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NEUROSCIENCE

Four decades of research on Viktor Mutt's neuropeptides with focus on galanin[1]

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Abstract. The aim of the article is to describe our work on peptides discovered in Dr. Mutt's laboratory, particularly galanin. Some personal recollections of meetings with Viktor Mutt and a brief overview of early neuropeptide research at Karolinska Institutet are provided. General aspects on neuropeptide signalling and neuropeptide–neurotransmitter coexistence are followed by the presentation of a possible involvement of the galanin system in pain and depression. Special emphasis is on the role of galanin in the rat and human locus coeruleus. Additional analyses of the human postmortem brains have given results on galanin and other peptides both in the normal prefrontal cortex as well as in different brain regions of depressed patients who have committed suicide and in control subjects. Possible options for developing treatment strategies for pain and depression based on galaninergic mechanisms are discussed. Finally, some recent drugs approved by the FDA for the treatment of conditions such as migraine, which target the signalling of other peptides, are highlighted. In conclusion, the aim of the article is to highlight the potential of the large group of neuropeptides as targets for the development of drugs that may further help patients with illnesses afflicting the nervous system.

Keywords: chemical neuroanatomy, neuropeptides, transmitter coexistence.

INTRODUCTION

My lecture at the Estonian Academy of Sciences has allowed me to remember and reflect on my interactions with Viktor and on our work on his peptides. Viktor Mutt's contribution to our understanding of the existence and role of hormonal peptides and neuropeptide transmitters in bodily functions represents a milestone in biomedical research. Viktor himself reviewed his work on two occasions, several decades ago, at a time when it was difficult to comprehend the full significance of neuropeptide signalling (still is!) (Mutt 1982; Mutt 1990). More recently, his work has been reviewed from a biochemical point of view by his close associates (Jörnvall et al. 2008).

Before the start of our collaboration, Viktor had reported on the structure of cholecystokinin (CCK) (Mutt and Jorpes 1968), and later, vasoactive intestinal peptide (VIP) (Said and Mutt 1970; Mutt and Said 1974) and gastrin releasing peptide (GRP) (McDonald et al. 1978) were discovered. The early 1980s then emerged as an

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[1] This article is based on the lecture presented by Tomas Hökfelt at a conference held at the Estonian Academy of Sciences, Tallinn, on 19 January 2024, commemorating the 100th birthday of Viktor Mutt. The first-person narrative is used when Tomas Hökfelt describes his personal recollections of Viktor Mutt and the events related to his legacy.

exciting and fruitful period in Viktor's laboratory, based on the strategy to search for C-terminally amidated peptides (Tatemoto and Mutt 1978), and, working with his PhD student Kazuhiko Tatemoto and other colleagues, several new peptides were discovered, including peptide HI (PHI) (Tatemoto and Mutt 1980), neuropeptide Y (NPY) (Tatemoto et al. 1982) and galanin (Tatemoto et al. 1983).

In this review, we will focus on the last peptide, galanin, and its three receptors GalR1–3 (Lang et al. 2015). Progress in the galanin field has been reported at three symposia: (i) Hökfelt et al. 1991; (ii) Hökfelt et al. 1998 (this symposium was held *before* Viktor passed away, so in his presence, but in the same year) (Fig. 1); (iii) Hökfelt and Crawley 2005; and in a book (Hökfelt 2010), as well as in review articles with a focus on galanin in the nervous system (only reviews from the last ten years are included) (Diáz-Cabiale et al. 2014; Holmes 2014; Freimann et al. 2015; Lang et al. 2015; Weinshenker et al. 2016; Millón et al. 2017; He et al. 2017; Šípková et al. 2017; Hökfelt et al. 2018; Genders et al. 2020; Oliveira Volpe et al. 2020; Demsie et al. 2020; Mills et al. 2021; Ullrich and Mac Gillavry 2021; Fonseca-Rodrigues 2022; Zhu et al. 2022).

In Hökfelt et al. (1991), Bertil Åberg (1925–1992), one of Sweden's leading figures in the biomedicine field as a scientist, as a pioneer in Swedish biotechnology and as an industrial leader, wrote a foreword to 'Viktor Mutt'. Similarly, in Hökfelt et al. (1998), Torvard Laurent (1930–2009), a distinguished professor in biochemistry at Uppsala University and Secretary of the Wenner-Gren Foundations, wrote an 'In Memoriam'. These texts represent personal and wonderful accounts of Viktor's life and achievements by two giants in the Swedish academic life. Their contributions will directly follow this article as Appendix A and B, respectively.

This said, neuropeptides are the most diverse group of signalling molecules in the nervous system, and there is a very large number of them (Burbach 2010; Wang et al. 2015), and there are even more receptors. In fact, peptides appear very early in the animal taxonomy. Thus, it has more recently been reported that the neurons of ctenophores, commonly known as comb jellies, which inhabit sea waters worldwide, lack monoamines (Moroz 2015), and that 'the first nervous system [that of ctenophores] evolved as nets of peptidergic cells' (Sachkova et al. 2021). A pioneer in

Fig. 1. Participants of the second galanin symposium in Stockholm, May 1998. Viktor is seen in the middle of the front row. He passed away in September of the same year. Photo: Stefan Zimmerman.

neuropeptide research was David de Wied of Utrecht University, who already in the late 1960s reported that vasopressin and vasopressin fragments have robust effects on brain functions, such as memory processes (de Wied 1969; de Wied 1977). Broad overviews on the purpose of neuropeptides, mechanisms involved in their actions and more can be found in several reviews (van den Pol 2012; Guillaumin and Burdakov 2021; Eiden et al. 2022). Textbooks on peptides have been written by Strand (1999) and edited by Kastin (2013).

Some personal memories of Viktor Mutt

Viktor was a very modest human being and professor (not always the case among the colleagues), which reflected at many levels, one being his office (Fig. 2). In those days, I had never visited a professor at Karolinska Institutet with such a small office. So, when Kjell Fuxe and I, or Tamas Bartfai and I, visited, we could not sit side-by-side. Instead, I had to sit behind Kjell or Tamas to look over their shoulder to say hello to Viktor and (to a very limited extent) participate in the discussion. And there is no student among those he lectured who has forgotten Professor Mutt. Why? Because he was so fascinating, perhaps? Because he knew the formulas of so many compounds by heart, possibly? But for certain, because he wrote the formulas with his right hand, and with the left he wiped them out. So, always a clean board. As a student, you really had to be alert. Because if not, you did not have a chance to take notes. It is, indeed, difficult to forget Viktor's lectures. Viktor may also be remembered for the naming of some peptides more recently discovered in his laboratory, such as galanin and NPY. To avoid the possibility that the name with regard to function might turn out to be a misnomer, he, as the biochemist and original individual he was, initiated a novel, objective principle: galanin stands for N-terminal **g**lycine and C-terminal **alanin**e, and NPY stands for **neuropeptide** with a C-terminal tyrosine, **Y**.

Fig. 2. Viktor Mutt in his (minute) office at Karolinska Institutet. Photographer unknown.

Neuropeptides at Karolinska Institutet

Neuropeptides have a long history at Karolinska: Ulf von Euler, the Swedish Nobel laureate, discovered substance P when he was working in the United Kingdom with John Gaddum (Euler and Gaddum 1931). Bengt Pernow, who later became Rector at Karolinska, wrote his thesis on substance P with von Euler as his supervisor (Pernow 1953a, 1953b). Until then the chemical nature of substance P had been unknown; however, in 1971, Susan Leeman and colleagues discovered that substance P is actually an undecapeptide (11 amino acids) (Chang et al. 1971). This started a worldwide 'race'. At Karolinska, Pernow initiated a new venture where, among others, Göran Nilsson and colleagues generated antibodies to substance P for radioimmunoassay and immunohistochemistry. My colleagues and I used the latter method and Göran's anti-substance P antibodies to trace the substance P systems in the brain and periphery (Hökfelt et al. 1975; Ljungdahl et al. 1978). Substance P antagonists were later tested for pain and depression, but large clinical trials eventually showed lack of efficacy. However, such an antagonist is now in the clinic for treatment of chemotherapy-induced nausea (Hargreaves et al. 2011) (see below).

It could also be mentioned that these substance P mapping studies were part of the development of a fairly new discipline, chemical neuroanatomy, defining neuronal systems on the basis of a chemical, such as the catecholamine systems (Carlsson et al. 1962; Dahlström and Fuxe 1964; Geffen et al. 1969). Chemically identified neurons then became a basis and prerequisite for the possibility to apply recent modern techniques, such as optogenetics and viral tracing, to experimental brain research.

General aspects on neuropeptide signalling and neurotransmitter coexistence

During the early work, many basic questions concerning neuropeptide signalling arose, questions that today appear somewhat trivial. For example: peptides are small proteins produced on ribosomes in the cell bodies and are released from nerve endings far away from the production site, perhaps even one meter in humans (of course, even more in the giraffe). How can a released transmitter be replaced when fast axonal transport over such a long distance may take days? A partial explanation came when the existence of dendritic release was introduced (Ludwig and Leng 2006); here the distance between synthesis and release is short. More recently, Erin Schumann and colleagues have shown that mRNA for peptides is actually present in nerve endings (Glock et al. 2017). So, maybe there is a local

synthesis of peptides in the terminal boutons, similar to monoamines, where the synthesizing enzymes are transported into the axon terminals.

In the 1980s, we studied many peripheral systems, such as autonomic and sensory ganglia. The most important finding, perhaps, was that neuropeptides are present in the same neurons that also produce the classic transmitters, such as noradrenaline, dopamine and more. The first example was noradrenaline together with somatostatin in sympathetic ganglia (Hökfelt et al. 1977). We followed up to show that coexistence also exists in the brain: substance P in serotonin neurons (Hökfelt et al. 1978; parallel to Chan-Palay et al. 1978) and CCK in dopamine neurons (Hökfelt et al. 1980a). So, it turned out to be a general principle: peptides always coexist with classic transmitters (Hökfelt et al. 1987).

In the subsequent work, my PhD student Jan Lundberg played an important role. (Jan, before finishing his thesis, moved to the Pharmacology Department, became a professor there, then Head of Global Research for AstraZeneca, and later on for Eli Lilly. A successful career, indeed.) To sum up, these studies, and data in the literature, showed that neuropeptide signalling is characterized as follows (Fig. 3): neuropeptides (i) are stored in special types of vesicles, large dense core vesicles (diameter ~1000 Å), and not in synaptic vesicles (diameter ~500 Å); (ii) are released when neurons fire at a high rate or in bursts (Lundberg et al. 1981; Andersson et al. 1982); (iii) are released extrasynaptically and act on receptors not embedded in the postsynaptic densities associated with Grey type 1 synapses, and thus, they may travel considerable distances in the extracellular space and act on distant re-

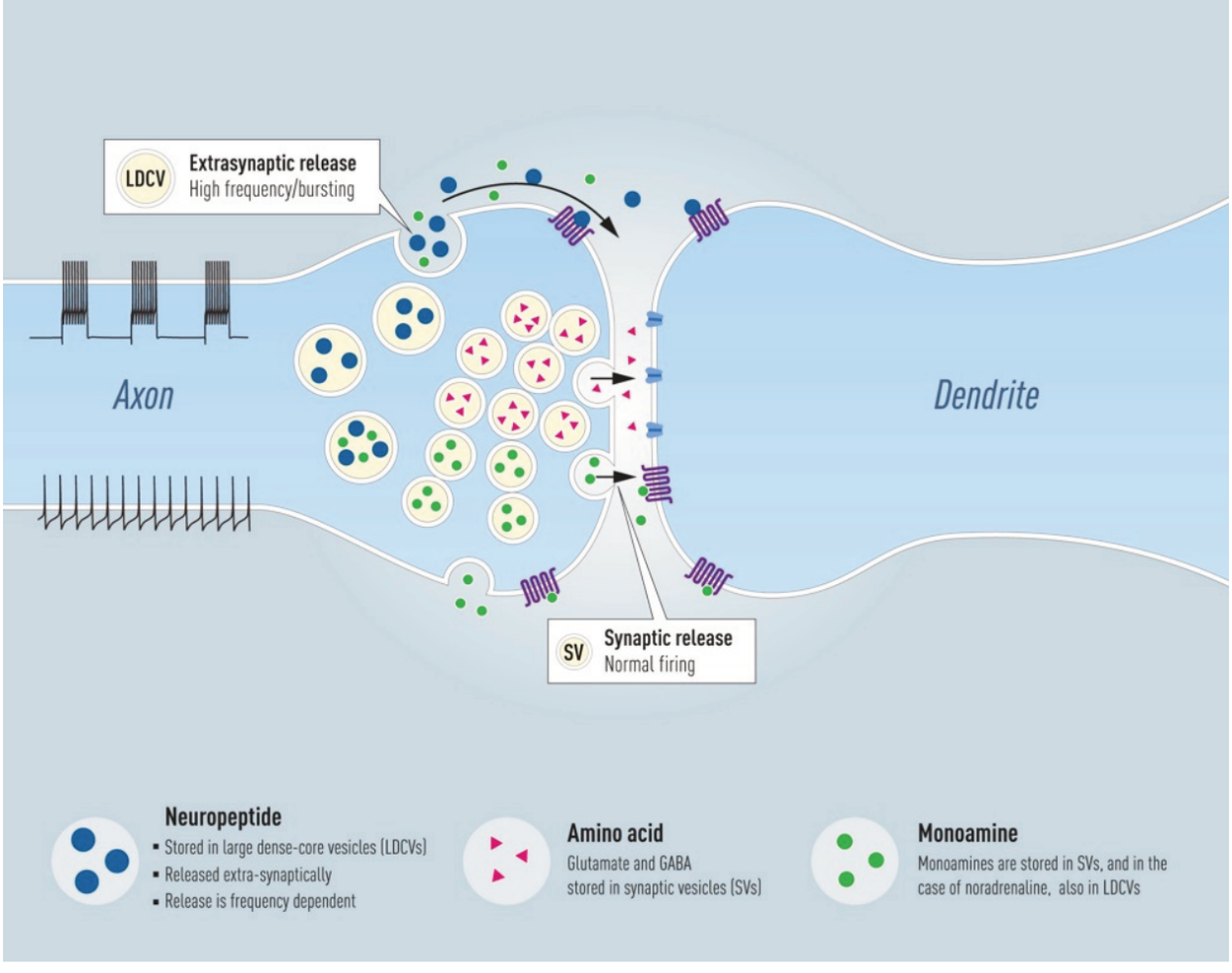

Fig. 3. Nerve ending with synapse and postsynaptic dendrite. Neuropeptides are stored in large dense core vesicles, released extrasynaptically after high frequency/burst firing, and can diffuse in the extracellular space to activate pre- and/or postsynaptic receptors of the G protein-coupled type. In contrast, small-molecule transmitters, such as GABA and glutamate, are stored in synaptic vesicles and released in the synapse after low frequency firing to, typically, activate postsynaptic ionotropic receptors. Monoamines such as noradrenaline, and perhaps also dopamine and serotonin, can be stored in both types of vesicles. Drawing by Mattias Karlén.

ceptors, which is called volume transmission (Fuxe et al. 2010); (iv) do not have a reuptake mechanism at the presynaptic membrane like the transporters for monoamines that terminate their action and replace depleted stores. Instead, peptides are enzymatically inactivated.

Publishing in *Nature* magazine

Our early work on neuropeptides led to an invitation to write a review in *Nature*, and the article, with my four PhD students as co-authors, appeared in 1980 with a photo of VIP neurons on the cover (Hökfelt et al. 1980b). It would turn out to be my most cited article.

Ten years later, I thought it was time to follow up with a new review summarizing what had happened since 1980. So, I submitted a draft to *Nature* and asked if they were interested in publishing a ten-year follow-up review. At the end of the submitted manuscript, I had written:

'If the '50s were the decade of acetylcholine, the '60s of catecholamines and serotonin, and the '70s of the inhibitory amino acid transmitters GABA and glycine, some of us had anticipated that the '80s would become the decade of the peptides. However, although a wealth of data was collected, a breakthrough in the understanding was not achieved. Instead, the **excitatory amino acids entered the scene** (authors' bolding) and grasped the attention with their implications for learning and memory and for involvement in brain pathology.'

After some time, I got a letter from a *Nature* editor, saying: 'First the bad news…We would not be able to offer to publish the article in its present form. Nevertheless, I was intrigued by your very last paragraph, on page 17 [see previous paragraph], that could be the germ of a completely re-written and much more compelling article. If, contrary to expectations, excitatory amino acids really were the big thing of the '80s, I feel that the review article should focus on these more or less exclusively [although I may be wrong here – I am very much a general reader…].'

Fortunately for me, the manuscript, after some important editing by Dr. Robert Elde, could be published in *Neuron* (Hökfelt 1991), actually owing to Dr. Eric Kandel, who also gave valuable advice.

Emerging sceptic attitudes to neuropeptides

However, dark clouds started to appear in the peptide sky. Doubts were expressed: are neuropeptides of interest at all, do they really have a function? Are they perhaps reminiscences from primitive organisms (comb jellies, see above)? I already mentioned the failure with substance P (neurokinin, NK) antagonists for treatment of pain and depression, a major disappointment and setback for neuropeptide research. Moreover, many small-molecule antagonists, developed with the aim to block signalling of other important neuropeptides, failed because of side effects, especially liver toxicity. In animal studies, knockout mice lacking a specific peptide were generated, but it was difficult to define a clear phenotype. (For me this was not a surprise because, as discussed above, peptides are not released under normal conditions but only when the animals are exposed to a situation when certain neuron systems fire at a high rate or in bursts (Hökfelt 1991)). On top of that, Chauncey Bowers published a review with the title 'Superfluous neurotransmitters?' (Bowers 1994). The cover of the *TINS* issue illustrated the idea of the author: GABA and monoamines were players in the field, whereas neuropeptides were sitting on the bench, doing nothing. Again, that was fully in line with our view: peptides are sitting on the bench as kind of reserve players until something important happens, e.g. a nerve injury. Then peptides are 'called in' to help 'solve the problem(s)'. In any case, these negative reports and attitudes were not helpful when it came to getting grants for neuropeptide research. One could say that, at the end of the millennium, the boom in neuropeptide research appeared to be over, and grants were difficult to get. In fact, grant applications to support research on galanin were later turned down in the UK, Australia and USA, even if submitted by some of the best laboratories in the galanin field.

However, neuropeptides have been useful as markers for neuron populations, e.g. interneurons in the cortex, although without exploring the functional role of the peptide. Fortunately, thanks to the recent introduction of novel methodologies, such as optogenetics, virus tracing and more, it has become possible to pin down the function of co-existing peptides. There are now many examples, but only a few very recent ones are mentioned here: (i) oxytocin from retrochiasmatic supraoptic nucleus activates oxytocin receptors in the anterior subdivision of the ventromedial hypothalamus, the ventrolateral part, and potentiates a response to aggressor cues in mice (Osakada et al. 2024); (ii) substance P from the principal nucleus of the bed nucleus of stria terminalis activates TACR1 receptors in the preoptic area and triggers mating behaviour (Bayless et al. 2023).

Neuropeptides and pain: focus on galanin

The failure of substance P antagonists to ameliorate pain allowed us to turn to another peptide: galanin (Tatemoto et al. 1983). This peptide is conserved among species and acts, as mentioned, via three receptors, GalR1, GalR2 and GalR3 (Lang et al. 2015). Today, we talk about a galanin family that also includes the galanin message-associated peptide (GMAP) (Rökaeus and Brownstein 1986), galanin-like peptide (Ohtaki et al. 1999), alarin (Santic et al. 2007) and spexin (Mirabeau et al. 2007). The reason

for selecting galanin for further studies on pain was a small paper that we published in *Neuroscience Letters* (Hökfelt et al. 1987), soon followed by more detailed immunohistochemical/biochemical studies (Villar et al. 1989; Villar et al. 1991). Here we show that, after transection of the sciatic nerve, there is a dramatic increase in galanin levels in the cell bodies of the corresponding dorsal root ganglion (DRG) neurons (L4 and L5 level) (Fig. 4). Just a year before, this had also been shown for VIP (Shehab and Atkinson 1986) and was later reported for NPY (Wakisaka et al. 1991). So, all these three 'Mutt peptides' react in a similar way to nerve injury of sensory ganglia. Absolutely essential for our work on galanin (and NPY) was our collaboration with Zsuzsanna Wiesenfeld-Hallin and her team, who carried out functional/electrophysiological experiments on galanin (and NPY) (Wiesenfeld-Hallin et al. 1988; Wiesenfeld-Hallin et al. 1989).

We and others have tried to understand the significance of the dramatic upregulation of galanin expression, and we now believe that this process is important both for pain signalling (Xu et al. 2000; Kerr et al. 2000; Liu and Hökfelt 2002; Holmes et al. 2005; Wiesenfeld-Halllin et al. 2005; Xu et al. 2010; Fonseca-Rodrigues et al. 2022; Hökfelt and Wiesenfeld-Hallin 2024) and nerve regeneration (Hobson et al. 2010). Based on pain studies in *rats*, we believe, in brief, that galanin in DRG neurons is part of an *endogenous* defence system against neuropathic pain. This pain is evoked by nerve injury and responds less well to treatment with morphine. The pain relief is exerted via the activation of GalR1 on glutamatergic interneurons in the spinal dorsal horn (Landry et al. 2006). Here a GalR1 *agonist* would reduce (neuropathic) pain. However, initially after the nerve injury, galanin also has an excitatory effect, exerted via GalR2 receptors on DRG neurons, a warning signal (Liu et al. 2001; Liu and Hökfelt 2002). Thus, GalR2 is pronociceptive, and the pain could in principle be attenuated by a GalR2 antagonist. Interestingly, two other peptides, substance P and calcitonin gene-related peptide (CGRP), which are excitatory and produce pain, are downregulated. There are additionally hundreds of other molecules in rodent DRG neurons that change in response to nerve injury (Costigan et al. 2002; Wang et al. 2002; Xiao et al. 2002; Wang et al. 2021). So, galanin is not the only molecule involved, but other molecules also participate in this endogenous pain defence system intrinsic to DRG neurons. There are extensive studies showing that NPY may be one such molecule (Nelson and Taylor 2021).

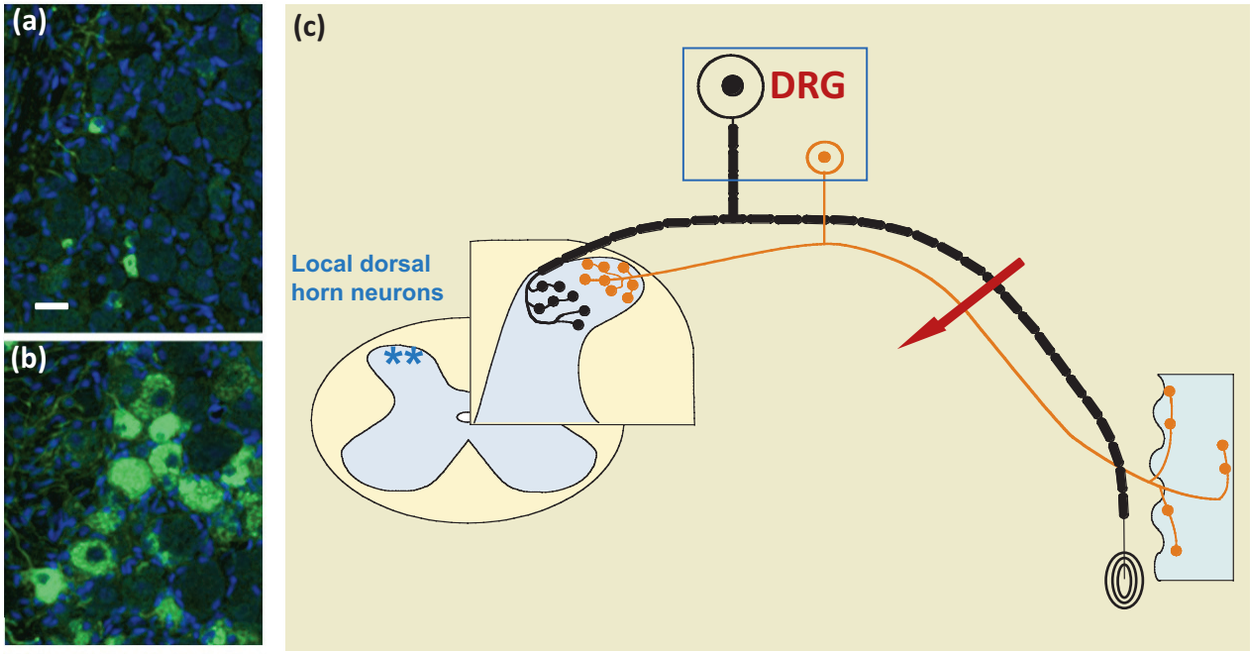

Fig. 4. Immunohistochemical micrographs of dorsal root ganglia (DRGs) and a drawing showing sensory neurons and a spinal cord. (a,b) Only a few galanin-positive neurons are seen in the contralateral DRG (a) versus numerous, strongly immunoreactive cells in the ipsilateral ganglion (b) after unilateral transection of the sciatic nerve in mice. Scalebar indicates 25 µM. (c) A population of small to medium-sized DRG neurons (orange) upregulate galanin expression after nerve transection. We have proposed that galanin represents an endogenous antinociceptive molecule combating *neuropathic* pain. Interneurons in the dorsal horn expressing e.g. opioid peptides, combat *inflammatory* pain. Drawing by Mattias Karlén.

It was already known that there is another endogenous pain defence system, which is associated with interneurons expressing opiate receptors, (see e.g. Fig. 4c) (Dubner and Ruda 1992). One natural ligand for these receptors is enkephalin, and interneurons expressing this neuropeptide intermingling with substance P afferents have been demonstrated in the dorsal horn (Hökfelt et al. 1977). Such opiate receptors are the targets for the efficacious treatment of 'common' pain with morphine and morphine-related drugs.

In conclusion, our studies on the galanin system after nerve injury suggest that both GalR1 and GalR2 receptors could be the targets for pain treatment: a GalR2 antagonist acting on DRG neurons could have an effect on the initial pain, and, more importantly, treatment with a GalR1 agonist acting on dorsal interneurons may reduce neuropathic pain. However, all these results are based on studies on *rats*. That these findings, however, may also be clinically relevant is supported by studies showing that galanin is dramatically upregulated in DRG neurons after transection of the sciatic nerve also in monkeys (Zhang et al. 1993). Moreover, around >10% of the neuron profiles in human control DRGs are galanin-positive versus >30% in patients suffering from severe brachial plexus injury (Landry et al. 2003). Thus, this endogenous defence system may also exist in primates, including man. However, recent studies suggest that human DRGs either only express GalR3 (Tavares-Ferreira et al. 2022) or no galanin receptors at all (Yu et al. 2023), but GalR1 is expressed in the human spinal cord, at least during development (Li et al. 2023). So, in the case of humans, either intrathecal injection of a GalR1 agonist or an agonist passing the blood-brain-barrier (BBB) may reduce pain. Alternatively, a peripherally acting GalR2 antagonist may have an analgesic effect, since our own, old qPCR studies do show the presence of both GalR2 and GalR3 transcripts in human DRGs (Barde and Hökfelt unpublished). Apparently, there are considerable species differences that have to be considered when discussing treatment of pain with drugs acting on the galanin system in humans. Clearly, the situation is complex and needs further exploration.

The galanin system in the rat brain: focus on locus coeruleus

The early mapping of the galanin neurons in the rat brain in our group was carried out by Tor Melander using antibodies raised by Åke Rökaeus (Rökaeus et al. 1984; Melander et al. 1986b), and in parallel by Skofitsch and Jacobowitz (1985). Tor noticed that galanin coexists with classic transmitters in several cases: with acetylcholine in basal forebrain neurons (Melander et al. 1985) and with noradrenaline in the locus coeruleus (LC), with serotonin in the dorsal raphe nuclei and more (Melander et al. 1986a).

We became particularly interested in the noradrenaline–galanin coexistence in LC (Fig. 5a–c), in view of the earlier-described wide distribution and manifold functions of the noradrenergic LC neurons, including their possible role in mood and behaviour (Foote et al. 1983; Aston-Jones et al. 1996). Our first approach was electrophysiology, and we could show that galanin inhibits the spontaneous firing of the noradrenergic neurons (Pieribone et al. 1995), confirming previously published results (Seutin et al. 1989; Sevcik et al. 1993). Further analysis indicated that this inhibition is mediated via GalR1 (Fig. 5d) (Ma et al. 2001). On the basis of the mentioned experiments with vasopressin and oxytocin (Ludwig and Leng 2006) and of our own studies on galanin (Vila-Porcile et al. 2009), we then developed the hypothesis that this inhibition is exerted by dendritic release of galanin acting on GalR1 autoreceptors, a mechanism possibly aiming at preventing overexcitation and possibly being part of the resilience machinery (Hökfelt et al. 2018), in agreement with other studies (Sciolino et al. 2015). A more recent report on the mouse brain, including single cell analysis, has revealed that LC neurons, in addition to galanin, express almost 20 further peptides and more than 30 neuropeptide receptors (Caramia et al. 2023). So, galanin is not the only interesting peptide – perhaps there are even more interesting

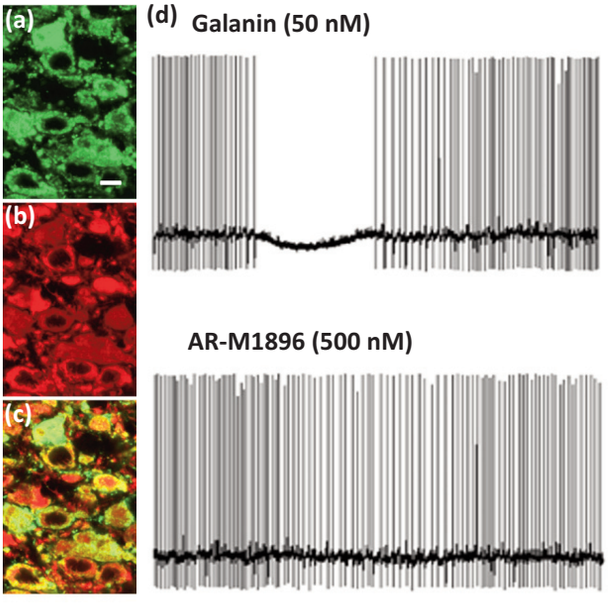

Fig. 5. Locus coeruleus (LC): immunohistochemical staining and electrophysiological recordings. (a–c) Double-staining with antibodies to galanin (a) and tyrosine hydroxylase (TH) (b). (c) shows the merged photo. Many neurons are both TH- and galanin-positive. Scalebar indicates 8 μM. (d) Galanin (top) inhibits spontaneous firing of LC neurons, but no effect can be seen after administration of the GalR2 agonist AR-M1896. (d) from Ma et al. 2001, with permission.

peptides than galanin. It is, however, important to point out that galanin, together with noradrenaline, is also transported into the terminal ramification of the LC neurons in the cortex and hippocampus (Xu et al. 1998), and that galanin receptors also exist in the forebrain. Galanin can thus influence signalling in such forebrain regions, as well.

The galanin system in the human locus coeruleus

After analysing rodent brains for many decades, we decided some 15 years ago to turn to the human brain. That seemed to be a relevant step, especially when diseases and their treatment are concerned. Our first target was the human LC (Le Maître et al. 2013). Here we observed transcripts for tyrosine hydroxylase (TH) (Fig. 6a,b), the first enzyme in the noradrenaline synthesis, i.e. in noradrenergic neuron, for galanin (Fig. 6c,d) and, surprisingly, for GalR3 (Fig. 6e,f). Interestingly, however, we could see these autoradiographic expression patterns after very different exposure times (Fig. 6b,d,f): TH mRNA was observed after several days (less than one week), galanin mRNA after around two weeks and GalR3 only after eight weeks – the last exclusively in brains with a very short postmortem time (one or two hours). TH and GalR3 mRNAs seemed to be present in virtually all cells and at about similar levels. In contrast, the galanin transcript levels varied considerably between individual cells. This fits with the fact that galanin is a molecule that is released depending on the activity of the individual cells, whereas the other transcripts produce proteins that are 'stable' and have a long half-life. But the GalR3 signal is still an uncertain finding. Interestingly, we prepared the GalR3 probe from human DRG mRNA. We have in a recent single-cell study on human LC neurons not been able to detect the GalR3 transcript, but instead GalR1 mRNA was observed in many neurons (Barde et al. 2024). In that study, we could, like in the mouse LC (Caramia et al. 2023), detect a large number of further neuropeptides and neuropeptide receptor transcripts in LC neurons (Barde et al. 2024).

Galanin and many other peptide systems in the human prefrontal cortex

Galanin has in rodent studies been considered to be a brain stem peptide with few galanin-positive neurons in the cortex and hippocampus (Merchenthaler et al. 1993), contrasting for example with NPY, which is expressed in many interneurons in these regions. It seems relevant to explore whether that is also the case in the human brain. Prefrontal cortex (PFC) occupies the most rostral part of the frontal lobe of the cerebral cortex and constitutes close to 20% of the total brain. It is divided into more than 15 subregions (Brodmann areas) and is involved in a large range of higher brain functions, such as planning, decision-making, memory and emotions.

We have analysed 17 microdissected subregions of the human PFC and three reference cortices using RNA sequencing (RNAseq), which includes the analysis of nearly 20 000 genes (Zhong et al. 2022). In this paper, we particularly focused on the transcripts for neuropeptides (78 were found) and their receptors (83). This allowed us to systematically explore the expression landscape of peptides and their receptors in the precisely punched samples from various PFC regions, and a larger number of neuropeptides and receptors were detected. For example, adrenomedullin, which has not been reported before, was robustly expressed in our study. Moreover, by integrating the single-nucleic RNAseq data from the PFC region, we have identified a considerable number of, what we called, interneuron and projection neuron 'candidates', apparently not detected in the single cell/nucleus studies (Zhong et al. 2022). The principle was: presence of a peptide *and* a receptor suggested an *interneuron candidate*; presence of a peptide *but no* receptor suggested a *projection neuron candidate*. An example of the former is CGRP, of the latter, thyrotropin releasing hormone (TRH).

The most abundant peptide was CCK, as was already shown in many studies in rats, where this peptide is expressed both in interneurons (de Felipe et al. 2013) and pyramidal neurons (Burgunder and Young III 1988). Also, in humans, CCK is expressed both in GABAergic interneurons (Bouras et al. 1986; Hodge et al. 2019) and glutamatergic pyramidal projection neurons (Hodge et al. 2019), now also shown with in situ hybridization in PFC (Zhong et al. 2022) (Fig. 7a). Additionally, the vesicular glutamate transporter 1 (VGLUT1) (Fig. 7b) and the vesicular GABA transporter (VGAT) (Fig. 7c) could be observed, partly with a similar distribution. As of particular interest, we detected several peptide receptors but not the corresponding peptide, suggesting that these receptors could be activated by blood-borne peptides. Such peptides include adiponectin (from fat), leptin (from fat) and gastric inhibitory peptide (GIP) (from K cells in the small intestine). This hypothesis is based on the concept that peptides can penetrate the BBB, which in fact has been shown to occur (Banks 2023). Only small amounts of peptide need to pass the BBB since the peptide receptor affinity is high (low nanomolar range).

The presence of a galanin system in the PFC was exciting for us. In rats, galanin expression in the cortex is limited (Merchenthaler et al. 1993) and is mainly present in noradrenergic afferents originating in the LC (Xu et al. 1998). However, in humans we detected galanin (and galanin receptors) in both glutamatergic and GABAergic neurons in the PFC (Fig. 8a,b) (Zhong et al. 2022). The presence of CCK in glutamatergic pyramidal neurons, which release an excitatory peptide and an excitatory

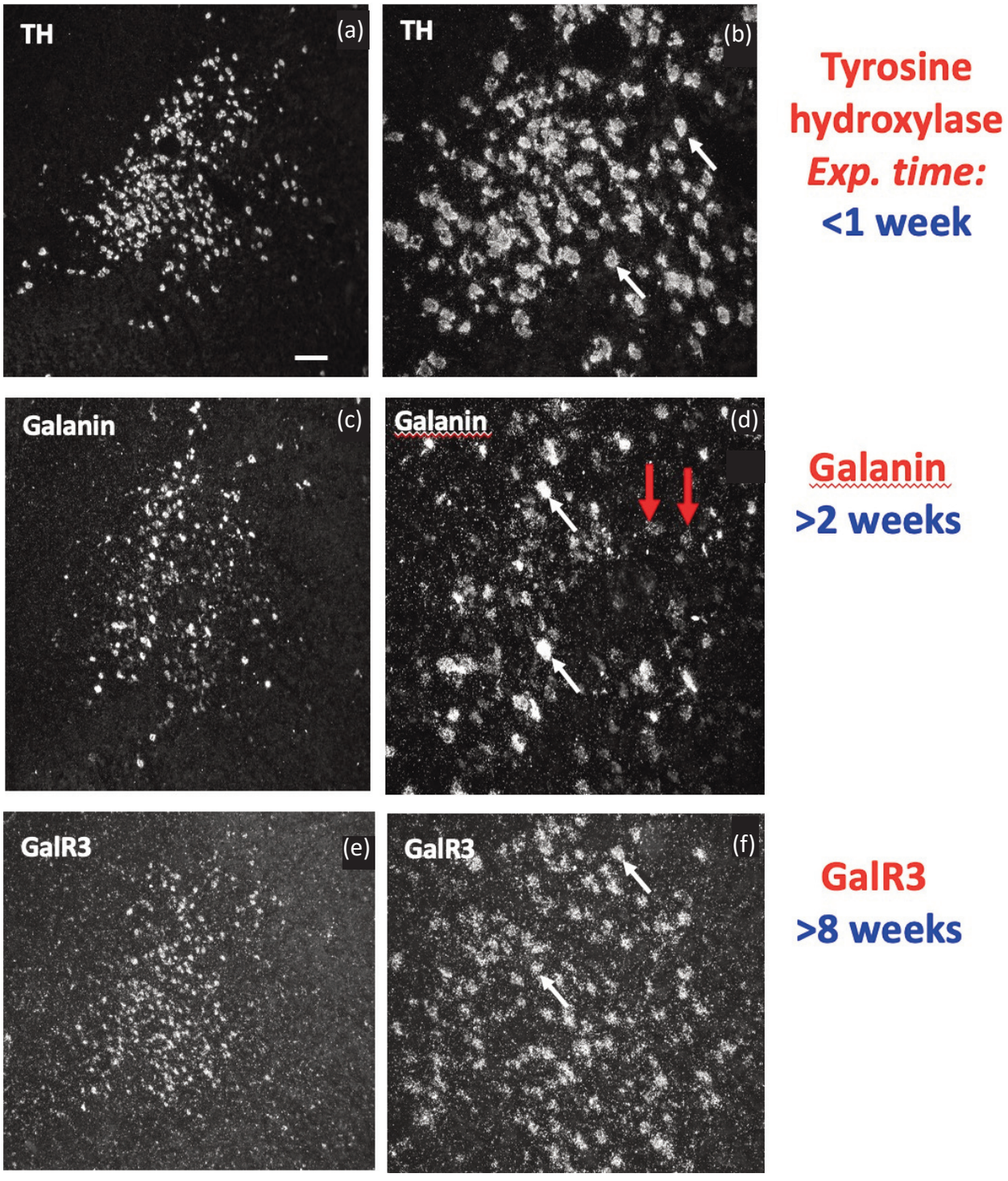

Fig. 6. In situ hybridization micrographs showing three transcripts in the human LC. Transcripts for TH (a,b), galanin (c,d) and the galanin type 3 receptor (GalR3) (e,f) are robustly expressed in the LC. The signal for both TH and GalR3 is similarly strong in all cells (arrows in b and f), whereas the galanin intensity varies between neurons (d), some strongly (white arrows), others (red arrows) weakly labelled. Scalebar indicates 200 µM (a) and 50 µM (b). From Le Maître et al. 2013, with permission.

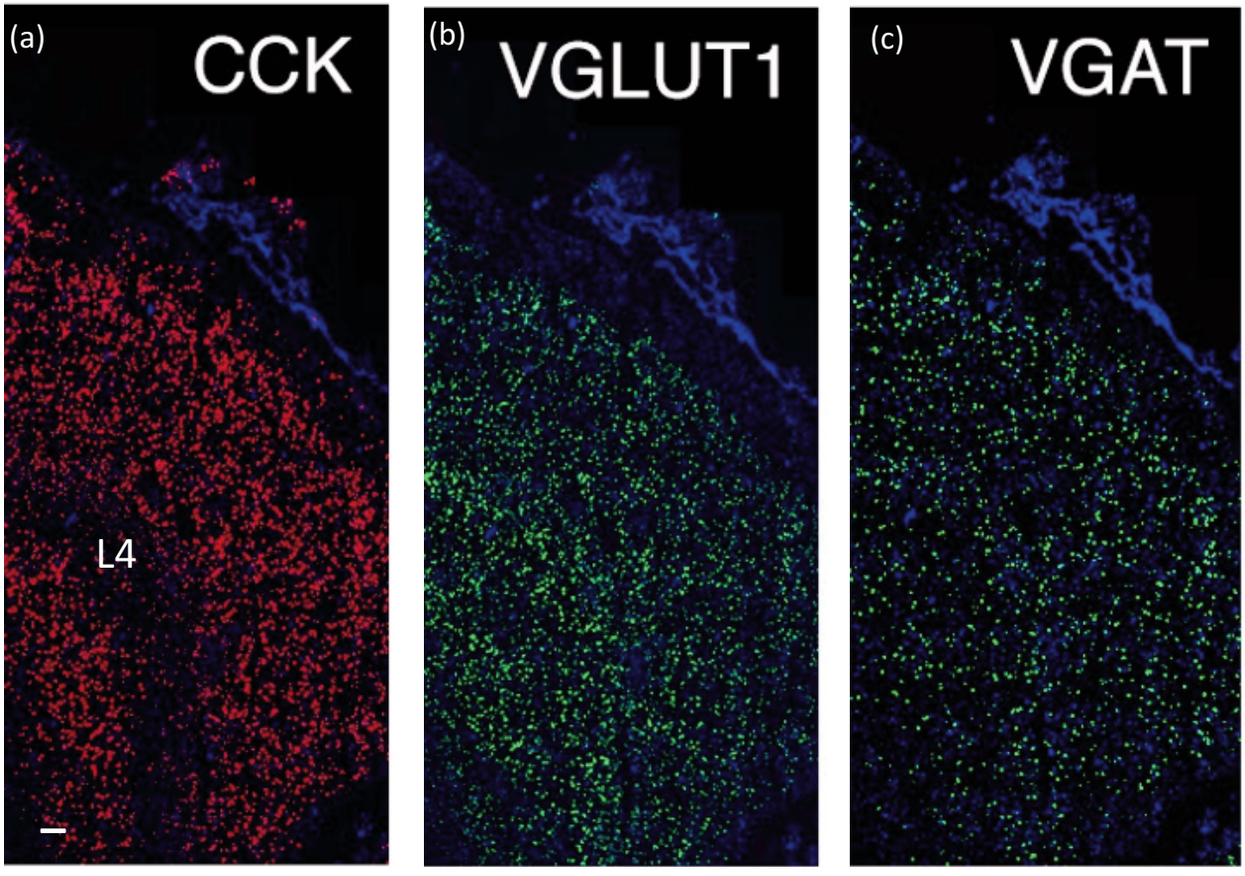

Fig. 7. RNAscope histochemistry (triple-labelling) of the human prefrontal cortex (PFC). The distribution of transcripts for cholecystokinin (CCK) (a), vesicular glutamate transporter1 (VGLUT1) (b) and vesicular GABA transporter (VGAT) (c) are shown. Note the high density of CCK-positive neurons in all layers except layer 4 (L4). Glutamatergic pyramidal neurons and GABAergic interneurons are evenly distributed, the former being more abundant than the latter. Detailed, high-power analysis reveals that the CCK transcripts are expressed both in glutamatergic and GABAergic cells (not shown). Scalebar indicates 100 μM. From Zhong et al. 2022, with permission.

principal transmitter, is an example of transmitter *co-agonism*, possibly *synergism*, i.e. these messengers may work together. Our findings suggest that galanin perhaps is more important in the human than in the rodent cortex, i.e. it participates in cognitive and other higher cortical functions in humans not only via release from noradrenergic nerve terminals but also by being released from pyramidal projection neurons and interneurons. In Fig. 8c, we have sketched a circuitry for the galaninergic systems in the human PFC. As mentioned, we have hypothesized that galanin may be involved in resilience and protect against glutamatergic over-excitation, thus being neuroprotective (Zhong et al. 2022). Moreover, we have speculated that the galanin system, in view of its expression in glutamatergic pyramidal neurons (Zhong et al. 2022), may offer a further target for the therapy of major depressive disorder (MDD) with the N-methyl-D-aspartate (NMDA) antagonist ketamine (Kadriu et al. 2019; Krystal et al. 2019).

Galanin: a possible role in depression

The presence of the galanin system both in the LC and PFC neurons may be an indication of an involvement also in mental disorders. Many animal experiments suggest that the galanin system plays a role in stress-related disorders, such as depressive-like behaviour and more (Bing et al. 1993; Fuxe et al. 1998; Lu et al. 2005; Weiss et al. 2005; Swanson et al. 2005; Kuteeva et al. 2008; Christiansen et al. 2011; Saar et al. 2013; Barnabas et al. 2016; Kawa et al. 2016; de Souza et al. 2018; Tillage et al. 2020; Yang et al. 2021; Flores-Burgess et al. 2022), of which reviews have been published (Wrenn and Crawley 2001; Holmes et al. 2003; Holmes and Picciotto 2006; Lu et al. 2007; Kuteeva et al. 2010; Weinshenker and Holmes 2016; Millón et al. 2017; Hökfelt et al. 2018; Pérez de la Mora et al. 2022). There are reports on patients showing a relation to the galanin system, based on both genetic ana-

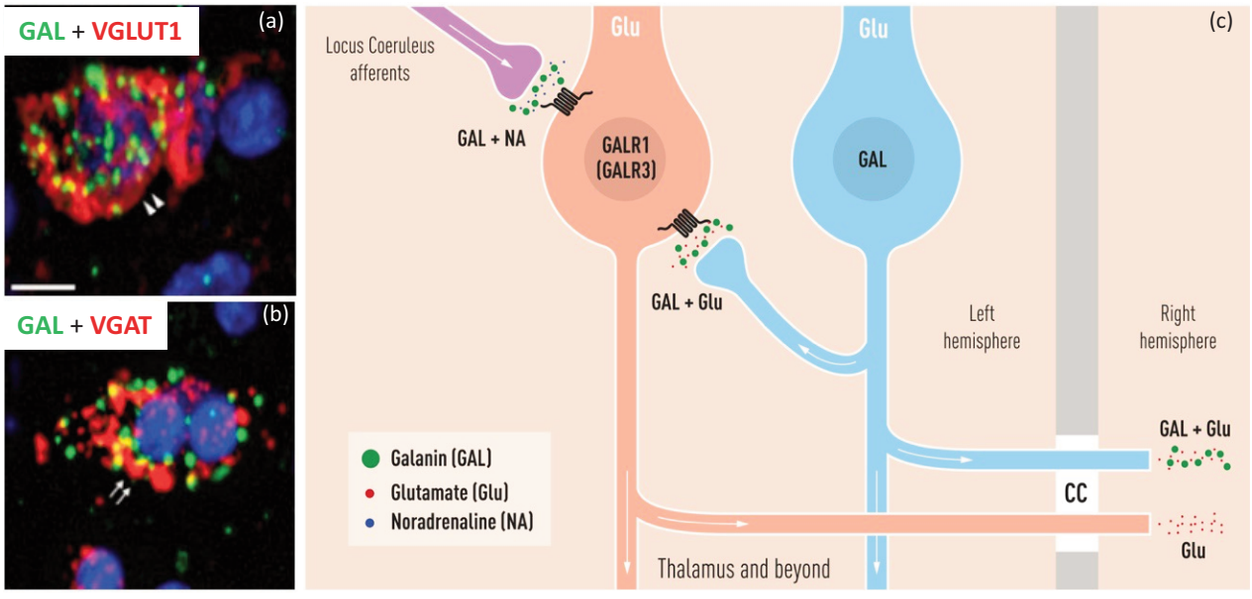

Fig. 8. Galanin systems in the human PFC. Galanin is expressed in: glutamatergic pyramidal neurons that may project contralaterally and/or to deeper parts of the brain/spinal cord (a,c); GABAergic interneurons (b, not shown in c); and noradrenergic nerve afferents originating in the LC (c). Regarding receptors, GalR1 and, at much lower levels, GalR3 are also expressed in glutamatergic pyramidal neurons (not shown). It is possible that galanin receptors are also present in noradrenergic afferents since both GalR1 and GalR2 transcripts have been found in human LC cell bodies (Barde et al. 2024). To sum up, there are at least three sources of galanin in human PFC: noradrenergic afferents (galanin is released together with noradrenaline), glutamatergic pyramidal neurons (galanin is released together with glutamate) and GABAergic interneurons (galanin is released together with GABA). Galanin released from these sources can target GalR1 (and GalR3) receptors on pyramidal cells. Interestingly, galanin in intra-telencephalic, crossing pyramidal neurons (c) could participate in the control of the contralateral cortex via an action on GalR1 expressing pyramidal neurons. CC – corpus callosum. Scalebar indicates 5 µM. Drawing by Mattias Karlén. From Zhong et al. 2022, with permission.

lyses (Unschuld et al. 2008; Unschuld et al. 2010; Wray et al. 2010; Davidson et al. 2011; Wang et al. 2013; da Conceição Machado et al. 2018; Keszler et al. 2019) and on other types of approaches (Murck et al. 2004; Wang et al. 2014). We have pursued this concept in several publications, both by analysing MDD patients (Barde et al. 2016; Barde et al. 2024) as well as carrying out genetic studies (Juhasz et al. 2014; Gonda et al. 2018).

In the biochemical studies (Table 1), we first focused on the galanin system and monitored the transcript levels (by quantitative PCR), DNA methylation status (by bisulphite pyrosequencing) and GAL peptide levels by radioimmunoassay (RIA) in postmortem brains from depressed persons who committed suicide ('depressed suicides') and controls (Barde et al. 2016). This study on five important human brain regions (anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), dorsal raphe nucleus (DRN), LC and, as a control, the medullary raphe nuclei (MRN)) provides detailed information on the galanin peptide levels, the transcript levels for galanin and GalR1–3 and the levels of DNA methylation of these four genes.

Both peptide and transcript levels showed marked regional variations, and there was a good correlation between them. This correlation is of some importance, since the issue is often discussed in the context of the many published single-cell/nucleus studies that are only based on transcripts/genes, questioning whether all these transcripts are really translated into protein/peptide. Our findings suggest that, concerning galanin, there is a correlation. For example, galanin peptide levels (pM/mG) in male controls are roughly 4–5 in DLPFC and ACC, 60 in DRN, 30 in LC and 15 in MRN. So, they are six times higher in LC and 12 times higher in DRN than in DLPFC/ACC. The corresponding raw cycle threshold values for transcripts (all samples pooled) are 27 for DLPFC and ACC, 26 for DRN, 25 for LC and MRN. Thus, LC and MRN have two times higher transcript levels than DRN and four times higher levels than DLPFC and ACC. The difference between DRN and LC probably reflects the fact that in the DRN the *galanin peptide* is mainly present in nerve endings, whereas the LC neuronal cell bodies mainly have the *galanin transcript*. Interestingly, the GalR1 transcript levels are as high or higher in DLFPC than in the lower brain

stem regions, suggesting an important role for galanin signalling in the human PFC. We have summarized the findings on differences between 'depressed suicides' and controls reported in Barde et al. (2016) in Table 1. Briefly, regarding the galanin system in MDD, the most pronounced transcript changes were found in three regions: decreased levels of galanin and GalR3 in the male DLPFC, and increased levels of galanin in the male and female DRN and LC. DNA methylation changed in the opposite direction for galanin in the male DLPFC and for GalR3 in the female DRN and LC.

A subsequent study included the substance P, NPY and CCK systems, but was *based only on qPCR* (Barde et al. 2024). The changes in transcript levels were modest: an increase in the female tachykinin in DLPFC and the male NPY in LC. Minor changes were seen in the female DLPFC and the male and female LC (Table 1). No changes were seen for the CCK system. To sum up, the galanin system seems to be the most promising target for the development of novel antidepressants, whereby galanin/galanin agonists and possibly GalR3 antagonists may act at the level of the lower brain stem (DRN and LC), whereas in the PFC, agonists may be an alternative. Also, the tachykinin receptors may be a target, as already indicated in the clinical trials reported by Kramer et al. (1998). Even if not strongly implicated in our study, there are many reports of an association between NPY and depression in the literature (Heilig 2004; Morales-Medina et al. 2010; Wu et al. 2011; Kautz et al. 2017; Mathé et al. 2020; Nahvi and Sabban 2020).

In the two original papers above, we have discussed the following limitations: (i) these studies were primarily bulk studies (not single-cell/nucleus), (ii) in many cases it was not known if transcripts studied were translated into peptide/protein, (iii) postmortem times were variable, (iv) the fact that the 'Barde et al. 2024 study' was carried out on the same samples as the 'Barde et al. 2016 study' but several years later, and more. One issue we did *not* raise was the surprising findings in the two PFC regions: *all differences* in transcript levels between 'depressed suicides' and controls were observed *in DLFPC*, whereas *none were found in ACC*. From studies in the literature, we would have expected that MDD is mainly associated with ACC (Drevets et al. 2008; Pizzagalli et al. 2018).

Our *genetic* approach also supports an involvement of the galanin system. In a candidate gene study, we analysed a European white population cohort totalling 2361 from Manchester, the United Kingdom, and Budapest, Hungary (Juhasz et al. 2014). We found that variants in genes for

Table 1. Summary of differences in transcript levels of three peptide systems in five brain regions between controls and depressed patients who committed suicide

	DLPFC		ACC		DRN		LC		MRN	
	M	F	M	F	M	F	M	F	M	F
Galanin	↓↓	↑	–	–	↑↑	↑↑	↑↑	↑↑	↑	↑
GalR1	↑	↑	–	–	↑	–	↑	–	–	–
GalR2	–	–	–	–	–	–	–	–	↓↓	–
GalR3	↓↓	–	–	–	↑	↑↑	↑	↑↑	↑	↑
SP	↑	↑	–	–	↑	–	↑	↑	–	–
TAC1	–	↑↑	–	–	–	–	–	↑	–	–
TACR1	–	–	–	–	–	–	↑	↑	–	–
TACR2	–	↑	–	–	–	–	–	↑	–	–
TACR3	–	↑	–	–	–	–	↑	↑	–	–
NPY	–	–	–	–	–	–	↑↑	↑	–	–
NPY1R	↑	↑	–	–	–	–	↑	–	–	–
NPY2R	–	–	–	–	–	–	–	–	–	–
NPY5R	–	–	–	–	–	–	–	–	–	–

The arrows represent statistical significance, where the upward arrow signifies the increase in gene expression and methylation status and vice versa. One arrow signifies $P < 0.05$ and two arrows stand for $P < 0.01$. Abbreviations: DLPFC – dorsolateral prefrontal cortex, ACC – anterior cingulate cortex, DRN – dorsal raphe nucleus, LC – locus coeruleus, MRN – medullary raphe nucleus, SP – substance P, NPY – neuropeptide Y.

galanin (GAL) and its three receptors (GALR1, GALR2, GALR3) conferred an increased risk of depression and anxiety in people who experienced childhood adversity or recent negative life events. This was achieved using Bayesian multivariate analysis that revealed a greater relevance of galanin system genes in highly stressed subjects compared to subjects with moderate or low life stress. This effect was observed in an independent analysis of the Manchester and Budapest subpopulations, and both in males and females. In a further study of functional polymorphisms, strong relevance of the GALR2 gene was found but only in moderate and/or high stress exposure groups (Gonda et al. 2018). In conclusion, these results further indicate that galanin pathways play an important role in the pathogenesis of depression in humans by increasing the vulnerability to early and recent psychosocial stress. This said, genome-wide association studies (GWAS) have not provided compelling evidence for the involvement of genes of the galanin system in MDD.

CONCLUDING REMARKS

One way to define the beginning of the history of mammalian neuropeptides is perhaps to identify the first publication of the amino acid sequence of such a molecule. If so, the report of the structure of vasopressin and oxytocin (Du Vigneaud 1954) is the starting point. Originally, these hormones were known to be expressed in the hypothalamic, magnocellular neurons projecting to the *posterior* pituitary, where vasopressin and oxytocin are released into the *systemic* circulation. Today we know that these peptides and their receptors are expressed in brain neurons and are involved in many functions beyond their peripheral actions. Also, the next wave of discovery involved hypothalamic hormones: the releasing/inhibitory hormones secreted into the *portal* vessels and controlling *anterior* pituitary functions, such as thyrotropin-releasing hormone (TRH), luteinizing hormone-releasing hormone (LHRH) and somatostatin (Guillemin 1978; Schally et al. 1978). Again, it was subsequently reported that these three peptides and their receptors are expressed, often widely, in the brain. In parallel, peptides like substance P (see above) and CCK, extracted from other sources, such as the gastro-intestinal tract, appeared on the scene. And here Viktor Mutt's laboratory made a major contribution. In parallel, a strong neuropeptide research developed in Japan, where also early on molecular biological methods were employed (Nakanishi, Noda, Numa), discovering new neuropeptides and neuropeptide receptors. In fact,

Table 2. 'Peptide' drugs approved by FDA and now on the market

- Anti-CGRP antibodies/receptor antibodies/antagonists for treatment of **migraine**
  - Emgality, galcanezumab, Eli Lilly; Ajovy, fremanezumab, Teva
  - Aimovig, erenumab, Novartis; Vyepti, eptinezumab, Lundbeck
- Orexin antagonists for treatment of **insomnia**
  - Belsomra, suvorexant, Merck; Dayvigo, lemborexant, Eisai
  - Qvivique, daridorexant, Idorsia
- NK1 antagonist for treatment of **emesis**
  - Emend, aprepitant, Merck
- GLP-1R agonists for treatment of **diabetes**, **obesity (and Alzheimer?)**
  - Victoza, liraglutid, Ozempic, Wegovy, Rybelsus (tablets), Amycretin, all semaglutide, all Novo Nordisk; Mounjaro, Zepbound, both tirzepatide, Trulicity, dulaglutide, all Eli Lilly
- NK3 antagonists for treatment of **flushes**
  - Veozah, fezolinetant, Astella

Shigetada Nakanishi's team cloned the first peptide receptor (Masu et al. 1987) – a breakthrough since up till then there had been some doubts about whether neuropeptides indeed had their own receptors. This said, an abundance of peptides was also discovered in non-mammalian species, in particular by Vittorio Erspamer and colleagues (Erspamer et al. 1978).

In this review, we have sketched the work on neuropeptides in our laboratory over the last five decades, selecting galanin as an example. Our hope, and we assume that of other galanin researchers as well, has been to eventually develop compounds that may help ameliorate illnesses of various kinds. It is evident in this review that we, targeting pain and mood disorders via galaninergic mechanisms, have failed, and that our hopes have not materialized. Early on, Tamas Bartfai, Ülo Langel and colleagues generated the first receptor selective ligands (Bartfai et al. 1992), and they rapidly became important tools for galanin researchers. However, they were mainly chimeric peptides and did not penetrate the BBB. In fact, only a few small-molecule BBB-penetrating antagonists have been developed for the galanin system, one being a group of GalR3 antagonists (Konkel et al. 2006; Swanson et al. 2005). This contrasts with the situation for many other neuropeptide receptors for which several efficacious antagonists are available, such as tachykinin receptor antagonists (Griebel and Holsboer 2012). Against this background, it is somewhat comforting that there are now several FDA approved drugs on the market that are based on peptidergic mechanisms (Table 2). There is hope that this is just the beginning, and that more compounds acting via neuropeptidergic mechanisms will be generated for treatment of diseases.

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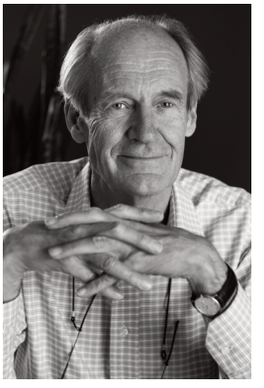

Photo: private collection

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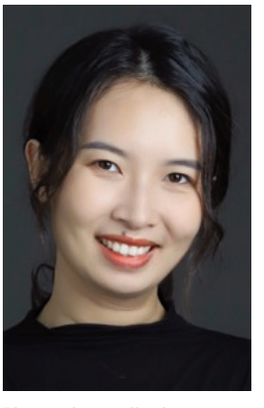

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APPENDIX A

Viktor Mutt (1991)

In 1946 the Department of Chemistry II at the Karolinska Institutet in Stockholm was housed in a building dating from the late 1850s. The head of the department was Erik Jorpes, who had fled from Finland during the First World War (he had been Minister of Health in the red revolutionary government of Finland, overthrown after the Civil War when Finland became an independent state). The same building also housed the departments led by Einar Hammarsten, Hugo Theorell and Ragnar Casparsson.

Jorpes had personally become rich by his association with the pharmaceutical house AB Vitrum, to which he had transferred his techniques for the production of bovine heparin and porcine insulin. This work in turn had aroused Jorpes' interest in 'natural' substances with a physiological action.

Jorpes had had a rough time during his first years in Sweden as a 'red' refugee, had often been hungry and had finished his medical studies chiefly thanks to his iron will. A long working day was natural to him, and he expected the same from all his employees. The standard 'nine to five' days was, according to Jorpes for 'bureaucrats and customs officials' and not for scientists. When Jorpes was in the lab, everybody should be in the lab – Christmas, Easter, Sundays and night mattered little. Several of us were paid by Vitrum and many received pay or bonuses from Jorpes' own pocket. Many were refugees from the Second World War, and Jorpes, remembering his early days in Sweden, tried in every way to help them along. One of the refugees was Viktor Mutt.

My first glimpse of Viktor Mutt was of a young man with a boyish face, who in a corner of the 'big laboratory' was, as a laboratory technician, handling an enormous mass of animal organs. Viktor had fled from Estonia (he was born in Tartu in 1923) and had the same working habits as the Boss.

Very early in the morning he cycled to the slaughterhouse in the south of Stockholm (about 5 miles) with samples of heparin plus a glass bead in test-tubes kept in a wooden rack. Fresh bovine blood was poured into the tubes; they were then stoppered, and coagulation time was measured with a stopwatch. An increase in coagulation time was a measure of heparin activity (Jalling, Jorpes and Linden 1946; *Quart. J. Pharmacol.,* **19**, 96). The results were brought back by Viktor to the laboratory and formed the basis for the morning discussion with Jorpes.

The importance of measuring activity when purifying a substance was a lesson from Jorpes to Viktor Mutt. Later – when Jorpes feared lack of raw material for insulin production and turned to whale pancreas as a raw material – Viktor started to extract enormous masses of organ material. Turning to pig intestines, he isolated secretin and cholecystokinin, purified them and had them transferred to pharmaceuticals (also sold by Vitrum, who were bought by Kabi, who 3 years ago sold the secretin to Ferring in Malmö).

Viktor realized that the intestinal extracts were a 'gold mine' for various peptides of which nothing was known about their structure or action in the body. Historically all Viktor's marvellous achievements in the field of the intestinal peptides are based on his early work with animal organ extracts.

Viktor would have remained a technician but he realized early on that in order to be able to do what he wanted in the future he must have an academic degree. I admit that I am breathless with admiration when I consider that he took an MB at the Karolinska Institutet in 1953 and defended a thesis for MD in 1959, nearly all the time keeping up hard laboratory work. Viktor was loyal to his old employer and benefactor, Erik Jorpes, until Erik died, and, luckily for the advancement of science, he has to this day been loyal to the field of intestinal peptides.

In Viktor's laboratory he and his guest scientists have isolated chymodenin, GIP (**G**astric **I**nhibitory – or **G**lucose dependent **I**nsulinotropic – **P**olypeptide), motilin, oxyntomodulin, a previously unknown form of somatostatin, VIP (**V**asoactive **I**ntestinal **P**eptide) and sorbin. In collaboration with T. J. McDonald, the bombesin-related GRP (**G**astric **R**eleasing **P**eptide) was isolated from porcine non-antralgastric tissue. In collaboration with K. Tatemoto, a chemical method was worked out for the determination of hormonal peptides with a C-terminal amidated alpha structure. This technique in turn led to the discovery of PHI (**P**eptide with N-terminal **H**istidine and C-terminal **I**soleucine amide), NPY (neuropeptide Y (tyrosine)) and galanin (a peptide with N-terminal **g**lycine and C-terminal **alanin**e amide). At Viktor Mutt's laboratory, species varieties were studied (e.g. human secretin, bovine, GIP), using radioreceptor assay techniques. Recently Viktor, together with Agerberth, Z.-W., Chen, Efendic and Östensson, have isolated from pig intestine a 60-residue polypeptide **PEC**-60 (**p**eptide with N-terminal glutamic acid (**E**) and C-terminal cysteine (**C**)). PEC-60 has a depressant activity on insulin secretion and a structural similarity to PSTI (**p**ancreatic **s**ecretory **t**rypsin **i**nhibitor). Several antibacterial peptides have been isolated from pig intestine by Viktor Mutt in collaboration with Hans Boman and his coworkers. One of these has been characterized as a cecropin, a type of antibacterial peptide until now only known to occur in insects (Cecropia). The amino acid sequence determinations of Viktor's various peptides are largely carried out in collaboration with Hans Jörnvall.

Viktor Mutt's lifelong work with animals and human organ extracts has indeed opened a door to a vast field of physiologically active peptides of interest, not only to the field of gastroenterology but also to that of the central nervous system. Many of the peptides will surely be shown in the near future to be of value for our understanding of the biochemical background of psychiatric diseases.

Bertil Åberg, MD, DVMhc, MDhc
President
Skandigen AB
Stockholm

APPENDIX B

In Memoriam (1998)

At the Wenner-Gren Symposium on 'Galanin: Basic Research Discoveries and Therapeutic Implications', which was held in Stockholm in the beginning of May 1998, the participants honoured Viktor Mutt, the discoverer of galanin. Mutt had then worked in peptide research for 50 years, it was 15 years since he had published the first paper on galanin, and he was going to celebrate his 75th birthday at the end of the year. We, who were present at the symposium, are glad that we at that time could express our appreciation. Viktor Mutt died on September 9 after an active day and after attending a meeting at the Royal Swedish Academy of Sciences.

Viktor Mutt came to Sweden from Estonia during the Second World War. He was employed as a technician by Professor Erik Jorpes at the Karolinska Institute in 1944. After a few years' work on heparin, he was asked by Jorpes to purify secretin for clinical use. To this was later added the purification of cholecystokinin and pancreozymin, which Mutt showed to be two activities of the same peptide. It was painstaking work; each step of purification could take years and each fraction had to be assayed on living cats. As a fellow graduate student in the same department during the 1950s it was with admiration that I followed Mutt's work. It would take him close to 20 years to isolate the pure peptides and to determine their structures. A graduate student of today will hardly accept that it takes 15 years of full-time work to get material for a doctoral thesis, which was the time it took Viktor Mutt. The work could never have been finished without his working capacity, tenacity, and patience.

The success with secretin and cholecystokinin merited for Mutt a chair in Biochemistry at the Karolinska Institute, which he held for two decades, and thereafter he worked as an emeritus. During this period, he designed methods to search for new active intestinal peptides, and a large number with important functions were discovered in his laboratory. One of them was galanin. Viktor Mutt was devoted to science. He was always meticulous in his work and scrupulous in his judgements. By his work he laid the foundation for modern neuropeptide research. He received honours but he never liked to be in the limelight. He has always been unobtrusive and humble, and it has sometimes been difficult to imagine that he held the eminent position that he did in the scientific community. He was a great man and a great scientist.

Torvard C. Laurent
Science Secretary
Wenner-Gren Foundation

REFERENCES

Andersson, P.-O., Bloom, S. R, Edwards, A. V. and Järhult, J. 1982. Effects of stimulation of the chorda tympani in bursts on submaxillary responses in the cat. *J. Physiol.*, **322**(1), 469–483. https://doi.org/10.1113/jphysiol.1982.sp014050

Aston-Jones, G., Rajkowski, J., Kubiak, P., Valentino, R. J. and Shipley, M. T. 1996. Role of the locus coeruleus in emotional activation. *Prog. Brain Res.*, **107**, 379–402. https://doi.org/10.1016/S0079-6123%2808%2961877-4

Banks, W. A. 2023. Viktor Mutt lecture: Peptides can cross the blood–brain barrier. *Peptides*, **169**, 171079. https://doi.org/10.1016/j.peptides.2023.171079

Barde, S. and Hökfelt, T. qPCR analysis of postmortem human dorsal root ganglia. Unpublished results.

Barde, S., Rüegg, J., Prud'homme, J., Ekström, T. J., Palkovits, M., Turecki, G. et al. 2016. Alterations in the neuropeptide galanin system in major depressive disorder involve levels of transcripts, methylation, and peptide. *Proc. Natl. Acad. Sci. U. S. A.*, **113**(52), E8472–E8481. https://doi.org/10.1073/pnas.1617824113

Barde, S., Aguila, J., Zhong, W., Solarz, A., Mei, I., Prud'homme, J. et al. 2024. Substance P, NPY, CCK and their receptors in five brain regions in major depressive disorder with transcriptomic analysis of locus coeruleus neurons. *Eur. Neuropsychopharmacol.* **78**, 54–63. https://doi.org/10.1016/j.euroneuro.2023.09.004

Barnabas, K., Zhang, L., Wang, H., Kirouac, G. and Vrontakis, M. 2016. Changes in galanin systems in a rat model of post-traumatic stress disorder (PTSD). *PLOS ONE*, **11**(12), e0167569. https://doi.org/10.1371/journal.pone.0167569

Bartfai, T., Fisone, G. and Langel, Ü. 1992. Galanin and galanin antagonists: molecular and biochemical perspectives. *Trends Pharmacol. Sci.*, **13**(8), 312–317. https://doi.org/10.1016/0165-6147(92)90098-Q

Bayless, D. W., Davis, C. O., Yang, R., Wei, Y., de Andrade Carvalho, V. M., Knoedler, J. R. et al. 2023. A neural circuit for male sexual behavior and reward. *Cell*, **186**(18), 3862–3881. https://doi.org/10.1016/j.cell.2023.07.021

Bing, O., Möller, C., Engel, J. A., Söderpalm, B. and Heilig, M. 1993. Anxiolytic-like action of centrally administered galanin. *Neurosci. Lett.*, **164**(1–2), 17–20. https://doi.org/10.1016/0304-3940(93)90846-D

Bouras, C., Magistretti, P. J. and Morrison, J. H. 1986. An immunohistochemical study of six biologically active peptides in the human brain. *Hum. Neurobiol.*, **5**(4), 213–226.

Bowers, C. W. Superfluous neurotransmitters? 1994. *Trends Neurosci.*, **17**(8), 315–320. https://doi.org/10.1016/0166-2236(94)90168-6

Burbach, J. P. H. 2010. Neuropeptides from concept to online database www.neuropeptides.nl. *Eur. J. Pharmacol.*, **626**(1), 27–48. https://doi.org/10.1016/j.ejphar.2009.10.015

Burgunder, J.-M. and Young, W. S. III. 1988. The distribution of thalamic projection neurons containing cholecystokinin messenger RNA, using in situ hybridization histochemistry and retrograde labeling. *Mol. Brain Res.*, **4**(3), 179–189. https://doi.org/10.1016/0169-328X(88)90024-1

Caramia, M., Romanov, R. A., Sideromenos, S., Hevesi, Z., Zhao, M., Krasniakova, M. et al. 2023. Neuronal diversity of neuropeptide signaling, including galanin, in the mouse locus coeruleus. *Proc. Natl. Acad. Sci. U. S. A.*, **120**(31), e2222095120. https://doi.org/10.1073/pnas.2222095120

Carlsson, A., Falck, B. and Hillarp, N. A. 1962. Cellular localization of brain monoamines. *Acta Physiol. Scand. Suppl.*, **56**(196), 1–28.

Chang, M. M., Leeman, S. E. and Niall, H. D. 1971. Amino-acid sequence of substance P. *Nat. New Biol.*, **232**, 86–87. https://doi.org/10.1038/newbio232086a0

Chan-Palay, V., Jonsson, G. and Palay, S. L. 1978. Serotonin and substance P coexist in neurons of the rat's central nervous system. *Proc. Natl. Acad. Sci. U. S. A.*, **75**(3), 1582–1586. https://doi.org/10.1073/pnas.75.3.1582

Christiansen, S. H., Olesen, M. V., Wörtwein, G. and Woldbye, D. P. 2011. Fluoxetine reverts chronic restraint stress-induced depression-like behaviour and increases neuropeptide Y and galanin expression in mice. *Behav. Brain Res.*, **216**(2), 585–591. https://doi.org/10.1016/j.bbr.2010.08.044

Costigan, M., Befort, K., Karchewski, L., Griffin, R. S., D'Urso, D., Allchorne, A. et al. 2002. Replicate high-density rat genome oligonucleotide microarrays reveal hundreds of regulated genes in the dorsal root ganglion after peripheral nerve injury. *BMC Neurosci.*, **3**, 16. https://doi.org/10.1186/1471-2202-3-16

da Conceição Machado, F., de Souza, L. V., Rangel, M., Jara, Z. P. and do Carmo Franco, M. 2018. Implication of galanin gene *rs948854* polymorphism in depressive symptoms in adolescents. *Horm. Behav.*, **97**, 14–17. https://doi.org/10.1016/j.yhbeh.2017.10.001

Dahlström, A. and Fuxe, K. 1964. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand. Suppl*., **232**, 1–55.

Davidson, S., Lear, M., Shanley, L., Hing, B., Baizan-Edge, A., Herwig, A. et al. 2011. Differential activity by polymorphic variants of a remote enhancer that supports galanin expression in the hypothalamus and amygdala: implications for obesity, depression and alcoholism. *Neuropsychopharmacology*, **36**(11), 2211–2221. https://doi.org/10.1038/npp.2011.93

DeFelipe, J., López-Cruz, P. L., Benavides-Piccione, R., Bielza, C., Larrañaga, P., Anderson, S. et al. 2013. New insights into the classification and nomenclature of cortical GABAergic interneurons. *Nat. Rev. Neurosci.*, **14**, 202–216. https://doi.org/10.1038/nrn3444

Demsie, D. G., Altaye, B. M., Weldekidan, E., Gebremedhin, H., Alema, N. M., Tefera, M. M. et al. 2020. Galanin receptors as drug target for novel antidepressants: review. *Biologics*, **14**, 37–45. https://doi.org/10.2147/BTT.S240715

de Souza, M. M., Silote, G. P., Herbst, L. S., Funck, V. R., Joca, S. R. L. and Beijamini, V. 2018. The antidepressant-like effect of galanin in the dorsal raphe nucleus of rats involves GAL_2 receptors. *Neurosci. Lett.*, **681**, 26–30. https://doi.org/10.1016/j.neulet.2018.05.029

De Wied, D. 1969. Effects of peptide hormones on behavior. In *Frontiers in Neuroendocrinology* (Ganong, W. F. and Martini, L., eds). Oxford University Press, New York, 97–140.

De Wied D. 1977. Peptides and behavior. *Life Sci.*, **20**(2), 195–204. https://doi.org/10.1016/0024-3205(77)90313-7

Díaz-Cabiale, Z., Flores-Burgess, A., Parrado, C., Narváez, M., Millón, C., Puigcerver, A. et al. 2014. Galanin receptor/

neuropeptide Y receptor interactions in the central nervous system. *Curr. Protein Pept. Sci.*, **15**(7), 666–672. https://doi.org/10.2174/1389203715666140901111709

Drevets, W. C., Savitz, J. and Trimble, M. 2008. The subgenual anterior cingulate cortex in mood disorders. *CNS Spectr.*, **13**(8), 663–681. https://doi.org/10.1017/s1092852900013 754

Dubner, R. and Ruda, M. A. 1992. Activity-dependent neuronal plasticity following tissue injury and inflammation. *Trends Neurosci.*, **15**(3), 96–103. https://doi.org/10.1016/0166-2236(92)90019-5

Du Vigneaud, V. 1954. Hormones of the posterior pituitary gland: oxytocin and vasopressin. *Harvey Lect.*, **50**, 1–26.

Eiden, L. E., Hernández, V. S., Jiang, S. Z. and Zhang, L. 2022. Neuropeptides and small-molecule amine transmitters: cooperative signaling in the nervous system. *Cell. Mol. Life Sci.*, **79**, 492. https://doi.org/10.1007/s00018-022-04451-7

Erspamer, V., Melchiorri, P., Erspamer, C. F. and Negri, L. 1978. Polypeptides of the amphibian skin active on the gut and their mammalian counterparts. *Adv. Exp. Med. Biol.*, **106**, 51–64. https://doi.org/10.1007/978-1-4684-7248-6_6

Euler, U. S. v. and Gaddum, J. H. 1931. An unidentified depressor substance in certain tissue extracts. *J. Physiol.*, **72**(1), 74–87. https://doi.org/10.1113/jphysiol.1931.sp002763

Flores-Burgess, A., Millón, C., Gago, B., García-Durán, L., Cantero-García, N., Puigcerver, A. et al. 2022. Galanin (1–15) enhances the behavioral effects of fluoxetine in the olfactory bulbectomy rat, suggesting a new augmentation strategy in depression. *Int. J. Neuropsychopharmacol.*, **25**(4), 307–318. https://doi.org/10.1093/ijnp/pyab089

Fonseca-Rodrigues, D., Almeida, A. and Pinto-Ribeiro, F. 2022. A new gal in town: a systematic review of the role of galanin and its receptors in experimental pain. *Cells*, 2022, **11**(5), 839. https://doi.org/10.3390/cells11050839

Foote, S. L., Bloom, F. E. and Aston-Jones, G. 1983. Nucleus locus ceruleus: new evidence of anatomical and physiological specificity. *Physiol. Rev.*, **63**(3), 844–914. https://doi.org/10.1152/physrev.1983.63.3.844

Freimann, K., Kurrikoff, K. and Langel, Ü. 2015. Galanin receptors as a potential target for neurological disease. *Expert Opin. Ther. Targets*, **19**(12), 1665–1676. https://doi.org/10.1517/14728222.2015.1072513

Fuxe, K., Jansson, A., Diaz-Cabiale, Z., Andersson, A., Tinner, B., Finnman, U. B. et al. 1998. Galanin modulates 5-hydroxytryptamine functions. Focus on galanin and galanin fragment/5-hydroxytryptamine$_{1A}$ receptor interactions in the brain. *Ann. N. Y. Acad. Sci.*, **863**, 274–290. https://doi.org/10.1111/j.1749-6632.1998.tb10702.x

Fuxe, K., Dahlström, A. B., Jonsson, G., Marcellino, D., Guescini, M., Dam, M. et al. 2010. The discovery of central monoamine neurons gave volume transmission to the wired brain. *Prog. Neurobiol.*, **90**(2), 82–100. https://doi.org/10.1016/j.pneurobio.2009.10.012

Geffen, L. B., Livett, B. G. and Rush, R. A. 1969. Immunohistochemical localization of protein components of catecholamine storage vesicles. *J. Physiol.*, **204**(3), 593–605. https://doi.org/ 10.1113/jphysiol.1969.sp008934

Genders, S. G., Scheller, K. J. and Djouma, E. 2020. Neuropeptide modulation of addiction: focus on galanin. *Neurosci. Biobehav. Rev.*, **110**, 133–149. https://doi.org/10.1016/j.neubiorev.2018.06.021

Glock, C., Heumüller, M. and Schuman, E. M. 2017. mRNA transport & local translation in neurons. *Curr. Opin. Neurobiol.*, **45**, 169–177. https://doi.org/10.1016/j.conb.2017.05.005

Gonda, X., Hullam, G., Antal, P., Eszlari, N., Petschner, P., Hökfelt, T. G. et al. 2018. Significance of risk polymorphisms for depression depends on stress exposure. *Sci. Rep.*, **8**, 3946. https://doi.org/10.1038/s41598-018-22221-z

Griebel, G. and Holsboer, F. 2012. Neuropeptide receptor ligands as drugs for psychiatric diseases: the end of the beginning? *Nat. Rev. Drug Discov.,* **11**, 462–478. https://doi.org/10.1038/nrd3702

Guillaumin, M. C. C. and Burdakov, D. 2021. Neuropeptides as primary mediators of brain circuit connectivity. *Front. Neurosci.*, **15**, 644313. https://doi.org/10.3389/fnins.2021.644313

Guillemin, R. 1978. Control of adenohypophysial functions by peptides of the central nervous system. *Harvey Lect.*, **71**, 71–131. https://pubmed.ncbi.nlm.nih.gov/101483/

Hargreaves, R., Ferreira, J. C. A., Hughes, D., Brands, J., Hale, J., Mattson, B. et al. 2011. Development of aprepitant, the first neurokinin-1 receptor antagonist for the prevention of chemotherapy-induced nausea and vomiting. *Ann. N. Y. Acad. Sci.*, **1222**, 40–48. https://doi.org/10.1111/j.1749-6632.2011.05961.x

He, B., Fang, P., Guo, L., Shi, M., Zhu, Y., Xu, B. et al. 2017. Beneficial effects of neuropeptide galanin on reinstatement of exercise-induced somatic and psychological trauma. *J. Neurosci. Res.*, **95**(4), 1036–1043. https://doi.org/10.1002/jnr.23869

Heilig, M. 2004. The NPY system in stress, anxiety and depression. *Neuropeptides*, **38**(4), 213–224. https://doi.org/10.1016/j.npep.2004.05.002

Hobson, S.-A., Bacon, A., Elliot-Hunt, C. R., Holmes, F. E., Kerr, N. C. H., Pope, R. et al. 2010. Galanin acts as a trophic factor to the central and peripheral nervous systems. In *Galanin. Experientia Supplematum* (Hökfelt, T., ed.). Springer, Basel, **102**, 25–38.

Hodge, R. D., Bakken, T. E., Miller, J. A., Smith, K. A., Barkan, E. R., Graybuck, L. T. et al. 2019. Conserved cell types with divergent features in human versus mouse cortex. *Nature*, **573**, 61–68. https://doi.org/10.1038/s41586-019-1506-7

Hökfelt, T. 1991. Neuropeptides in perspective: the last ten years. *Neuron*, **7**(6), 867–879. https://doi.org/10.1016/0896-6273(91)90333-u

Hökfelt, T. (ed.). 2010. *Galanin. Exp. Suppl.*, **102**. Springer, Basel.

Hökfelt, T. and Crawley, J. (eds). 2005. *Special Issue on Galanin.* In *Proceedings of the Third International Symposium on Galanin and Its Receptors, San Diego, California, USA, 21–22 October 2004.* Neuropeptides, **39**, 125–362.

Hökfelt, T. and Wiesenfeld-Hallin, Z. 2024. The therapeutic potential of galanin in the management of pain – a review article. *Dan. Med. J.*, **71**(2), A10230653. https://doi.org/10.61409/a10230653

Hökfelt, T., Kellerth, J. O., Nilsson, G. and Pernow, B. 1975. Substance P: localization in the central nervous system and in some primary sensory neurons. *Science*, **190**(4217), 889–890. https://doi.org/10.1126/science.242075

Hökfelt, T., Ljungdahl, A., Terenius, L., Elde, R. and Nilsson, G. 1977. Immunohistochemical analysis of peptide pathways

possibly related to pain and analgesia: enkephalin and substance P. *Proc. Natl. Acad. Sci. U. S. A.*, **74**(7), 3081–3085. https://doi.org/10.1073/pnas.74.7.3081

Hökfelt, T., Ljungdahl, A., Steinbusch, H., Verhofstad, A., Nilsson, G., Brodin, E. et al. 1978. Immunohistochemical evidence of substance P-like immunoreactivity in some 5-hydroxytryptamine-containing neurons in the rat central nervous system. *Neuroscience*, **3**(6), 517–538. https://doi.org/10.1016/0306-4522(78)90017-9

Hökfelt, T., Rehfeld, J. F., Skirboll, L., Ivemark, B., Goldstein, M. and Markey, K. 1980a. Evidence for coexistence of dopamine and CCK in meso-limbic neurones. *Nature*, **285**, 476–478. https://doi.org/10.1038/285476a0

Hökfelt, T., Johansson, O., Ljungdahl, Å., Lundberg, J. M. and Schultzberg, M. 1980b. Peptidergic neurones. *Nature*, **284**, 515–521. https://doi.org/10.1038/284515a0

Hökfelt, T., Millhorn, D., Seroogy, K., Tsuruo, Y., Ceccatelli, S., Lindh, B. et al. 1987. Coexistence of peptides with classical neurotransmitters. *Experientia*, **43**, 768–780. https://doi.org/10.1007/BF01945354

Hökfelt, T., Bartfai, T., Jacobowitz, D. and Ottoson, D. (eds). 1991. Galanin. A New Multifunctional Peptide in The Neuro-Endocrine System. *Wenner-Gren Symposium*, **58**. Macmillan, London. https://doi.org/10.1007/978-1-349-12664-4

Hökfelt, T., Bartfai, T. and Crawley, J. (eds). 1998. Galanin: Basic Research Discoveries and Therapeutic Implications. *Ann. N. Y. Acad. Sci.*, **863**(1), 1–469.

Hökfelt, T., Barde, S., Xu, Z. D., Kuteeva, E., Rüegg, J., Le Maitre, E. et al. 2018. Neuropeptide and small transmitter coexistence: fundamental studies and relevance to mental illness. *Front. Neural Circuits*, **12**, 106. https://doi.org/10.3389/fncir.2018.00106

Holmes, P. V. 2014. Trophic mechanisms for exercise-induced stress resilience: potential role of interactions between BDNF and galanin. *Front. Psychiatry*, **5**, 90. https://doi.org/10.3389/fpsyt.2014.00090

Holmes, A. and Picciotto, M. R. 2006. Galanin: a novel therapeutic target for depression, anxiety disorders and drug addiction? *CNS Neurol. Disord. Drug Targets*, **5**(2), 225–232. http://dx.doi.org/10.2174/187152706776359600

Holmes, A., Heilig, M., Rupniak, N. M. J., Steckler, T. and Griebel, G. 2003. Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. *Trends Pharmacol. Sci.*, **24**(11), 580–588. https://doi.org/10.1016/j.tips.2003.09.011

Holmes, F. E., Mahoney, S.-A. and Wynick, D. 2005. Use of genetically engineered transgenic mice to investigate the role of galanin in the peripheral nervous system after injury. *Neuropeptides*, **39**(3), 191–199. https://doi.org/10.1016/j.npep.2005.01.001

Jörnvall, H., Agerberth, B. and Zasloff, M. 2008. Viktor Mutt: a giant in the field of bioactive peptides. *Compr. Biochem.*, **46**, 397–416. https://doi.org/10.1016/S0069-8032(08)00006-5

Juhasz, G., Hullam, G., Eszlari, N., Gonda, X., Antal, P., Anderson, I. M. et al. 2014. Brain galanin system genes interact with life stresses in depression-related phenotypes. *Proc. Natl. Acad. Sci. U. S. A.*, **111**(16), E1666–1673. https://doi.org/10.1073/pnas.1403649111

Kadriu, B., Musazzi, L., Henter, I. D., Graves, M., Popoli, M. and Zarate, C. A., Jr. 2019. Glutamatergic neurotransmission: pathway to developing novel rapid-acting antidepressant treatments. *Int. J. Neuropsychopharmacol.*, **22**(2), 119–135. https://doi.org/10.1093/ijnp/pyy094

Kastin, A. J. (ed.). 2013. *Handbook of Biologically Active Peptides.* 2nd ed. Academic Press/Elsevier, San Diego, London, Waltham, MA. https://doi.org/10.1016/C2010-0-66490-X

Kautz, M., Charney, D. S. and Murrough, J. W. 2017. Neuropeptide Y, resilience, and PTSD therapeutics. *Neurosci. Lett.*, **649**, 164–169. https://doi.org/10.1016/j.neulet.2016.11.061

Kawa, L., Barde, S., Arborelius, U. P., Theodorsson, E., Agoston, D., Risling, M. et al. 2016. Expression of galanin and its receptors are perturbed in a rodent model of mild, blast-induced traumatic brain injury. *Exp. Neurol.*, **279**, 159–167. https://doi.org/10.1016/j.expneurol.2016.02.019

Kerr, B. J., Wynick, D., Thompson, S. W. and McMahon, S. B. 2000. The biological role of galanin in normal and neuropathic states. *Prog. Brain Res.*, **129**, 219–230. https://doi.org/10.1016/s0079-6123(00)29016-x

Keszler, G., Molnár, Z., Rónai, Z., Sasvári-Székely, M., Székely, A. and Kótyuk, E. 2019. Association between anxiety and non-coding genetic variants of the galanin neuropeptide. *PLOS ONE*, **14**(12), e0226228. https://doi.org/10.1371/journal.pone.0226228

Konkel, M. J., Lagu, B., Boteju, L. W., Jimenez, H., Noble, S., Walker, M. W. et al. 2006. 3-arylimino-2-indolones are potent and selective galanin GAL3 receptor antagonists. *J. Med. Chem.*, **49**(13), 3757–3758. https://doi.org/10.1021/jm060001n

Kramer, M. S., Cutler, N., Feighner, J., Shrivastava, R., Carman, J., Sramek, J. J. et al. 1998. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science*, **281**(5383), 1640–1645. https://doi.org/10.1126/science.281.5383.1640

Krystal, J. H., Abdallah, C. G., Sanacora, G., Charney, D. S. and Duman, R. S. 2019. Ketamine: a paradigm shift for depression research and treatment. *Neuron*, **101**(5), 774–778. https://doi.org/10.1016/j.neuron.2019.02.005

Kuteeva, E., Wardi, T., Lundström, L., Sollenberg, U., Langel, Ü., Hökfelt, T. et al. 2008. Differential role of galanin receptors in the regulation of depression-like behavior and monoamine/stress-related genes at the cell body level. *Neuropsychopharmacology*, **33**(11), 2573–2585. https://doi.org/10.1038/sj.npp.1301660

Kuteeva, E., Hökfelt, T., Wardi, T. and Ogren, S. O. 2010. Galanin, galanin receptor subtypes and depression-like behaviour. *Exp. Suppl.*, **102**, 163–181. https://doi.org/10.1007/978-3-0346-0228-0_12

Landry, M., Åman, K., Dostrovsky, J., Lozano, A. M., Carlstedt, T., Spenger, C. et al. 2003. Galanin expression in adult human dorsal root ganglion neurons: initial observations. *Neuroscience*, **117**(4), 795–809. https://doi.org/10.1016/S0306-4522(02)00965-X

Landry, M., Bouali-Benazzouz, R., André, C., Shi, T. J. S., Léger, C., Nagy, F. et al. 2006. Galanin receptor 1 is expressed in a subpopulation of glutamatergic interneurons in the dorsal horn of the rat spinal cord. *J. Comp. Neurol.*, **499**(3), 391–403. https://doi.org/10.1002/cne.21109

Lang, R., Gundlach, A. L., Holmes, F. E., Hobson, S. A., Wynick, D., Hökfelt, T. et al. 2015. Physiology, signaling, and pharmacology of galanin peptides and receptors: three

decades of emerging diversity. *Pharmacol. Rev.*, **67**(1), 118–175. https://doi.org/10.1124/pr.112.006536

Le Maître, E., Barde, S. S., Palkovits, M., Diaz-Heijtz, R. and Hökfelt, T. G. 2013. Distinct features of neurotransmitter systems in the human brain with focus on the galanin system in locus coeruleus and dorsal raphe. *Proc. Natl. Acad. Sci. U. S. A.*, **110**(6), E536–545. https://doi.org/10.1073/pnas.1221378110

Li, X., Andrusivova, Z., Czarnewski, P., Langseth, C. M., Andersson, A., Liu, Y. et al. 2023. Profiling spatiotemporal gene expression of the developing human spinal cord and implications for ependymoma origin. *Nat. Neurosci.*, **26**, 891–901. https://doi.org/10.1038/s41593-023-01312-9

Liu, H.-X. and Hökfelt, T. The participation of galanin in pain processing at the spinal level. 2002. *Trends Pharmacol. Sci.*, **23**(10), 468–474. https://doi.org/10.1016/S0165-6147(02)02074-6

Liu, H.-X., Brumovsky, P., Schmidt, R., Brown, W., Payza, K., Hodzic, L. et al. 2001. Receptor subtype-specific pronociceptive and analgesic actions of galanin in the spinal cord: selective actions via GalR1 and GalR2 receptors. *Proc. Natl. Acad. Sci. U. S. A.*, **98**(17), 9960–9964. https://doi.org/10.1073/pnas.161293598

Ljungdahl, Å., Hökfelt, T. and Nilsson, G. 1978. Distribution of substance P-like immunoreactivity in the central nervous system of the rat—I. Cell bodies and nerve terminals. *Neuroscience*, **3**(10), 861–943. https://doi.org/10.1016/0306-4522(78)90116-1

Lu, X., Barr, A. M., Kinney, J. W., Sanna, P., Conti, B., Behrens, M. M. et al. 2005. A role for galanin in antidepressant actions with a focus on the dorsal raphe nucleus. *Proc. Natl. Acad. Sci. U. S. A.* **102**(3), 874–879. https://doi.org/10.1073/pnas.0408891102

Lu, X., Sharkey, L. and Bartfai T. 2007. The brain galanin receptors: targets for novel antidepressant drugs. *CNS Neurol. Disord. Drug Targets*, **6**(3), 183–192. https://doi.org/10.2174/187152707780619335

Ludwig, M. and Leng, G. Dendritic peptide release and peptide-dependent behaviours. *Nat. Rev. Neurosci.*, **7**, 126–136. https://doi.org/10.1038/nrn1845

Lundberg, J. M., Anggård, A., Emson, P., Fahrenkrug, J. and Hökfelt, T. 1981. Vasoactive intestinal polypeptide and cholinergic mechanisms in cat nasal mucosa: studies on choline acetyltransferase and release of vasoactive intestinal polypeptide. *Proc. Natl. Acad. Sci. U. S. A.*, **78**(8), 5255–5259. https://doi.org/10.1073/pnas.78.8.5255

Ma, X., Tong, Y. G., Schmidt, R., Brown, W., Payza, K., Hodzic, L. et al. 2001. Effects of galanin receptor agonists on locus coeruleus neurons. *Brain Res.*, **919**(1), 169–174. https://doi.org/10.1016/s0006-8993(01)03033-5

Masu, Y., Nakayama, K., Tamaki, H., Harada, Y., Kuno, M. and Nakanishi, S. 1987. cDNA cloning of bovine substance-K receptor through oocyte expression system. *Nature*, **329**, 836–838. https://doi.org/10.1038/329836a0

Mathé, A. A., Michaneck, M., Berg, E., Charney, D. S. and Murrough, J. W. 2020. A randomized controlled trial of intranasal neuropeptide Y in patients with major depressive disorder. *Int. J. Neuropsychopharmacol.*, **23**(12), 783–790. https://doi.org/10.1093%2Fijnp%2Fpyaa054

McDonald, T. J., Nilsson, G., Vagne, M., Ghatei, M., Bloom, S. R. and Mutt, V. 1978. A gastrin releasing peptide from the porcine nonantral gastric tissue. *Gut*, **19**(9), 767–774. https://doi.org/ 10.1136/gut.19.9.767

Melander, T., Staines, W. A., Hökfelt, T., Rökaeus, Å., Eckenstein, F., Salvaterra, P. M. et al. 1985. Galanin-like immunoreactivity in cholinergic neurons of the septum-basal forebrain complex projecting to the hippocampus of the rat. *Brain Res.*, **360**(1–2), 130–138. https://doi.org/10.1016/0006-8993(85)91228-4

Melander, T., Hökfelt, T., Rökaeus, A., Cuello, A. C., Oertel, W. H., Verhofstad, A. et al. 1986a. Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. *J. Neurosci.*, **6**(12), 3640–3654. https://doi.org/10.1523/jneurosci.06-12-03640.1986

Melander, T., Hökfelt, T. and Rökaeus, A. 1986b. Distribution of galaninlike immunoreactivity in the rat central nervous system. *J. Comp. Neurol.*, **248**(4), 475–517. https://doi.org/10.1002/cne.902480404

Merchenthaler, I., López, F. J. and Negro-Vilar, A. 1993. Anatomy and physiology of central galanin-containing pathways. *Prog. Neurobiol.*, **40**(6), 711–769. https://doi.org/10.1016/0301-0082(93)90012-h

Millón, C., Flores-Burgess, A., Narváez, M., Borroto-Escuela, D. O., Gago, B., Santín, L. et al. 2017. The neuropeptides galanin and galanin(1–15) in depression-like behaviours. *Neuropeptides*, **64**, 39–45. https://doi.org/10.1016/j.npep.2017.01.004

Mills, E. G., Izzi-Engbeaya, C., Abbara, A., Comninos, A. N. and Dhillo, W. S. 2021. Functions of galanin, spexin and kisspeptin in metabolism, mood and behaviour. *Nat. Rev. Endocrinol.*, **17**(2), 97–113. https://doi.org/10.1038/s41574-020-00438-1

Mirabeau, O., Perlas, E., Severini, C., Audero, E., Gascuel, O., Possenti, R. et al. 2007. Identification of novel peptide hormones in the human proteome by hidden Markov model screening. *Genome Res.*, **17**(3), 320–327. https://doi.org/10.1101/gr.5755407

Morales-Medina, J. C., Dumont, Y. and Quirion, R. 2010. A possible role of neuropeptide Y in depression and stress. *Brain Res.*, **1314**, 194–205. https://doi.org/10.1016/j.brainres.2009.09.077

Moroz, L. L. 2015. Convergent evolution of neural systems in ctenophores. *J. Exp. Biol.*, **218**(Pt 4), 598–611. https://doi.org/10.1242/jeb.110692

Murck, H., Held, K., Ziegenbein, M., Künzel, H., Holsboer, F. and Steiger, A. 2004. Intravenous administration of the neuropeptide galanin has fast antidepressant efficacy and affects the sleep EEG. *Psychoneuroendocrinology*, **29**(9), 1205–1211. https://doi.org/10.1016/j.psyneuen.2004.02.006

Mutt, V. 1982. Chemistry of the gastrointestinal hormones and hormone-like peptides and a sketch of their physiology and pharmacology. *Vitam. Horm.*, **39**, 231–427. https://doi.org/10.1016/s0083-6729(08)61138-3

Mutt, V. 1990. Recent developments in the chemistry of gastrointestinal peptides. *Eur. J. Clin. Invest.*, **20**(1), 2–9. https://doi.org/10.1111/j.1365-2362.1990.tb01770.x

Mutt, V. and Jorpes, J. E. 1968. Structure of porcine cholecystokinin-pancreozymin. Cleavage with thrombin and with trypsin. *Eur. J. Biochem.*, **6**(1), 156–162. https://doi.org/10.1111/j.1432-1033.1968.tb00433.x

Mutt, V. and Said, S. I. 1974. Structure of the porcine vasoactive intestinal octacosapeptide. The amino-acid sequence. Use of kallikrein in its determination. *Eur. J. Biochem.*, **42**(2), 581–589. https://doi.org/10.1111/j.1432-1033.1974.tb03373.x

Nahvi, R. J. and Sabban, E. L. 2020. Sex differences in the neuropeptide Y system and implications for stress related disorders. *Biomolecules*, **10**(9), 1248. https://doi.org/10.3390/biom10091248

Nelson, T. S. and Taylor, B. K. 2021. Targeting spinal neuropeptide Y1 receptor-expressing interneurons to alleviate chronic pain and itch. *Prog. Neurobiol.*, **196**, 101894. https://doi.org/10.1016/j.pneurobio.2020.101894

Ohtaki, T., Kumano, S., Ishibashi, Y., Ogi, K., Matsui, H., Harada, M. et al. 1999. Isolation and cDNA cloning of a novel galanin-like peptide (GALP) from porcine hypothalamus. *J. Biol. Chem.*, **274**(52), 37041–37045. https://doi.org/10.1074/jbc.274.52.37041

Oliveira Volpe, C. M., Vaz, T., Rocha-Silva, F., Villar-Delfino, P. H. and Nogueira-Machado, J. A. 2020. Is galanin a promising therapeutic resource for neural and nonneural diseases? *Curr. Drug Targets*, **21**(9), 922–929. https://doi.org/10.2174/1389450121666200225112055

Osakada, T., Yan, R., Jiang, Y., Wei, D., Tabuchi, R., Dai, B. et al. 2024. A dedicated hypothalamic oxytocin circuit controls aversive social learning. *Nature*, **626**, 347–356. https://doi.org/10.1038/s41586-023-06958-w

Pérez de la Mora, M., Borroto-Escuela, D. O., Crespo-Ramírez, M., Rejón-Orantes, J. D. C., Palacios-Lagunas, D. A., Martínez-Mata, M. K. et al. 2022. Dysfunctional heteroreceptor complexes as novel targets for the treatment of major depressive and anxiety disorders. *Cells*, **11**(11), 1826. https://doi.org/10.3390/cells11111826

Pernow, B. 1953a. Distribution of substance P in the central and peripheral nervous system. *Nature*, **171**(4356), 746. https://doi.org/10.1038/171746a0

Pernow, B. 1953b. Studies on substance P: purification, occurrence and biological actions. *Acta Physiol. Scand. Suppl.*, **29**(105), 1–89.

Pieribone, V. A., Xu, Z. Q., Zhang, X., Grillner, S., Bartfai, T. and Hökfelt, T. 1995. Galanin induces a hyperpolarization of norepinephrine-containing locus coeruleus neurons in the brainstem slice. *Neuroscience*, **64**(4), 861–874. https://doi.org/10.1016/0306-4522(94)00450-j

Pizzagalli, D. A., Webb, C. A., Dillon, D. G., Tenke, C. E., Kayser, J., Goer, F. et al. 2018. Pretreatment rostral anterior cingulate cortex theta activity in relation to symptom improvement in depression: a randomized clinical trial. *JAMA Psychiatry*, **75**(6), 547–554. https://doi.org/10.1001/jamapsychiatry.2018.0252

Rökaeus, A. and Brownstein, M. J. 1986. Construction of a porcine adrenal medullary cDNA library and nucleotide sequence analysis of two clones encoding a galanin precursor. *Proc. Natl. Acad. Sci. U. S. A.*, **83**(17), 6287–6291. https://doi.org/10.1073/pnas.83.17.6287

Rökaeus, A., Melander, T., Hökfelt, T., Lundberg, J. M., Tatemoto, K., Carlquist, M. et al. 1984. A galanin-like peptide in the central nervous system and intestine of the rat. *Neurosci. Lett.*, **47**(2), 161–166. https://doi.org/10.1016/0304-3940(84)90423-3

Saar, I., Runesson, J., Järv, J., Kurrikoff, K. and Langel, Ü. 2013. Novel galanin receptor subtype specific ligand in depression like behavior. *Neurochem Res.*, **38**(2), 398–404. https://doi.org/10.1007/s11064-012-0933-8

Sachkova, M. Y., Nordmann, E.-L., Soto-Àngel, J. J., Meeda, Y., Górski, B., Naumann, B. et al. 2021. Neuropeptide repertoire and 3D anatomy of the ctenophore nervous system. *Curr. Biol.*, **31**(23), 5274–5285. https://doi.org/10.1016/j.cub.2021.09.005

Said, S. I. and Mutt, V. 1970. Polypeptide with broad biological activity: isolation from small intestine. *Science*, **169**(3951), 1217–1218. https://doi.org/10.1126/science.169.3951.1217

Santic, R., Schmidhuber, S. M., Lang, R., Rauch, I., Voglas, E., Eberhard, N. et al. 2007. Alarin is a vasoactive peptide. *Proc. Natl. Acad. Sci. U. S. A.*, **104**(24), 10217–10222. https://doi.org/10.1073/pnas.0608585104

Schally, A. V., Coy, D. H. and Meyers, C. A. 1978. Hypothalamic regulatory hormones. *Annu. Rev. Biochem.*, **47**, 89–128. https://doi.org/10.1146/annurev.bi.47.070178.000513

Sciolino, N. R., Smith, J. M., Stranahan, A. M., Freeman, K. G., Edwards, G. L., Weinshenker, D. et al. 2015. Galanin mediates features of neural and behavioral stress resilience afforded by exercise. *Neuropharmacology*, **89**, 255–264. https://doi.org/10.1016/j.neuropharm.2014.09.029

Seutin, V., Verbanck, P., Massotte, L. and Dresse, A. 1989. Galanin decreases the activity of locus coeruleus neurons in vitro. *Eur. J. Pharmacol.*, **164**(2), 373–376. https://doi.org/10.1016/0014-2999(89)90481-0

Sevcik, J., Finta, E. P. and Illes, P. 1993. Galanin receptors inhibit the spontaneous firing of locus coeruleus neurones and interact with μ-opioid receptors. *Eur. J. Pharmacol.*, **230**(2), 223–230. https://doi.org/10.1016/0014-2999(93)90806-s

Shehab, S. A. and Atkinson, M. E. 1986. Vasoactive intestinal polypeptide (VIP) increases in the spinal cord after peripheral axotomy of the sciatic nerve originate from primary afferent neurons. *Brain Res.*, **372**(1), 37–44. https://doi.org/10.1016/0006-8993(86)91456-3

Šípková, J., Kramáriková, I., Hynie, S. and Klenerová, V. 2017. The galanin and galanin receptor subtypes, its regulatory role in the biological and pathological functions. *Physiol. Res.*, **66**(5), 729–740. https://doi.org/10.33549/physiolres.933576

Skofitsch, G. and Jacobowitz, D. M. 1985. Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. *Peptides*, **6**(3), 509–546. https://doi.org/10.1016/0196-9781(85)90118-4

Strand, F. L. 1999. *Neuropeptides: Regulators of Physiological Processes*. MIT Press, Cambridge, MA. https://doi.org/10.7551/mitpress/4950.001.0001

Swanson, C. J., Blackburn, T. P., Zhang, X., Zheng, K., Xu, Z.-Q., Hökfelt, T. et al. 2005. Anxiolytic- and antidepressant-like profiles of the galanin-3 receptor (Gal_3) antagonists SNAP 37889 and SNAP 398299. *Proc. Natl. Acad. Sci. U. S. A.*, **102**(48), 17489–17494. https://doi.org/10.1073/pnas.0508970102. Erratum in: *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**(14), 5632. Erratum in: *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**(40), 14978.

Tatemoto, K. and Mutt, V. 1978. Chemical determination of polypeptide hormones. *Proc. Natl. Acad. Sci. U. S. A.*, **75**(9), 4115–4119. https://doi.org/10.1073/pnas.75.9.4115

Tatemoto, K. and Mutt, V. 1980. Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. *Nature*, **285**(5764), 417–418. https://doi.org/10.1038/285417a0

Tatemoto, K., Carlquist, M. and Mutt, V. 1982. Neuropeptide Y – a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature*, **296**(5858), 659–660. https://doi.org/10.1038/296659a0

Tatemoto, K., Rökaeus, Å., Jörnvall, H., McDonald, T. J. and Mutt, V. 1983. Galanin – a novel biologically active peptide from porcine intestine. *FEBS Lett.*, **164**(1), 124–128. https://doi.org/10.1016/0014-5793(83)80033-7

Tavares-Ferreira, D., Shiers, S., Ray, P. R., Wangzhou, A., Jeevakumar, V., Sankaranarayanan, I. et al. 2022. Spatial transcriptomics of dorsal root ganglia identifies molecular signatures of human nociceptors. *Sci. Transl. Med.*, **14**(632), eabj8186. https://doi.org/10.1126/scitranslmed.abj8186

Tillage, R. P., Sciolino, N. R., Plummer, N. W., Lustberg, D., Liles, L. C., Hsiang, M. et al. 2020. Elimination of galanin synthesis in noradrenergic neurons reduces galanin in select brain areas and promotes active coping behaviors. *Brain Struct. Funct.*, **225**(2), 785–803. https://doi.org/10.1007/s00429-020-02035-4

Ullrich, D. and Mac Gillavry, D. W. 2021. Mini-review: a possible role for galanin in post-traumatic stress disorder. *Neurosci. Lett.*, **756**, 135980. https://doi.org/10.1016/j.neulet.2021.135980

Unschuld, P. G., Ising, M., Erhardt, A., Lucae, S., Kohli, M., Kloiber, S. et al. 2008. Polymorphisms in the galanin gene are associated with symptom-severity in female patients suffering from panic disorder. *J. Affect. Disord.*, **105**(1–3), 177–184. https://doi.org/10.1016/j.jad.2007.05.006

Unschuld, P. G., Ising, M., Roeske, D., Erhardt, A., Specht, M., Kloiber, S. et al. 2010. Gender-specific association of galanin polymorphisms with HPA-axis dysregulation, symptom severity, and antidepressant treatment response. *Neuropsychopharmacology*, **35**, 1583–1592. https://doi.org/10.1038/npp.2010.30

van den Pol, A. N. 2012. Neuropeptide transmission in brain circuits. *Neuron*, **76**(1), 98–115. https://doi.org/10.1016/j.neuron.2012.09.014

Vila-Porcile, E., Xu, Z.-Q., Mailly, P., Nagy, F., Calas, A., Hökfelt, T. et al. 2009. Dendritic synthesis and release of the neuropeptide galanin: morphological evidence from studies on rat locus coeruleus neurons. *J. Comp. Neurol.*, **516**(3), 199–212. https://doi.org/10.1002/cne.22105

Villar, M. J., Cortés, R., Theodorsson, E., Wiesenfeld-Hallin, Z., Schalling, M., Fahrenkrug, J. et al. 1989. Neuropeptide expression in rat dorsal root ganglion cells and spinal cord after peripheral nerve injury with special reference to galanin. *Neuroscience*, **33**(3), 587–604. https://doi.org/10.10 16/0306-4522(89)90411-9

Villar, M. J., Wiesenfeld-Hallin, Z., Xu, X. J., Theodorsson, E., Emson, P. C. and Hökfelt, T. 1991. Further studies on galanin-, substance P-, and CGRP-like immunoreactivities in primary sensory neurons and spinal cord: effects of dorsal rhizotomies and sciatic nerve lesions. *Exp. Neurol.*, **112**(1), 29–39. https://doi.org/10.1016/0014-4886(91)90111-o

Wakisaka, S., Kajander, K. C. and Bennett, G. J. 1991. Increased neuropeptide Y (NPY)-like immunoreactivity in rat sensory neurons following peripheral axotomy. *Neurosci. Lett.*, **124**(2), 200–203. https://doi.org/10.1016/0304-3940(91)900 93-9

Wang, H., Sun, H., Della Penna, K., Benz, R. J., Xu, J., Gerhold, D. L. et al. 2002. Chronic neuropathic pain is accompanied by global changes in gene expression and shares pathobiology with neurodegenerative diseases. *Neuroscience*, **114**(3), 529–546. https://doi.org/10.1016/s0306-4522(02)00341-x

Wang, Y.-J., Li, H., Yang, Y.-T., Tie, C.-L., Li, F., Xu, Z.-Q. et al. 2013. Association of galanin and major depressive disorder in the Chinese Han population. *PLOS ONE*, **8**(5), e64617. https://doi.org/10.1371/journal.pone.0064617

Wang, Y.-J., Yang, Y.-T., Li, H., Liu, P.-Z., Wang, C.-Y. and Xu, Z.-Q. 2014. Plasma galanin is a biomarker for severity of major depressive disorder. *Int. J. Psychiatry Med.,* **48**(2), 109–119. https://doi.org/10.2190/pm.48.2.d

Wang, Y., Wang, M., Yin, S., Jang, R., Wang, J., Xue, Z. et al. 2015. NeuroPep: a comprehensive resource of neuropeptides. *Database*, **2015**, bav038. https://doi.org/10.1093/database/bav038

Wang, K., Wang, S., Chen, Y., Wu, D., Hu, X., Lu, Y. et al. 2021. Single-cell transcriptomic analysis of somatosensory neurons uncovers temporal development of neuropathic pain. *Cell Res.*, **31**(8), 904–918. https://doi.org/10.1038/s41422-021-00479-9. Erratum in: *Cell Res.*, 2021, **31**(8), 939–940.

Weinshenker, D. and Holmes, P. V. 2016. Regulation of neurological and neuropsychiatric phenotypes by locus coeruleus-derived galanin. *Brain Res.*, **1641**(Pt B), 320–337. https://doi.org/10.1016/j.brainres.2015.11.025

Weiss, J. M., Boss-Williams, K. A., Moore, J. P., Demetrikopoulos, M. K., Ritchie, J. C. and West, C. H. K. 2005. Testing the hypothesis that locus coeruleus hyperactivity produces depression-related changes via galanin. *Neuropeptides*, **39**(3), 281–287. https://doi.org/10.1016/j.npep.2004.12.028

Wiesenfeld-Hallin, Z., Villar, M. J. and Hökfelt, T. 1988. Intrathecal galanin at low doses increases spinal reflex excitability in rats more to thermal than mechanical stimuli. *Exp. Brain Res.*, **71**(3), 663–666. https://doi.org/10.1007/bf00248760

Wiesenfeld-Hallin, Z., Xu, X.-J., Villar, M. J. and Hökfelt, T. 1989. The effect of intrathecal galanin on the flexor reflex in rat: increased depression after sciatic nerve section. *Neurosci. Lett.*, **105**(1–2), 149–154. https://doi.org/10.1016/0304-3940(89)90027-x

Wiesenfeld-Hallin, Z., Xu, X.-J., Crawley, J. N. and Hökfelt, T. 2005. Galanin and spinal nociceptive mechanisms: recent results from transgenic and knock-out models. *Neuropeptides*, **39**(3), 207–210. https://doi.org/10.1016/j.npep.2004.12.017

Wray, N. R., Pergadia, M. L., Blackwood, D. H. R., Penninx, B. W. J. H., Gordon, S. D., Nyholt, D. R. et al. 2010. Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. *Mol. Psychiatry*, **17**(1), 36–48. https://doi.org/10.1038/mp.2010.109

Wrenn, C. C. and Crawley, J. N. 2001. Pharmacological evidence supporting a role for galanin in cognition and affect. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **25**(1), 283–299. https://doi.org/10.1016/s0278-5846(00)00156-1

Wu, G., Feder, A., Wegener, G., Bailey, C., Saxena, S., Charney, D. et al. 2011. Central functions of neuropeptide Y in mood and anxiety disorders. *Expert Opin. Ther. Targets*, **15**(11), 1317–1331. https://doi.org/10.1517/14728222.2011.628314

Xiao, H.-S., Huang, Q.-H., Zhang, F.-X., Bao, L., Lu, Y.-J., Guo, C. et al. 2002. Identification of gene expression profile of dorsal root ganglion in the rat peripheral axotomy model of neuropathic pain. *Proc. Natl. Acad. Sci. U. S. A.*, **99**(12), 8360–8365. https://doi.org/10.1073/pnas.122231899

Xu, X. J., Hökfelt, T., Bartfai, T. and Wiesenfeld-Hallin, Z. 2000. Galanin and spinal nociceptive mechanisms: recent advances and therapeutic implications. *Neuropeptides*, **34**(3–4), 137–147. https://doi.org/10.1054/npep.2000.0820

Xu, X. J., Hökfelt, T. and Wiesenfeld-Hallin, Z. 2010. Galanin and spinal pain mechanisms: past, present, and future. In *Galanin. Experientia Supplementum* (Hökfelt, T. ed.). Springer, Basel, **102**, 39–50. https://doi.org/10.1007/978-3-0346-0228-0_4

Xu, Z. Q., Shi, T. J. and Hökfelt, T. 1998. Galanin/GMAP- and NPY-like immunoreactivities in locus coeruleus and noradrenergic nerve terminals in the hippocampal formation and cortex with notes on the galanin-R1 and -R2 receptors. *J. Comp. Neurol.*, **392**(2), 227–251. https://doi.org/10.1002/(sici)1096-9861(19980309)392:2%3C227::aid-cne6%3E3.0.co;2-4

Yang, Y., Li, Y., Liu, B., Li, C., Liu, Z., Deng, J. et al. 2021. Involvement of Scratch2 in GalR1-mediated depression-like behaviors in the rat ventral periaqueductal gray. *Proc. Natl. Acad. Sci. U. S. A.*, **118**(24), e1922586118. https://doi.org/10.1073/pnas.1922586118

Yu, H., Usoskin, D., Nagi, S., Hu, Y., Kupari, J., Bouchatta, O. et al. 2023. Single-soma deep RNA sequencing of human DRG neurons reveals novel molecular and cellular mechanisms underlying somatosensation. *bioRxiv*, preprint. https://doi.org/10.1101/2023.03.17.533207

Zhang, X., Ju, G., Elde, R. and Hökfelt, T. 1993. Effect of peripheral nerve cut on neuropeptides in dorsal root ganglia and the spinal cord of monkey with special reference to galanin. *J. Neurocytol.*, **22**(5), 342–381. https://doi.org/10.1007/bf01195558

Zhong, W., Barde, S., Mitsios, N., Adori, C., Oksvold, P., von Feilitzen, K. et al. 2022. The neuropeptide landscape of human prefrontal cortex. *Proc. Natl. Acad. Sci. U. S. A.*, **119**(33), e2123146119. https://doi.org/10.1073/pnas.2123146119

Zhu, S., Hu, X., Bennett, S., Charlesworth, O., Qin, S., Mai, Y. et al. Galanin family peptides: molecular structure, expression and roles in the neuroendocrine axis and in the spinal cord. *Front. Endocrinol.*, **13**, 1019943. https://doi.org/10.3389/fendo.2022.1019943

Neli aastakümmet Viktor Muti avastatud neuropeptiidide uuringuid, keskendudes galaniinile

Tomas Hökfelt, Swapnali Barde, Wen Zhong, Zhi-Qing David Xu ja Pēteris Alberts

Artikkel põhineb Tomas Hökfelti peetud ettekandel professor Viktor Muti 100. sünniaastapäevale pühendatud pidulikul konverentsil Eesti Teaduste Akadeemias 2024. aasta jaanuaris. Artiklis kirjeldatakse Viktor Muti avastatud neuropeptiididega tehtud uurimistöö tulemusi, pöörates erilist tähelepanu neuropeptiid galaniini uuringutele. Lisatud on ka mõned Hökfelti isiklikud mälestused kohtumistest Viktor Mutiga ja lühike ülevaade varasematest Karolinska instituudis tehtud neuropeptiidide uuringutest. Neuropeptiidide toimemehhanismide ning neurotransmitterite ja neuropeptiidide kooseksisteerimise üldise kirjelduse järel analüüsitakse galaniini signalisatsioonisüsteemi võimalikku seotust valu- ja depressiooninähtudega. Erilist tähelepanu pööratakse galaniini esinemisele ja toimele roti ja inimese *locus coeruleus*'es. Surmajärgsed inimaju-uuringud on võimaldanud iseloomustada galaniini ja teiste peptiidide rolli normaalses prefrontaalses ajukoores, aga ka enesetapu sooritanud depressiooniga patsientide erinevates ajupiirkondades. Arutletakse täiendavate võimaluste üle valu ja depressiooni ravimeetodite arendamiseks galaniinergiliste mehhanismide abil. Ühtlasi käsitletakse mõningaid USA toidu- ja ravimiameti heaks kiidetud migreeniravimeid, mis põhinevad muudel bioaktiivsetel peptiididel. Kokkuvõttes avaldatakse lootust, et neuropeptiidide alaste uuringute edusammud võivad edaspidi aidata kaasa närvisüsteemihaiguste ravile.