mdb biosciences.

Post Surgical Pain Model in Swine: New Findings

Castel D.[2] Hifi O[1]. Doron G[1]. Reichstein A[1]. Schauder A[1]. Oren S[1]. Abu-Zer S[1]. and Meilin S[1].

1. MD Biosciences Neuro; R&D Department. 4 Eli Horovitz Str. Rehovot, Israel

2. Sheba Medical Center, Tel Aviv University, Israel.

Introduction

Animal models for post-surgical pain are commonly used to study new therapies for acute pain. The most cited model is the Brennan rat model for post-surgical pain first published in 1996 (Brennan et al., Pain 64(3):493-502, 1996). Classical evaluation of nociception includes Von Frey and paw pressure tests to assess mechanical hyperalgesia and the hot plate test to assess thermal hyperalgesia. However, one of the major disadvantages of the rodent model is the limitation in assessing topical and localized treatments. Furthermore, a paper published by Segelcke et al. in 2023 suggests that proteome profiling of human skin after incision reveals protein-protein interactions correlated with pain and hyperalgesia. These protein-protein interactions were differentially modulated in mice compared to humans, raising questions regarding the translatability of rodent models to human (Segelcke et al., Br J Anaesth 2023 130(3):331-34).

We have developed two post-surgical models in swine (Castel et al., EJP 2014;18(4):496-505; Castel et al., Anesth Surg Care, 2021; 1: 1-3.). Recently, HTX-011 (ZYNRELEF™) was approved by the FDA following preclinical research and development using our translational post operative pain model in pigs.

Methods

The methods are fully described in Castel et al., EJP 2014;18(4). Briefly, Danish Landrace × Large White cross-bred weaned male and female pigs from the domestic herd at Lahav Labs, Negev, Israel, were used. A full-skin incision of 6–7 cm was made through only the skin and fascia, keeping the muscle intact. The incision was made on the left side of the lower back towards the caudal end, approximately 2 cm lateral to the spine line of the pig (Figure 1).
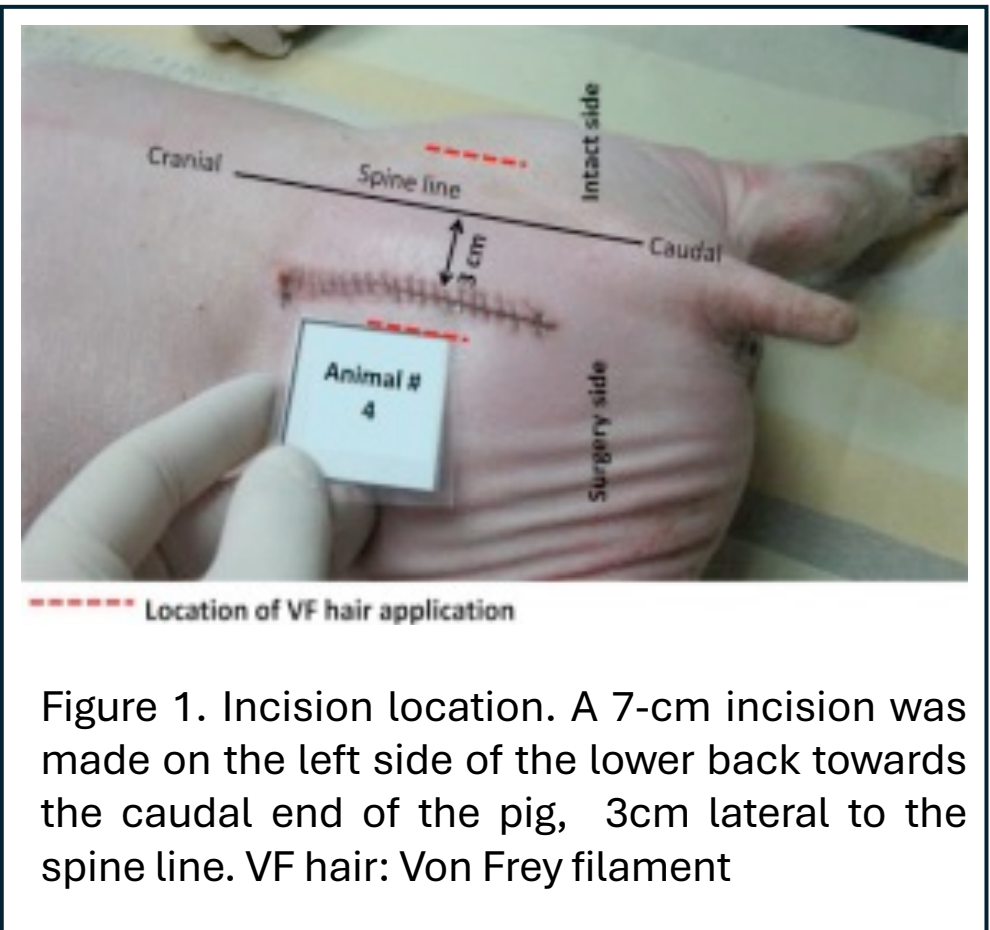

Figure 1. Incision location. A 7-cm incision was made on the left side of the lower back towards the caudal end of the pig, 3cm lateral to the spine line. VF hair: Von Frey filament
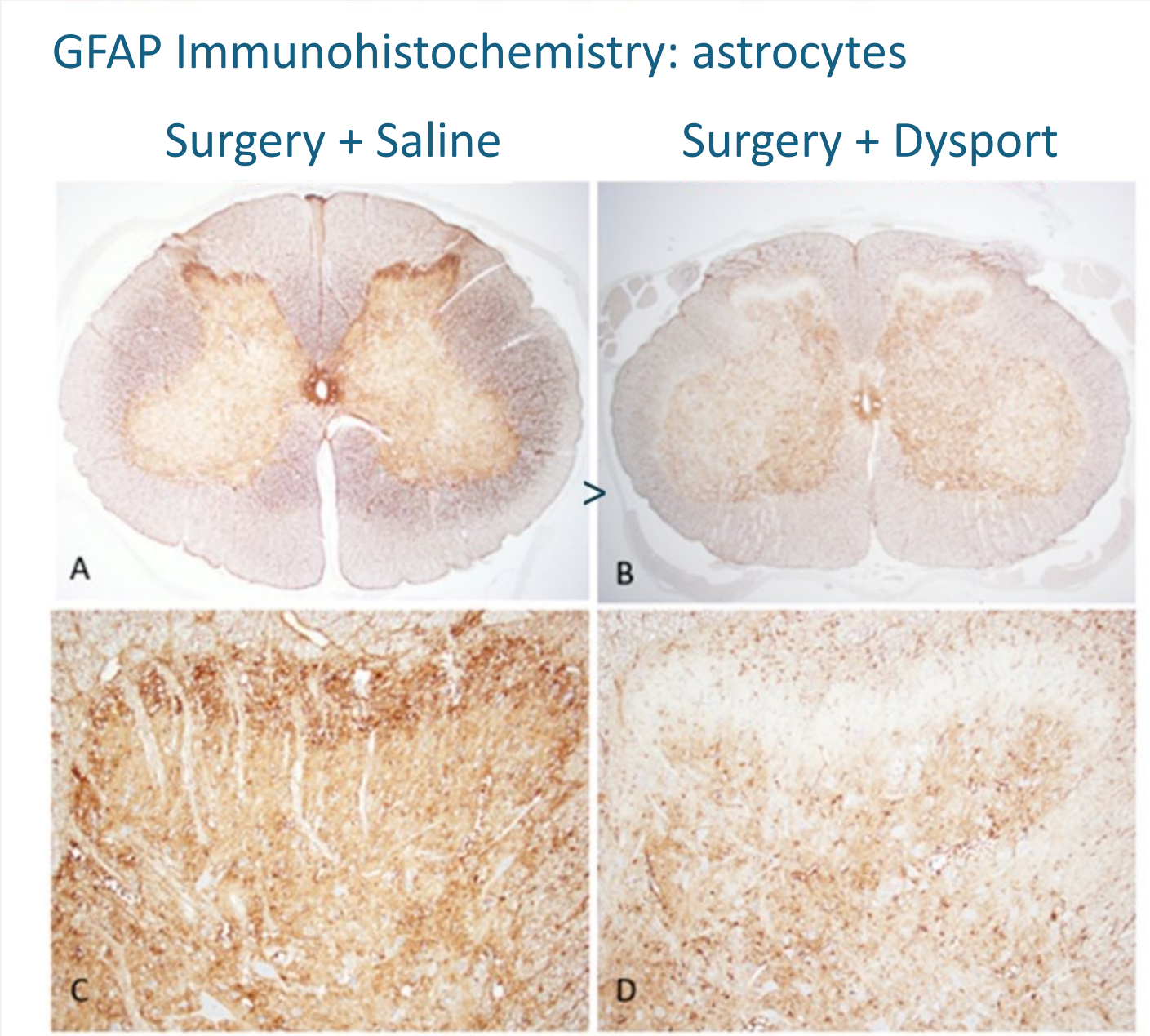
Pain Assessment:
- Mechanical sensitivity using Von Frey filaments
- Response to thermal stimuli
- Behavior testing:
  - Approaching test
  - Behavior score (table below)

Distress behavior score (DBS)

Scoring category	Parameter	Score
Category 1.	Avoiding standing (lying down)	1
	Standing	0
Category 2.	Avoiding walking	1
	Walking	0
Category 3.	Guarding behavior (protecting the wound side while walking)	1
	Acting normal	0
Category 4.	Moving away when approached by the investigator	1
	Not moving away when approached by investigator	0
Category 5.	Restlessness	1
	Normal	0
Category 6.	Staying in isolation from other animals	1
	Staying together with other animals	0
Category 7.	High pitch/distress vocalization	1
	Normal vocalization	0

Immunohistochemistry:
- IENF: The naïve skin of female and male animals was cut and stained for PGP9.5.
- Microglia and astrocytes: The spinal cord was harvested and stained for microglia (Ib-1A) and astrocytes (GFAP).

Results

Below are four examples of the post operative pain model in pigs in basic science and applied science.

Block 1: Translational Model

ZYNRELEF (HTX-011)study; FDA approval, 2021

Translational Model

Figure 2. On the left, the synergistic activity of Meloxicam and Bupivacaine (HTX-011) in pigs. On the right, the synergistic activity of HTX-011 in humans.
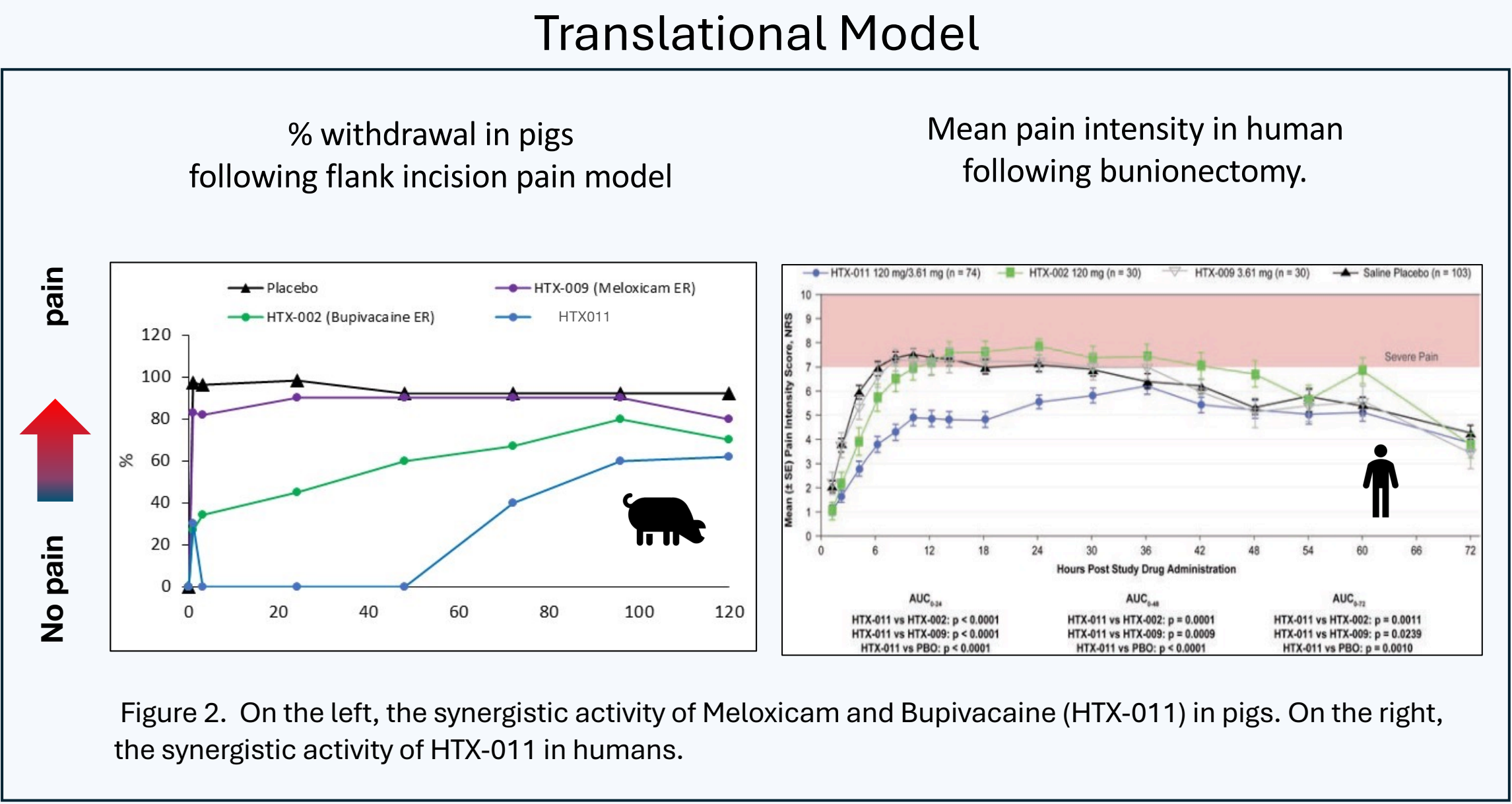
MoA hypothesis testing

Figure 3. In vivo measurement of wound pH shows an increase in the pH following treatment with HTX-011.

MoA
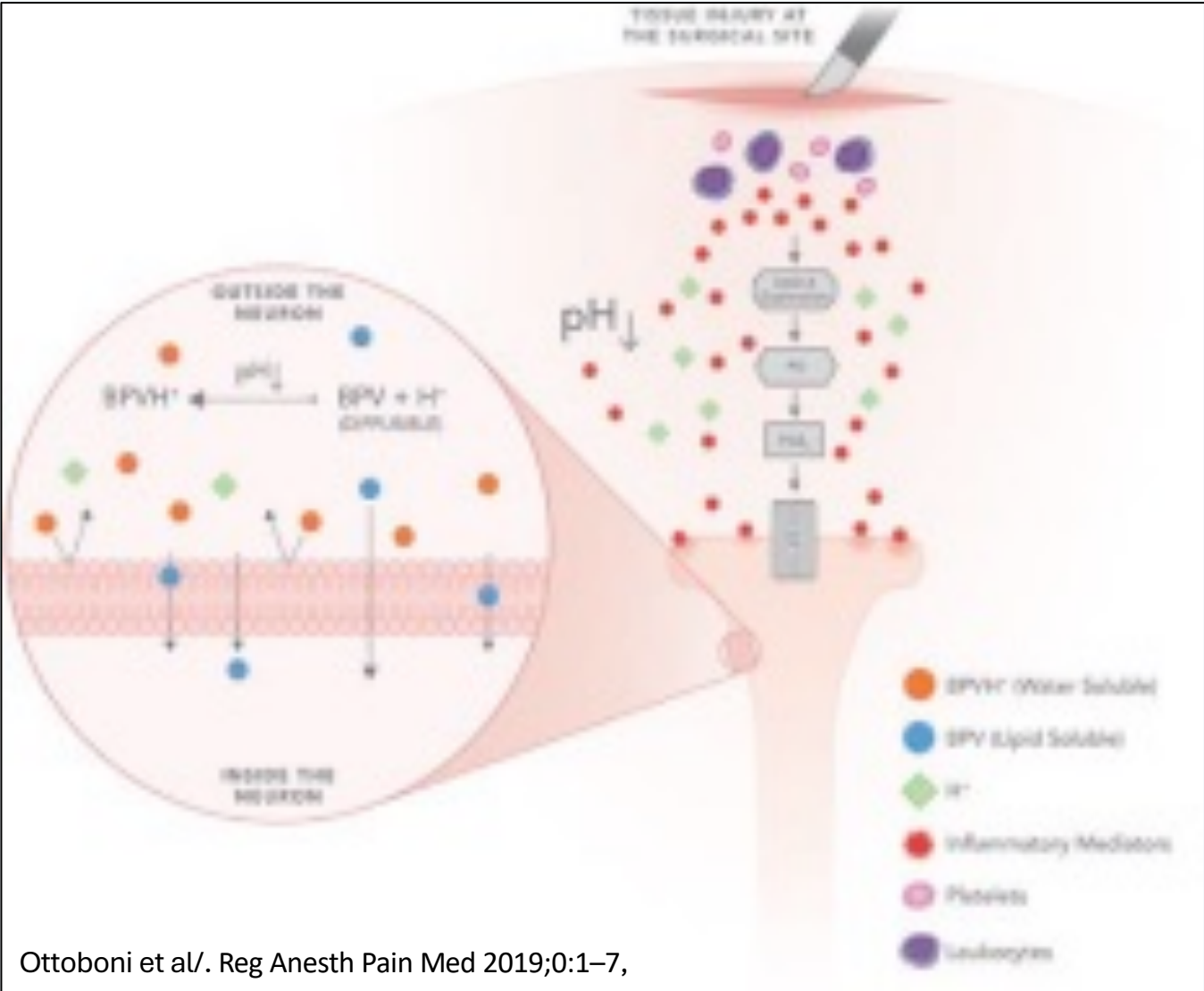
Ottoboni et al. Reg Anesth Pain Med 2019;0:1–7,
Figure 4. Graphic illustration of HTX-11 mode of action.

Block 2: Typical Behavior Readouts Following Post Operative Pain in Pigs

Mechanical Allodynia | DBS | Approaching Test

Figure 5. A-C: Effects of aboBoNT-A, saline, and liposomal bupivacaine on the number of animals exhibiting postsurgical pain-related versus normal responses in each group. Mechanical sensitivity/withdrawal force (**A**),DBS (**B**) and approaching time (**C**) in animals that received a full-skin and muscle incision and retraction surgery (Day 0).

Cornet et al., Nature, Sci Rep. 2022 Dec 13;12(1):21555.

Block 3: Immunohistochemistry

Dysport Effect of Biomarkers in the Spinal Horn

GFAP Immunohistochemistry: astrocytes
Surgery + Saline | Surgery + Dysport
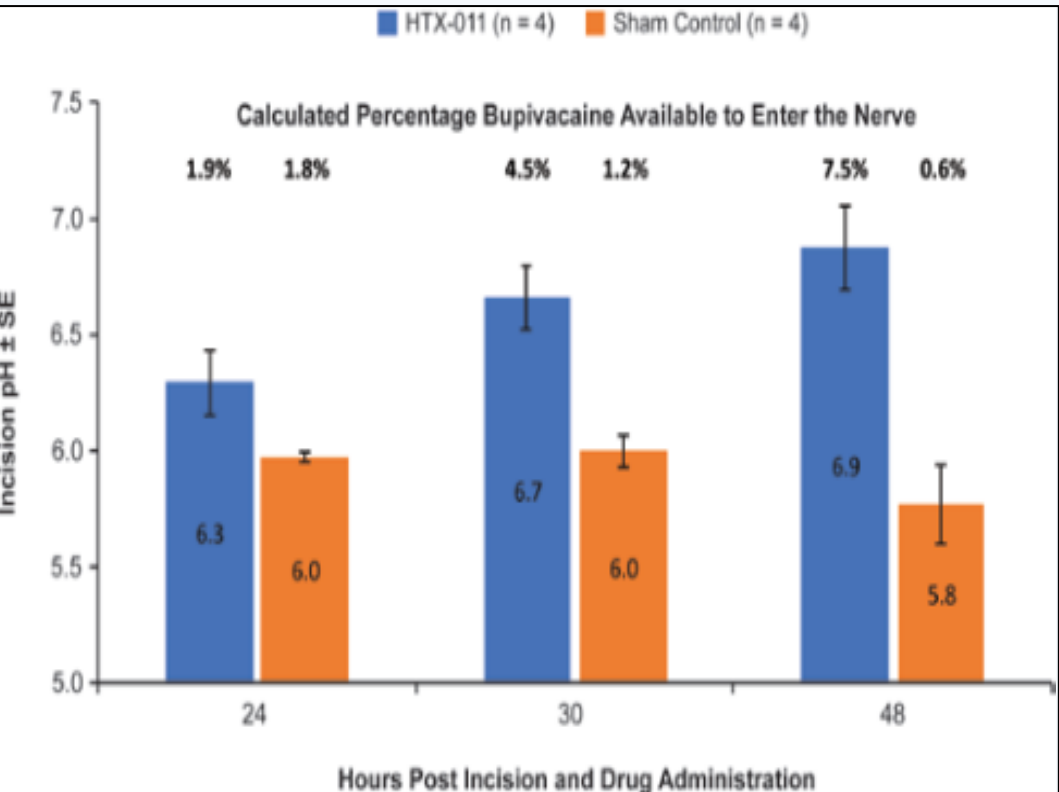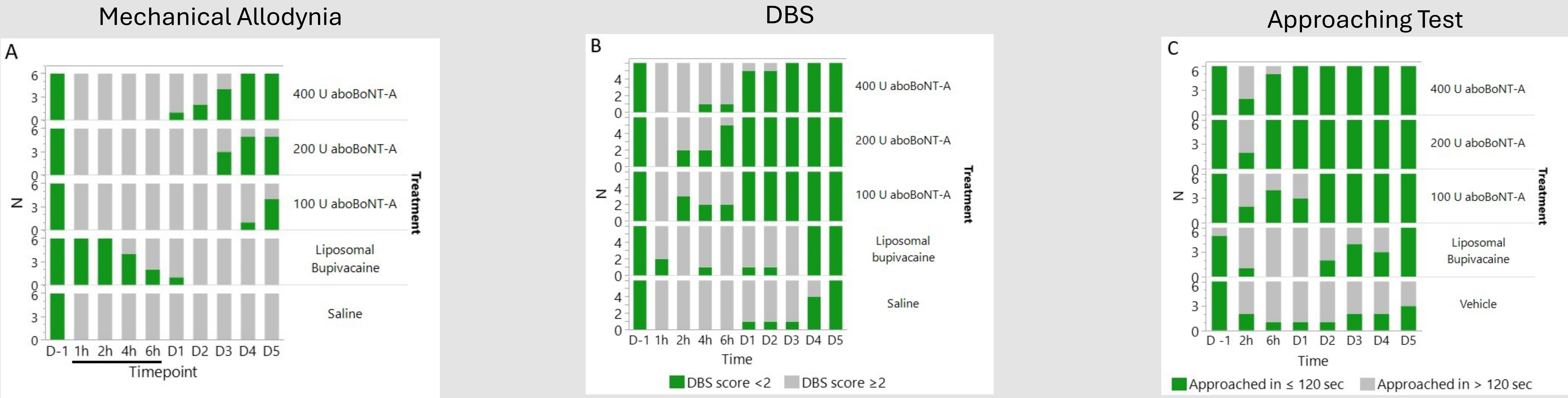
Figures 6. Dysport reduces astrocytes in the spinal horn.

Figure 7. The effect of Dysport was route of administration related.

Block 4: Sex Differences in Post Operative Pain in Pigs

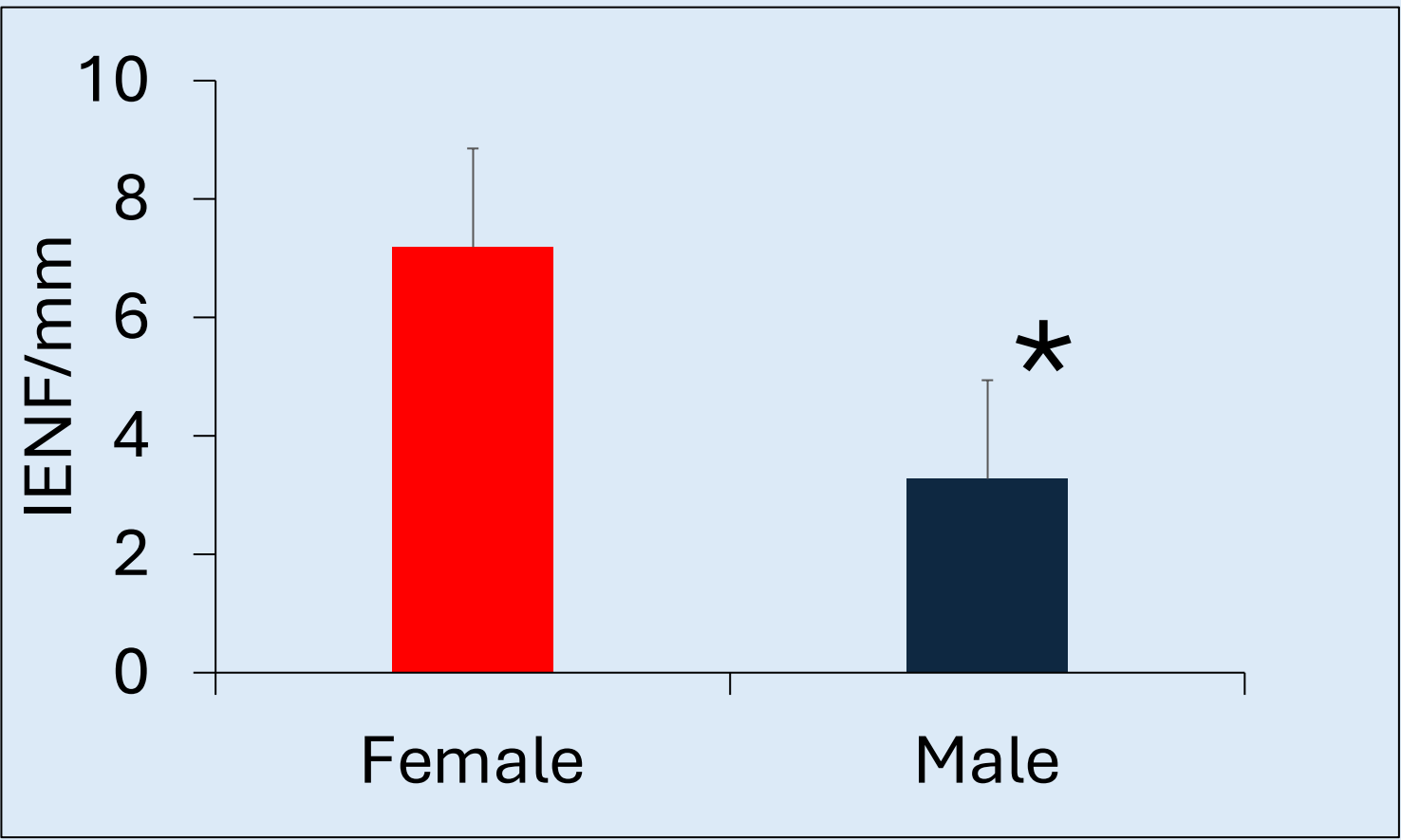#### B-Tubulin 3 Expression in the Skin: Marker for Intra Epidermis Nerve Fibers

Figure 8. Female pigs express higher density of IENF.