral cerebral ventricle,[45] or locally into the arcuate nucleus via a lentivirus constitutively expressing and secreting the PTH2R antagonist HYWY-TIP39 from the locally infected cells decreased suckling-induced prolactin secretion.[14] In line with these data, maternal absence of the PTH2R hindered postnatal pup development.[29]

While prolactin is critically important for lactation and contributes to maternal behaviours,[47] maternal behaviours are present even in the absence of prolactin suggesting independent routes for control of maternal behaviour.[48] Indeed, it has been shown that neurons activated in mothers by pups include those which are not sensitive to prolactin.[49] The preoptic area of the hypothalamus is the area most critical for maternal behaviours in rodents.[50] Since TIP39 fibres and PTH2R are abundant in this brain region,[51] the effect of TIP39 on maternal behaviours has been addressed. Local administration of PTH2R antagonist into the preoptic area, performed by the PTH2R antagonist HYWY-TIP39 expressing lentivirus described above, reduced pup-induced place preference, suggesting a role of TIP39 in maternal attachment and motivation.[45] A possible target of TIP39 is galanin-containing preoptic neurons as these cells, known to govern some aspect of maternal behaviours including retrieving of the pups to the nest,[52] are innervated by TIP39 fibres.[25] Additional cell types in the preoptic area also participate in the control of maternal behaviours,[53,54] which may also contribute to the mediation of TIP39 action.

10 | SOCIAL INTERACTIONS

Recent studies confirm the role of TIP39 in the control of social interactions between conspecifics. The role of TIP39 (PTH2) was described in both tracking and interacting with the social environment of zebrafish.[55] The level of TIP39 increased after social exposure in previously isolated fish and decreased after isolation in socially reared fish. The sensory modality that controls expression of TIP39 was to be mechanical not visual origin and was induced by the movements of neighbouring fish received via mechanoreceptors in the lateral line organ.[55] It was also demonstrated that the expression level of TIP39 in the PIL is significantly higher in rats kept in social environment compared with chronically isolated rats.[56] PIL neurons are activated following social encounters of female rats.[24] It was also demonstrated that nerve fibre terminals containing TIP39 closely appose the cell

bodies of oxytocin neurons in the paraventricular hypothalamic nucleus (Figure 2). These observations suggest that the PIL and its neurons containing TIP39, is a relay station for socially relevant sensory information which conveys the input of social touch from the spinal cord to oxytocin-secreting neurons, which it activates.[24]

The chemogenetic stimulation of PIL neurons resulted in a significant increase in the duration of direct social contact (such as mounting or side-to-side contact) and social grooming between the rats.[56] Specific chemogenetic manipulations of the PIL-preoptic area pathway suggest a role of this thalamo-hypothalamic pathway in the control of affiliative social touch.[56]

11 | THERMOREGULATION

The brain centre for thermoregulation is located in the median preoptic nucleus (MnPO) in the hypothalamic preoptic area, which regulates brown adipose tissue thermogenesis and the cutaneous vascular tone via descending projections.[57] Thermal information on environmental temperature sensed by skin thermoreceptors is received by the MnPO GABAergic neurons which integrate this information with local thermal influences and project to the dorsomedial hypothalamic nucleus and the rostral medullary raphe region.[58] A high density of TIP39 terminals and PTH2R expression were demonstrated in the MnPO.[16,33] Therefore, investigation of the functional involvement of the TIP39-PTH2R system in thermoregulation was conducted. The core temperature of WT mice was increased following the administration of TIP39 into the lateral ventricle. TIP39 injection did not have such an effect in PTH2R knockout mice, which excluded the possibility of a nonspecific action of TIP39.[33] The TIP39-PTH2R system may have a significant role in the response to cold, since PTH2R knockout mice had impaired heat production upon cold exposure, but not in a hot environment, and also had no change in basal temperature.[33] Temperature sensation was also normal in PTH2R knockout mice, therefore PTH2R may play a role in the heat production signal or heat production ability.[43] Since the local administration of TIP39 via implanted cannulas into the MnPO resulted in a larger body temperature increase for longer periods of time than injection of the same amount of TIP39 into the lateral cerebral ventricle, it is suggested that the MnPO is involved in the thermoregulatory effect of TIP39. Supporting this idea, local administration of TIP39 into the dorsomedial hypothalamic nucleus did not affect body temperature.

12 | POTENTIAL ROLES OF TIP39 IN AUDITORY RESPONSES

The position of the MPL immediately next to the nuclei of the lateral lemniscus and its bilateral anatomical connections with auditory brain regions[22,59] suggest that paralemniscal TIP39 neurons are likely to play a role in some auditory functions. Based on previous studies performed in bats, a brain site medial to the intermediate nucleus of the lateral lemniscus was demonstrated to be responsive to ultrasound emitted by conspecifics and is involved vocalization of this species.[23,60,61] There are less data available in rodents. It has been reported that TIP39 neurons in the paralemniscal express increased c-Fos in response to loud white noise.[62,63] Consequently, auditory inputs can potentially stimulate TIP39 neurons in mothers. Pups, when feeling distress, do vocalize at specific ultrasonic frequencies.[64] These distress calls have a role in warning the mothers who turn to their pups.[65–67] Nevertheless, our knowledge is limited regarding the neuronal tracts by which these specific auditory signals arrive at limbic and hypothalamic brain regions to alter behavioural and potentially hormonal responses of mothers. It is assumed that the distress calls emitted by the pups can stimulate TIP39 cells in the paralemniscal regions in dams. Furthermore, these cells might potentially convey this input to the upper brain regions to affect maternal responses.

Stereotaxic lesion studies addressing the projections of paralemniscal TIP39 neurons suggest projections to nontonotopic auditory brainstem regions.[15] Tract tracing experiments demonstrated that some hypothalamic brain regions including the paraventricular nucleus are approached by axons emanating from the paralemniscal nucleus.[62] This, and other nontonotopic organized auditory areas, which are also targets of neurons of the paralemniscal nucleus[15] could potentially affect some auditory centres of the dams to be more sensitive towards the distress calls of their pups.[62]

13 | ENDOCRINE EFFECTS OF TIP39

The PTH2R is highly expressed in some neuroendocrine centres of the hypothalamus such as the preoptic area, periventricular, paraventricular and arcuate nuclei.[16,43,51] TIP39-positive fibres are also abundant in these brain regions. The role of the TIP39/PTH2R system has previously been investigated in TIP39 and PTH2R knockout mice and by administration of TIP39 and an antagonist of the PTH2R.

TIP39-positive fibres are abundant around the somatostatin neurons in the periventricular hypothalamic nucleus. Furthermore, PTH2R has also been demonstrated in somatostatin neurons in the rat[51,68] and in man.[69] TIP39 administration had a strong effect on the secretion of GH from the pituitary and was completely blocked for 3 h following intracerebroventricular TIP39 injection.[2] The effect of TIP39 may be via activation of PTH2Rs on periventricular neurons and release of somatostatin, which in turn inhibits GH secretion.

TIP39 might have an effect on the release of additional pituitary hormones including arginine-vasopressin (AVP). TIP39 administration into to lateral ventricle resulted in a reduced plasma AVP level.[70] In addition, the AVP increase following dehydration by water deprivation for 48 h, hyperosmolality following i.p. injection of hypertonic saline, and hypovolemia following i.p. injection of polyethylene glycol were suppressed by TIP39 injection.[70] Further results suggest that TIP39 does not change AVP via an hemodynamic or osmotic influence, since the administration of the peptide resulted in a fall in mean arterial blood pressure, which would stimulate AVP secretion rather than decrease it. It has also been suggested that TIP39 inhibits AVP by a central action and possibly via an opioid system, since the opioid

receptor antagonist naloxone significantly reversed the inhibitory effect of TIP39 on dehydration-induced AVP release.[70] TIP39 and PTH2R expression is low in the supraoptic nucleus and the region of the PVN where magnocellular AVP neurons are located. Thus, it has been suggested that the hypothalamic arcuate nucleus is responsible for the AVP release-inhibitory effect of TIP39. TIP39 fibres and PTH2R are abundant in the arcuate nucleus[16] along with many opioid neurons, which participate in the regulation of AVP release.[71]

The TIP39-PTH2R system participates in the regulation of stress at different levels. TIP39 activates corticotropin-releasing hormone-containing neurons in the hypothalamic paraventricular nucleus and thereby can evoke corticosterone release.[26,69] An in vitro study revealed that corticotropin-releasing hormone (CRH) secretion from medial basal hypothalamic explants is increased by bath application of TIP39.[72] In addition, the plasma adrenocorticotropic (ACTH) was dose-dependently increased in rat following the intracerebroventricular injection of TIP39.[72]

TIP39 is highly expressed in the postpartum period and participates in the release of prolactin during lactation as discussed. Its possible effect on ocytocin secretion has also been described and this may be one of the ways by which TIP39 affects social behaviour.

14 | CONCLUSIONS

TIP39 affects multiple behaviours via a variety of mechanisms (Figure 3). At present it is not known if and how they are related to each other. Many of the effects are advantageous in mothers during lactation when TIP39 is upregulated. However, TIP39 has several different actions in nonmother, even male rodents, some of them are probably unrelated to the maternal actions. Another intriguing question is how TIP39 can exert its various effects. One possibility is by altering pituitary hormone levels, which in turn affect the brain. In most cases, however, the behavioural actions of TIP39 are better explained by actions within the diverse target areas of projections of TIP39 neurons. It is likely that PTH2R mediates the behavioural actions of TIP39, based on both pharmacological and anatomical studies. Therefore, the PTH2R may be a valuable target for future drug development in order to exert specific influences on pathological behaviours.

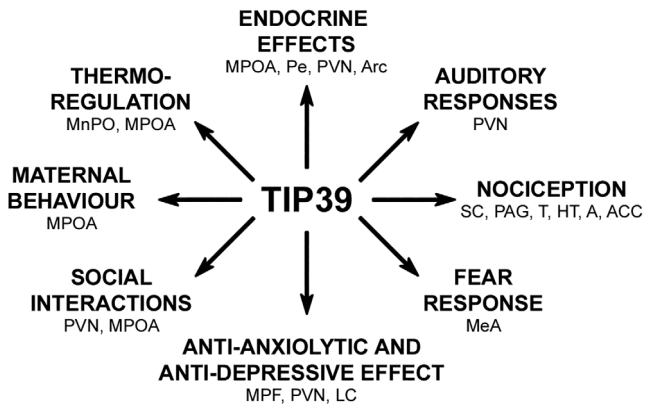

FIGURE 3 Summary of the effects of TIP39 neuropeptide and the affected brain regions. A, Amygdala; ACC, anterior cingulate cortex; Arc, arcuate nucleus; HT, hypothalamus; LC, locus coeruleus; MeA, medial nucleus of the amygdala; MnPO, median preoptic nucleus; MPF, medial prefrontal cortex; MPOA, medial preoptic nucleus; PAG, periaqueductal grey; Pe, periventricular hypothalamic nucleus; PVN, paraventricular hypothalamic nucleus; SC, spinal cord; T, thalamus

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AUTHOR CONTRIBUTIONS

Dávid Keller: Conceptualization; data curation; funding acquisition; investigation; methodology; visualization; writing – original draft; writing – review and editing. **Mumeko C Tsuda:** Investigation; methodology; writing – original draft; writing – review and editing. **Ted B. Usdin:** Conceptualization; funding acquisition; writing – original draft; writing – review and editing. **Arpád Dobolyi:** Conceptualization; funding acquisition; project administration; supervision; writing – original draft; writing – review and editing.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

1. Usdin TB, Gruber C, Bonner TI. Identification and functional expression of a receptor selectively recognizing parathyroid hormone, the PTH2 receptor. *J Biol Chem*. 1995;270:15455-15458.

2. Usdin TB, Bonner TI, Hoare SR. The parathyroid hormone 2 (PTH2) receptor. *Receptors Channels*. 2002;8:211-218.
3. Usdin TB, Hoare SR, Wang T, Mezey E, Kowalak JA. TIP39: a new neuropeptide and PTH2-receptor agonist from hypothalamus. *Nat Neurosci*. 1999;2:941-943.
4. Goold CP, Usdin TB, Hoare SR. Regions in rat and human parathyroid hormone (PTH) 2 receptors controlling receptor interaction with PTH and with antagonist ligands. *J Pharmacol Exp Ther*. 2001;299:678-690.
5. Della Penna K, Kinose F, Sun H, Koblan KS, Wang H. Tuberoinfundibular peptide of 39 residues (TIP39): molecular structure and activity for parathyroid hormone 2 receptor. *Neuropharmacology*. 2003;44:141-153.
6. Piserchio A, Usdin T, Mierke DF. Structure of tuberoinfundibular peptide of 39 residues. *J Biol Chem*. 2000;275:27284-27290.
7. Usdin TB, Bonner TI, Harta G, Mezey E. Distribution of parathyroid hormone-2 receptor messenger ribonucleic acid in rat. *Endocrinology*. 1996;137:4285-4297.
8. Hoare SR, Bonner TI, Usdin TB. Comparison of rat and human parathyroid hormone 2 (PTH2) receptor activation: PTH is a low potency partial agonist at the rat PTH2 receptor. *Endocrinology*. 1999;140:4419-4425.
9. Clark JA, Bonner TI, Kim AS, Usdin TB. Multiple regions of ligand discrimination revealed by analysis of chimeric parathyroid hormone 2 (PTH2) and PTH/PTH-related peptide (PTHrP) receptors. *Mol Endocrinol*. 1998;12:193-206.
10. Hoare SR, Clark JA, Usdin TB. Molecular determinants of tuberoinfundibular peptide of 39 residues (TIP39) selectivity for the parathyroid hormone-2 (PTH2) receptor. N-terminal truncation of TIP39 reverses PTH2 receptor/PTH1 receptor binding selectivity. *J Biol Chem*. 2000;275:27274-27283.
11. Kuo J, Usdin TB. Development of a rat parathyroid hormone 2 receptor antagonist. *Peptides*. 2007;28:887-892.
12. Dobolyi A, Palkovits M, Usdin TB. The TIP39-PTH2 receptor system: unique peptidergic cell groups in the brainstem and their interactions with central regulatory mechanisms. *Prog Neurobiol*. 2010;90:29-59.
13. Brenner D, Bago AG, Gallatz K, Palkovits M, Usdin TB, Dobolyi A. Tuberoinfundibular peptide of 39 residues in the embryonic and early postnatal rat brain. *J Chem Neuroanat*. 2008;36:59-68.
14. Cservenak M, Szabo ER, Bodnar I, et al. Thalamic neuropeptide mediating the effects of nursing on lactation and maternal motivation. *Psychoneuroendocrinology*. 2013;38:3070-3084. doi:10.1016/j.psyneuen.2013.09.004
15. Dobolyi A, Palkovits M, Bodnar I, Usdin TB. Neurons containing tuberoinfundibular peptide of 39 residues project to limbic, endocrine, auditory and spinal areas in rat. *Neuroscience*. 2003;122:1093-1105.
16. Faber CA, Dobolyi A, Sleeman M, Usdin TB. Distribution of tuberoinfundibular peptide of 39 residues and its receptor, parathyroid hormone 2 receptor, in the mouse brain. *J Comp Neurol*. 2007;502:563-583.
17. Hokfelt T, Phillipson O, Goldstein M. Evidence for a dopaminergic pathway in the rat descending from the A11 cell group to the spinal cord. *Acta Physiol Scand*. 1979;107:393-395.
18. Nandi D, Aziz T, Carter H, Stein J. Thalamic field potentials in chronic central pain treated by periventricular gray stimulation – a series of eight cases. *Pain*. 2003;101:97-107.
19. Dobolyi A, Irwin S, Makara G, Usdin TB, Palkovits M. Calcitonin gene-related peptide-containing pathways in the rat forebrain. *J Comp Neurol*. 2005;489:92-119.
20. Barsy B, Kocsis K, Magyar A, et al. Associative and plastic thalamic signaling to the lateral amygdala controls fear behavior. *Nat Neurosci*. 2020;23:625-637. doi:10.1038/s41593-020-0620-z
21. Coolen LM, Veening JG, Petersen DW, Shipley MT. Parvocellular subparafascicular thalamic nucleus in the rat: anatomical and functional compartmentalization. *J Comp Neurol*. 2003;463:117-131.
22. Varga T, Palkovits M, Usdin TB, Dobolyi A. The medial paralemniscal nucleus and its afferent neuronal connections in rat. *J Comp Neurol*. 2008;511:221-237.
23. Fenzl T, Schuller G. Periaqueductal gray and the region of the paralemniscal area have different functions in the control of vocalization in the neotropical bat, *Phyllostomus discolor*. *Eur J Neurosci*. 2002;16:1974-1986.
24. Cservenak M, Keller D, Kis V, et al. A thalamo-hypothalamic pathway that activates oxytocin neurons in social contexts in female rats. *Endocrinology*. 2017;158:335-348. doi:10.1210/en.2016-1645
25. Cservenak M, Kis V, Keller D, et al. Maternally involved galanin neurons in the preoptic area of the rat. *Brain Struct Funct*. 2017;222:781-798. doi:10.1007/s00429-016-1246-5
26. Dimitrov E, Usdin TB. Tuberoinfundibular peptide of 39 residues modulates the mouse hypothalamic-pituitary-adrenal axis via paraventricular glutamatergic neurons. *J Comp Neurol*. 2010;518:4375-4394.
27. LaBuda CJ, Dobolyi A, Usdin TB. Tuberoinfundibular peptide of 39 residues produces anxiolytic and antidepressant actions. *Neuroreport*. 2004;15:881-885.
28. Dobolyi A, Ueda H, Uchida H, Palkovits M, Usdin TB. Anatomical and physiological evidence for involvement of tuberoinfundibular peptide of 39 residues in nociception. *Proc Natl Acad Sci USA*. 2002;99:1651-1656.
29. Coutellier L, Logemann A, Rusnak M, Usdin TB. Maternal absence of the parathyroid hormone 2 receptor affects postnatal pup development. *J Neuroendocrinol*. 2011;23:612-619. doi:10.1111/j.1365-2826.2011.02149.x
30. Gauriau C, Bernard JF. Pain pathways and parabrachial circuits in the rat. *Exp Physiol*. 2002;87:251-258. doi:10.1113/eph8702357
31. Dafny N. Pain tracts and sources. In: Byrne JH, ed. *Electronic Textbook for the Neurosciences*. Neuroscience Online; 2020.
32. Dimitrov EL, Petrus E, Usdin TB. Tuberoinfundibular peptide of 39 residues (TIP39) signaling modulates acute and tonic nociception. *Exp Neurol*. 2010;226:68-83.
33. Dimitrov EL, Kim YY, Usdin TB. Regulation of hypothalamic signaling by tuberoinfundibular peptide of 39 residues is critical for the response to cold: a novel peptidergic mechanism of thermoregulation. *J Neurosci*. 2011;31:18166-18179. doi:10.1523/JNEUROSCI.2619-11.2011
34. Fegley DB, Holmes A, Riordan T, et al. Increased fear- and stress-related anxiety-like behavior in mice lacking tuberoinfundibular peptide of 39 residues. *Genes Brain Behav*. 2008;7:933-942.
35. Tsuda MC, Yeung HM, Kuo J, Usdin TB. Incubation of fear is regulated by TIP39 peptide signaling in the medial nucleus of the amygdala. *J Neurosci*. 2015;35:12152-12161. doi:10.1523/JNEUROSCI.1736-15.2015
36. Coutellier L, Usdin TB. Enhanced long-term fear memory and increased anxiety and depression-like behavior after exposure to an aversive event in mice lacking TIP39 signaling. *Behav Brain Res*. 2011;222:265-269. doi:10.1016/j.bbr.2011.02.043
37. Andrews B, Brewin CR, Philpott R, Stewart L. Delayed-onset posttraumatic stress disorder: a systematic review of the evidence. *Am J Psychiatry*. 2007;164:1319-1326. doi:10.1176/appi.ajp.2007.06091491
38. Siegmund A, Wotjak CT. A mouse model of posttraumatic stress disorder that distinguishes between conditioned and sensitised fear. *J Psychiatr Res*. 2007;41:848-860. doi:10.1016/j.jpsychires.2006.07.017
39. Pickens CL, Golden SA, Nair SG. Incubation of fear. *Curr Protoc Neurosci*. 2013;64:6.27.1–6.27.11. doi:10.1002/0471142301.ns0627s64:
40. LeDoux J. The emotional brain, fear, and the amygdala. *Cell Mol Neurobiol*. 2003;23:727-738.
41. Baisley SK, Cloninger CL, Bakshi VP. Fos expression following regimens of predator stress versus footshock that differentially affect prepulse inhibition in rats. *Physiol Behav*. 2011;104:796-803. doi:10.1016/j.physbeh.2011.08.001

42. Yoshida M, Takayanagi Y, Onaka T. The medial amygdala-medullary PrRP-synthesizing neuron pathway mediates neuroendocrine responses to contextual conditioned fear in male rodents. *Endocrinology*. 2014;155:2996-3004. doi:10.1210/en.2013-1411
43. Dobolyi A, Dimitrov E, Palkovits M, Usdin TB. The neuroendocrine functions of the parathyroid hormone 2 receptor. *Front Endocrinol*. 2012;3:121. doi:10.3389/fendo.2012.00121
44. Gellen B, Zelena D, Usdin TB, Dobolyi A. The parathyroid hormone 2 receptor participates in physiological and behavioral alterations of mother mice. *Physiol Behav*. 2017;181:51-58. doi:10.1016/j.physbeh.2017.09.005
45. Cservenak M, Bodnar I, Usdin TB, Palkovits M, Nagy GM, Dobolyi A. Tuberoinfundibular peptide of 39 residues is activated during lactation and participates in the suckling-induced prolactin release in rat. *Endocrinology*. 2010;151:5830-5840.
46. Varga T, Mogyorodi B, Bago AG, et al. Paralemniscal TIP39 is induced in rat dams and may participate in maternal functions. *Brain Struct Funct*. 2012;217:323-335. doi:10.1007/s00429-011-0357-2
47. Brown RSE, Aoki M, Ladyman SR, et al. Prolactin action in the medial preoptic area is necessary for postpartum maternal nursing behavior. *Proc Natl Acad Sci USA*. 2017;114:10779-10784. doi:10.1073/pnas.1708025114
48. Pereira M. Structural and functional plasticity in the maternal brain circuitry. *New Dir Child Adolesc Dev*. 2016;2016:23-46.
49. Olah S, Cservenak M, Keller D, et al. Prolactin-induced and neuronal activation in the brain of mother mice. *Brain Struct Funct*. 2018;223:3229-3250. doi:10.1007/s00429-018-1686-1
50. Bridges RS. The behavioral neuroendocrinology of maternal behavior: past accomplishments and future directions. *Horm Behav*. 2020;120:104662. doi:10.1016/j.yhbeh.2019.104662
51. Dobolyi A, Irwin S, Wang J, Usdin TB. The distribution and neurochemistry of the parathyroid hormone 2 receptor in the rat hypothalamus. *Neurochem Res*. 2006;31:227-236.
52. Wu Z, Autry AE, Bergan JF, Watabe-Uchida M, Dulac CG. Galanin neurons in the medial preoptic area govern parental behaviour. *Nature*. 2014;509:325-330. doi:10.1038/nature13307
53. Scott N, Prigge M, Yizhar O, Kimchi T. A sexually dimorphic hypothalamic circuit controls maternal care and oxytocin secretion. *Nature*. 2015;525:519-522. doi:10.1038/nature15378
54. Moffitt JR, Bambah-Mukku D, Eichhorn SW, et al. Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region. *Science*. 2018;362:eaau5324. doi:10.1126/science.aau5324
55. Anneser L, Alcantara IC, Gemmer A, Mirkes K, Ryu S, Schuman EM. The neuropeptide Pth2 dynamically senses others via mechanosensation. *Nature*. 2020;588:653-657. doi:10.1038/s41586-020-2988-z
56. Keller D, Láng T, Cservenak M, et al. A thalamo-preoptic pathway promoting social touch. *bioRxiv*. 2022. doi:10.1101/2022.01.11.475648
57. Nakamura K, Morrison SF. Central efferent pathways for cold-defensive and febrile shivering. *J Physiol*. 2011;589:3641-3658. doi:10.1113/jphysiol.2011.210047
58. Morrison SF, Nakamura K. Central neural pathways for thermoregulation. *Front Biosci*. 2011;16:74-104.
59. Dobolyi A, Palkovits M, Usdin TB. Expression and distribution of tuberoinfundibular peptide of 39 residues in the rat central nervous system. *J Comp Neurol*. 2003;455:547-566.
60. Metzner W. An audio-vocal interface in echolocating horseshoe bats. *J Neurosci*. 1993;13:1899-1915.
61. Metzner W. Anatomical basis for audio-vocal integration in echolocating horseshoe bats. *J Comp Neurol*. 1996;368:252-269.
62. Palkovits M, Dobolyi A, Helfferich F, Usdin TB. Localization and chemical characterization of the audiogenic stress pathway. *Ann N Y Acad Sci*. 2004;1018:16-24.
63. Palkovits M, Helfferich F, Dobolyi A, Usdin TB. Acoustic stress activates tuberoinfundibular peptide of 39 residues neurons in the rat brain. *Brain Struct Funct*. 2009;214:15-23.
64. Hofer MA. Multiple regulators of ultrasonic vocalization in the infant rat. *Psychoneuroendocrinology*. 1996;21:203-217.
65. Hashimoto H, Saito TR, Furudate S, Takahashi KW. Prolactin levels and maternal behavior induced by ultrasonic vocalizations of the rat pup. *Exp Anim*. 2001;50:307-312.
66. Terkel J, Damassa DA, Sawyer CH. Ultrasonic cries from infant rats stimulate prolactin release in lactating mothers. *Horm Behav*. 1979;12:95-102.
67. Febo M, Stolberg TL, Numan M, Bridges RS, Kulkarni P, Ferris CF. Nursing stimulation is more than tactile sensation: it is a multisensory experience. *Horm Behav*. 2008;54:330-339. doi:10.1016/j.yhbeh.2008.02.024
68. Wang T, Palkovits M, Rusnak M, Mezey E, Usdin TB. Distribution of parathyroid hormone-2 receptor-like immunoreactivity and messenger RNA in the rat nervous system. *Neuroscience*. 2000;100:629-649.
69. Bago AG, Dimitrov E, Saunders R, et al. Parathyroid hormone 2 receptor and its endogenous ligand tuberoinfundibular peptide of 39 residues are concentrated in endocrine, viscerosensory and auditory brain regions in macaque and human. *Neuroscience*. 2009;162:128-147. doi:10.1016/j.neuroscience.2009.04.054
70. Sugimura Y, Murase T, Ishizaki S, et al. Centrally administered tuberoinfundibular peptide of 39 residues inhibits arginine vasopressin release in conscious rats. *Endocrinology*. 2003;144:2791-2796.
71. Antunes-Rodrigues J, de Castro M, Elias LL, Valenca MM, McCann SM. Neuroendocrine control of body fluid metabolism. *Physiol Rev*. 2004;84:169-208. doi:10.1152/physrev.00017.2003
72. Ward HL, Small CJ, Murphy KG, Kennedy AR, Ghatei MA, Bloom SR. The actions of tuberoinfundibular peptide on the hypothalamo-pituitary axes. *Endocrinology*. 2001;142:3451-3456.

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