UNITED STATES
SECURITIES AND EXCHANGE COMMISSION
Washington, D.C. 20549

FORM 8-K

CURRENT REPORT

Pursuant to Section 13 or 15(d) of the Securities Exchange Act of 1934

Date of report (date of earliest event reported): June 21, 2022

TONIX PHARMACEUTICALS HOLDING CORP.

(Exact name of registrant as specified in its charter)

Nevada	**001-36019**	**26-1434750**
(State or Other Jurisdiction of Incorporation)	**(Commission File Number)**	**(IRS Employer Identification No.)**

26 Main Street, Chatham, New Jersey 07928
(Address of principal executive offices) (Zip Code)

Registrant's telephone number, including area code: (862) 904-8182

Check the appropriate box below if the Form 8-K filing is intended to simultaneously satisfy the filing obligation of the registrant under any of the following provisions (see General Instruction A.2. below):

☐ Written communications pursuant to Rule 425 under the Securities Act (17 CFR 230.425)
☐ Soliciting material pursuant to Rule 14a-12 under the Exchange Act (17 CFR 240.14a-12)
☐ Pre-commencement communications pursuant to Rule 14d-2(b) under the Exchange Act (17 CFR 240.14d-2(b))
☐ Pre-commencement communications pursuant to Rule 13e-4(c) under the Exchange Act (17 CFR 240.13e-4(c))

Securities registered pursuant to Section 12(b) of the Act:

Title of each class	Trading Symbol(s)	Name of each exchange on which registered
Common Stock	TNXP	The NASDAQ Capital Market

Indicate by check mark whether the registrant is an emerging growth company as defined in Rule 405 of the Securities Act of 1933 (§ 230.405 of this chapter) or Rule 12b-2 of the Securities Exchange Act of 1934 (§ 240.12b-2 of this chapter).

Emerging growth company ☐

If an emerging growth company, indicate by check mark if the registrant has elected not to use the extended transition period for complying with any new or revised financial accounting standards provided pursuant to Section 13(a) of the Exchange Act. ☐

Item 7.01 Regulation FD Disclosure.

On June 21, 2022, Tonix Pharmaceuticals Holding Corp. (the "Company") announced the publication of a paper entitled "Impact of Magnesium on Oxytocin Receptor Function," in the journal *Pharmaceutics* (the "Paper"), that includes data showing the enhancing effects of magnesium ("Mg^{2+}") on the activity of intranasal oxytocin in an animal model of craniofacial pain. A copy of the press release which discusses this matter and the Paper, which may contain material, nonpublic information, are furnished hereto as Exhibits 99.01 and 99.02, respectively, and incorporated herein by reference.

The information in this Item 7.01 of this Current Report on Form 8-K, including Exhibits 99.01 and 99.02 attached hereto, shall not be deemed "filed" for purposes of Section 18 of the United States Securities Exchange Act of 1934 (the "Exchange Act") or otherwise subject to the liabilities of that section, nor shall they be deemed incorporated by reference in any filing under the United States Securities Act of 1933 or the Exchange Act, except as shall be expressly set forth by specific reference in such a filing.

Item 8.01. Other Events.

On June 21, 2022, the Company announced the publication of the Paper that described results from a research team led by Professor David Yeomans. The paper includes data showing the enhancing effects of Mg^{2+} on the activity of intranasal oxytocin in an animal model of craniofacial pain. The Mg^{2+} enhanced formulation of intranasal oxytocin is the basis for the Company's TNX-1900[2] drug candidate in development to prevent migraine headaches in chronic migraineurs, and TNX-2900, which is in development to treat hyperphagia in adolescent and young adult patients with Prader-Willi syndrome. Professor Yeomans was the scientific founder of Trigemina, Inc. from which Tonix acquired rights to the Mg^{2+}enhanced oxytocin technology. Professor Yeomans is a consultant to Tonix and the research described in the paper was funded in part by Tonix. The data evidences the role Mg^{2+} plays in the effect of oxytocin on pain reduction both *in vitro* and *in vivo* in an animal model of head pain, and that adding Mg^{2+} to intranasal oxytocin reduces or eliminates high dose inhibition such that analgesia increases with higher doses of oxytocin. The Company expects that the Mg^{2+} enhanced formulation of intranasal oxytocin in TNX-1900 and TNX-2900 may provide consistent effects to the extent that the Mg^{2+} component of the formulation reduces or eliminates high dose inhibition and may also allow for using higher oxytocin doses. Intranasal oxytocin with Mg^{2+} demonstrated augmented craniofacial analgesia in animal models, and,

under test conditions, oxytocin has the potential to allow for the use of higher oxytocin doses. The clinical significance of these observations is that the formulation of oxytocin plus Mg^{2+} in the Company's TNX-1900 and TNX-2900 product candidates has the potential to enhance oxytocin efficacy for pain and other uses.

The Company believes that TNX-1900 has the potential to be a non-addicting, non-constipating and easy to administer alternative to opioids to treat migraine and craniofacial pain, and that targeted delivery of oxytocin could translate into selective blockade of the neurotransmitter calcitonin gene-related peptide ("CGRP") in release in the trigeminal ganglion and not throughout the body, which could be a potential safety advantage over systemic CGRP inhibition.

Forward- Looking Statements

This Current Report on Form 8-K contains certain forward-looking statements within the meaning of Section 27A of the Securities Act of 1933 and Section 21E of the Securities Exchange Act of 1934 and Private Securities Litigation Reform Act, as amended, including those relating to the development of TNX-102 SL, the Company's product development, clinical trials, clinical and regulatory timelines, market opportunity, competitive position, possible or assumed future results of operations, business strategies, potential growth opportunities and other statement that are predictive in nature. These forward-looking statements are based on current expectations, estimates, forecasts and projections about the industry and markets in which we operate and management's current beliefs and assumptions.

These statements may be identified by the use of forward-looking expressions, including, but not limited to, "expect," "anticipate," "intend," "plan," "believe," "estimate," "potential," "predict," "project," "should," "would" and similar expressions and the negatives of those terms. These statements relate to future events or our financial performance and involve known and unknown risks, uncertainties, and other factors which may cause actual results, performance or achievements to be materially different from any future results, performance or achievements expressed or implied by the forward-looking statements. Such factors include those set forth in the Company's filings with the SEC. Prospective investors are cautioned not to place undue reliance on such forward-looking statements, which speak only as of the date of this press release. The Company undertakes no obligation to publicly update any forward-looking statement, whether as a result of new information, future events or otherwise.

Item 9.01 Financial Statements and Exhibits.

(d)

Exhibit No.	Description.
99.01	Press release of the Company, dated June 21, 2022
99.02	Impact of Magnesium on Oxytocin Receptor Function
104	Cover Page Interactive Data File (embedded within the Inline XBRL document)

SIGNATURE

Pursuant to the requirement of the Securities Exchange Act of 1934, the registrant has duly caused this report to be signed on its behalf by the undersigned thereunto duly authorized.

TONIX PHARMACEUTICALS HOLDING CORP.

Date: June 21, 2022

By: /s/ Bradley Saenger
Bradley Saenger
Chief Financial Officer

Exhibit 99.01

Tonix Pharmaceuticals Announces Publication of Paper in *Pharmaceutics* on the Enhancing Effect That Magnesium Contributes to *in vivo* Intranasal Oxytocin Analgesia

Intranasal Oxytocin with Magnesium Demonstrated Augmented Craniofacial Analgesia in an Animal Model

Enhanced Effect of Mg^{2+} is the Core Patented Technology of TNX-1900 for Migraine

TNX-2900 Orphan Drug Designated Product for Prader-Willi Syndrome Also Contains Mg^{2+}

Issued Patents Expected to Provide Exclusivity Until 2036

CHATHAM, N.J., June 21, 2022 – Tonix Pharmaceuticals Holding Corp. (Nasdaq: TNXP), a clinical-stage biopharmaceutical company, announced the publication of a paper, entitled "Impact of Magnesium on Oxytocin Receptor Function," in the journal *Pharmaceutics*, that described results from a research team led by Professor David Yeomans[1]. The paper includes data showing the enhancing effects of magnesium (Mg^{2+}) on the activity of intranasal oxytocin in an animal model of craniofacial pain. The Mg^{2+} enhanced formulation of intranasal oxytocin is the basis for the Company's TNX-1900[2] drug candidate in development to prevent migraine headaches in chronic migraineurs, and TNX-2900[2] which is in development to treat hyperphagia (over-eating) in adolescent and young adult patients with Prader-Willi syndrome. Professor Yeomans was the scientific founder of Trigemina, Inc. from which Tonix acquired rights to the Mg^{2+}enhanced oxytocin technology. Professor Yeomans is a consultant to Tonix and the research described in the paper was funded in part by Tonix.

"This new paper further evidences the important role Mg^{2+} plays in the effect of oxytocin on pain reduction both *in vitro* and *in vivo* in an animal model of head pain," said Seth Lederman, M.D., Chief Executive Officer of Tonix Pharmaceuticals. "Prior to this work, studies of intranasal oxytocin on pain yielded inconsistent results because the analgesic effect of oxytocin decreased with higher doses, a phenomenon called 'high dose inhibition' or colloquially, as an 'inverted U' shaped dose response. Professor Yeomans and his team have shown that adding Mg^{2+} to intranasal oxytocin reduces or eliminates the 'high dose inhibition', such that analgesia increases with higher doses of oxytocin. Consequently, we expect that the Mg^{2+} enhanced formulation of intranasal oxytocin in TNX-1900 and TNX-2900 may provide consistent effects to the extent that the Mg^{2+} component of the formulation reduces or eliminates the high dose inhibition and may also allow for using higher oxytocin doses."

Professor Yeomans said, "Mg^{2+} levels modulate the affinity of oxytocin receptor for oxytocin *in vitro*. We have now confirmed that at above physiological concentrations Mg^{2+} increases the activity of oxytocin for oxytocin receptor *in vivo.* Intranasal oxytocin with Mg^{2+} demonstrated augmented craniofacial analgesia in animal models. Critically, we have also demonstrated that, under test conditions, oxytocin has an 'inverted U' shaped dose response which Mg^{2+} can overcome, potentially allowing for the use of higher oxytocin doses. The potential clinical significance of these observations is that the formulation of oxytocin plus Mg^{2+} in Tonix's TNX-1900 and TNX-2900 has the potential to enhance oxytocin efficacy for pain as well as for other uses."

"Tonix is excited to develop Mg^{2+}-enhanced formulation of intranasal oxytocin as a non-addictive treatment for migraine and craniofacial pain and for the treatment of Prader-Willi Syndrome," added Dr. Lederman. "TNX-1900 will enter Phase 2 for prophylaxis of chronic migraine in the second half of 2022 and TNX-2900 is in development for treating hyperphagia in Prader Willi syndrome. The preclinical data that we have seen to date are promising and show that oxytocin, a natural hormone, is capable of blocking the release of the neurotransmitter calcitonin gene-related peptide (CGRP) in the brain coverings and trigeminal ganglia, thus potentially modulating a key step in the causation of migraine. It has been shown that intranasally delivered oxytocin selectively reaches the trigeminal ganglia with low systemic absorption. Overall, we believe that TNX-1900 has the potential to be a non-addicting, non-constipating and easy to administer alternative to opioids to treat migraine and craniofacial pain. We believe targeted delivery of oxytocin could translate into selective blockade of CGRP release in the trigeminal ganglion and not throughout the body, which could be a potential safety advantage over systemic CGRP inhibition. In addition, daily dosing is more quickly reversible, in contrast to monthly or quarterly dosing, giving physicians and their patients greater control."

About Intranasal Oxytocin

Oxytocin is a naturally occurring human hormone that acts as a neurotransmitter in the brain. Oxytocin has no recognized addiction potential. Oxytocin is approved by the U.S. Food and Drug Administration (FDA) as Pitocin®[3], an intravenous infusion or intramuscular injection drug, for use in pregnant women to induce labor. An intranasal form of oxytocin was marketed in the U.S. by Novartis to assist in the production of breast milk as Syntocinon®[4] (oxytocin nasal 40 units/ml), but the product was discontinued, and the New Drug Application (NDA) has been withdrawn.

About Migraine

Migraine is a neurological condition that manifests in throbbing headache, often on one side of the head, that lasts at least four hours. It can also be accompanied by nausea, vomiting, visual disturbances, and sensitivity to bright light, strong smells, and loud noises[5]. Epidemiological studies indicate that globally, approximately 1.2 billion individuals suffer from migraines annually.[6] Approximately 39 million Americans suffer from migraines and among these individuals, approximately four million experience chronic migraines (15 or more headache days per month).[6]

About TNX-1900[2]

TNX-1900 (intranasal potentiated oxytocin) is a proprietary formulation of oxytocin and Mg^{2+} in development as a candidate for prophylaxis of chronic migraine and for the treatment of craniofacial pain, insulin resistance and related conditions. In 2020, TNX-1900 was acquired from Trigemina, Inc. TNX-1900 is a drug-device combination product, based on an intranasal actuator device that delivers oxytocin and Mg^{2+} into the nose. It has been observed that low oxytocin levels in the body can lead to increase in migraine headache frequency, and that increased oxytocin levels can relieve migraine headaches. Certain other chronic pain conditions are also associated with decreased oxytocin levels. Migraine attacks are caused, in part, by the activity of pain-sensing trigeminal nerve cells which, when activated, release of CGRP which binds to receptors on other nerve cells and starts a cascade of events that is believed to result in headache. Oxytocin when delivered via the nasal route, concentrates in the trigeminal system[8] resulting in binding of oxytocin to receptors on neurons in the trigeminal system, inhibiting transmission of pain signals and the release of CGRP[9]. Blocking CGRP release is a distinct mechanism compared with CGRP antagonist and anti-CGRP antibody drugs, which block the binding of CGRP to its receptor. With TNX-1900, the addition

of magnesium to the oxytocin formulation enhances oxytocin receptor binding[10] as well as its effects on trigeminal neurons and craniofacial analgesic effects in animal models[1]. Intranasal oxytocin has been well tolerated in several clinical trials in both adults and children[11]. Targeted nasal delivery results in low systemic exposure and lower risk of non-nervous system, off-target effects which could potentially occur with systemic CGRP antagonists such as anti-CGRP antibodies[12]. For example, CGRP has roles in dilating blood vessels in response to ischemia, including in the heart. Tonix has licensed technology from the University of Geneva to use TNX-1900 for the treatment of insulin resistance and related conditions.

About Prader Willi Syndrome

Prader-Willi syndrome is a rare genetic disorder of failure to thrive in infancy and uncontrolled appetite and obesity in childhood and adulthood with no approved treatments available that occurs in approximately one in 15,000 births. Prader-Willi syndrome results in physical, mental and behavioral problems. A key feature of Prader-Willi syndrome in infants is a lack of suckling and poor muscle strength which leads to malnutrition and failure to thrive. However, paradoxically in children and adults, the key feature of Prader-Willi syndrome is a constant sense of hunger (hyperphagia), which leads to severe obesity. Intranasal oxytocin improves suckling in newborn animals but also suppresses feeding behaviors in adult animal models.

About TNX-2900[5]

TNX-2900 (intranasal potentiated oxytocin) is a proprietary formulation of oxytocin and Mg^{2+} in development as a candidate for treatment of hyperphagia in Prader-Willi syndrome. TNX-2900 is a drug-device combination product, based on an intranasal actuator device that delivers oxytocin and Mg^{2} into the nose. Tonix licensed technology to treat Prader Willi Syndrome and non-organic failure to thrive disease from Inserm (the French National Institute of Health and Medical Research). The licensing agreement was negotiated and signed by Inserm Transfert, the private subsidiary of Inserm, on behalf of Inserm, Aix-Marseille Université and Centre Hospitalier Universitaire of Toulouse. The co-exclusive license allows Tonix to expand its intranasal potentiated oxytocin development program to the treatment of Prader-Willi syndrome. The patents covering the technology are expected to provide market exclusivity for the co-licensees in the U.S. and Europe through 2031, which exclusivity could be extended after marketing authorization by a Supplemental Protection Certificate in Europe or a Patent Term Extension in the U.S., independent of other Tonix-held patents covering the formulation and oxytocin potentiation technologies for intranasal administration.

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[1]Bharadwaj VN, et al., *Pharmaceutics*. 2022; 14(5):1105. https://doi.org/10.3390/pharmaceutics14051105
[2]TNX-1900 and TNX-2900 are investigational new drugs and have not been approved for any indication.
[3]Pitocin® is a trademark of Par Pharmaceutical, Inc.
[4]Syntocinon® is a trademark of BGP Products Operations GmbH.
[4]https://www.mayoclinic.org/diseases-conditions/migraine-headache/symptoms-causes/syc-20360201
[6]Burch et al., *Migraine: Epidemiology, Burden, and Comorbidity,* Neurol Clin 37 (2019) 631–649.
[7]Yeomans, DC et al. 2017. US patent US2017368095.
[8]Yeomans DC, et al. *Transl Psychiatry*. 2021. 11(1):388.
[9]Tzabazis A, et al. *Cephalalgia*. 2016. 36(10):943-50.
[10]Antoni FA and Chadio SE. *Biochem J.* 1989. 257(2):611-4.
[11]Cai Q, et al., *Psychiatry Clin Neurosci*. 2018. Mar;72(3):140-151.
[12]MaassenVanDenBrink A, et al. *Trends Pharmacol Sci.* 2016. 37(9):779-788.

About Tonix Pharmaceuticals Holding Corp.*

Tonix is a clinical-stage biopharmaceutical company focused on discovering, licensing, acquiring and developing therapeutics to treat and prevent human disease and alleviate suffering. Tonix's portfolio is composed of central nervous system (CNS), rare disease, immunology and infectious disease product candidates. Tonix's CNS portfolio includes both small molecules and biologics to treat pain, neurologic, psychiatric and addiction conditions. Tonix's lead CNS candidate, TNX-102 SL (cyclobenzaprine HCl sublingual tablet), is in mid-Phase 3 development for the management of fibromyalgia with a new Phase 3 study launched in the second quarter of 2022 and interim data expected in the first quarter of 2023. TNX-102 SL is also being developed to treat Long COVID, a chronic post-acute COVID-19 condition. Tonix expects to initiate a Phase 2 study in Long COVID in the third quarter of 2022. TNX-1300 (cocaine esterase) is a biologic designed to treat cocaine intoxication that is Phase 2 ready and has been granted Breakthrough Therapy Designation by the FDA. TNX-1900 (intranasal potentiated oxytocin), a small molecule in development for chronic migraine, is expected to enter the clinic with a Phase 2 study in the second half of 2022. Tonix's rare disease portfolio includes TNX-2900 (intranasal potentiated oxytocin) for the treatment of Prader-Willi syndrome. TNX-2900 has been granted Orphan-Drug Designation by the FDA. Tonix's immunology portfolio includes biologics to address organ transplant rejection, autoimmunity and cancer, including TNX-1500 which is a humanized monoclonal antibody targeting CD40-ligand being developed for the prevention of allograft and xenograft rejection and for the treatment of autoimmune diseases. A Phase 1 study of TNX-1500 is expected to be initiated in the second half of 2022. Tonix's infectious disease pipeline consists of a vaccine in development to prevent monkeypox and smallpox called TNX-801, next-generation vaccines to prevent COVID-19, and a platform to make fully human monoclonal antibodies to treat COVID-19. Tonix's lead vaccine candidates for COVID-19 are TNX-1840 and TNX-1850, which are live virus vaccines based on Tonix's recombinant pox vector (RPV) live virus vaccine platform.

**All of Tonix's product candidates are investigational new drugs or biologics and none have been approved for any indication*

This press release and further information about Tonix can be found atwww.tonixpharma.com.

Forward Looking Statements

Certain statements in this press release are forward-looking within the meaning of the Private Securities Litigation Reform Act of 1995. These statements may be identified by the use of forward-looking words such as "anticipate," "believe," "forecast," "estimate," "expect," and "intend," among others. These forward-looking statements are based on Tonix's current expectations and actual results could differ materially. There are a number of factors that could cause actual events to differ materially from those indicated by such forward-looking statements. These factors include, but are not limited to, risks related to the failure to obtain FDA clearances or approvals and noncompliance with FDA regulations; delays and uncertainties caused by the global COVID-19 pandemic; risks related to the timing and progress of clinical development of our product candidates; our need for additional financing; uncertainties of patent protection and litigation; uncertainties of government or third party payor reimbursement; limited research and development efforts and dependence upon third parties; and substantial competition. As with any pharmaceutical under development, there are significant risks in the

development, regulatory approval and commercialization of new products. Tonix does not undertake an obligation to update or revise any forward-looking statement. Investors should read the risk factors set forth in the Annual Report on Form 10-K for the year ended December 31, 2021, as filed with the Securities and Exchange Commission (the "SEC") on March 14, 2022, and periodic reports filed with the SEC on or after the date thereof. All of Tonix's forward-looking statements are expressly qualified by all such risk factors and other cautionary statements. The information set forth herein speaks only as of the date thereof.

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pharmaceutics

MDPI

Article

Impact of Magnesium on Oxytocin Receptor Function

Vimala N. Bharadwaj [1], Justin Meyerowitz [1], Bende Zou [2], Michael Klukinov [1], Ni Yan [2], Kaustubh Sharma [2], David J. Clark [1,3], Xinmin Xie [2] and David C. Yeomans [1,*]

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Simple Summary: What is already known: Mg^{2+} levels modulate the affinity of oxytocin receptors for oxytocin in vitro, low serum Mg^{2+} is correlated with migraine headache onset. What this study adds: Electrophysiologic and behavioral assays demonstrate that Mg^{2+} increases the efficacy of oxytocin; oxytocin efficacy is limited by Mg^{2+} availability. Clinical significance: Modulating Mg^{2+} levels may enhance oxytocin efficacy for pain, other uses, and endogenous processes.

Abstract: Background and Purpose: The intranasal administration of oxytocin (OT) reduces migraine headaches through activation of the oxytocin receptor (OTR). Magnesium ion (Mg^{2+}) concentration is critical to the activation of the OTR, and a low serum Mg^{2+} concentration is predictive of a migraine headache. We, therefore, examined the functional impact of Mg^{2+} concentration on OT-OTR binding efficacy using two complimentary bioassays. Experimental Approach: Current clamp recordings of rat trigeminal ganglia (TG) neurons measured the impact of Mg^{2+} on an OT-induced reduction in excitability. In addition, we assessed the impact of Mg^{2+} on intranasal OT-induced craniofacial analgesia in rats. Key Results: While OT alone dose-dependently hyperpolarized TG neurons, decreasing their excitability, the addition of 1.75 mM Mg^{2+} significantly enhanced this effect. Similarly, while the intranasal application of OT produced dose-dependent craniofacial analgesia, Mg^{2+} significantly enhanced these effects. Conclusions and Implications: OT efficacy may be limited by low ambient Mg^{2+} levels. The addition of Mg^{2+} to OT formulations may improve its efficacy in reducing headache pain as well as for other OT-dependent processes.

Keywords: oxytocin; magnesium; pain; analgesia; headaches; agonist

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Citation: Bharadwaj, V.N.; Meyerowitz, J.; Zou, B.; Klukinov, M.; Yan, N.; Sharma, K.; Clark, D.J.; Xie, X.; Yeomans, D.C. Impact of Magnesium on Oxytocin Receptor Function. *Pharmaceutics* **2022**, *14*, 1105. https://doi.org/10.3390/pharmaceutics14051105

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1. Introduction

Oxytocin (OT) is a nine-residue, cyclized peptide which, through to the oxytocin receptor (OTR), acts as both a hormone, regulating diverse processes such as glucose metabolism, social bonding, lactation, and uterine contraction, as well as a neurotransmitter, modulating neural processes including pain [1–4]. Inadequate OTR activity is associated with postpartum hemorrhage, inadequate milk production, autism spectrum disorder (ASD), Prader–Willi syndrome, and migraine headaches [5–8]. Studies in both animal models and patients have demonstrated that intranasal administration of OT can relieve core symptoms in ASD, schizophrenia, migraine, and anxiety disorders [9–11]. Several factors have been found to alter OTR activity including OT levels, OTR expression, and OTR affinity for its ligand [8]. The last of these, OTR affinity, is closely modulated by the local concentration of the magnesium cation (Mg^{2+}) which acts as an essential cofactor, dramatically increasing the affinity of OTR for OT [8,12,13]. Recently, Meyerowitz et al. [14] published the structure of the OT-OTR-Mg^{2+} coordination complex as identified using

cryo-electron microscopy, demonstrating the critical role that Mg^{2+} plays in OT binding to OTR. Thus, alterations of serum or exogenously applied Mg^{2+} levels should play a critical role in OTR activity and consequently the physiological and psychological functions listed above.

Our previous work showed that OT decreases craniofacial pain in rodent models and headache severity and frequency in patients with migraine [10]. Not surprisingly, low serum Mg^{2+} levels strongly correlate with the frequency of headache attacks in patients with migraine [15–17]. However, the functional consequence of Mg^{2+} control of OT binding has not been adequately examined, particularly with regard to the function of OTR activity and pain. The purpose of this study was, therefore, to examine the impact of Mg^{2+} on OT-OTR binding efficacy by examining these effects in neuronal electrophysiology and pain behavior. The tools selected to address our question included both current clamp experiments on freshly dissociated rat trigeminal ganglia (TG) neurons to determine OT effects on neuronal excitability as well as the in vivo impact of the addition of Mg^{2+} to intranasally administered OT in a rat model of craniofacial pain.

2. Materials and Methods

2.1. Experimental Design

The objective of this study was to examine the impact of Mg^{2+} on OT-OTR binding efficacy by examining the effects on two key physiologic assays. We have previously demonstrated that OTR levels in trigeminal neurons are dependent, in part, on the presence of inflammatory cytokines [18]. Therefore, in this study, rats were pretreated with a complete Freund's adjuvant (CFA) injection into the temporomandibular joint (TMJ) in order to produce a robust inflammation of trigeminally innervated tissue, inducing OTR upregulation approximately 24 h prior to harvesting and dissociating TG neurons. An electrophysiological recording was used to measure the change in membrane potential of TG neurons due to vehicle/OT treatment. In a separate group of animals, approximately 24 h after CFA injection, withdrawal latencies in response to noxious heat applied to the cheek were determined. Increased withdrawal latencies was indicative of analgesia/anti-nociception. Sample sizes were chosen on their basis to detect statistically significant results, with statistical analysis detailed throughout the study.

2.2. Animals

All animal care and experimental procedures complied with the laws of the United States and regulations of the Department of Agriculture and were approved by the Stanford University (Stanford, CA, USA) Institutional Animal Care and Use Committee, in accordance with the 2011 National Institute of Health Guide for the Care and Use of Laboratory Animals. Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010; McGrath & Lilley, 2015). Ethical permission (A3213-01 on 3 November 2020)

Rats (male, 250–330 g, Envigo, Indianapolis, IN, USA) were maintained two per cage in a controlled environment (temperature: 21.5 ± 4.5 °C/relative humidity: 35–55%) under a standard 12 h light/12 h dark lighting cycle (lights on at 7:00, no twilight). Cage changes occurred twice a week, using standard bedding. Food and water were provided ad libitum. Estrogen levels have been shown to drive OTR expression levels and so could drive substantial variability in response to OT treatment. Thus, we chose to use male animals for these studies to minimize this factor. We are currently pursuing separate experiments intended to explore this issue with regard to Mg^{2+} effects. Initial sample sizes were approximated by Power analysis, with animals assigned to groups randomly. Drug treatment experiments were conducted in a blinded fashion.

2.3. Sample Preparation

2.3.1. Solutions for Electrophysiological Recording

OT (Grindeks, Riga, Latvia) was dissolved in distilled water as 1 mM stock and diluted for external solutions. Different concentrations of OT (1.0, 3.0, 10, 30, 100, 300, or 1000 nM) were applied from the reservoir using gravity feeding through a 27G tip-blunted needle with an opening placed about 200 µm away from the recorded cell. The stream of solution covered the cell well when the switch was turned on. The cells were physically stable during perfusion.

2.3.2. Solutions for Behavior Studies

The appropriate amount of test compound was accurately weighed out using a calibrated electrical balance and placed into microcentrifuge tubes. Solutions were prepared for an administration volume of 50 µL containing vehicle or one solution for one of five doses of OT (0.5, 1.0, 4.0, 8.0, or 32.0 µg) plus or minus 300 mM magnesium citrate. These doses and concentrations were selected based on the results of prior studies [10,19,20]. Ten groups of 10 rats each were randomly assigned to receive a vehicle or one of the five doses of OT or OT plus 300 mM Mg^{2+}. Each solution was coded by the sponsor and the experimenter performed the experiment in a strictly blinded manner, including drug administration and data analysis.

2.4. Electrophysiology

2.4.1. Induction of Inflammation

Briefly, rats (male, 250–330 g, Envigo, n = 10) were placed in an anesthesia chamber and anesthetized with 2.5% isoflurane. Prior to TMJ injection, the rat's mouths were propped open to palpate the target area. In this position, an oval-shaped groove located in the center of the cheek and above the mandible can be distinctly felt. With the syringe positioned at a 30-degree angle from the rat's cheek, the tip of the needle was inserted just under the articular disc (approximately 1.5 mm in diameter and 1.0 mm deep). Thereafter, 50 µL of CFA (DIFCO; Sigma Aldrich, St. Louis, MO, USA) was injected (1 mL syringe with a 25G 5/8-inch needle) into the left TMJ to produce robust and prolonged orofacial inflammation. After CFA injection, rats were returned to home cages. Approximately 24 h later, rats were euthanized by decapitation after induction of deep anesthesia with isoflurane.

2.4.2. Tissue Processing

The rat's TG were carefully dissected from the surrounding connective tissues and minced into small pieces with an iris scissor. The TG were digested in 0.5 mL of mixed enzyme solution: (*w*/*v*, final concentration) 0.1% trypsin (Sigma, T9201), 0.1% collagenase Sigma, C1764), and 0.01% DNase (Sigma, D5025) diluted in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) (Sigma, St. Louis, MO, USA). The tissue pieces were then incubated at 32 °C with a water bath for 55 min. Following digestion, tissue fragments were mechanically dissociated using a series of glass Pasteur pipettes with decreasing internal diameter. Dissociated cells were centrifuged at 180× *g* for 3 min, the supernatant was removed, and the cells were gently re-suspended in an external recording solution. Cells were then plated onto poly-L-lysine (Sigma, St. Louis, MO, USA) coated cover slips (Chemglass Life Sciences Vineland, NJ, USA.

2.4.3. Current Clamp Recording

Whole-cell voltage-clamp recordings were performed using the MultiClamp 700B amplifier (Molecular Devices, San Jose, CA, USA) and analyzed offline with pCLAMP10.4 software (Molecular Devices, San Jose CA, USA). The external solution was composed of (in mM) NaCl (130), N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid (HEPES)-Na (10), KCl (5), $CaCl_2$ (1), and Glucose (10), pH adjusted to 7.3–7.4 using HCl, with or without 1.75 mM $MgCl_2$. The electrode internal solution was composed of (in mM) KF (120),

HEPES (10), ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) (11), $CaCl_2$ (1), $MgCl_2$ (1), KCl (10), and KOH (11), pH adjusted to 7.3–7.4 using KOH. Patch-pipettes were fabricated from 1.5 mm outside diameter (OD) borosilicate capillary glass (Warner Instruments, Hamden, CT, USA) using a micropipette puller (Model P-87, Sutter Instrument, Novato, CA, USA). NaCl, HEPES-Na, KCl, CaCl2, Glucose, HCl, MgCl2, KF, HEPES, EGTA, KOH were purchased from Sigma (St. Louis, MO, USA). Glass pipettes filled this intracellular saline with a resistance of 3–5 MΩ. Whole-cell patch recordings had series resistances of <25 MΩ after whole-cell configuration and were periodically checked with the seal test voltage step (10 mV, 10 ms) to monitor series resistances throughout the recordings. Hyperpolarizing current pulses (about −0.3 nA, 500 ms) were delivered every 5 s throughout the experiment, unless otherwise specified, in order to monitor membrane input resistance and stabilize membrane potential in control external solution.

Measurement of change in membrane potential: After successful current clamp recording, the effect of the vehicle external solution application to cells on membrane potential was measured. After the membrane potential had stabilized for at least 10 s, a solution containing OT plus or minus 1.75 mM Mg^{2+} was applied for 2–5 min until the membrane potential stabilized further (on a new level) for at least 10 s.

2.5. Behavioral Analgesia

2.5.1. Withdrawal Latency

Rats (male, 250–330 g, Envigo, n = 10 per group) were used and treated with CFA injection into the TMJ as described above in order to produce a robust inflammation of trigeminally innervated tissue. Approximately 24 h after CFA injection, withdrawal latencies in response to noxious heat applied to the depilated (NAIR® hair removal cream; Church & Dwight Co., Ewing, NJ, USA) and blackened (with India ink (Chartpak Inc., Leeds, MA, USA)) cheek were determined. Latency to withdrawal response was used as an indicator of nociceptive responsiveness. We have previously demonstrated, using single-fiber peripheral nerve recordings, that low intensity (slow ramp) skin heating evokes withdrawal responses mediated by the activation of C- (unmyelinated) nociceptive fibers; higher intensity skin heating (rapid ramp) selectively elicits responses mediated by A-delta (myelinated) thermonociceptors [21,22]. Briefly, to assess C fiber mediated responses, heat intensity was adjusted by altering the supply voltage (35–55 V) of the focused lamp until a withdrawal response was observed to occur with latency between 7.5 and 8.5 s. In order to reduce the potential for tissue damage, a cut-off latency of 15 s was implemented after which the stimulus was terminated. Rats not responding (within 10 s) to a supply voltage of 55 V during baseline testing were excluded from the study. For A-delta fiber testing, the heat intensity was adjusted by altering the supply voltage (60–85 V) of the focused lamp until withdrawal responses were observed to occur with a latency of between 2.5 and 3.5 s. The intensity applied to achieve such latencies was noted for each animal and used to assess withdrawal latencies prior to and following nasal administration of the test agent. To reduce the potential for tissue damage, a cut-off latency of 5 s was implemented during A-delta fiber testing. Rats not responding (within 3.5 s) to a supply voltage of 85 V were excluded from the study. A total of five rats were excluded from further testing by not reaching these criteria. Baseline withdrawal latencies were determined for each fiber type prior to nasal application of the test agent.

To deliver intranasal OT or vehicle the rats were anesthetized in a chamber using isoflurane (2%). They were then placed on a heating pad in a supine position as the anesthesia was continued with a nose cone. This horizontal position of the head was maintained throughout the procedure preventing drainage of the drug solution to the trachea and esophagus. The total volume of 50 µL solutions was administered by pipette in 6–7 µL drops in alternating naris every two min, over a total of 14 min. The drop was placed at the naris opening while occluding the opposite naris allowing the animal to snort the drop into the nasal cavity. The rats were allowed to wake up in a separate cage on a heating pad. Rats were then returned to their home cage. Withdrawal latencies in response

to A-delta or C fiber cheek stimulation were then remeasured at 60 min following dosing. At the end of the testing session, rats were euthanized by CO_2 inhalation.

2.5.2. Efficacy Evaluation

Withdrawal latencies in response to thermal stimuli (noxious heat) were recorded as an index of thermal pain sensitivity. Increased withdrawal latencies were considered indicative of analgesia/anti-nociception.

2.6. Data and Statistical Analysis

All data are presented as means ±SD with significance set at $p < 0.05$. Statistical analysis was undertaken only for data sets where each group size was at least n = 5. All results were analyzed using the GraphPad Prism 9.0 software (GraphPad Software Inc., San Diego, CA, USA. RRID:SCR_008).

2.6.1. Electrophysiology

Data were acquired using Clampfit—V10.4, Molecular Devices (San Jose, CA, USA) and data sheets were constructed in Excel (Microsoft) and GraphPad Prism Software. Two-way ANOVA were performed to compare the overall significance of the difference between OT and OT + Mg^{2+}. Sidak's multiple comparison test was used to determine differences at individual concentrations. Significance was set at $p < 0.05$.

2.6.2. Withdrawal Latency

All data are expressed as mean withdrawal latency ±SE at 60 min post-dosing. Data generated during the testing of each fiber type (e.g., A-delta and C fibers) were analyzed, tabulated, and graphed separately. Statistical analyses were conducted using GraphPad Prism statistical software. All tests were conducted at the 0.05 level of statistical significance. Data generated were assessed using separate 2-way repeated-measures ANOVAs to determine if nasal OT produced dose-dependent significant increases in withdrawal latencies for A-delta or C fiber mediated responses and whether the addition of Mg^{2+} would significantly increase that response as determined by a significant difference between dose-response curves. Sidak's multiple comparison tests were used for subsequent pairwise comparisons to pretreatment latencies.

3. Results

3.1. Electrophysiology Recording: Effect of Different Doses of OT with and without Mg^{2+} on Membrane Potential

After a successful current clamp of TG neurons, the vehicle was applied as a control to the external solution. Both with or without 1.75 mM Mg^{2+}, perfusion of cells with vehicle increased membrane potential insignificantly from -59.4 ± 1.9 mV to -60.6 ± 1.9 mV ($p = 0.13$, n = 9, student paired *t*-test). Figure 1A,B shows an example of a typical current clamp trace recording of a TG neuron from a CFA-inflamed rat. OT dose (3 nM) alone did not induce any change in the membrane potential (Figure 1A). However, the addition of 1.75 mM Mg^{2+} resulted in the hyperpolarization of membrane potential (Figure 1B). While OT alone dose-dependently induced membrane hyperpolarization, a consistently larger hyperpolarization of membrane potential was concentration-dependently observed with the addition of 1.75 mM Mg^{2+} (Figure 1C). Two-way ANOVA analysis showed the effect of OT on hyperpolarization was significantly different between the OT and OT + Mg^{2+} groups overall (2-way ANOVA, ($p < 0.05$)). A post-hoc multiple comparison (Sidak's) test showed significant difference between OT and OT + Mg^{2+} groups at 1, 3, 10 ($p < 0.05$), and 1000 nM OT concentrations ($p < 0.05$).

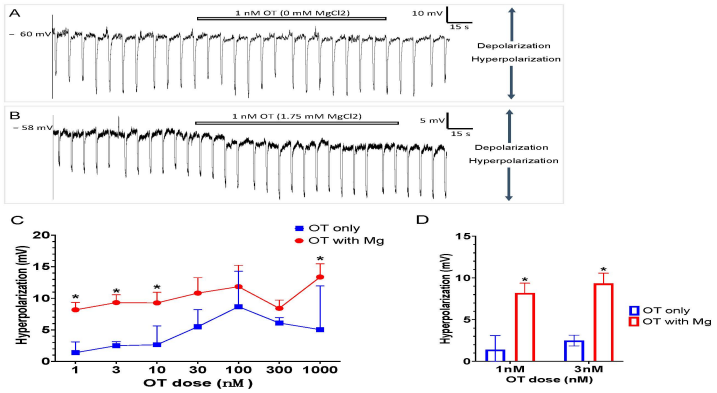

Figure 1. Effect of addition of Mg^{2+} to the OT-induced decrease in excitability of TG neurons from CFA-inflamed rats. (**A**,**B**) Examples of current clamp traces of TG neurons from CFA-inflamed rat; (**A**) no change in membrane potential when treated with 3 nM OT in a 0 mM $MgCl_2$ buffer and (**B**) hyperpolarization observed when treated with 3 nM OT in a 1.75 mM $MgCl_2$ buffer. (**C**,**D**) OT alone dose-dependently hyperpolarizes TG cell membranes, decreasing excitability; the addition of 1.75 mM Mg^{2+} significantly (* $p < 0.05$, ANOVA) potentiates the capacity of OT to hyperpolarize TG cell membranes (**C**). A specific example of this is observed in (**D**), where the addition of Mg^{2+} significantly (* $p < 0.05$) increased the induced membrane hyperpolarization of 1 nM or 3 nM OT from −1.4 ± 1.6 mV (n = 6) and −2.5 ± 0.6 mV (n = 6) for OT alone to −8.1 ± 1.2 mV (n = 7) and −9.3 ± 1.2 mV (n = 7) for OT plus Mg^{2+}, respectively. Subsequent pairwise comparisons indicated significant (* $p < 0.05$) differences from OT alone for OT plus Mg^{2+} at 1, 3, 10, and 1000 nM OT concentrations. This enhanced hyperpolarization is emblematic of decreased excitability and thus, decreased capacity to carry pain signals to the central nervous system for pain perception. Error bars show ± S.D.

In fact, while 1 or 3 nM OT had a minimal membrane potential effect (−1.4 ± 1.6 mV (n = 6) and −2.5 ± 0.6 mV (n = 6) hyperpolarization for 1 and 3 nM OT, respectively), the addition of 1.75 mM $MgCl_2$ to the external solution induced a strong hyperpolarization, −8.1 ± 1.2 mV (n = 7 for 1 nM OT) and −9.3 ± 1.2 mV (n = 7 for 3 nM OT), respectively (Figure 1D). Two-way ANOVA analysis showed the overall effect of OT on hyperpolarization were significantly different between the OT and OT + Mg^{2+} groups (2-way ANOVA, ($p < 0.05$)). A post-hoc multiple comparison (Sidak's) test showed a significant difference between OT and OT + Mg^{2+} groups at 1 ($p < 0.05$) and 3 nM OT concentrations ($p < 0.05$).

3.2. Behavioral Analgesia: Effect of Different Doses of OT with and without Mg^{2+} on Withdrawal Latency

Baseline testing revealed stable head withdrawal response latencies for A-delta (2.6–3.2 s) and C-fiber (7.5–8.1 s) radiant heat stimulation of the cheek (Figure 2A,B). The intranasal

application of OT produced a dose-dependent increase in withdrawal latency up to the highest dose (32 µg), where efficacy was seen to dramatically decrease for both A-delta and C fiber mediated pain responses. Application of the same doses of OT in the presence of Mg^{2+} however, produced a significant ($p < 0.0001$, 2-way ANOVA) increase in withdrawal latency compared to efficacy in the absence of Mg^{2+} for both A-delta (Figure 2A) and C-fiber (Figure 2B) testing. Follow-on analysis revealed significant differences ($p < 0.05$) between OT and OT + Mg^{2+} for A-delta testing at OT doses of 0.5, 1.0, and 32 µg OT; C-fiber responses were significantly different at 0.5, 4.0, 8.0, and 32 µg OT.

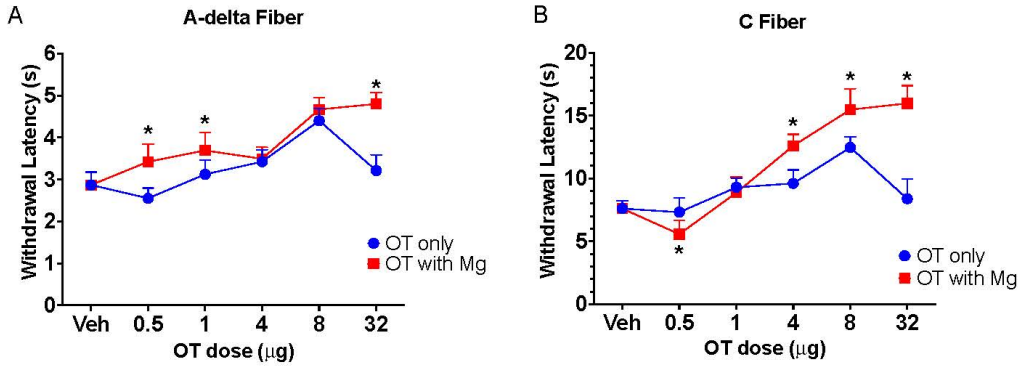

Figure 2. Effect of the addition of Mg^{2+} on intranasal OT-induced craniofacial analgesia. For both A-delta (**A**) and C fiber (**B**) mediated withdrawal responses to noxious heat stimulation of the cheek of pre-inflamed rats, intranasally applied OT produced a dose-dependent analgesic effect as evidenced by significant ($p < 0.05$, ANOVA) increases in withdrawal latency at the 60 min time point after administration, n = 10. However, the addition of 300 mM Mg^{2+} to the treatment significantly increased OT analgesia for both stimulus types ($p < 0.05$). Subsequent pairwise comparisons indicated significant (* = $p < 0.05$) differences from OT alone for OT plus Mg^{2+} for A-delta testing at OT doses of 0.5, 1.0, and 32 µg ($p < 0.05$); C-fiber responses were significantly different (* = $p < 0.05$) at 0.5, 4.0, 8.0, and 32 µg OT. Interestingly, while the efficacy of the highest OT dose (32 µg) demonstrated a decrease in efficacy when compared to lower doses, this dose-response inversion was prevented by the addition of Mg^{2+}.

4. Discussion and Conclusions

The requirement of Mg^{2+} for OT's high affinity for its receptor has long been known [13]. More recently, the precise architecture of this Mg^{2+} coordination site has been elucidated in the high-resolution cryo-electron microscopy structure of OT bound to its receptor, revealing that OT and OTR together form an octahedral Mg^{2+} coordination site between the receptor and ligand [14]. However, the functional consequence of Mg^{2+} control of OT binding has not been adequately examined, particularly with regard to how Mg^{2+} levels could affect the impact of OT-OTR binding on pain as well as other phenomena. The results of the second messenger study described in [14] demonstrate that Mg^{2+} is an essential cofactor for full OT-OTR agonism and that Mg^{2+} concentration-dependently increases G_q and G_{11} activation in OTR-transfected HEK cells. Interestingly, this study also demonstrated that OT-OTR binding did not reach maximal efficacy at physiologically relevant Mg^{2+} concentrations [14]. Consistent with these findings, while OT alone dose-dependently hyperpolarized TG neurons, decreasing their excitability, the addition of a supraphysiological (1.75 mM) concentration of Mg^{2+} significantly enhanced this effect, implying a supportive impact of Mg^{2+} on OT craniofacial analgesia. In a demonstration of this support, while intranasal application of OT produced dose-dependent craniofacial analgesia, the addition of 300 mM Mg^{2+} to the administered OT significantly enhanced this analgesia.

The trigeminal nerve provides pain signaling from the head to the central nervous system for the perception of craniofacial pain. Thus, decreases in the excitability of these neurons should produce decreases in craniofacial pain sensitivity. The results of the current study are consistent with our previous finding [23] that OT decreases the excitability of TG neurons in vitro as evidenced by a robust increased (hyperpolarized) cell membrane potential. This work also suggested that this decrease in neuronal excitability is likely mediated, at least in part, by an increase in voltage-gated K^+ channel (Kv) current density [23]. As with the second messenger findings, the addition of 1.75 mM Mg^{2+} to the applied OT in the same concentration range used in the second messenger study produced a significant increase in the degree of hyperpolarization of the membrane. In the absence of Mg^{2+}, the maximal efficacy of OT is not reached, indicating the necessity of Mg^{2+} for the full agonism of OT. Interestingly, while the efficacy of the highest OT dose (32 µg) demonstrated a decrease in efficacy when compared to lower doses, this inversion was prevented by the addition of Mg^{2+}. These findings indicate that the addition of Mg^{2+} produces a more robust decrease in cell excitability, consistent with a stronger analgesic effect than that observed with OT alone.

Using autoradiography and tissue scintillation counts, we have previously demonstrated that radiolabeled OT, when applied nasally, concentrates in the trigeminal nerve and ganglia [20,24]; an approximately 10–20 times higher concentration of radiolabeled OT was detected in the trigeminal system compared to other tissue regions [20,24]. We have also shown that intranasal OT inhibits the transmission of pain messages to the central nervous system [10], inducing analgesia in rodent craniofacial pain models [10,19] and relief from headaches in patients with migraine [10]. The current study demonstrates that, as with the in vitro assays, the addition of Mg^{2+} to OT significantly enhances these analgesic effects. Previously, intravenous Mg^{2+} has been shown to abort continuous migraines and, when given as an oral supplement, reduce their frequency [25]. We have hypothesized that these effects might be mediated, in part, by a Mg^{2+} induced increase in the affinity of OTR for endogenous OT, thereby decreasing the excitability of trigeminal nociceptive neurons. Similarly, we have hypothesized that the decrease in serum Mg^{2+} during pre-menstruation and menstruation might help explain the phenomena of menstrual migraine [8].

In addition to the menstrual cycle effects on Mg^{2+} and migraine, serum estrogen levels, which vary over the menstrual cycle, have been shown to drive OTR expression levels [26,27] and have been hypothesized to underly, in part, the pathogenesis of menstrual migraine [8]. The variability of serum estrogen in females could also drive substantial variability in response to intranasal OT treatment. Thus, we chose to use male animals in these studies to minimize this factor. Because of the variability of serum Mg^{2+} and estrogen, it is likely that the effects of OT in females may vary significantly from those in males [8]. We are currently pursuing separate experiments intended to explore this issue with regard to the impact of Mg^{2+} on OT analgesia in females across the menstrual cycle.

The lowest OT dose (0.5 µg), supplemented with Mg^{2+} produced shorter withdrawal latencies compared to the OT group without Mg^{2+}. One explanation for this observation is based on our preliminary electrophysiological studies that show 1.75 mM Mg^{2+}, in the absence of OT, is in fact, depolarizing in some cells. Thus, with a very low concentration of OT, it is likely that this depolarizing effect overwhelms any minimal hyperpolarizing effect of the OT. Interestingly, while the efficacy of the highest OT dose (32 µg) demonstrated a decrease in efficacy when compared to lower doses, this inversion was prevented by the addition of Mg^{2+}. In a separate ongoing study (and so not directly comparable), we have similarly found that 128 µg demonstrates a significant drop in efficacy compared to lower doses that were preventable by the addition of Mg^{2+}. Inverted U dose responses have been widely reported for various systems, including social recognition [28], opioid-induced respiratory depression [29], and, very recently, in an autism spectrum clinical trial [30]. Although it is not unknown for peptide neurotransmitters to have an inverted U dose-response, the specific reason for a decrease in withdrawal latency at a higher OT dose is still unclear. One explanation could be off-target effects where OT, at high

enough concentrations, begins acting on a different receptor. For example, OT has a very high affinity for the V1a receptor and so it is possible that the effects of OT on this or other receptors might counteract those on the OT receptor. This decrease in efficacy at higher doses of OT may be instrumental in the difficulty in demonstrating clear efficacy of intranasal OT in many clinical studies, where a moderate effect is seen with low doses, but higher doses do not show an improvement. The addition of Mg^{2+} to an OT formulation should allow the use of higher doses, overcoming this barrier for a number of indications. The second messenger study by Meyerowitz et al. [14] showed that the inversion of OT dose-response was not observed, which is likely due to the simplified milieu of the transfected cells versus that of whole neurons or in vivo.

Taken together, the results of these two sets of experiments suggest that Mg^{2+} is required for the full agonism of OT and that, for pain and in many other therapeutic or disease settings, the efficacy of OT may be limited by the availability of Mg^{2+}. Thus, the addition of Mg^{2+} to OT formulations or the development of novel OT analogs based on recently elucidated OTR structural biology [14] that obviate the need for Mg^{2+} may enable enhanced OTR efficacy.

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Institutional Review Board Statement: All animal care and experimental procedures complied with the laws of the United States and regulations of the Department of Agriculture, and were approved by Stanford University (Stanford, CA, USA) Institutional Animal Care (A3213-01 on 3 November 2020) and Use Committee, in accordance with the 2011 National Institute of Health Guide for the Care and Use of Laboratory Animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest: D.C.Y. is a consultant for Tonix Pharmaceuticals. The remaining authors have no conflict of interest to declare.

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