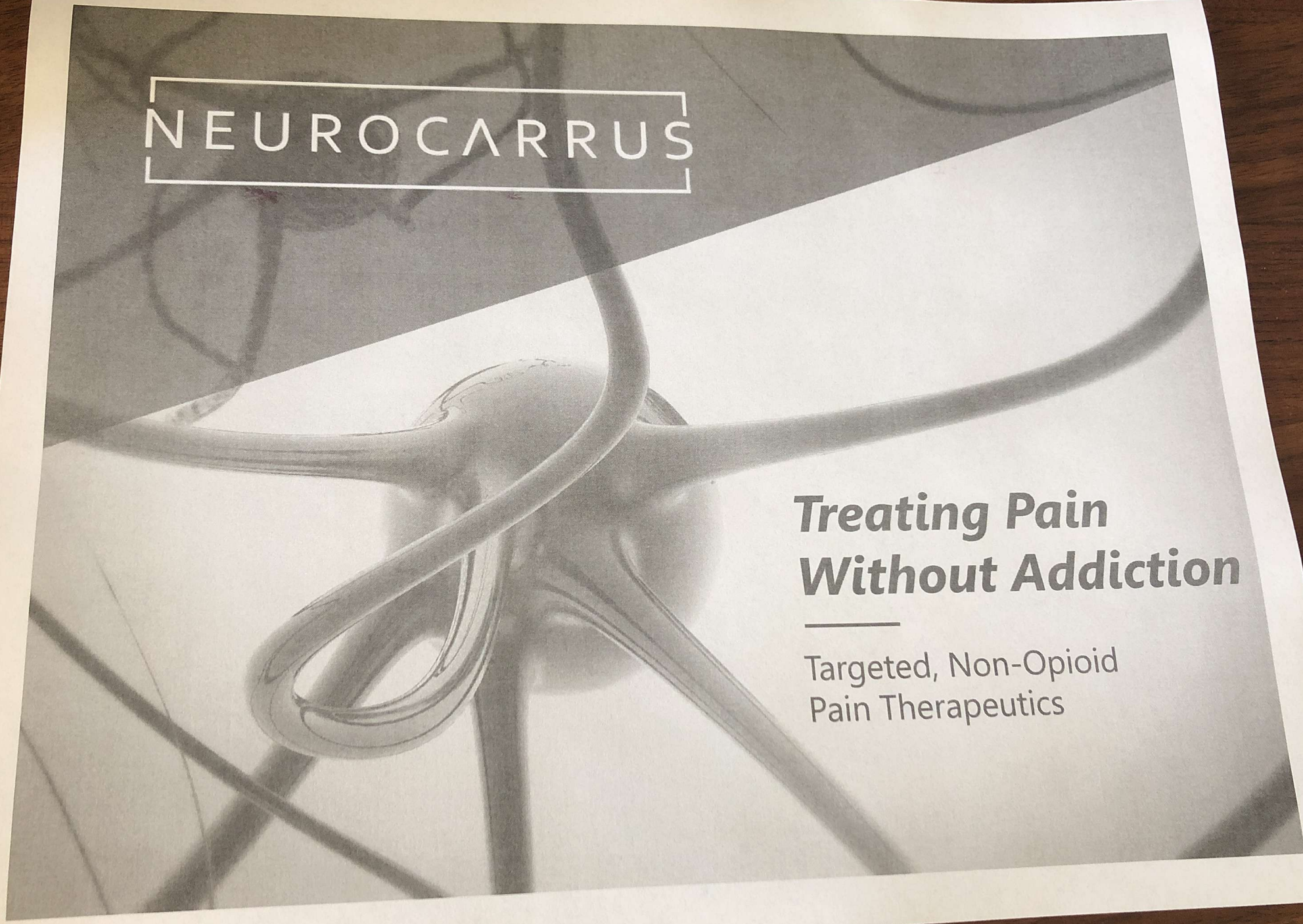
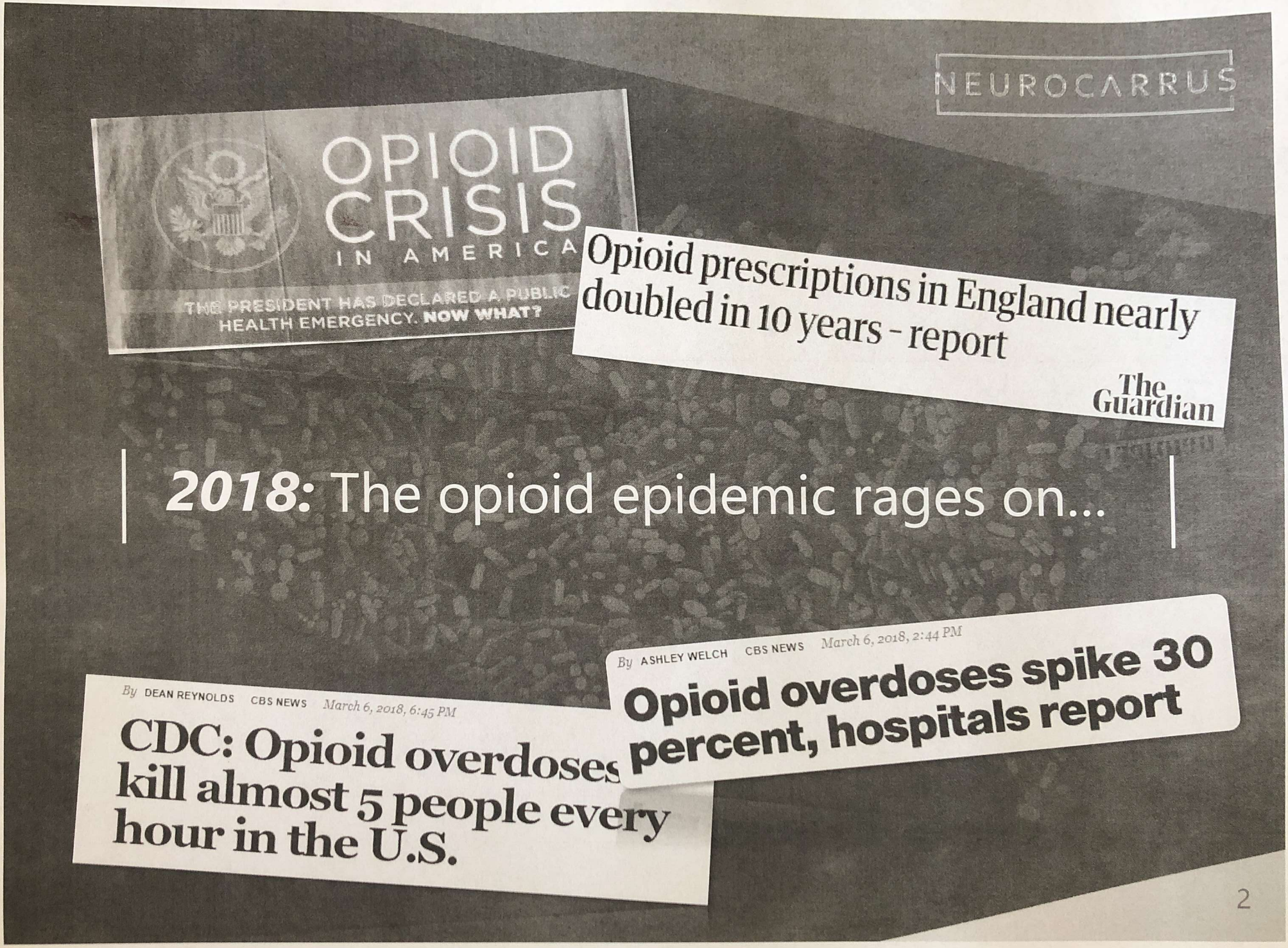
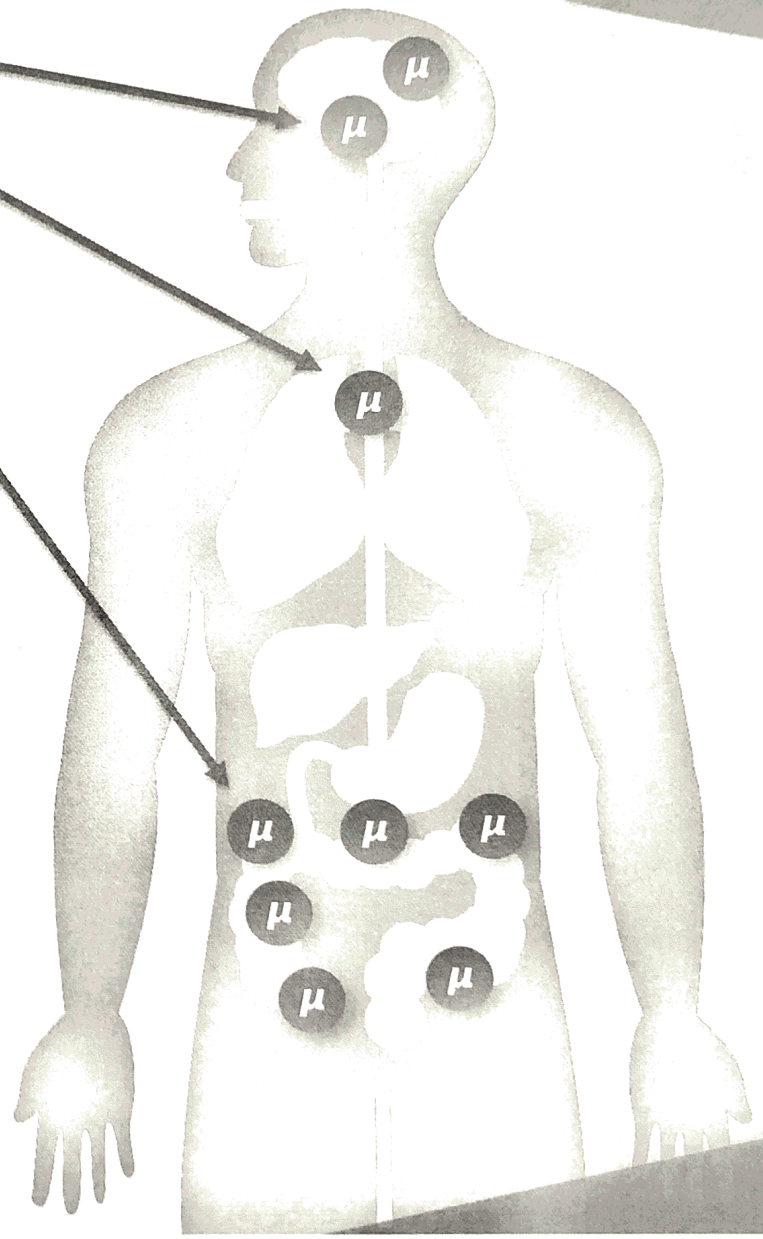

NEUROCARRUS
Treating Pain
Without Addiction
Targeted, Non-Opioid
Pain Therapeutics

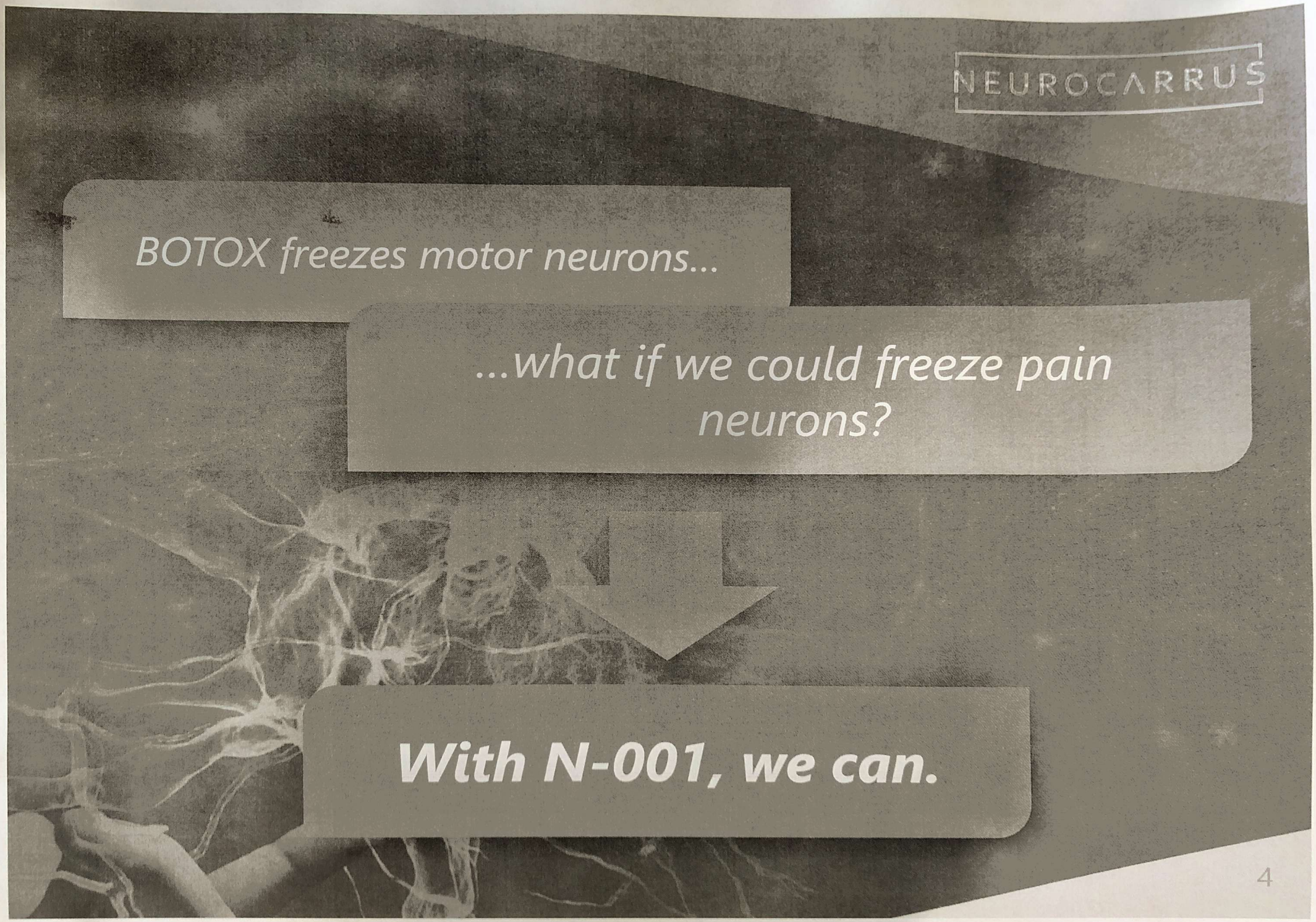

NEUROCARRUS
OPIOID CRISIS IN AMERICA
THE PRESIDENT HAS DECLARED A PUBLIC HEALTH EMERGENCY. NOW WHAT?
Opioid prescriptions in England nearly doubled in 10 years - report
The Guardian
2018: The opioid epidemic rages on...
By ASHLEY WELCH CBS NEWS March 6, 2018, 2:44 PM
Opioid overdoses spike 30 percent, hospitals report
By DEAN REYNOLDS CBS NEWS March 6, 2018, 6:45 PM
CDC: Opioid overdoses kill almost 5 people every hour in the U.S.

Opioids block pain through interaction with ***μ(mu)-receptors***.

But interaction with mu-receptors in ***the brain*** leads to an ***addictive euphoria***, among other side effects.

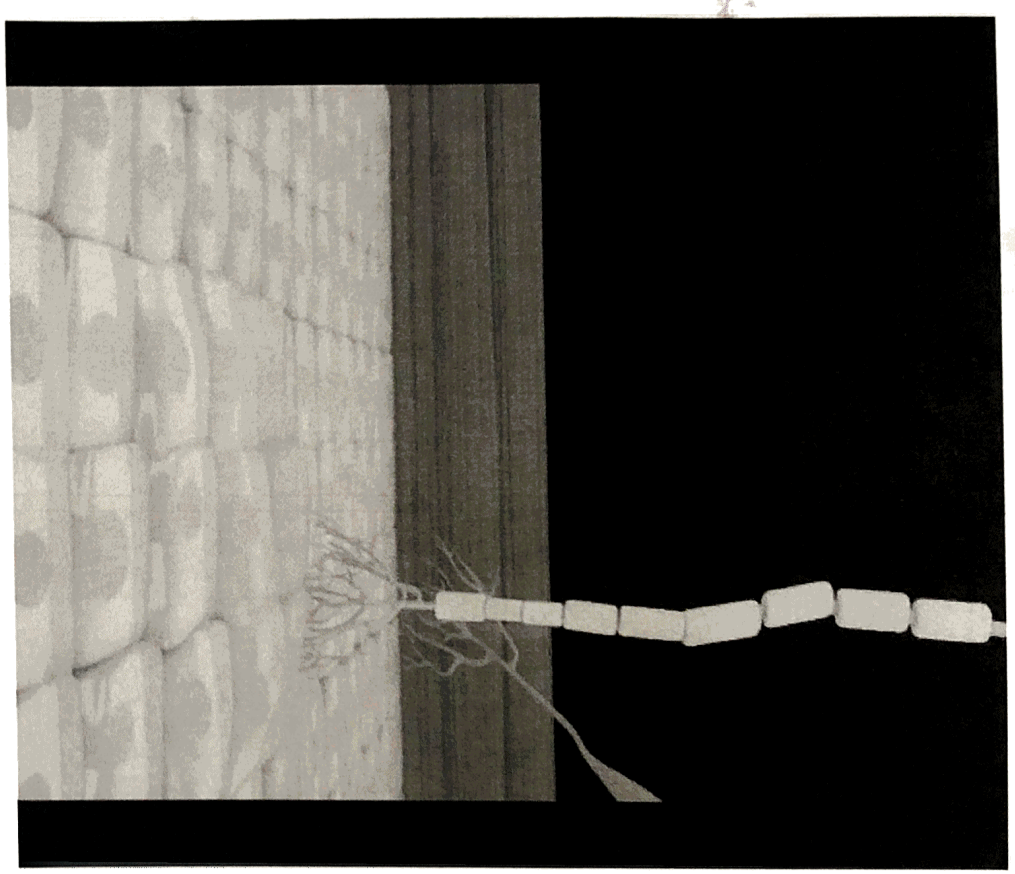
Clearly, we need a ***new treatment*** for ***pain.***



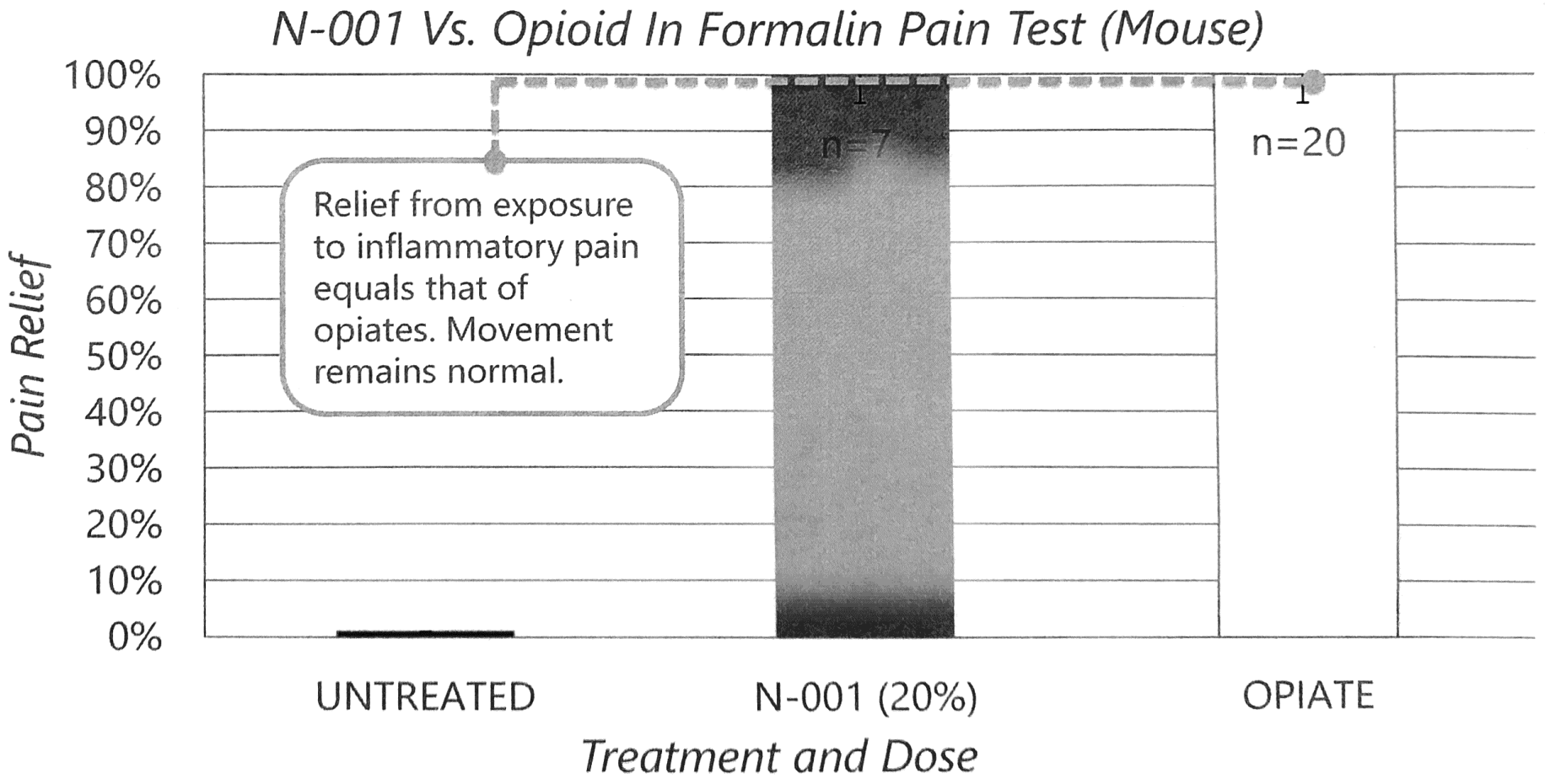

NEUROCARRUS
BOTOX freezes motor neurons...
...what if we could freeze pain neurons?
With N-001, we can.

N-001 Profile

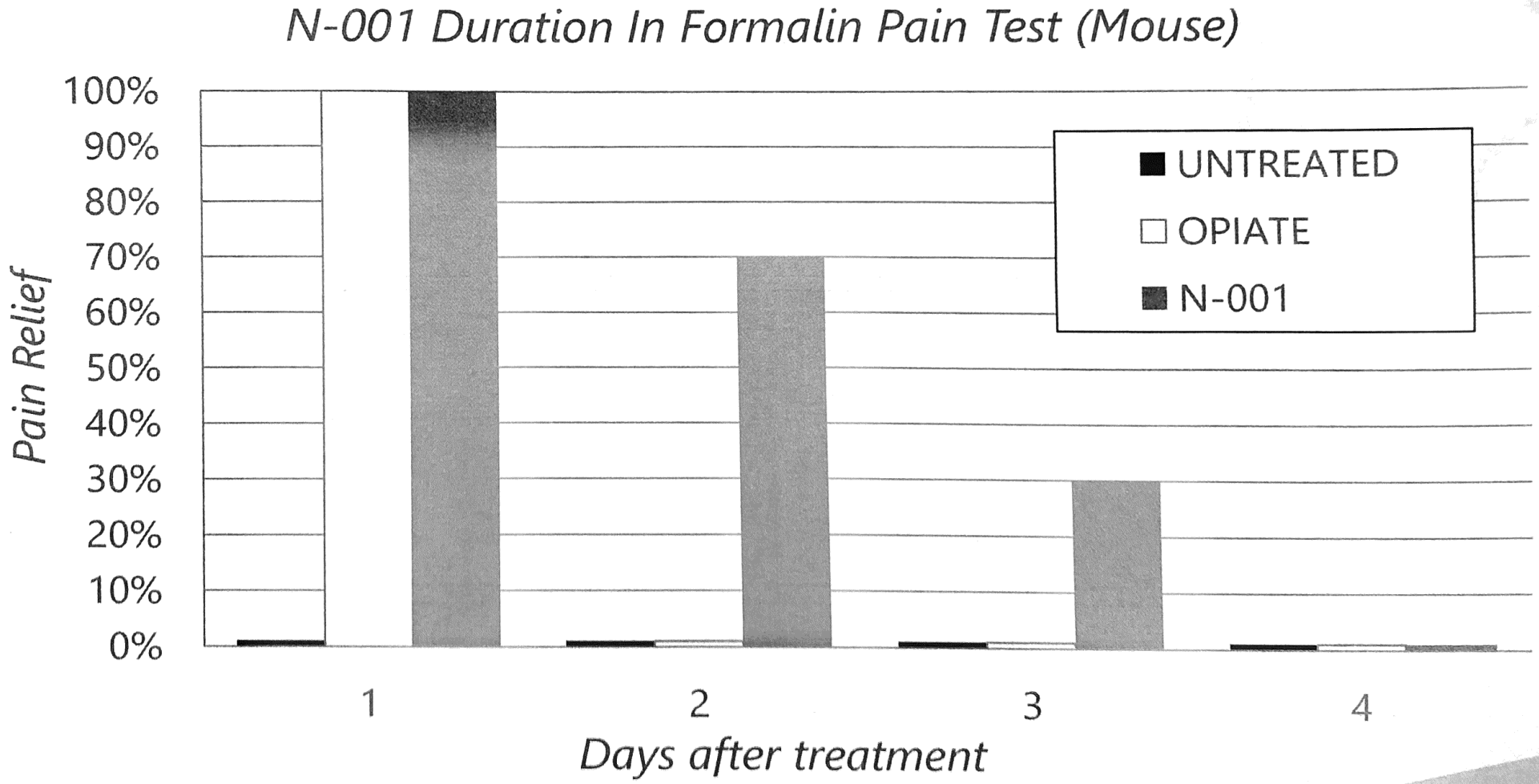
- A rationally designed protein that targets pain (sensory) neurons
- Disrupts pain signaling in neuronal axon through modification of cytoskeletal actin
- Locally administered at site of pain by injection or topical application
- Uses our novel drug delivery system, capable of transporting other drugs to targeted neurons



N-001 ***eliminates*** acute pain.

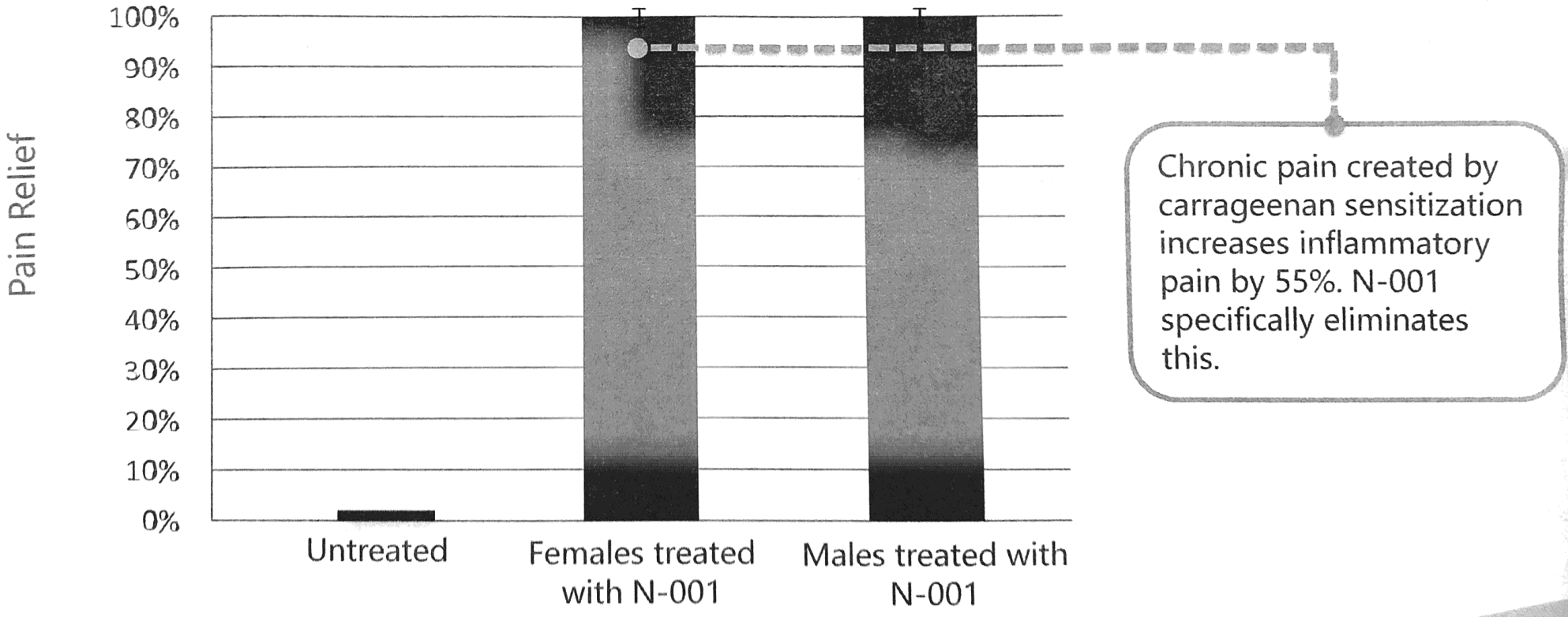


N-001 decreases pain for ***longer*** than opiates.



N-001 ***eliminates*** chronic pain.

N-001 Chronic Pain Test (Mouse), Carageenan Sensitized



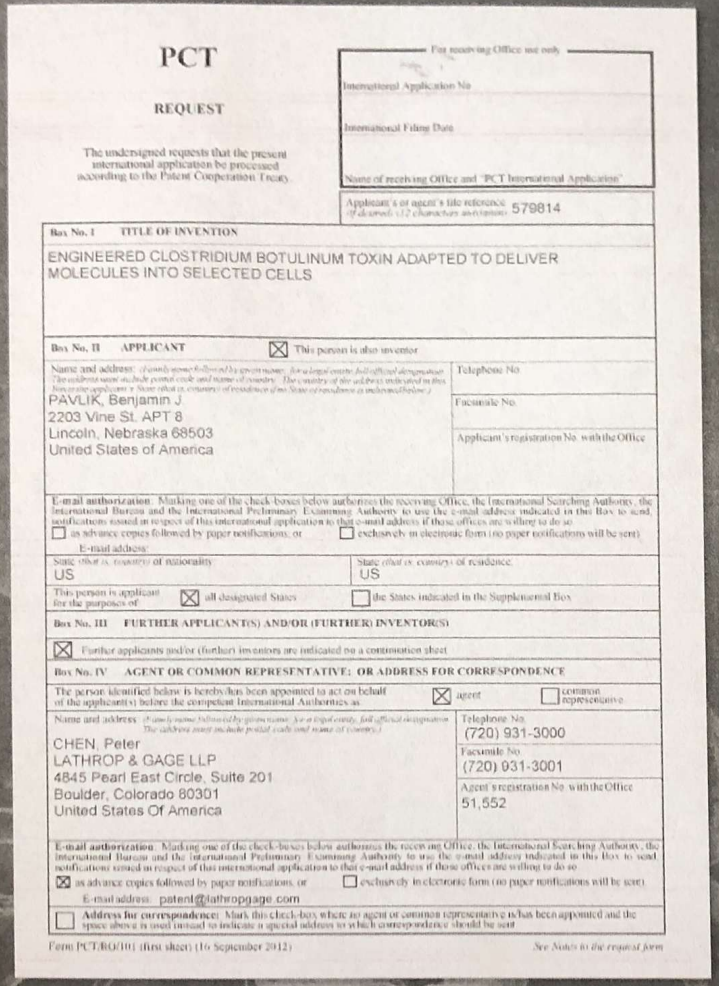

NEUROCARRUS
Global Pain Treatment Market (Pharmaceutical):
$32 billion
U.S. Diabetic Neuropathy patient population:
15 million
U.S. Osteoarthritis patient population:
30 million

NEUROCARRUS

Intellectual Property

Exclusive license for all fields of use (drug and delivery system)

PCT filed in 8 countries



PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference (If desired, ≤12 characters are suggested): 579814

Box No. I TITLE OF INVENTION

ENGINEERED CLOSTRIDIUM BOTULINUM TOXIN ADAPTED TO DELIVER MOLECULES INTO SELECTED CELLS

Box No. II APPLICANT ☒ This person is also inventor

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

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Facsimile No.

Applicant's registration No. with the Office

E-mail authorization: Marking one of the check-boxes below authorizes the receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority to use the e-mail address indicated in this Box to send notifications issued in respect of this international application to that e-mail address if these offices are willing to do so.

☐ as advance copies followed by paper notifications, or ☐ exclusively in electronic form (no paper notifications will be sent)

E-mail address:

State (that is, country) of nationality: US

State (that is, country) of residence: US

This person is applicant for the purposes of: ☒ all designated States ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

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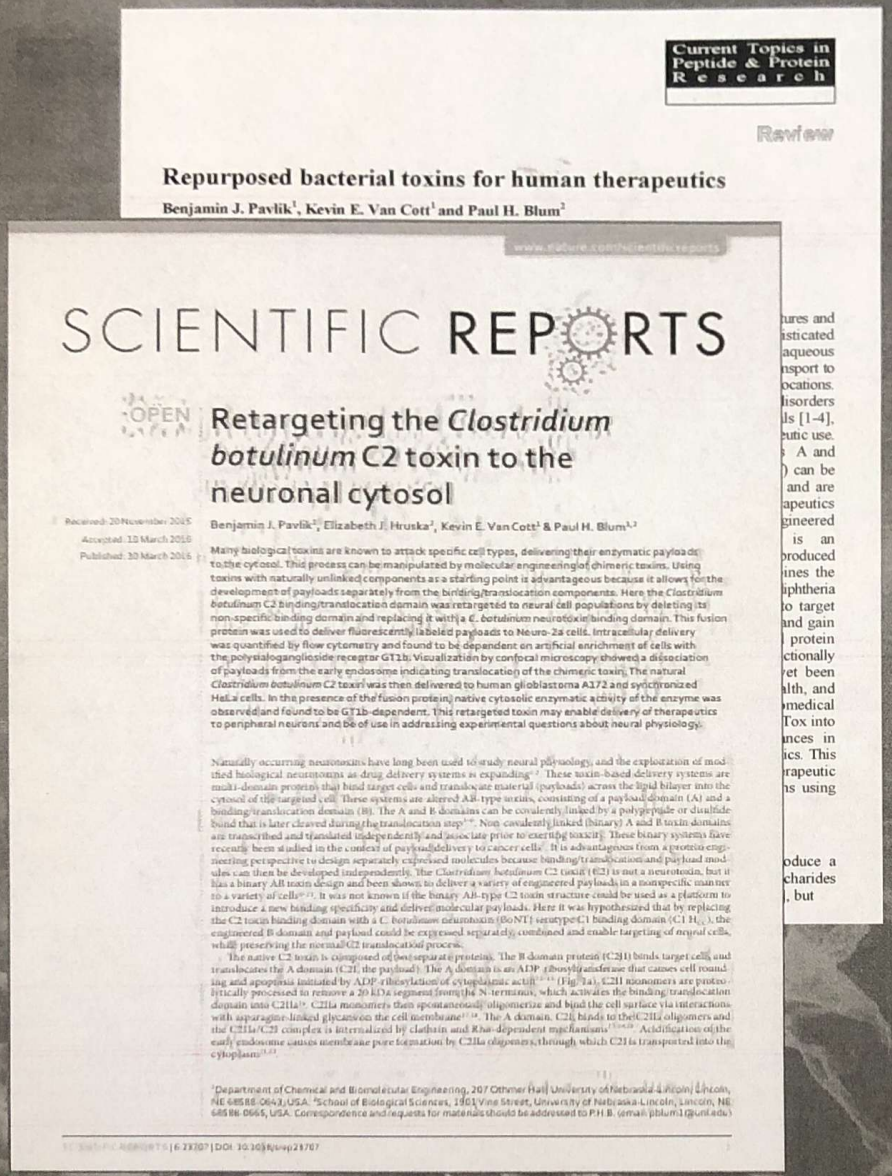
Form PCT/RO/101 (first sheet) (16 September 2012) *See Notes to the request form*



Current Topics in Peptide & Protein Research

Review

Repurposed bacterial toxins for human therapeutics

Benjamin J. Pavlik[1], Kevin E. Van Cott[1] and Paul H. Blum[2]

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Retargeting the *Clostridium botulinum* C2 toxin to the neuronal cytosol

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Benjamin J. Pavlik[1], Elizabeth J. Hruska[1], Kevin E. Van Cott[1] & Paul H. Blum[1,2]

Many biological toxins are known to attack specific cell types, delivering their enzymatic payloads to the cytosol. This process can be manipulated by molecular engineering of chimeric toxins. Using toxins with naturally unlinked components as a starting point is advantageous because it allows for the development of payloads separately from the binding/translocation components. Here the *Clostridium botulinum* C2 binding/translocation domain was retargeted to neural cell populations by deleting its non-specific binding domain and replacing it with a *C. botulinum* neurotoxin binding domain. This fusion protein was used to deliver fluorescently labeled payloads to Neuro-2a cells. Intracellular delivery was quantified by flow cytometry and found to be dependent on artificial enrichment of cells with the polysialoganglioside receptor GT1b. Visualization by confocal microscopy showed a dissociation of payloads from the early endosome indicating translocation of the chimeric toxin. The natural *Clostridium botulinum* C2 toxin was then delivered to human glioblastoma A172 and synchronized HeLa cells. In the presence of the fusion protein, native cytosolic enzymatic activity of the enzyme was observed and found to be GT1b-dependent. This retargeted toxin may enable delivery of therapeutics to peripheral neurons and be of use in addressing experimental questions about neural physiology.

Naturally occurring neurotoxins have long been used to study neural physiology, and the exploration of modified biological neurotoxins as drug delivery systems is expanding[1–4]. These toxin-based delivery systems are multi-domain proteins that bind target cells and translocate material (payloads) across the lipid bilayer into the cytosol of the targeted cell. These systems are altered AB-type toxins, consisting of a payload domain (A) and a binding/translocation domain (B). The A and B domains can be covalently linked by a polypeptide or disulfide bond that is later cleaved during the translocation step[5–8]. Non-covalently linked (binary) A and B toxin domains are transcribed and translated independently and associate prior to exerting toxicity. These binary systems have recently been studied in the context of payload delivery to cancer cells[9]. It is advantageous from a protein engineering perspective to design separately expressed molecules because binding/translocation and payload modules can then be developed independently. The *Clostridium botulinum* C2 toxin (C2) is not a neurotoxin, but it has a binary AB toxin design and been shown to deliver a variety of engineered payloads in a nonspecific manner to a variety of cells[9–12]. It was not known if the binary AB-type C2 toxin structure could be used as a platform to introduce a new binding specificity and deliver molecular payloads. Here it was hypothesized that by replacing the C2 toxin binding domain with a *C. botulinum* neurotoxin (BoNT) serotype C1 binding domain (C1 H_{CC}), the engineered B domain and payload could be expressed separately, combined and enable targeting of neural cells, while preserving the normal C2 translocation process.

The native C2 toxin is composed of two separate proteins. The B domain protein (C2II) binds target cells and translocates the A domain (C2I, the payload). The A domain is an ADP ribosyltransferase that causes cell rounding and apoptosis initiated by ADP-ribosylation of cytoplasmic actin[13–15] (Fig. 1a). C2II monomers are proteolytically processed to remove a 20 kDa segment from the N-terminus, which activates the binding/translocation domain into C2IIa[16]. C2IIa monomers then spontaneously oligomerize and bind the cell surface via interactions with asparagine-linked glycans on the cell membrane[17–19]. The A domain, C2I, binds to the C2IIa oligomers and the C2IIa/C2I complex is internalized by clathrin and Rho-dependent mechanisms[17,18,20]. Acidification of the early endosome causes membrane pore formation by C2IIa oligomers, through which C2I is transported into the cytoplasm[21,22].

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Publications

Nature

Current Topics in Peptide and Protein Research

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Partners

NIH National Institute on Drug Abuse

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INDIE BIO ACCELERATING BIOLOGY

SOSV

NEBRASKA DEPARTMENT OF ECONOMIC DEVELOPMENT

innoture MEDICAL TECHNOLOGY

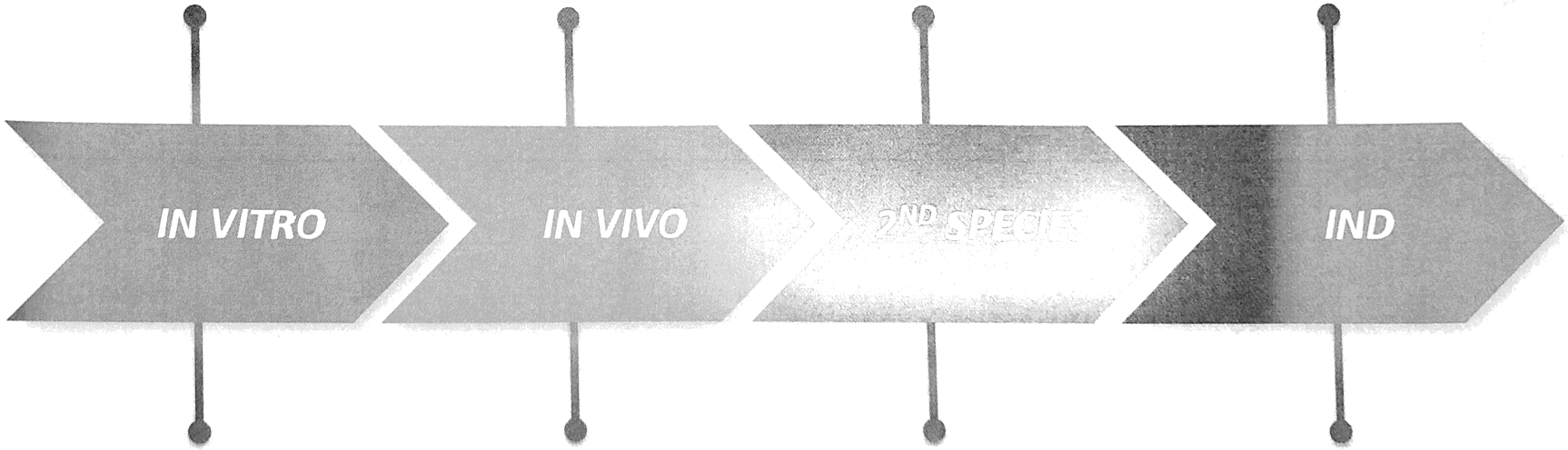
Pre-Clinical Development

Dosing
Neural Signaling
Manufacturing

Efficacy, Mechanism
Pharmacokinetics Toxicity,
Formulation

Advanced
Model of Pain
Indication

FDA - Center for
Biologics Evaluation and
Research



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NEBRASKA
DEPARTMENT OF
ECONOMIC DEVELOPMENT

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paul@neurocarrus.com