The Next Generation of Anti-Itch Treatment: Progress in the Elucidation of an Itch-Specific Sensory Pathway

Abstract: Itch is a common irritating sensation for almost everyone, but it is also a clinical symptom for many patients with uremia, atopic eczema, diabetes and malaria (patients with malaria are commonly given chloroquine, a drug that induces intense itching). There are three classifications of itch: Neuropathic itch results from damage to neurons, psychogenic itch is psychiatric in origin, and pruritoceptive itch involves activation of peripheral nerve fibers, e.g., poison ivy or mosquito bites (Yosipovitch and Samuel 2008). Pruritoceptive itch sensation (henceforth called itch) involves the detection of an environmental stimulus, a pruritogen, leading to an aversive response. There are many unanswered questions about itch, including the existence of a somatosensory pathway dedicated to sensing itch (*an itch-specific pathway*) and its underlying molecular mechanism. In 2007, the first molecule implicated to be a primary neurotransmitter in an itch-specific somatosensory pathway was identified in mice, it is called *gastrin-releasing peptide* (GRP) (Sun et al., 2007; 2009). Recently, there has been controversy regarding both its itch-specificity and proposed role in the somatosensory pathway (Fleming et al 2012). Now, Mishra and Hoon (2013) have provided additional *in vivo* data that may resolve the controversy. Their data suggest that *GRP* may be directly or indirectly downstream of another molecule called *Natriuretic polypeptide b* (Nppb). While there is still controversy over the role GRP and Nppb in *itch-specific* sensory signaling, the identification and characterization of GRP and Nppb as important molecules in itch sensation still represents a great leap in the development of anti-itch drugs effective for a variety of itch conditions.

Introduction

Itch is possibly one of the most common symptoms experienced by healthy individuals acutely via insect bites or poison ivy and clinically/chronically by people with conditions such as atopic eczema, uremia, diabetes, or scabies mite infection. Despite nearly a century of research on itch, an effective therapy has yet to be developed (Patel and Dong 2010; McNeil and Dong 2012).

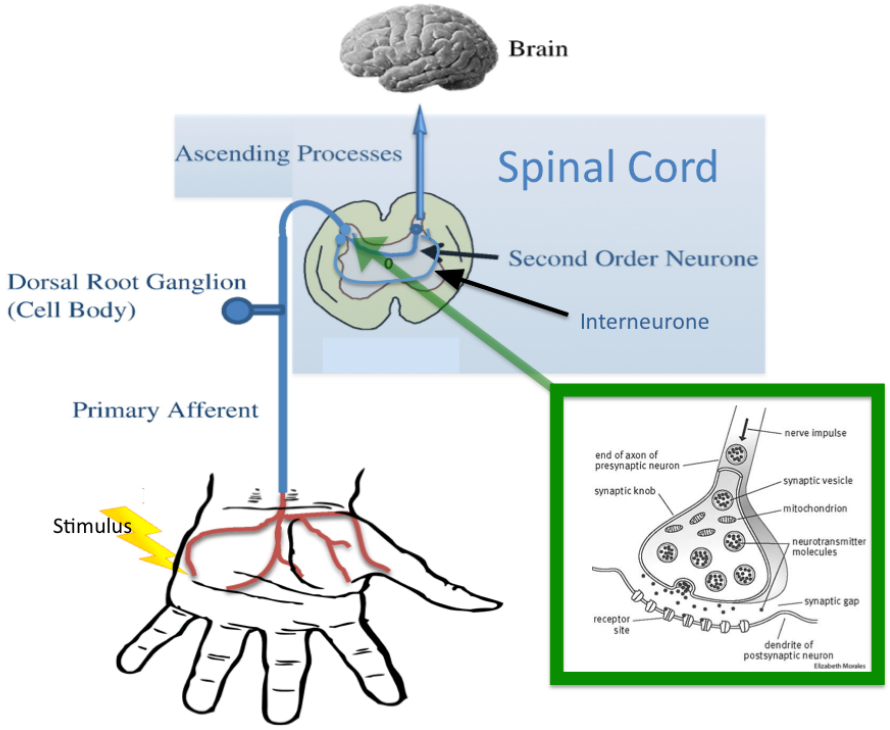

Figure 1. The Somatosensory pathway and the synapse. Pruritogens bind pruritoceptors (sensory receptors) on primary (first-order) sensory neurons, causing the release of a primary neurotransmitter into the synapse with the secondary (second order) neuron in the dorsal horn of the spinal cord. Secondary neurons can send axonal projects to either spinal interneurons (allowing for more modulation of the itch-signal) or to the brain, where final processing of the stimulus takes place. (Layman 2011)

Sensory information, including itch, is detected by the Peripheral Nervous System, conveyed by neurons to the Central Nervous system, and processed in the somatosensory cortex of the brain. The general Somatosensory pathway (Figure 1) is comprised of primary and secondary sensory neurons. Primary sensory neurons, or Dorsal root ganglion (DRG) sensory neurons, as they are more often called, have membrane-bound receptors that are activated by environmental stimuli. DRG sensory neurons synthesize primary neurotransmitters and upon stimulation release them into synapses with secondary neurons in the dorsal horn of the spinal cord. These *primary* neurotransmitters bind receptors on secondary sensory neurons, activating them. Secondary neurons then release neurotransmitters into synapses with either other spinal neurons (i.e. interneurons), or with neurons in the brain. Ultimately, the brain will process the environmental stimulus, combine it with other sensory information, develop a perception, and consider whether a physical response is necessary. In the case of itch, the environmental stimulus that initiates this somatosensory signaling cascade is a pruritogen. The perception created in the brain by activation of this somatosensory pathway is one of aversion, and the physical response is to scratch the area of the body that itches.

The somatosensory pathway is complicated; there is great variability at multiple stages in the pathway. For instance, at the initial stage of activation in the somatosensory system, the DRG primary sensory neurons can have multiple types of itch-detecting receptors activate them, leaden to the signaling cascade mentioned above. One such receptor is the histamine-receptor. When histamine binds the receptor, it activates DRG primary sensory neurons and the somatosensory pathway as previously described. One would like to think that histamine receptor antagonists (antihistamines) would then relieve itch sensation. Indeed, antihistamines do relieve

itch-sensation for many patients. However, since DRG primary sensory neurons can have more than one itch-sensing receptor type, there are histamine dependent and independent itch pathways and so antihistamines are not effective at inhibiting all types of itch. The complexity of the somatosensory pathway for itch has made it difficult to develop a single effective therapy for multiple itch-inducing conditions. Therefore, future studies that aim to mollify chronic itch conditions ought to consider searching for a molecular factor more universal to multiple subtypes of itch (e.g. histamine dependent *and* independent). The studies discussed in this paper follow such a strategy; they have identified and characterized the Gastrin-releasing peptide (GRP) and Natriuretic polypeptide b (Nppb) as itch-specific primary neurotransmitters.

This paper will introduce these seminal studies, and address current controversy over the itch-specificity of GRP and Nppb and the merit of current models for itch-specific sensory pathways. Despite the controversy, the discovery of these new molecules represent the first major step in the field towards developing an accurate model of itch-specific sensory pathways, and effective anti-itch treatment for a variety of itch conditions (recall that primary neurotransmitters are released by DRG primary sensory neurons into synapses with spinal cord neurons. There are likely fewer primary neurotransmitters than receptors for pruritogens, so drugs that target them will likely have a much broader effect on itch sensation.

1. The Gastrin-releasing peptide as part of an itch-specific sensory pathway

1.1 Why investigate Gastrin-releasing peptide in itch sensation?

Gastrin-releasing peptide (GRP) is structurally homologous to a molecule called bombesin. This information led Sun and Chen to investigate the possibility that GRP and

possibly its receptor, gastrin-releasing peptide receptor (GRPR), are involved in itch sensation (Sun and Chen 2007). Intracerebral administration of bombesin elicits grooming behavior in rodents, thus bombesin has been hypothesized to be a mediator of pruritus (Cowan et al., 1985; Gmerek and Cowan 1983). While structural homology may not be the best preliminary evidence, this is how Sun and Chen describe their reasoning for investigating GRP in itch-sensation.

Prior literature implicates unmyelinated neurons in the spinal cord to be part of an itch-specific sensory pathway, i.e. one dedicated tot he sneezing of itch and no other types of sensation (Andrew and Craig 2001). Sun and Chen's immunocytochemistry (ICC) data shows GRPR stains to be colocalized with a marker for unmyelinated neurons in the spinal cord and have no overlap with the myelinated markers. This ICC datum suggests that GRPR protein is localized in itch-specific neurons, providing support for the possible involvement of GRP/GRPR in itch sensation (Andrew and Craig 2001; Sun and Chen 2007).

Several itch-inducing pathways involve the *Transient Receptor Potential cation channel subfamily V member one* (TRPV1) ion channel (Shim et al., 2007; Imachi et al., 2009). Silencing of TRPV1-expressing neurons leads to profound loss of itch, thermosensation, and pain response (Imachi et al., 2009; Cavanaugh et al., 2009). The ICC data from Sun and Chen shows an overlap between GRP and TRPV1 stains, further supporting the hypothesis that GRP is involved in itch sensation. Though, TRPV1 is also involved in thermosensation and pain, so the data could also represent a role for GRP in these sensations.

1.2 Gastrin-releasing peptide is part of an itch-specific somatosensory pathway

In order to determine whether the GRP and its receptor, GRPR, are part of a somatosensory pathway dedicated to itch sensation, Sun and Chen created a GRPR knockout (KO) mouse strain. They compared this KO strain to wild-type mice for thermal, mechanical, inflammatory pain-sensation (nociception) by exposing them to sources of heat, applying mechanical pressure, and injecting the inflammatory agent formalin, respectively. They found that the GRPR KO strain mice were not significantly different from wild-type mice in their sensitivity to pain, indicating that the GRPR is not involved in pain sensation.

To determine whether GRP is involved in itch sensation, Sun and Chen (2007) compare the ability of their somatosensory pathways to convey a sensory signal for itch by exposing the skin of wild-type and GRPR KO mice to a range of pruritogens. They found that GRPR KO mice had a significantly lower number of scratching bouts. Furthermore, injection of GRP into the spinal cord (intrathecal injection) induced scratching in a dose-dependent manner. Co-injection of GRP with GRPR antagonist (which binds and deactivates the GRPR) inhibited GRP-induced scratching significantly, suggesting that GRP binds to GRPR to induce itching. Intrathecal injection of GRPR antagonist prior to exposure to pruritogenic stimuli significantly reduced scratching. Neither GRPR antagonist nor GRP intrathecal injection affected pain sensitivity. Collectively, these results suggest that GRP/GRPR are involved in a itch-specific sensory pathway.

So far, this paper has introduced data suggesting that GRPR is a receptor involved in itch-specific sensation (McNeil and Dong 2012). Since antagonism (i.e. inactivation) of GRPR

inhibited itch sensation induced by all tested pruritogens, GRPR may be a prime target for future itch therapies.

1.3 Where GRP might fit in a model for itch sensation

In situ hybridization (ISH) and *Immunocytochemistry* (ICC) data from Sun and Chen (2007) show that the neurotransmitter GRP mRNA is expressed by DRG primary sensory neurons and that GRP protein is localized in synapses between DRG primary sensory neurons, suggesting that GRP is primary neurotransmitter— synthesized in DRG primary sensory neurons.

Surgical ablation of DRG neurons, called DRG rhizotomy, drastically decreases the presence of DRG-derived molecules in the spinal cord. Sun and Chen found that mice given DRG rhizotomy had decreased ICC stain intensity for GRP in the spinal cord, indicating that GRP is synthesized in DRG neurons and released at the spinal cord (Sun and Chen 2007).

The ISH, ICC and ablation experiments support the hypothesis that that GRP is a primary neurotransmitter released by DRG primary sensory neurons onto spinal cord neurons in an itch-specific sensory pathway.

2. Controversy over the role of GRP/GRPR in an itch-specific sensory pathway

In 2012, Fleming et al. published a paper with data that contradicts the conclusions of Sun and Chen (2007). In doing so, Fleming et al have brought the role of GRP in itch-sensation into controversy. The following sections (2.1, 2.2, and 2.3) will describe and discuss the

controversy regarding 1) the role of GRP as a primary neurotransmitter in itch-sensation, and 2) the itch-specificity of GRPR, and 3) the related model of itch-specific sensation.

2.1 Differences in ISH and ICC data cast doubt on the hypothesis that GRP is synthesized in DRG primary sensory neurons

Fleming et al (2012) used RT-PCR (reverse-transcriptase PCR), PCR and ISH to measure GRP mRNA levels in DRG primary sensory neurons and in spinal cord neurons. They found that DRG primary sensory neurons had extremely low levels of GRP mRNA whereas spinal cord neurons had high levels. Fleming et al's data suggest that GRP is not expressed in DRG primary sensory neurons, and instead might be expressed in the spinal cord. A neuron that does not synthesize a molecule itself most often does not release it as a neurotransmitter. This contradicts Sun and Chen's expression profile of GRP, thus the proposed role for GRP as a primary neurotransmitter (a neurotransmitter released by DRG primary sensory neurons) has come under question.

Sun and Chen did not publish any methods for their ISH data, making a comparison and analysis of the data difficult. Even so, it is worth noting that by using multiple techniques (RT-PCR, PCR, and ISH) Fleming et al appear to be more methodologically thorough in their conclusion that the mRNA expression pattern of GRP. Additionally, previous literature that uses ISH supports Fleming et al (2012).

For ICC, both Fleming et al. and Sun and Chen used the same GRP antibody from ImmunoStar, so it is possible to analyze why these two laboratories have such different data. Fleming et al. states that the difference in detection of GRP could be due to differences in tissue

fixation conditions prior to staining. Indeed, it is possible that the differences in data are due to differences in the age or strain of the mice used, but a lack of detail in the methods of Sun and Chen (2007) makes further analysis quite hard.

Fleming et al have brought into question the GRP mRNA expression and protein localization experiments of Sun and Chen and subsequently made their hypothesized role for GRP as a primary neurotransmitter controversial.

2.2 Differences in DRG rhizotomy data cast further doubt on the hypothesis that GRP is a primary neurotransmitter in itch sensation

As mentioned previously, Sun and Chen saw such a decrease in ICC GRP stain intensity in the spinal cord following DRG rhizotomy, suggesting that GRP is a DRG primary sensory neuron-derived (a.k.a. primary) neurotransmitter. Fleming et al (2012) had contradictory results. Using ICC to determine protein levels as Sun and Chen did, they saw an insignificant decrease in GRP stain intensity (relative to control stains), suggesting that GRP is not derived from DRG primary sensory neuron and therefore not a primary sensory neurotransmitter.

Again, a lack of methodological detail on the part of Sun and Chen prevents thorough comparison of two data sets. Like before, Fleming et al appear to have been more scientifically thorough; in addition to staining for GRP, Fleming et al. stained for a panel of markers synthesized in the DRG and spinal cord to compare and control for changes of intensity. Such additional stains control for confounding factors. For example, the death of the DRG primary sensory neurons could somehow have affected the levels of spinal cord-derived markers, leading to false changes in the stain intensity of spinal cord-derived markers. By also measuring the stain

intensity of these known spinal cord-derived markers, Fleming et al have created the appropriate controls to which they can compare changes of GRP stain intensity and accurately infer the neuronal source of GRP.

Fleming et al. found that following DRG rhizotomy, the intensity of GRP staining decreased, but that the decrease was not significantly different from the decrease in intensity of the dorsal spinal cord marker and *was* significantly different from the percent decrease of DRG markers. These data suggest that GRP is not derived from DRG primary sensory neurons, so it is not likely a primary neurotransmitter (Fleming et al 2012).

In a more recent paper from Dr. Chen's laboratory (Zhao et al., 2013), they recapitulate their DRG rhizotomy data and add more of the appropriate controls. Their data show that mice given DRG rhizotomy have a GRP stain intensity significantly lower compared to mice that did not undergo the rhizotomy, and that the difference between GRP and DRG marker stain intensity is not statistically significant (Zhao et al 2013). While Zhao et al did use more appropriate controls for the DRG rhizotomy experiments, they still did not have all the necessary controls (as Fleming et al did). Zhao et al did not provide DRG rhizotomy data for any spinal-cord-derived markers, thus they have not controlled for confounding possibilities. For instance, the DRG rhizotomy could somehow affect spinal cord neurons and lead to a decrease in spinal cord derived molecules, so if the data suggested GRP is derived from the spinal cord, the experimenters would not be able to tell whether their data is a false positive or not.

While Zhao et al. acknowledge that their results differ from Fleming et al, they offer no explanation. It is possible that difference in data from the two laboratories is due to them using

different strains of mice. However, a lack of methodological detail in Sun and Chen 2007 and Zhao et al 2013 has prevented further investigation of this possibility.

Fleming et al show mRNA-expression data contradictory to that of Sun and Chen; their data suggest that GRP is expressed primarily in the spinal cord and not in DRG neurons. If true, this would imply that GRP is synthesized in the spinal cord and that the GRP/GRPR might be part of spinal cord interneuronal synapses. Such a conclusion is supported by previous literature identifying a subset of spinal interneurons involved in itch (Ross et al., 2010). It would be interesting to see if there is any overlap in the subpopulation of GRP-expressing spinal neurons identified by Fleming et al (2012) and the itch-associated neurons identified by Ross et al., (2010). If there was to be an overlap, it would further support the hypothesis that GRP is in fact synthesized locally in the spinal cord, and suggest an alternative hypothesis that GRP/GRPR are involved in spinal-neuron to spinal-neuron communication, not DRG primary sensory neuron to spinal neuron communication (as was proposed by Sun and Chen).

2.3 Questioning whether GRPR is part of exclusively itch-sensing neuronal pathways

McNeil and Dong 2012 noted another issue with Sun and Chen's work: that GRP is likely not the only ligand that can bind and activate the GRPR. Neuromedin B, a structural sibling to GRP in the bombesin-like family of peptides, has mRNA expressed in pain-sensing and itch-sensing neurons. It can bind to GRPR, and intrathecal injection of NMB can induce itching behavior (Fleming et al., 2012; Jensen et al., 2008; Su and Ko 2011). These studies cast further doubt on the implication that GRP and GRPR are part of an itch-specific pathway.

3. Natriuretic polypeptide b as a neurotransmitter in Itch-specific sensation

Thus far, this paper has introduced GRP and GRPR as molecules in an itch-specific sensory pathway and discussed related controversies. Now, it will consider the most recent contribution to the controversy, the work of Mishra and Hoon (2013). Their work does not support Sun and Chen's hypothesized role for GRP as an itch-specific primary neurotransmitter. However, Mishra and Hoon's model for itch-specific sensation still incorporates GRP.

3.1 mRNA expression patterns of Natriuretic polypeptide b suggest an important role in itch-signaling

As stated previously, several itch-inducing pathways involve the TRPV1 ion channel, and

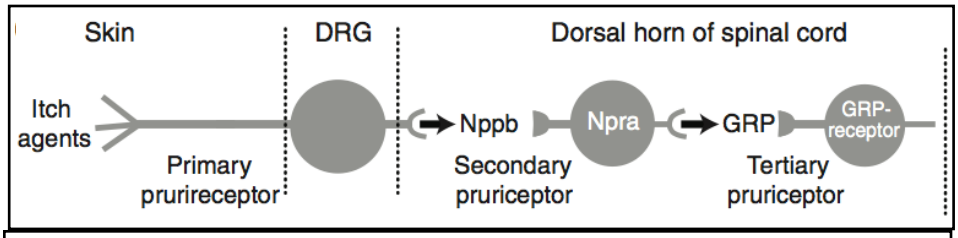

Figure 2. The itch-specific pathway proposed by Mishra and Hoon 2013. Itch agents stimulate primary sensory/DRG neurons, leading to the release of Nppb into dorsal horn synapses, where it binds the secondary pruritoceptor, Npra on a secondary sensory neuron. The activation of Npra directly or indirectly stimulates release of GRP onto a tertiary sensory neuron in the spinal cord. A direct stimulation would imply that Npra and GRP are expressed by the same neuron, so activation of this neuron via Npra receptor binding would cause that same neuron to release GRP. An indirect relationship would imply that Npra expressing neurons stimulate GRP-expressing neurons to release GRP (Mishra and Hoon 2013)

silencing of TRPV1-expressing neurons leads to profound loss of itch, thermosensation, and pain response (Shim et al., 2007; Imachi et al., 2009; Cavanaugh et al., 2009). Mishra and Hoon

(2013) used microarray-based differential screening in mice with ablated TRPV1$^+$ neurons to identify candidate molecules that mediate itch signaling. Among the candidates identified in the microarray was Natriuretic polypeptide b (Nppb). ISH data showed that Nppb mRNA to be expressed in a subset of DRG neurons, and that 70% of these neurons also expressed mRNA expression for pruritogen receptors (Mishra and Hoon 2013). One possible reason why the overlap is not 100% is that Nppb might not be the only primary neurotransmitter involved in itch sensation. These data suggest that it could be a primary neurotransmitter specific to itch sensation.

3.2 Nppb is part of an itch-specific somatosensory pathway

Nppb knockout mice had normal responses to thermally and mechanically painful stimuli, but significantly fewer scratching bouts when exposed to pruritic stimuli. Intrathecal injection of Nppb induced scratching behavior in wild-type and Nppb knockout mice. The receptor for Nppb, Npra, is shown via ISH to be expressed primarily in lamina I of the dorsal horn. These results and the expression pattern of Nppb suggest it might be responsible for the deficit of pruritic response seen in the knockout mice, and possibly an itch-specific primary neurotransmitter.

3.3 Fitting Nppb with in a model of itch-specific sensory signaling

To see how Nppb might be related to GRP in a model for itch-specific sensation, Mishra and Hoon conducted a series of mechanistic experiments. First, they showed that GRP-induced scratching was unchanged by knocking out Nppb or ablating Npra. Second, they

pharmacologically inhibited GRPR, and found that it attenuated the scratching response of wild-type mice to histamine, GRP, and Nppb. Attenuation of the scratching responses was also seen following ablation of $GRPR^+$ (i.e. GRPR-expressing) neurons. The expression pattern of Npra completely overlaps with that of GRP, and ablation of $Npra^+$ neurons significantly reduces the number of GRP^+ cells, as shown by ISH (Mishra and Hoon, 2013). This indicates that 1) GRP and Npra are synthesized by the same neurons, and 2) that GRP may be released as a result of stimulation of the Npra receptor. These data suggest that GRP is (directly or indirectly) downstream of Nppb in an itch-specific sensory pathway (Figure 2), and support the hypothesis that GRP is not a primary neurotransmitter in an itch-specific sensory pathway.

4. Controversy over the role of Nppb/Npra in an itch-specific sensory pathway

This paper has introduced the discovery of the first molecules proposed to be part of an itch-specific pathway, described and discussed controversy regard the molecules, and provided the most current model for an itch-specific sensory pathway. The current section will discuss the more recent controversy over the role of Npra in itch-specific somatosensory perception and its related model for itch-specific sensation.

GRPR is not the only receptor whose role in itch-signaling has been questioned. Npra has been suggested to be part of an inflammatory nociceptive pathway (Zhang et al., 2010; Zhao et al., 2013 unpublished). Despite having likely seen the publication from Zhang et al., as it was published three years earlier, Mishra and Hoon did not publish data measuring the inflammatory pain sensation of either Nppb knockout or $Npra^+$-ablated mice. By doing so, they leave themselves open to criticism. Notably, the contradictory data (from Zhang et al) was obtained

using rats, and there is variability in how different species detect pruritogens (Zhang et al., 2010; Patel and Dong 2010). For example, histamine induces itch in mice and humans but not rats, and mice appear to have overlap in histaminergic and nonhistaminergic itch pathways, whereas human and non-human primates do not (Patel and Dong 2010). Assuming that there is no species-based variation for this particular case, the contradictory data casts doubt on Mishra and Hoon's proposed mechanism for an itch-specific sensory pathway. Furthermore, Mishra and Hoon provide data showing that Npra and GRP are colocalized in spinal cord neurons, suggesting that they might be expressed in the same neurons. By challenging the itch specificity of Npra, Zhao et al. have also challenged the itch-specific of their (laboratory, i.e. Dr. Chen's) own champion molecule, GRP.

While the role of Nppb/Npra and GRP/GRPR as itch-specific mediators of sensation have not been thoroughly established, these two sets of molecules represent great progress in itch-research. With further research the role of these molecules in an itch-specific somatosensory pathway will become more clear, and could lead to the development of more effective therapies for people suffering from itch-related conditions.

5. Evaluating the candidacy of GRPR and Npra as targets for future anti-itch therapies: why their discovery remains a 'great leap forward'

While the itch-specificity of GRP and Npra receptors may be questioned, they remain viable targets for future anti-itch therapies; the possibility that they might be involved in pain sensation does not completely detract from the fact that antagonism of the GRP and Npra blocks multiple kinds of induced itch. Hypothetically, if a clinical drug that antagonizes GRPR or Npra

were to be developed, the controversy over its itch-specificity would suggest that the drug may have pain-related side effects. While these side effects are not known, it is possible that they are manageable with or without painkillers. Thus the GRPR and Npra cannot be excluded as candidate targets for further development of anti-itch treatments.

In considering potential targets for future itch-therapies, it ought to be noted that GRP/GRPR and Nppb/Npra were found to be important in mouse models. Given that there can be species-specific differences in itch sensation (as previously described), their importance to murine itch sensation may not be seen in other mammals, including humans. Therefore scientists will not truly know the efficacy of anti-itch therapies that target GRP/GRPR or Nppb/Npra until clinical trials take place. Despite this shortcoming, GRP/GRPR and Nppb/Npra remain leading targets for future anti-itch therapy.

Conclusions

Itch is both a common, everyday, sensation and a clinical symptom for which current treatments are often not effective. Despite decades of research, the complexity of the somatosensory pathway for itch has made it difficult to develop a single effective therapy for multiple itch-inducing conditions. This is why the studies discussed in this paper are significant advancements. They searched for itch-specific neurotransmitters, the release of which is more common in multiple subtypes of itch (e.g. histamine dependent *and* independent). These studies have identified and characterized the Gastrin-releasing peptide and Natriuretic polypeptide b as primary neurotransmitters in an itch-specific sensory pathway. The latest model for an itch-specific somatosensory pathway suggests that pruritogens activate DRG primary sensory

neurons, leading them to release Nppb into a synapse with Npra-expressing neurons in the spinal cord. Then Npra-expressing neurons release GRP onto other spinal neurons, after which the signal for itch will ultimately reach the brain. This paper has also addressed controversy over the itch-specificity of GRP/GRPR and Nppb/Npra and the details of their accompanying models for itch-specific somatosensory pathways. While this controversy may bring into question the candidacy of these molecules as targets for anti-itch therapies, it does not exclude them from consideration. The discovery of these new molecules remains the first major advancement in the development of an accurate model of itch-specific sensory pathways, and next-generation anti-itch treatments. Future studies should consider testing the itch-specificity of GRP/GRPR and Nppb/Npra in other species such as rats to determine whether GRP/GRPR and/or Nppb/Npra are highly conserved. Given proof of a conserved nature for GRP/GRPR and/or Nppb/Npra, scientists ought to pursue clinical trials.

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Peer Review

2. **Sayback**

This review addresses the question of what roles GRP and Nppb have in the molecular pathway for sensing pruritus, which is interesting because there are many subtypes of itch that use variations of the pathway so finding an molecule that is specific to the itch pathway but common to all types of itch would allow for a ubiquitous itch treatment. The types of evidence discussed in the review are recent studies on GTP and Nppb and current models of the pruritogenic sensory pathway.

I am convinced that this is an interesting paper because it seems like itch is a common problem and not enough is understood about it to develop a treatment. I think it would help to give brief descriptions of GTP and Nppb beyond being "itch-specific primary neurotransmitters" and to summarize the actual evidence found by the seminal studies mentioned in the introduction because it would provide a better preview of the content discussed in the body of the paper.

After reading the headings, I would describe this review to a layperson as an article about the molecules used by brain cells to signal the sensation of itching, their role in the signaling pathway, and whether they are used exclusively for signaling itch.

My peer was in agreement.

3. **Reader Response –** within paper

4. **Criteria-Based Response**

Title: Brief, factual, and informative **GRP and Nppb could be named in the title because they are the main topic and only their part of the pathway (initial signaling) is discussed. The title suggests a general survey of the entire pathway.**

Abstract:

Summarizes the paper in 150-250 words **yes – exactly 250 words!**

Contains a statement of the question and its importance **not explicitly stated but topic is clear**

Summarizes the types of evidence that will be covered **vague, but the nature of the evidence makes it hard to summarize evidence without going into detail**

Provides a concluding sentence **yes**

Introduction:

Contains a statement of the question **yes**

Discusses information necessary to understand the question **yes**

Describes why the question is important **yes**

Is no more than 2-3 paragraphs long **4 paragraphs but the first one is really short and can easily be combined with the next one**

It would be helpful if the labels in the diagram of the somatosensory pathway were tailored to reflect itch-specific components – pruritogen, DRG neuron, dorsal horn of spinal cord. Location of primary neurotransmitters could also be highlighted because that is the proposed function of the two main molecules, GRP and Nppb.

Body of the paper:

Describes, in more detail than in the introduction, the information necessary to understand the question and the answer **yes**

Contains all of the information necessary to explain the answer to the question **I wish DRG neurons were more clearly defined – most of my confusion stemmed from this. I also do not have a clear understanding of rhizotomy in the context of DRG neurons and what its results mean.**

Describes, in detail, what is known about the answer **yes, except section 2.1- There is a 3 paragraph discussion about the fact that Fleming's data contradicts Sun's data and casts doubt on its validity, but Fleming's actual procedure and results are not given so the reader has to trust that the author is correct without being able to compare the experiments and see what parts conflict and why they create controversy.**

Contains multiple sections headed by brief, informative headers **yes – The headings provided smooth guidance through the course of the review.**

Separates different parts of the answer into different sections **yes – Subsections with individual titles made it much easier to keep track of large amounts of new information.**

Conclusion:

Summarizes the answer to the question **yes**

Includes a short discussion of what is still not understood
and future areas of study **kind of – It just says that the proposed roles of the two molecules are possibly true but possibly not true and future studies should continue to look for molecules ubiquitous in itch sensing. It could be more speculative instead of fixating on things that have already been extensively discussed.**

Overall

Information is clearly correct, and linked to the question/answer **yes**

Terms and abbreviations are defined, and concepts that are not universally understood (eg: gravity) are explained conceptually.

Information and concepts are ordered in a way that introduces information as it is needed **yes**

Tense usage is appropriate to a review article in the Biological Sciences **There were several instances where a description of experiments switched to present tense, but in most of the paper the tenses made sense.**

References:

All quotations, paraphrases/summaries, and primary data are referenced in the text **yes**

All in-text references are referenced in the end-of-text citations list **yes**

References are formatted according to *Cell's* conventions **yes**

There was one web page in the reference list, and *Cell* does not specify how non-journal sources should be cited.

Narrative Description

This paper is probably somewhere between a B and an A and could easily be an A with minor adjustments to the content of several sections. The majority of the content was adequately explained for a scientific audience to understand. I thought it was extremely well structured because each section fit into the next to tell the story. The writing style efficiently communicated the most important points, so even in parts where I was being presented large amounts of new information I did not feel overwhelmed. Each transition summarized the discussion to that point and gave a preview of the following section without being tedious, so I could have read the paper comfortably even without headings.

5. Action Plan

Give more clear definitions of DRG neurons and the prurireceptor pathway that make them distinct from the general somatosensory pathway
In section 2.1, give the actual results of Fleming's study
Consider adding the rationale for DRG rhizotomy
Proofread for grammatical errors and places where you described experiments in present tense (I think I saw 2 or 3)

Write a short letter or list, describing to yourself and a peer what concerns Dr. Benyajati had and what you decided to do about them

The main concern Dr. Benyajati had was organizational. She wanted me to better connect my body paragraphs and my main idea, specifically to adjust my main idea to be more what I described in my 'letter to the editor'. She also wanted me to not have so much detail on the data of each paper and to include more commentary.

I addressed each of these concerns, adjusting my main idea and providing small paragraphs throughout the paper explaining how the previous and current sections of text would relate to the main idea. I got rid of nearly all the specific descriptions of data and added more commentary.

Revisit the F_2013_BIO275W_Assignment_Prompt_Grading_Criteria one last time, and think about the narrative and checklist descriptions of the A paper. At the end of the paper, include a reflective section that explain how you met all of these elements of an effective paper, or explains why you do not think that you were able to achieve all of them. Propose a final grade for yourself, using the wording in the grading criteria.

I clearly identified that itch is a common problem for which there are often no effective treatments and that there was a gap in the our knowledge of itch-specific neural pathways. I have several paragraphs summarizing and explaining the significance of neighboring sections of text, particularly in reference to the main ideas.

I have all the structural requirements met (abstract, intro, body) and each section accomplishes what it is supposed to. I am 0.5 page over the upper limit, but that includes my two figures.

After my meeting with Tip I made many revisions, but I didn't get the chance to do that one exercise we did a few weeks ago. That said, the sentences should still be good, but I wish they were better.

I would give myself an A-/B+ . There seems to be a lot of repeated statements, bordering on poor word choice, and overall my writing style is a bit wordy. The content and structure are fine.