Sex Difference in Swine Post-Operative Pain Model

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Introduction

Post-operative pain accompanies any surgical procedure. In the last decade it has been shown that in many cases women are more susceptible to post-operative pain than men (Lau & Patil, 2004; Nadi et al., 2019; Zheng et al., 2017, Mogil 2020), meaning that woman show more sensitivity to pain and less tolerance to medicine compared to men. Yet, in a review from 2018 describing the preclinical research of post-operative pain, Pogatzki-Zhan et al., (Pogatzki et al., 2018) show that the majority of studies use male animals and not female animals. A decade ago Mogil and Bailey, (Mogil & Bailey, 2010) suggest three reasons for favoring male studies over female studies: 1. Inertia researchers do not tend to change their research subject in the middle of their career, fearing that they will not be able to compare the new data to the historical data collected over the years. 2. The expectation that hormonal cyclicity will add to experimental variability. 3. Pain researchers often think of sex differences in pain as small, contradictory, and inconsequential.

Recently Mogil published a review stating that "it is becoming increasingly clear that robust differences exist in the genetic, molecular, cellular and systems-level mechanisms of acute and chronic pain processing in male and female rodents and humans" (Mogil, 2020)

Methods

Animals:

Danish Landrace × Large White cross-bred weaned male pigs from the domestic herd at Lahav Labs, Negev, Israel, were used.

Surgery:

The surgery was previously described in Castel et al., 2014 (Castel et al., 2014). Briefly, skin/muscle incision/retraction (SMIR) model was performed as follows: incision of 6–7 cm was made through the skin, fascia and muscle on one side of the low back of the pig. Incisions were closed using 3-0 silk sutures (Assut UK Ltd, West Yorkshire, UK) and a continuous suturing technique.

Von-Frey Testing

Mechanical allodynia was assessed using von Frey filaments (Ugo Basile, Italy). The filaments were applied at approximately ~0.5 cm proximal to the incision line on the surface of the flank skin. As the gram number of filaments increased, the force on the flank's skin increased. The maximum force was 60 g. Filaments were applied until the animal withdrew from the stimuli. Each filament was applied 3 times. If withdrawal had not been achieved, a thicker filament was applied. If a withdrawal had been achieved, a thinner filament was. By alternating the filament thickness, the force required to achieve withdrawal reaction was determined and recorded.

Approaching test assay:

The normal behavior of the pigs when a person enters their pen is to move away from the intruder. As the animals become accustomed to the person entering the pen, the approaching time decreases. This basic natural behavior of the animals was tested following the SMIR surgery. The time for the animals to approach the intruder was measured in seconds with the high cutoff in both sexes at 120 seconds.

Open field:

The animals were introduced to the open field arena for a period of 5 minutes as was previously described (Castel et al.,). The total distance that the animals walked and the percentage of time that the animals spent in the central area were measured.

Distress behavior score:

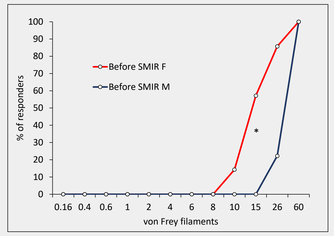
Following incision, the behavior of the animal changes. When approached the animals move away and guard the incision side, sometimes emitting vocalization. This is the main phenomenon observed following this surgery. In rare cases the animals become restless or show an isolation behavior. The distress behavior is scored from 0 (normal) to 7 (very distressed).

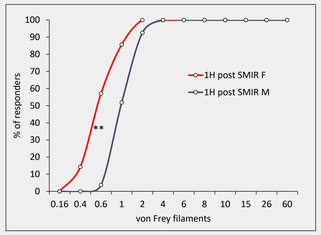
Tables 1: Total score was the sum of all sections below and serves as the animal's DBS.

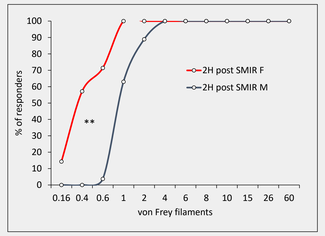
Scoring Section	Parameter	Score
Section 1.	Avoiding standing (the animals tend not to stand when food is served or when researcher is approaching)	1
	Standing	0
Section 2.	Avoiding walking (the animal is able to walk but is avoiding this action by leaning on the wall of the pen)	1
	Walking	0
Section 3.	Protecting the incision side while walking (the animal walks with the incision side is facing the pen walls)	1
	Acting normal	0
Section 4.	Moving away when approached by researcher (the animal does not show interest in the researcher and is running from the researcher when approached)	1
	Friendly behavior	0
Section 5.	Restlessness (the animal is passing in the pen and sometimes shows aggressiveness towards its pen mate)	1
	Normal	0
Section 6.	Isolation (the animal does not interact with its pen mate and is standing in one of the pen corner)	1
	Normal	0
Section 7.	Screaming (high vocalization) (the animal scream)	1
	Normal vocalization	0

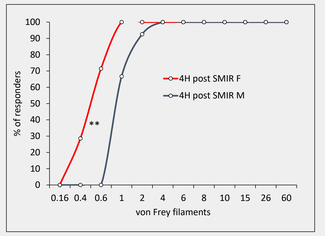
Results

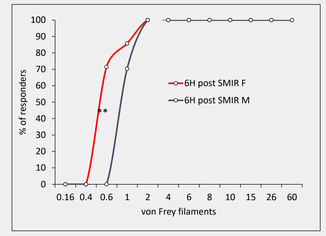
Von Frey testing:

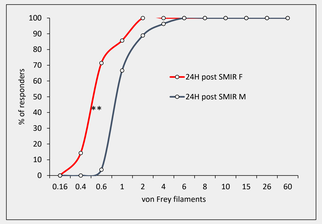


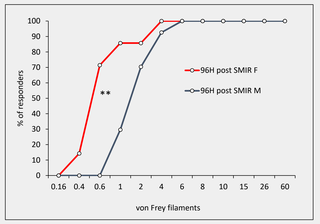












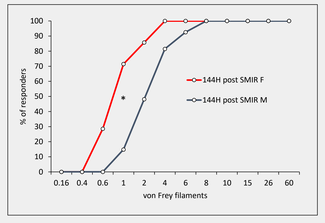
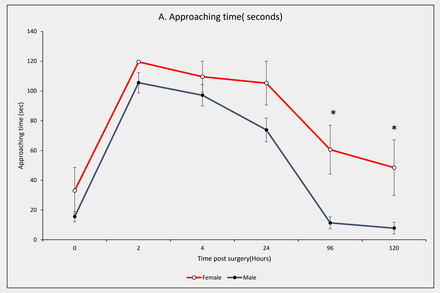


Figure 1: The response of female and male animals to von Frey filament stimulation. The x-axis represents the pressure of each filament applied on the skin (g). The y-axis is the percentage of animals that responded to a specific filament. Even before the SMIR surgery the female animals responded to a lower pressure then the male animals ($p<0.05$). Evidently, female animals initially responded to 10 g, while some male animals responded to 15 g. The majority of male pigs responded to 26-60 g.

After the surgery the female animals were clearly more sensitive to the von Frey stimulation as the red (female) line shifts toward the left part of the graphs at all timepoints. This suggests higher sensitivity to von Frey stimulation in female animals.

*$p<0.05$ female vs. male; **$p<0.01$ female vs. mail.

Approaching test



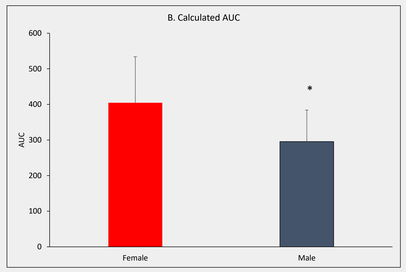


Figure 2 : Changes in approaching time following the SMIR surgery. The normal behavior of the animal is to move away from a person entering their pen. During the acclimation period the animal becomes accustomed to the researcher entering their pen and the approaching time is minimal (0-30 seconds). However, 2 hours post SMIR the approaching time increases with no difference between female and male animals. However, the recovery time is different. While there was no difference at baseline prior to surgery between males and females, changes were observed at 96 hours and 144 hours. At 96 hours there was no difference in approaching time vs. pre-surgery in male animals. In female animals the approaching time was longer at 96 hours and 144 hours compared to males.

*$p<0.05$ vs. Female

Open Field

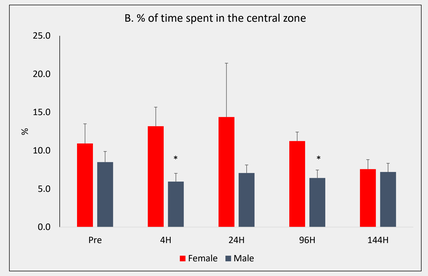


Figure 3: Changes in the walking distance (A) and the percentage of time spent in the central zone (B) following SMIR in female and male pigs. Male pigs walked the same distance before and after SMIR. In contrast, female pigs walked less at 4 and 24 hours post SMIR. There was no difference in the percentage of time that the female or male animals spent in the central zone after SIMR vs. pre-surgery. However, males spent less time in the central zone. Further studies should be performed to support or reject this set of data.

*$p<0.05$ vs. baseline prior to the surgery. #$p<0.05$ vs. female.

Distress Behaviour Score

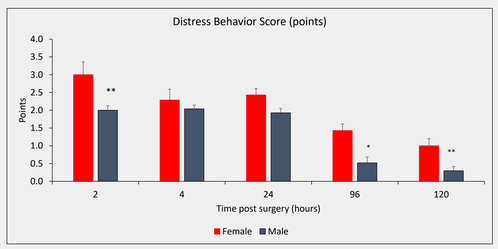


Figure 4: Changes in the distress score following SMIR. Female animals showed a higher distress score at 2 hours, 96 hours and 144 hours post-surgery. At 4 hours and 24 hours post SMIR no different was found between females and males.

*$p<0.05$ vs. female; **$p<0.01$ vs. female

IENF count

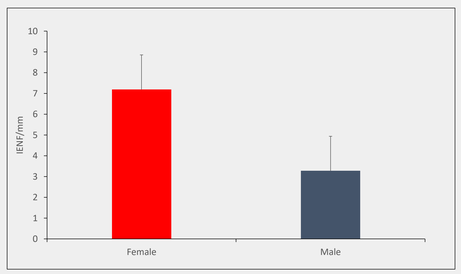


Figure 5: Intra Epidermis Nerve Fiber (IENF) count shows a significant difference in the nerve ending density between female and male.

**$p<0.05$ vs. female

Conclusions

1. **Naïve, untreated, young female pigs are more sensitive to mechanical stimuli than male pigs.**
2. **Female pigs, not sexual matured, are more sensitive to post-operative pain than male pigs.**
3. **Higher density of IENF was found in naïve female pigs. This might be part of the reasons for the female higher sensitivity to post-operative pain versus male.**
4. **These results highlight the importance of including females and males in the study of pain.**

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mdbioscences.