

MEDICAL TRAINING OF MINPIGS FOR STRESS CONTROL DURING WOUND HEALING RESEARCH.

INTRODUCTION

Pigs are often used as animal models for skin wound healing research because their skin and healing process closely resemble those observed in humans. For these studies, Göttingen minipigs are useful because of their small adult size, un-pigmented skin and gentle behavior. Stress has been proved to interfere wound healing and increase healing time, showing both statistical and clinical differences^{1,2,3}. Wound healing studies can be affected by stress, making the results less reliable and representative. Stress also decreases animal welfare and efforts must be made in order to minimize it.

Operant conditioning through positive reinforcement offers excellent results in animal training for scientific purposes (vital signs determination, sample obtaining, treatment application) and handling procedures (weight control, transport).

OBJECTIVE

The aim of this study was to develop and assess the efficacies of a medical training protocol in Göttingen minipigs during the conduct of a preclinical wound healing study in order to improve the animal welfare and the reliability of the study results.

MATERIAL AND METHODS

The study protocol was approved by the institutional Animal use and care committee. Six adult male Göttingen minipigs were used. Animals were housed at the animal housing facilities under controlled conditions (45-65% RH, 10-24°C, 12/12 light cycle) on HEPA-filtered specific rooms equipped with 3x2m individual pens and automatic water dispenser.

Besides pain, that was addressed with a strong analgesic protocol, Stress during individual housing and procedural handling were determined as main stressors during the wound healing study and were assessed individually in each animal using two predetermined charts of stress signs and intensity (Table 1 and 2). Stress signs were monitored daily since the beginning of the medical training plan, two weeks before the skin wound creation. After the wound creation, housing signs were monitored daily for two weeks, but handling signs were monitored according to bandage changes and treatment application two times per week during the same period. During this period, when an animal displayed signs of pain or stress during the bandage changing, the process were immediately stopped, signs were recorded and anesthesia was administered through a face mask in order to avoid unnecessary stress.

Housing stress was minimized by the use of chewy elements in the pen as environmental enrichment elements (Figure 1.-A) and by allowing the expression of social behaviors maintaining the animals in groups for 1h/day (Figure 1.-B). Handling stress during the approach and catching, sling transport and bandage change was minimized by the oral administration of diluted yogurt (Figure 1.-C) as a positive reinforcement when the animals kept calm during the procedures (Figure 1.-D) along the training and the research phases.

HANDLING RELATED STRESS					
ACTIONS	BEHAVIOR DURING PROCEDURES	HIGH	MEDIUM	LOW	ABSENT
APPROXIMATION, CATCHING AND SLING TRANSPORT	Hiding attempts	3	2	1	0
	Efforts to avoid being caught	3	2	1	0
	Vocalizations during approximation / manipulation / sling transport	3	2	1	0
DRESSING CHANGING WOUND CLEANING	Vocalizations during the procedure	3	2	1	0
	Evasion attempts during the procedure	3	2	1	0

Table 1.- Handling related stress signs and associated punctuation.

HOUSING RELATED STRESS					
SITUATION	SPONTANEOUS BEHAVIOR	HIGH	MEDIUM	LOW	ABSENT
GROUP ATTITUDE	Separation from the group / lack of social interaction	3	2	1	0
	Posture and/or movement alteration	3	2	1	0
INDIVIDUAL HOUSING ATTITUDE	Decreased food intake (>30%)	3	2	1	0
	Decreased water intake (>30%)	3	2	1	0
	Depression/Apathy	3	2	1	0

Table 2.- Housing related stress signs and associated punctuation.

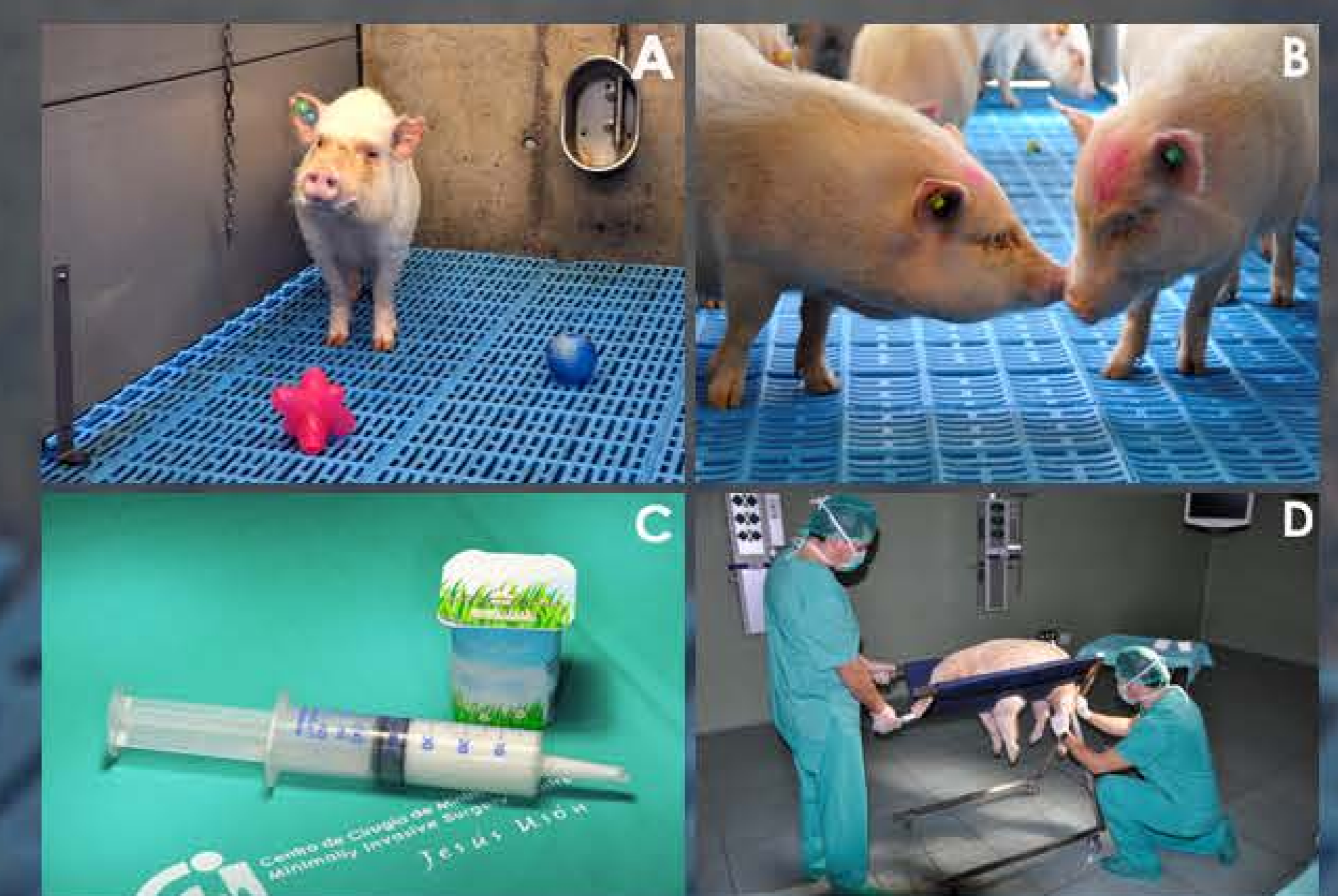


Figure 1.- Stress relief aspects. A: Environmental enrichment elements; B: Social interaction periods; C: Positive reinforcements reward; D: Bandage changing training.

RESULTS

Results show a significant decrease in the expression of stress signs since the second day of medical training (Figure 2). Once the ulceration model was created the stress signs detected raised up specially during follow up and bandage change days. Housing stress signs were slightly present during the first days after wound creation due to the discomfort originated by the ulceration model even in presence of a powerful analgesic treatment (Figure 3), however the detected signs were slight and only appeared during the first four days after the model creation. The figure 4 shows how after the wound creation the stress signs related to handling were mainly expressed during bandage changes while handling during catching and sling transport were well tolerated.

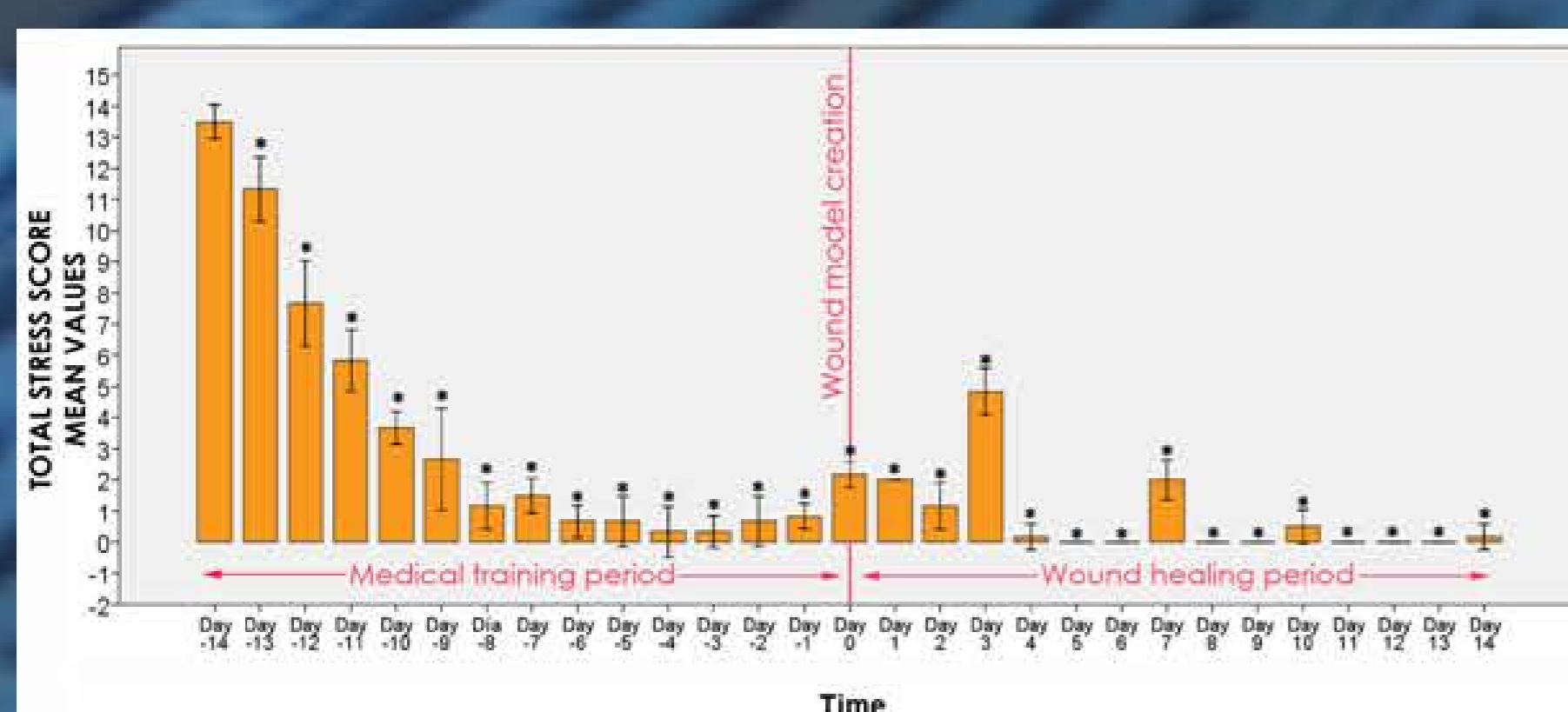


Figure 2.- Total stress score during the study. Mean ± Standard deviation. HSD-Tukey test was used to study changes with time. P<0,05 were considered significant.

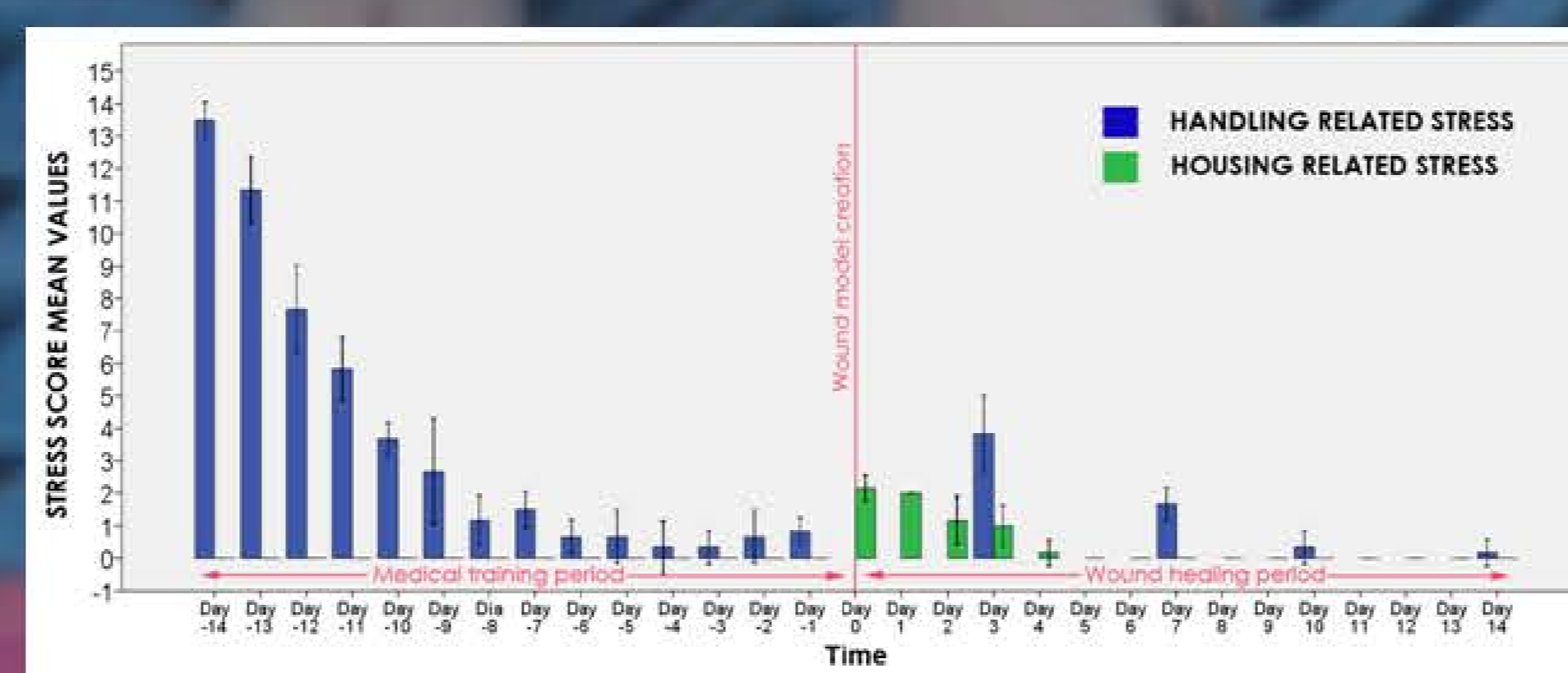


Figure 3.- Stress score mean values by stress source.

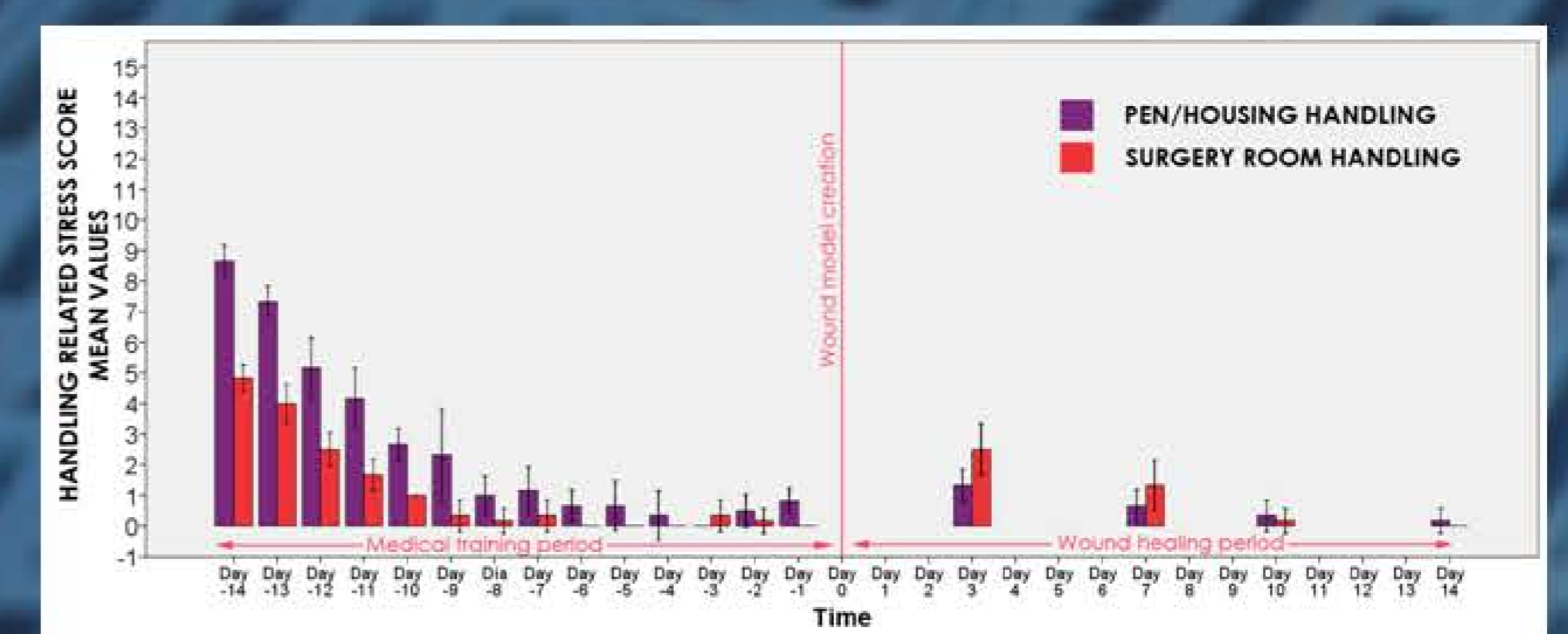


Figure 4.- Handling related stress score mean values by stress source.

CONCLUSIONS

Medical training through positive reinforcement significantly reduce the stress suffered by the animals on wound healing studies, improving animal welfare and contributing in obtaining more reliable results. It is hard to completely control the pain originated during wound cleaning and dressing changes, even in presence of a strong analgesic treatment, due to the hyperalgesic state of the wounded tissues. Preventive training of study animals in the procedures that are expected to be stressful or painful makes a difference in animal welfare and study results.

The use of medical training can therefore contribute in the refinement of almost every procedure performed in conscious laboratory animals, it's use can also contribute in reducing the costs of many studies by reducing the number of staff required for the procedures and minimizing the use of sedative / anaesthetic drugs. Although medical training can effectively minimize or avoid the stress suffered by the animals in many routine procedures, the nature of the procedure it self can require the mandatory use anaesthetic treatments to completely avoid animal suffering.

Bibliography

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