The Neuropeptide Y System Regulates Both Mechanical and Histaminergic Itch

Tianle Gao[1,2,3], Haisha Ma[1,3], Bo Xu[1], Jessica Bergman[1], Dan Larhammar[1] and Malin Charlotta Lagerström[1]

Itch is a somatosensory modality that serves to alert an organism to harmful elements removable by scratching, such as parasites and chemical irritants. Recently, ablation or silencing of neuropeptide Y (NPY)-expressing spinal interneurons was reported to selectively enhance mechanical itch, whereas chemical itch was unaffected. We examined the effect of activating the NPY/Y_1 receptor system on scratch behavior in mice. We found that intrathecal administration of the Y_1 agonist [Leu^{31},Pro^{34}]-NPY (LP-NPY) attenuated itch behavior induced by application of 0.07 g von Frey filament in the nape of the neck compared with saline treatment, indicating that activation of the spinal NPY/Y_1 system dampens mechanical itch. However, intrathecal administration of LP-NPY also attenuated chemically induced scratching provoked by intradermal application of histamine or the mast cell degranulator 48/80 (histaminergic itch), and the latter effect could be reversed by administration of the Y_1 antagonist BIBO3304. Intrathecal application of the native nonselective agonist NPY also attenuated histamine or 48/80-induced scratching. Our analyses emphasize the importance of including additional quantitative parameters to characterize the full spectrum of itch behavior and show that the NPY/Y_1 system dampens both mechanically and chemically induced scratching and hence is shared by the two submodalities of itch.

Journal of Investigative Dermatology (2018) **138,** 2405–2411; doi:10.1016/j.jid.2018.05.008

INTRODUCTION

The neurotransmitter neuropeptide Y (NPY) is expressed by spinal inhibitory interneurons that reside in lamina I–IV of the dorsal horn and receive input from Aβ and C fibers (Bourane et al., 2015; Rowan et al., 1993). NPY binds to the NPY receptor family, where Y_1 is expressed on excitatory interneurons in the spinal cord and in primary afferent neurons (Todd et al., 2003; Zhang et al., 1999), whereas Y_2 is mostly found on primary afferent neurons (Brumovsky et al., 2005). NPY receptor subtypes Y_4, Y_5, and Y_6 have very modest or negligible expression in the adult spinal cord (Naveilhan et al., 1998). The NPY receptors couple via $G\alpha_i$, and bath application of NPY or the Y_1 agonist [Leu^{31},Pro^{34}]-NPY (LP-NPY, selective for Y_1 versus Y_2 [Beck-Sickinger et al., 2018]) induces hyperpolarization in patch-clamped spinal lamina II interneurons (Miyakawa et al., 2005), indicating that NPY-expressing interneurons contribute to the inhibitory tone in the dorsal spinal cord via activation of Y_1.

Intrathecal delivery of LP-NPY reduces both mechanical and cold allodynia associated with neuropathic pain in the chronic constructive injury model (Malet et al., 2017). Moreover, intrathecal administration of the Y_1 antagonist BIBO3304 blocks the anti-hyperalgesic effects of NPY in the plantar incision model of postsurgical pain (Yalamuri et al., 2013) and in the spared nerve injury model (Intondi et al., 2008) and promotes hypersensitivity in the common peroneal and sural transection model of neuropathic pain (Solway et al., 2011), indicating that NPY/Y_1 has an analgesic role in the regulation of pain. Moreover, the spinal NPY system was recently reported to have a distinct role in itch transmission (Bourane et al., 2015).

Itch evokes the desire to scratch and serves to protect the organism from agents that can cause harm. The sensation of itch is transmitted from the periphery to the central nervous system by myelinated touch-associated (mechanical itch) (Bourane et al., 2015) or unmyelinated C (chemical itch) (Schmelz et al., 1997) primary afferents, suggesting separate transmission pathways. Mice partially ablated for their NPY-expressing spinal interneurons displayed an increased spontaneous itch behavior and alloknesis, whereas chemically induced scratch behavior, induced by the mast cell degranulator 48/80, was reported to be unaffected, suggesting that the spinal NPY system selectively gates mechanical itch (Bourane et al., 2015) and showing a spinal component to a plausible selective pathway for mechanical itch. However, the role of the Y_1 receptor was not addressed. We set out to investigate the role of Y_1 in this neuronal network using a targeted pharmacological approach.

RESULTS

The Y_1 receptor regulates mechanically induced scratching behavior

To investigate the role of the Y_1 in mechanical itch, mice were given an intrathecal injection of LP-NPY (10 nmol/L) or saline and their acute behavioral response, after application of a 0.07 g von Frey filament to the nape of the neck, was monitored. LP-NPY did not affect scratch episodes with regard to either duration ($P = 0.12$), number ($P = 0.11$), or

[1]*Department of Neuroscience, Uppsala University, Uppsala, Sweden; and* [2]*Institute of Materia Medica Chinese Academy of Medical Sciences, Beijing, China*

[3]*These authors contributed equally to this work.*

Correspondence: Malin Lagerström, Department of Neuroscience, Science for Life Laboratory, Uppsala University, Husargatan 3, Box 593, 751 24 Uppsala, Sweden. E-mail: Malin.Lagerstrom@neuro.uu.se

Abbreviations: LP-NPY, [Leu^{31},Pro^{34}]-neuropeptide Y; NPY, neuropeptide Y

Received 16 January 2018; revised 2 May 2018; accepted 16 May 2018; accepted manuscript published online 24 May 2018; corrected proof published online 27 July 2018

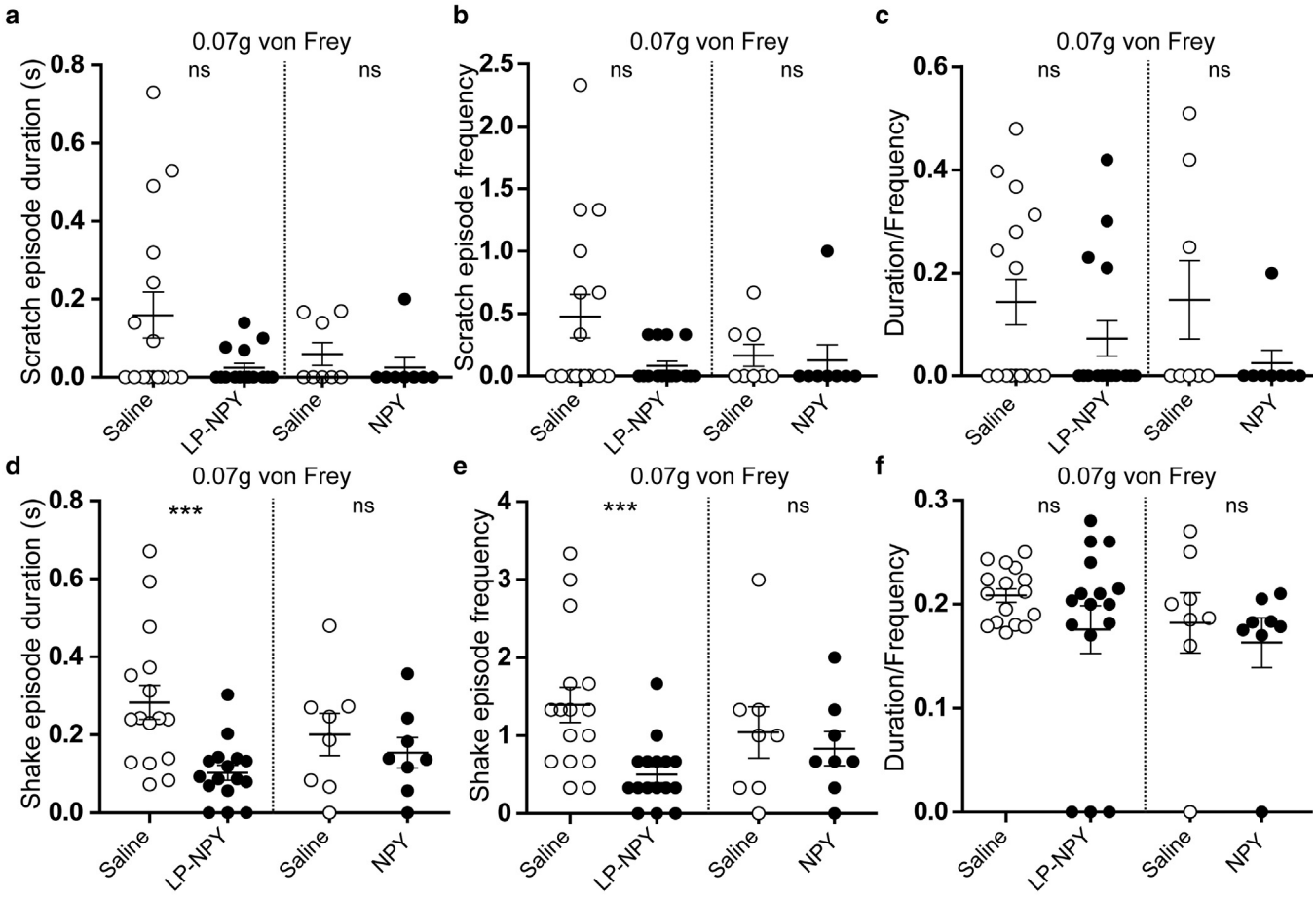

Figure 1. Activation of Y_1 reduces mechanical itch behavior. (**a**) The effect of LP-NPY (n = 16), NPY (n = 8), or saline (n = 16 and 8, respectively) on scratch episode duration induced by five applications of 0.07 g von Frey filament to the nape of the neck. (**b**) The effect of LP-NPY (n = 16), NPY (n = 8), or saline (n = 16 and 8, respectively) on scratch episode frequency induced by five applications of 0.07 g von Frey filament to the nape of the neck. (**c**) The effect of LP-NPY (n = 16), NPY (n = 8), or saline (n = 16 and 8, respectively) on mean length of scratch episode induced by five applications of 0.07 g von Frey filament to the nape of the neck. Mann-Whitney t-test (GraphPad Prism, La Jolla, CA) was used in **a**–**c**. (**d**) The effect of LP-NPY (n = 16), NPY (n = 8), or saline (N = 16 and 8, respectively) on shake episode duration induced by five applications of 0.07 g von Frey filament. Unpaired *t* test (passed D'Agostino and Pearson normality test [GraphPad Prism]) (**e**) The effect of LP-NPY (n = 16), NPY (n = 8), or saline (n = 16 and 8, respectively) on shake episode frequency induced by five applications of 0.07 g von Frey filament. LP-NPY, Mann-Whitney *t* test (GraphPad Prism); NPY, unpaired *t* test (passed D'Agostino and Pearson normality test [GraphPad Prism]). (**f**) The effect of LP-NPY (n = 16), NPY (n = 8), or saline (n = 16 and 8, respectively) on mean length of shake episode induced by five applications of 0.07 g von Frey filament. LP-NPY, unpaired *t* test (passed D'Agostino and Pearson normality test [GraphPad Prism]); NPY, Mann-Whitney *t* test (GraphPad Prism). ***$P < 0.001$, ns > 0.05. (**f**). LP-NPY, [Leu^{31},Pro^{34}] neuropeptide Y; NPY, neuropeptide Y; ns, not significant.

mean length ($P = 0.23$) compared with saline injection (Figure 1a–c). However, episodes of shaking behavior (defined in Table 1) were reduced both regarding duration ($P = 0.0007$) and number ($P = 0.001$) (Figure 1d–f), indicating a regulatory role for the NPY/Y_1 system in acute mechanically induced itch. Intrathecal injection of NPY affected neither scratching nor shaking behavior induced by 0.07 g von Frey filament ($P > 0.05$) (Figure 1a–f).

The Y_1 receptor regulates 48/80- and histamine-induced scratching behavior

To investigate the role of the NPY system in chemical itch, mice were injected intrathecally with NPY, LP-NPY, or saline, followed by an intradermal injection of the pruritogens chloroquine or 48/80 in the nape of the neck. Chloroquine and 48/80 were chosen as pruritogens because both agents induce pronounced scratching in the cheek model (Inoue et al., 2016; Liu et al., 2016) and limited wiping (Liu et al., 2016), thus inducing itch. Intrathecal injection of NPY (10 nmol/L) attenuated the duration of compound 48/80-induced scratch episodes ($P = 0.003$) (Figure 2a), whereas the number of scratch episodes was unchanged ($P = 0.78$) compared with vehicle injection (Figure 2b). The mean length of scratch episodes was shorter in NPY-treated mice ($P = 0.004$) (Figure 2c), and shorter scratch episodes were more frequent in NPY-treated mice (0.1- to 0.2-s interval, $P < 0.001$) (Figure 2d), indicating that NPY regulates chemically induced scratch behavior by shortening the time spent scratching.

Intrathecal administration of the Y_1 agonist LP-NPY also decreased the duration of 48/80-induced scratch episodes ($P = 0.002$) (Figure 2a) and, as with NPY, there was no difference between the number of scratch episodes between LP-NPY and saline ($P = 0.09$), although a trend to decreased number of scratch episodes was observed (Figure 2b). LP-NPY did not affect the mean length of the scratch episodes;

Table 1. Description of the terms used to describe the scratch behavior

Term	Description
Scratch episode	Targeted hind paw scratching toward the pruritogen-injected area from the time point when the paw was lifted until it was placed back on the ground
Shake episode	Rapid shaking of the upper back area, a behavior that correlates to scratch behavior (Jinks and Carstens, 2002)
Episode duration	Total time of episodes
Episode frequency	Total number of episodes
Mean length of episode	Duration/frequency

nevertheless, longer scratch episodes ($P < 0.05$) (0.4- to 0.5-s interval) were more frequent in controls compared with LP-NPY–injected mice (Figure 2e). The effect of LP-NPY on 48/80-induced scratch duration could be reversed by administration of the Y_1 antagonist BIBO3304 (Wieland et al., 1998) (100 nmol/L) 10 minutes before the LP-NPY injection ($P = 0.2$ and $P > 0.05$, respectively, compared with vehicle treatment) (Figure 2a and f). BIBO3304 alone did not affect 48/80-induced scratch duration ($P = 0.39$), number of scratch episodes ($P = 0.31$), or mean length of scratch episodes ($P = 0.48$) (Figure 2a–c), but the number of scratch episodes in the 0.3- to 0.4-s and 0.4- to 0.5-s intervals was increased ($P < 0.01$ and $P < 0.05$, respectively) (Figure 2g), indicating that antagonism of Y_1 prolongs the length of scratch episodes.

To further evaluate the effect of the NPY system in histaminergic itch, mice were intrathecally administrated with saline, NPY, or LP-NPY, after which the mice were intradermally injected with histamine (100 μg) in the nape of the neck. As in 48/80-induced itch, intrathecal injection of either NPY or LP-NPY decreased the duration only of histamine-induced scratch episodes ($P < 0.001$) (Figure 3a), keeping the number of scratch episodes unaffected ($P > 0.05$) compared with the saline-treated group (Figure 3b). The mean length of the scratch episodes in the groups treated with NPY and LP-NPY, respectively, was also reduced compared with the saline-treated group (Figure 3c) ($P < 0.001$), again indicating that NPY/Y_1 regulates histamine-related scratch behavior by shortening the time spent scratching. In fact, LP-NPY shifted the overall distribution of histamine-induced scratch episode intervals by increasing the amount of shorter scratch episodes in the 0.1- to 0.2-s and 0.2- to 0.3-s intervals ($P < 0.001$ and $P < 0.05$, respectively)

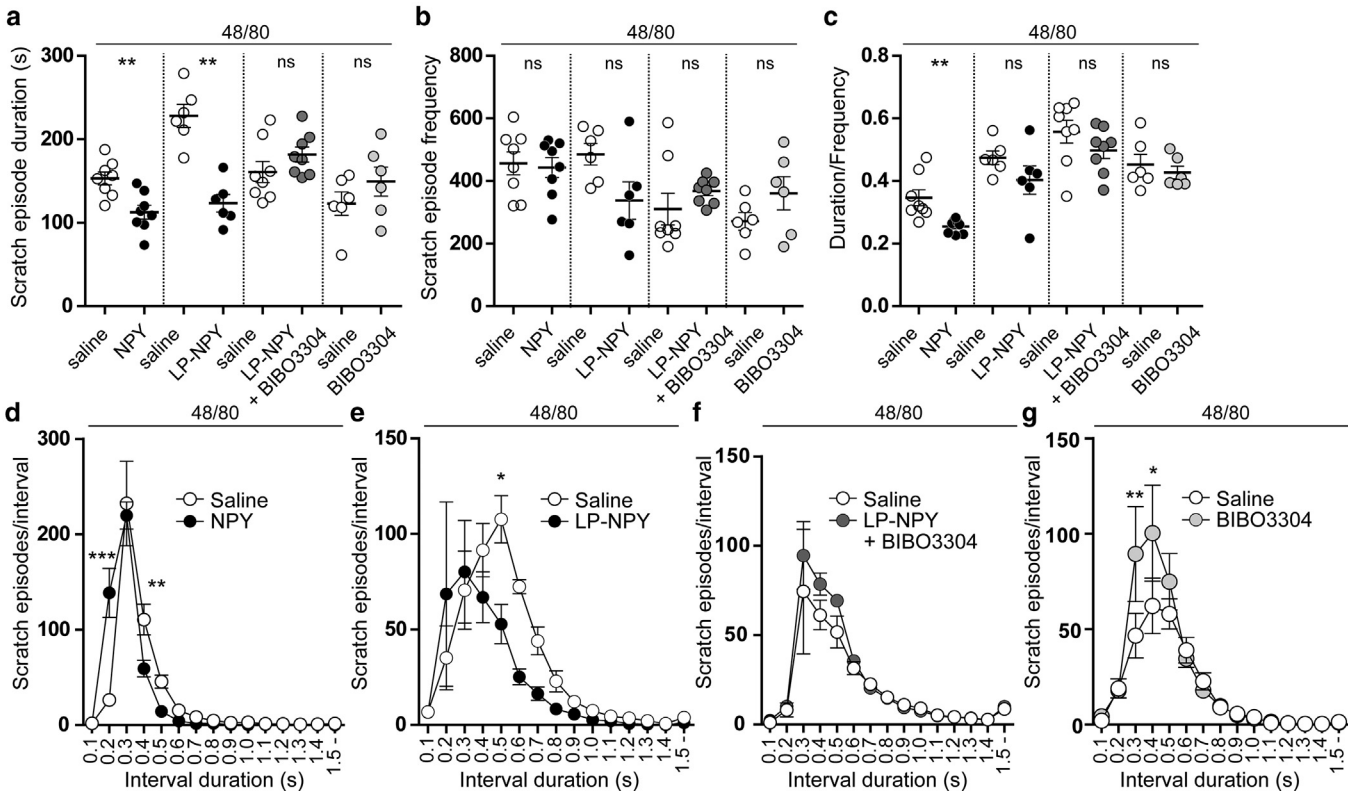

Figure 2. Activation of the NPY/Y_1 system regulates 48/80-induced chemical itch. (**a**) The effect of NPY (10 nmol/L) (n = 8), LP-NPY (10 nmol/L) (n = 6), BIBO3304 (100 nmol/L) (n = 8 and 6, respectively), or saline on 48/80 (100 μg)-induced scratch episode duration (see Table 1 for definitions). (**b**) The effect of NPY (n = 8), LP-NPY (n = 6), BIBO3304 (n = 8 and 6, respectively) or saline on 48/80-induced scratch episode frequency (Table 1). (**c**) The effect of NPY (n = 8), LP-NPY (n = 6), BIBO3304 (n = 8 and 6, respectively) or saline on 48/80-induced mean length of scratch episodes (Table 1). (**d**–**g**) The distribution of scratch episodes from short to longer (n as described in **a**–**c**). Interval duration: 0.1 second represents scratch episodes within the 0- to 0.1-s interval, 0.2 second represents scratch episodes within the 0.1- to 0.2-s interval, and so forth. 1.5 seconds represents scratch episodes within 1.4- to 1.5-s interval and longer. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns > 0.05. In **a**–**c**: LP-NPY and BIBO3304, Mann-Whitney test; NPY and BIBO3304 + LP-NPY, unpaired *t* test, passed D'Agostino and Pearson normality test. In **d**–**g**, two-way analysis of variance with Bonferroni posttest (GraphPad Prism, La Jolla, CA). LP-NPY, [Leu^{31},Pro^{34}] neuropeptide Y; NPY, neuropeptide Y; ns, not significant.

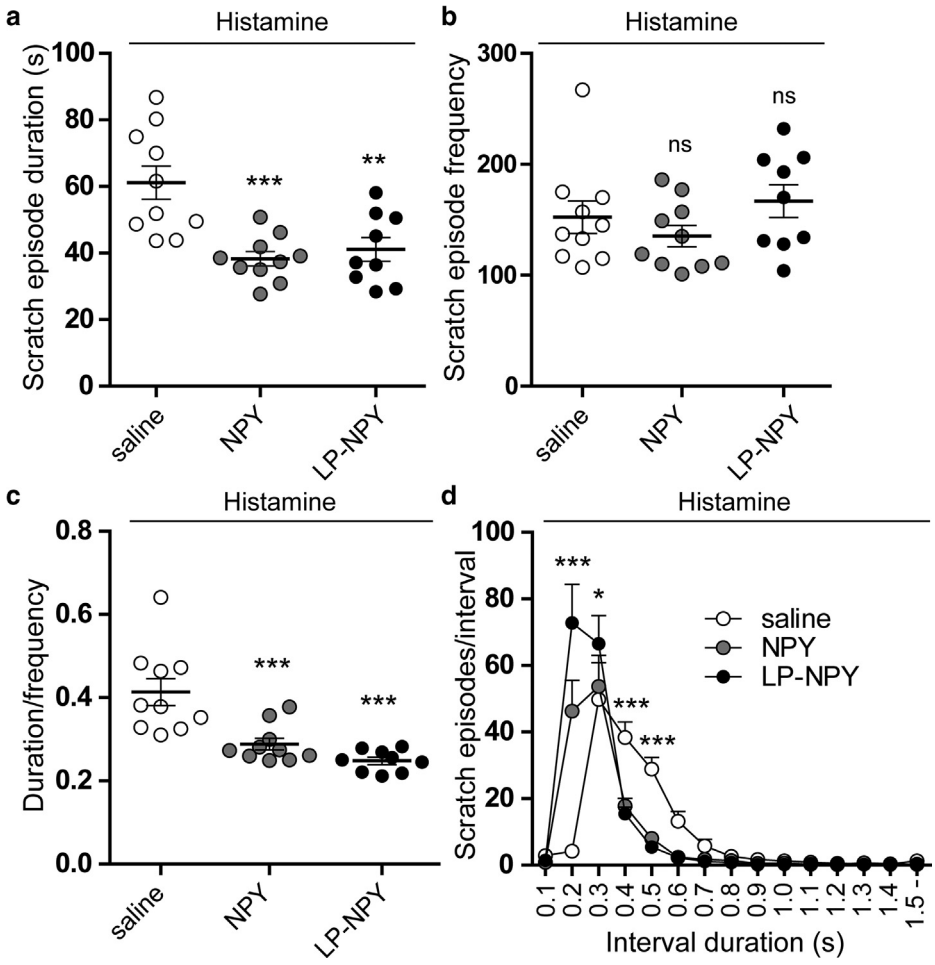

Figure 3. Activation of the NPY/Y_1 system attenuates histaminergic itch. (**a**) The effect of intrathecal injection of NPY (10 nmol/L) (n = 10), LP-NPY (10 nmol/L) (n = 9) or saline (n = 10) on histamine (100 μg)-induced scratch episode duration. (**b**) The effect of intrathecal injection of NPY (n = 10), LP-NPY (n = 9), or saline (n = 10) on histamine-induced scratch episode frequency. (**c**) The effect of intrathecal injection of NPY (n = 10), LP-NPY (n = 9), or saline (n = 10) on histamine-induced mean length of scratch episodes. (**d**) The distribution of scratch episodes from short to longer (n as described in **a**–**c**). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns > 0.05. In **a**–**c**, one-way analysis of variance with Bonferroni posttest (GraphPad Prism, La Jolla, CA). In **d**, two-way analysis of variance with Bonferroni posttest (GraphPad Prism). One animal was excluded from the LP-NPY–treated group because the digital camera malfunctioned during the recording. LP-NPY, [Leu^{31},Pro^{34}] neuropeptide Y; NPY, neuropeptide Y; ns, not significant.

whereas NPY and LP-NPY decreased the number of scratch episodes in the longer 0.3- to 0.4-s and 0.4- to 0.5-s intervals ($P < 0.001$) compared with saline-treated mice (Figure 3d).

To evaluate if the NPY/Y_1 system also regulates chemically induced scratching by a non-histaminergic component, the pruritic non-histaminergic anti-malaria drug chloroquine (10 mmol/L) was injected intradermally after an intrathecal injection of LP-NPY or saline. There was no difference in scratch episode duration ($P = 0.39$) (Figure 4a), number of scratch episodes ($P = 0.39$) (Figure 4b), or mean length of scratch episodes ($P = 0.39$) (Figure 4c) between LP-NPY and saline after the chloroquine injection, with the exception of the 0.5- to 0.6-s interval, which was more frequent in LP-NPY–treated mice ($P < 0.05$) (Figure 4d).

DISCUSSION

Our analysis shows that the spinal NPY/Y_1 system regulates acute mechanical itch because intrathecal administration of the Y_1 agonist LP-NPY could reduce the duration and number of shaking episodes after application of a von Frey filament to the nape of the neck. Itch is associated with scratching but also with shakes of the fur, which previously has been shown to correlate well with scratching behavior (Jinks and Carstens, 2002). Our finding is also in agreement with the findings of Bourane et al. (2015), who showed that spinal NPY interneurons gate mechanical itch.

NPY was also found to regulate chemical histamine or 48/80-induced itch by reducing the duration of scratch episodes without affecting the number of scratch episodes, resulting in a shortening of the mean length of scratch episodes. Also, LP-NPY reduced the duration of histamine or 48/80-induced scratch behavior, showing that the NPY/Y_1 system regulates both chemical (histaminergic) and mechanical itch. Our conclusions regarding chemical itch thus differ from the previous finding by Bourane et al. (2015), who concluded that 48/80-induced itch was not to affected by the NPY system. A plausible reason for this difference could be the way that the analysis of the itch behavior was performed. Bourane et al. focused their itch analysis on the number of scratch episodes per time period (frequency), that is, how often the animal started to scratch to remove the perceived irritant. However, behavioral research needs to consider the duration of a specific behavior (Brown and Bolivar, 2018), in this case how much time the animal spends scratching to remove the perceived irritant. The field of itch behavior analysis is still in its infancy, and according to our survey only 5% (5 out of the 100 most recent articles addressing itch behavior in mice) addressed both number and duration of scratch episodes or bouts. By considering the number, duration, and mean length of scratch episodes, a more complete picture of the behavior can be obtained (Table 1). In fact, our analysis shows that the number of 48/80-induced scratch episodes is unchanged

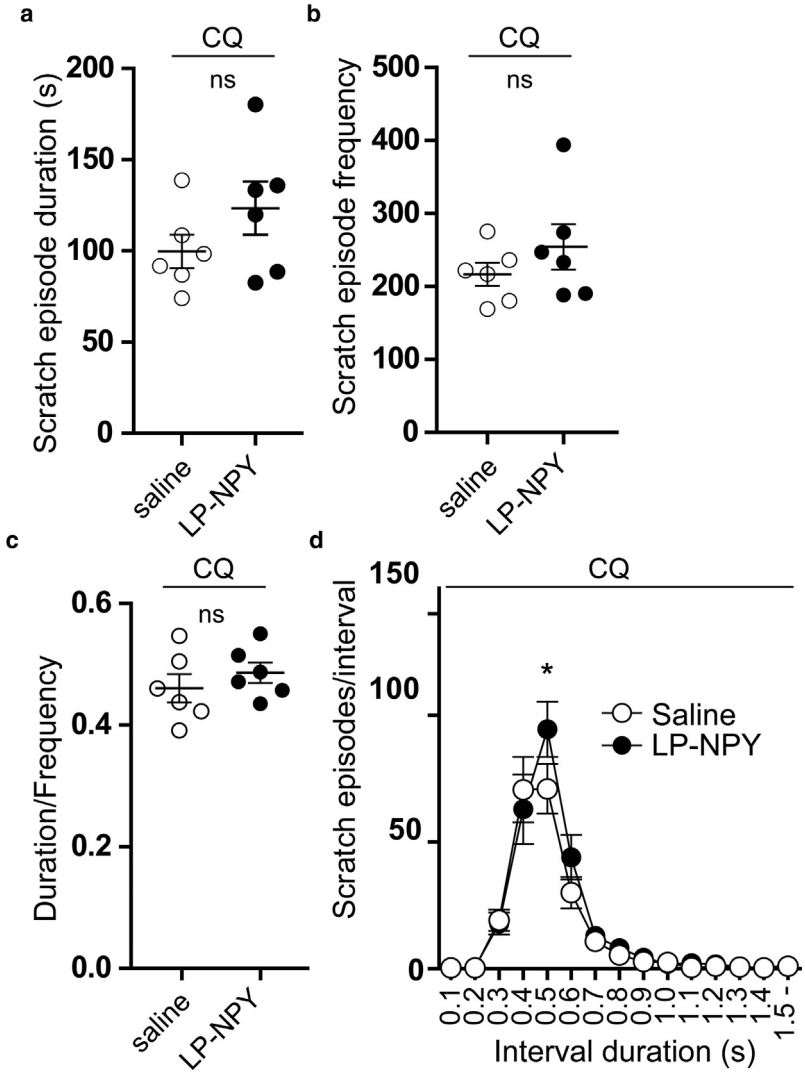

Figure 4. The effect of the NPY/Y_1 system on CQ-induced chemical itch. (**a**) The effect of LP-NPY (10 nmol/L) (n = 6) or saline on CQ-induced scratch episode duration (see Table 1 for definitions). (**b**) The effect of LP-NPY (n = 6) or saline on CQ-induced scratch episode frequency (Table 1). (**c**) The effect of LP-NPY (n = 6) or saline on CQ-induced mean length of scratch episodes (Table 1). (**d**–**g**) The distribution of scratch episodes from short to longer (n as described in **a**–**c**). *$P < 0.05$, ns > 0.05. In **a**–**c**, Mann-Whitney test. In **d**, two-way analysis of variance with Bonferroni posttest (GraphPad Prism, La Jolla, CA). CQ, chloroquine; LP-NPY, [Leu^{31},Pro^{34}] neuropeptide Y; NPY, neuropeptide Y; ns, not significant.

when NPY is administered intrathecally, which is consistent with the finding of Bourane et al., indicating that the NPY system is not affecting how often an itch stimulus is perceived (resulting in the initiation of a scratch response). However, we found that release of NPY and subsequent activation of the Y_1 reduces scratch episode duration, indicating that NPY/Y_1 mutes chemically induced itch behavior by reducing the time spent scratching.

Itch transmission can be regulated at the spinal level by a subpopulation of inhibitory interneurons expressing the transcription factor Bhlhb5 (a class B basic-helix-loop-helix transcription factor) (Ross et al., 2010) (Figure 5). Bhlhb5 neurons inhibit scratch behavior (Ross et al., 2010) and are activated by temperature counterstimuli, thus representing a pathway that may explain how heat/cold stimuli could attenuate itch (Kardon et al., 2014). NPY interneurons in turn are innervated by Aβ and C fibers (Bourane et al., 2015; Rowan et al., 1993) and are activated upon mechanical, chemical, and temperature counterstimuli (Liu et al., 2010; Polgar et al., 2013). Somatostatin has been found to potentiate chemically induced scratching by hyperpolarizing Bhlhb5 neurons through activation of the $G\alpha_i$-coupled somatostatin receptor 2A (Kardon et al., 2014), and consistently antagonizing the somatostatin receptor 2A has been found to reduce itch (Huang et al., 2018), emphasizing the role of somatostatin-expressing neurons in the regulation of itch. The Y_1 receptor is expressed by somatostatin-positive spinal interneurons (Zhang et al., 1999), and NPY causes hyperpolarization of spinal Y_1-expressing neurons (Miyakawa et al., 2005), which consequently leads to attenuated scratching behavior or, more specifically, to reduced time spent scratching (Figure 5), suggesting that NPY interneurons represent a plausible pathway for the counterstimulus mechanical pain's ability to inhibit itch. Combining our findings with those of Bourane et al. (2015) could suggest that when spinal NPY-expressing interneurons are ablated or silenced, the ability of the counterstimulus, scratch, to inhibit itch is diminished, resulting in an increased spontaneous itch behavior.

This study shows that the NPY/Y_1 system regulates both mechanical and chemical (histaminergic) itch and emphasizes the importance of using a broader spectrum of parameters when itch-induced responses are analyzed. This will enable separate analysis of the initiation/perception of itch and the regulatory aspects of the counterstimulus, scratch, thus refining future itch analysis.

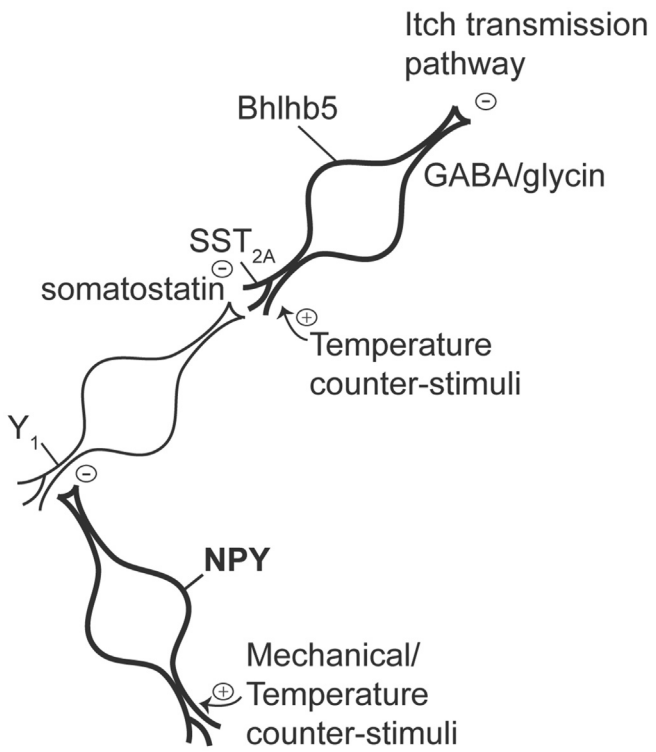

Figure 5. Schematic describing plausible transmission pathways for the regulation of itch. NPY interneurons are found in lamina I–IV of the spinal cord (Bourane et al, 2015), receive input from Aβ and C fibers (Rowan et al., 1993; Bourane et al., 2015) and are activated by mechanical, chemical, and temperature (capsaicin) counterstimuli (Polgar et al., 2013). NPY induces hyperpolarization in substantia gelatinosa interneurons through interactions with the Y1 receptor (Miyakawa et al., 2005). Y1 is co-localized with somatostatin (Zhang et al., 1999), which acts to hyperpolarize inhibitory interneurons expressing the transcription factor Bhlhb5 through interaction with the SST_{2A} receptor (Kardon et al., 2014). NPY inhibits the Y1 (Miyakawa et al., 2005)/somatostatin neurons, thereby plausibly potentiating the ability for Bhlhb5 neurons to attenuate the scratch behavior, resulting in shorter scratch episodes. NPY, neuropeptide Y; SST_{2A}, somatostatin receptor 2A.

MATERIALS AND METHODS

Animals

Animal procedures were approved by the local ethical committee in Uppsala and followed the Directive 2010/63/EU of the European Parliament and of the Council, The Swedish Animal Welfare Act (Djurskyddslagen: SFS 1988:534), The Swedish Animal Welfare Ordinance (Djurskyddsförordningen: SFS 1988:539), and the provisions regarding the use of animals for scientific purposes (DFS 2004:15 and SJVFS 2012:26). All behavior analyses were performed in a controlled environment at 20–24 °C, 45%–65% humidity, and during the light 12-hour day/night cycle.

Chemical itch

Adult C57BL6 mice (>7 weeks) were placed and acclimated for 5–10 minutes in a transparent cage with bedding before they were sedated using 3%–4% isoflurane, followed by 2% isoflurane during the injection. When fully sedated, the mice were slowly injected intrathecally (L5–L6) with 5 μl of BIBO3304 (100 nmol/L; Tocris Bioscience, Bristol, UK), LP-NPY (10 nmol/L, selective for Y_1 versus Y_2 ($Y_1 \approx Y_4 \approx Y_5 \approx Y_6 >> Y_2$ [Beck-Sickinger et al., 2018]) (Bachem, Bubendorf, Switzerland), NPY (10 nmol/L, Bachem), or saline (9 mg/ml, Apoteket, Sweden) using a 25-μl Hamilton syringe. The concentrations of BIBO3304, LP-NPY, and NPY were chosen based on the potencies of respective ligand to the Y_1 (Beck-Sickinger et al., 2018). Mice then recovered (normal motor behavior determined by visual inspection) and acclimated for 10 minutes in a transparent cage with bedding before being given an 50-μl intradermal injection in the nape of the neck of compound 48/80 (2 μg/μl; Sigma, St. Louis, MO) or histamine (2 μg/μl, Sigma) or chloroquine (10 mmol/L, Sigma) and were recorded for 60 minutes using a digital camera. The investigators were not present in the room during the recordings and were blinded to the intrathecal treatment given. To evaluate the selectivity of LP-NPY for Y_1, the Y_1-selective antagonist BIBO3304 or saline (control) was given intrathecally 10 minutes before LP-NPY or saline using a 25-μl Hamilton syringe (the same concentrations of LP-NPY or BIBO3304 were used as listed).

The itch behavior was scored manually using the software AniTracker, version 1.0 (www.rsutils.com/downloads.html), and the results were displayed as the mean number ± standard error of the mean of scratch episode duration, scratch episode frequency, scratch episode duration/frequency, and frequency of episode duration/time interval per group in 60 minutes (Table 1). All video analyses were done by observers blind to the intrathecal treatment given.

Mechanical itch

Adult C57BL6 mice (>7 weeks) were placed in a chamber supplied with constant flow of 3% isoflurane, 21% oxygen, and 78% nitrogen. When fully sedated, the mice were slowly injected intrathecally (L5–L6) with 5 μl LP-NPY (10 nmol/L), NPY (10 nmol/L), or saline (9 mg/ml) using a 25-μl Hamilton syringe. Mice were then acclimated for 10 minutes in a transparent cage with bedding and recorded with a digital camera. After acclimation, von Frey filaments (AgnThos, Lingö, Sweden) were applied on the nape of neck. Mechanical itch behaviors were recorded as number of scratch episodes and number of shaking episodes. A shake of the body upon von Frey stimuli was recorded as one episode of shaking (Table 1). Each mouse was given five consecutive mechanical stimuli at the frequency of 1/second by a 0.07 g von Frey filament. The mechanical itch behavior was reported as total numbers of scratching/shaking episodes per five von Frey stimuli. The itch behavior was scored manually using the software AniTracker, version 1.0, and the results were displayed as the mean number ± standard error of the mean of scratch or shake episode duration and scratch or shake episode frequency and mean length of scratch or shake episode (Table 1).

Statistical analysis

Gaussian distribution was tested using D'Agostino and Pearson normality test (GraphPad Prism, La Jolla, CA). One-way analysis of variance with Bonferroni posttest (GraphPad Prism) was used to compare three different treatments. Unpaired *t* test (GraphPad Prism) was used for the data sets that passed the normality test, and Mann-Whitney test (GraphPad Prism) was used for the other datasets except the episode duration intervals, which were analyzed using two-way analysis of variance with Bonferroni posttest (GraphPad Prism).

ORCID

Dan Larhammar: http://orcid.org/0000-0002-6736-0663
Malin Charlotta Lagerström: http://orcid.org/0000-0002-9086-2805

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We acknowledge Erika Roman and Klas Kullander for constructive comments on the manuscript. This work was supported by grants from the Swedish Research Council, Uppsala University, The Brain Foundation and the

foundations of Ragnar Söderberg and Byggmästare Olle Engkvist. MCL is a Ragnar Söderberg Fellow in Medicine.

AUTHOR CONTRIBUTIONS

TG and HM conducted the behavioral experiment. TG, HM, and JB analyzed the videos. TG, HM, and MCL conducted the statistical analysis. BX prepared and blinded the intrathecally given agonists. MCL designed the project. All authors contributed to the writing of the manuscript.

REFERENCES

Beck-Sickinger A, Colmers WF, Cox HM, Doods HN, Herzog H, Larhammar D, et al. Neuropeptide Y receptors: Y1 receptor, http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=305; 2018 (accessed 27 April 2018).

Bourane S, Duan B, Koch SC, Dalet A, Britz O, Garcia-Campmany L, et al. Gate control of mechanical itch by a subpopulation of spinal cord interneurons. Science 2015;350(6260):550–4.

Brown RE, Bolivar S. The importance of behavioural bioassays in neuroscience. J Neurosci Methods 2018;300:68–76.

Brumovsky P, Stanic D, Shuster S, Herzog H, Villar M, Hokfelt T. Neuropeptide Y2 receptor protein is present in peptidergic and nonpeptidergic primary sensory neurons of the mouse. J Comp Neurol 2005;489:328–48.

Huang J, Polgar E, Solinski HJ, Mishra SK, Tseng PY, Iwagaki N, et al. Circuit dissection of the role of somatostatin in itch and pain. Nat Neurosci 2018;21:707–16.

Inoue A, Uchida H, Nakazawa T, Yamamoto T, Ito S. Phosphorylation of NMDA receptor GluN2B subunit at Tyr1472 is important for trigeminal processing of itch. Eur J Neurosci 2016;44:2474–82.

Intondi AB, Dahlgren MN, Eilers MA, Taylor BK. Intrathecal neuropeptide Y reduces behavioral and molecular markers of inflammatory or neuropathic pain. Pain 2008;137:352–65.

Jinks SL, Carstens E. Responses of superficial dorsal horn neurons to intradermal serotonin and other irritants: comparison with scratching behavior. J Neurophysiol 2002;87:1280–9.

Kardon AP, Polgar E, Hachisuka J, Snyder LM, Cameron D, Savage S, et al. Dynorphin acts as a neuromodulator to inhibit itch in the dorsal horn of the spinal cord. Neuron 2014;82:573–86.

Liu T, Han Q, Chen G, Huang Y, Zhao LX, Berta T, et al. Toll-like receptor 4 contributes to chronic itch, alloknesis, and spinal astrocyte activation in male mice. Pain 2016;157:806–17.

Liu Y, Abdel Samad O, Zhang L, Duan B, Tong Q, Lopes C, et al. VGLUT2-dependent glutamate release from nociceptors is required to sense pain and suppress itch. Neuron 2010;68:543–56.

Malet M, Leiguarda C, Gaston G, McCarthy C, Brumovsky P. Spinal activation of the NPY Y1 receptor reduces mechanical and cold allodynia in rats with chronic constriction injury. Peptides 2017;92:38–45.

Miyakawa A, Furue H, Katafuchi T, Jiang N, Yasaka T, Kato G, et al. Action of neuropeptide Y on nociceptive transmission in substantia gelatinosa of the adult rat spinal dorsal horn. Neuroscience 2005;134:595–604.

Naveilhan P, Neveu I, Arenas E, Ernfors P. Complementary and overlapping expression of Y1, Y2 and Y5 receptors in the developing and adult mouse nervous system. Neuroscience 1998;87:289–302.

Polgar E, Sardella TC, Tiong SY, Locke S, Watanabe M, Todd AJ. Functional differences between neurochemically defined populations of inhibitory interneurons in the rat spinal dorsal horn. Pain 2013;154:2606–15.

Ross SE, Mardinly AR, McCord AE, Zurawski J, Cohen S, Jung C, et al. Loss of inhibitory interneurons in the dorsal spinal cord and elevated itch in Bhlhb5 mutant mice. Neuron 2010;65:886–98.

Rowan S, Todd AJ, Spike RC. Evidence that neuropeptide Y is present in GABAergic neurons in the superficial dorsal horn of the rat spinal cord. Neuroscience 1993;53:537–45.

Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjork HE. Specific C-receptors for itch in human skin. J Neurosci 1997;17:8003–8.

Solway B, Bose SC, Corder G, Donahue RR, Taylor BK. Tonic inhibition of chronic pain by neuropeptide Y. Proc Natl Acad Sci USA 2011;108:7224–9.

Todd AJ, Hughes DI, Polgar E, Nagy GG, Mackie M, Ottersen OP, et al. The expression of vesicular glutamate transporters VGLUT1 and VGLUT2 in neurochemically defined axonal populations in the rat spinal cord with emphasis on the dorsal horn. Eur J Neurosci 2003;17:13–27.

Wieland HA, Engel W, Eberlein W, Rudolf K, Doods HN. Subtype selectivity of the novel nonpeptide neuropeptide Y Y1 receptor antagonist BIBO 3304 and its effect on feeding in rodents. Br J Pharmacol 1998;125:549–55.

Yalamuri SM, Brennan TJ, Spofford CM. Neuropeptide Y is analgesic in rats after plantar incision. Eur J Pharmacol 2013;698(1–3):206–12.

Zhang X, Tong YG, Bao L, Hokfelt T. The neuropeptide Y Y1 receptor is a somatic receptor on dorsal root ganglion neurons and a postsynaptic receptor on somatostatin dorsal horn neurons. Eur J Neurosci 1999;11:2211–25.