Corso di base in materia di protezione degli animali utilizzati a fini scientifici

Pisa 5 novembre 2020

Segni clinici di malessere e interventi terapeutici

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Segni clinici di dolore, sofferenza e distress

Cosa è il dolore?

"Una sensazione ed una esperienza spiacevoli, associate con un danno tissutale **reale o potenziale**" IASP

Non si tratta di come si sente….ma di come ci fa sentire!!

(Prof Jacky Reid)

Poiché siamo nel territorio EMOZIONALE è da considerarsi una esperienza **unica** ed **individuale**

Valutazione del dolore: perché è importante?

- **Dovere etico e morale**

- **Buon fine della sperimentazione**

1) ridurre morbilità e mortalità

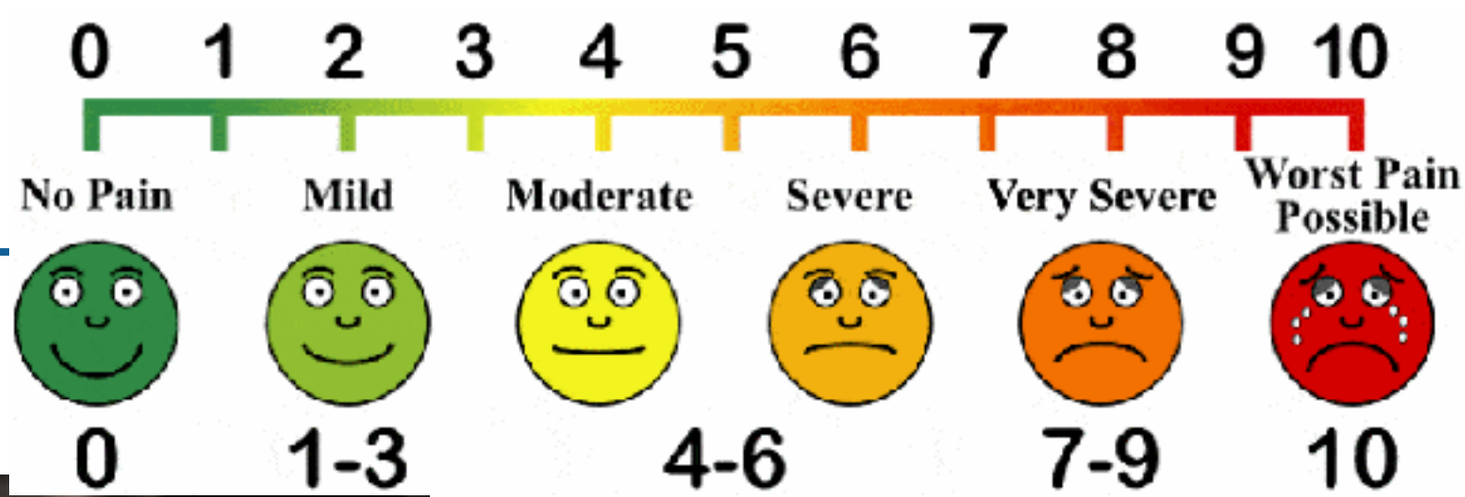
2) migliorare interazione con gli animali

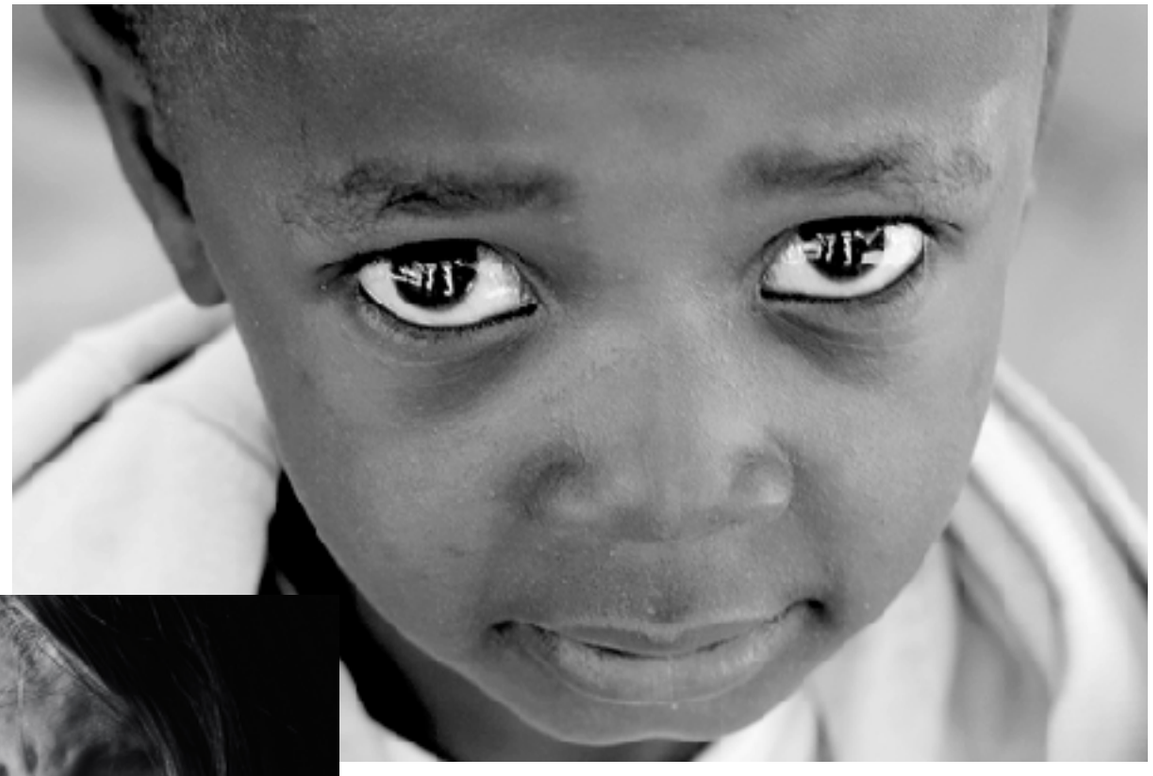
Segni clinici di dolore, sofferenza e distress

- alterazione del comportamento interattivo
- alterazione del comportamento abituale
- riduzione dell'appetito/defecazione
- riduzione/alterazione del movimento
- riduzione della toelettatura
- riduzione dell'attività ludica

Valutazione del dolore

- i segni clinici sono specie-specifici
- necessità di conoscere l'etologia della specie
- necessità di valutare gli animali prima dello stimolo nocicettivo
- monitorare le modificazioni nel tempo

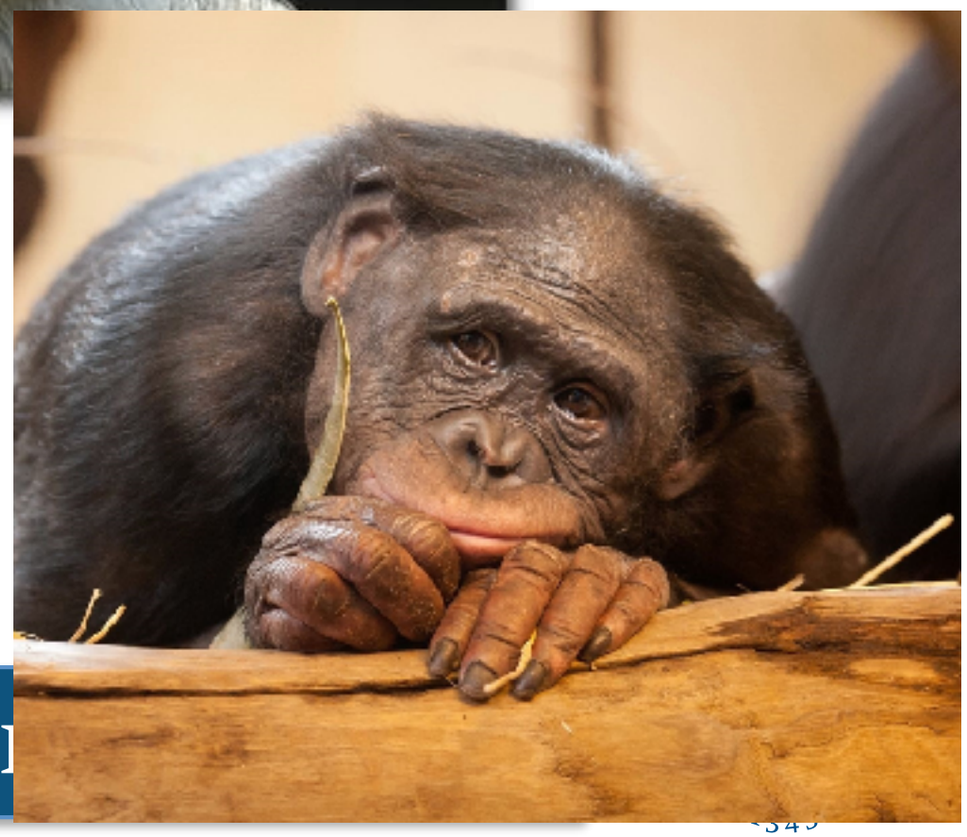

0 1 2 3 4 5 6 7 8 9 10
No Pain
Mild
Moderate
Severe
Very Severe
Worst Pain Possible
0
1-3
4-6
7-9
10



Segni Clinici: Primati non umani

- Segni clinici non evidenti: più facilmente riconoscibili mediante registrazione in assenza di persone
- segni di prostrazione, tristezza, occhi vitrei
- evitano il contatto visivo e l'interazione tra conspecifici
- anoressia/disoressia
- automutilazioni

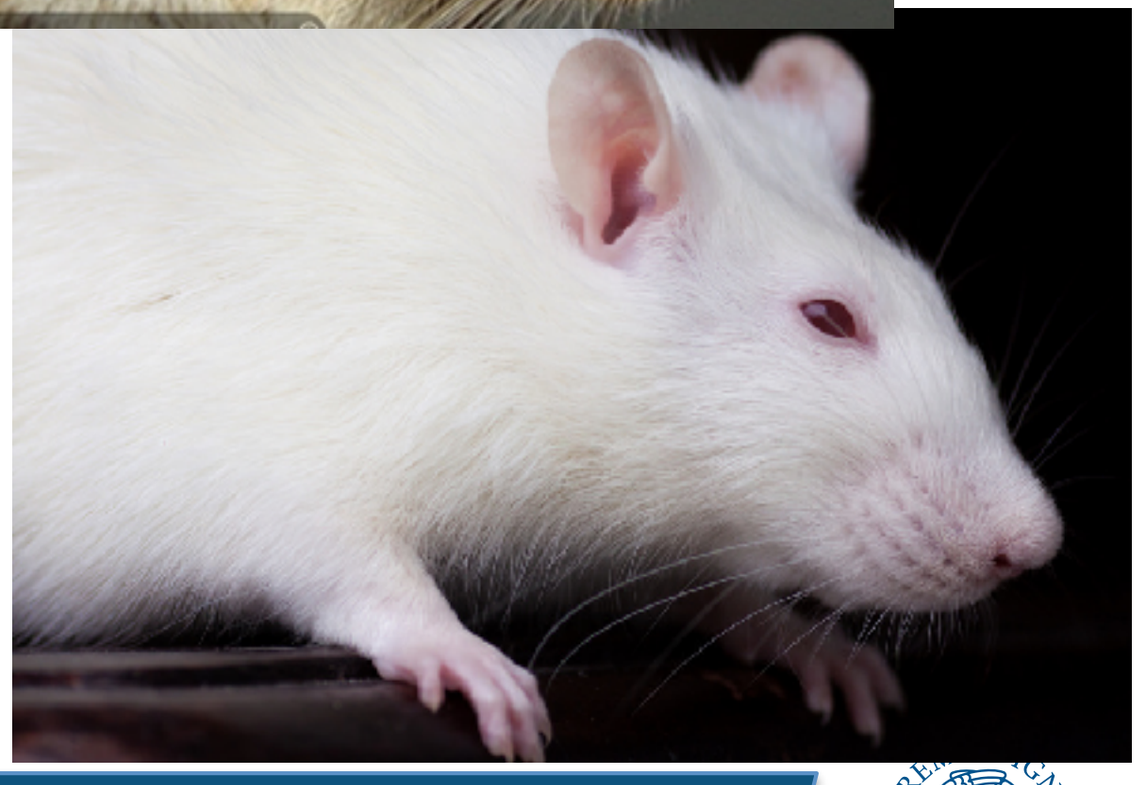
Segni Clinici: Primati non umani



Segni Clinici: Roditori

- vocalizzazioni (***spesso non udibili: ultrasuoni) e aumento dell'aggressività quando manipolati
- riduzione dell'appetito e disoressia
- alterazione dell'interazione tra conspecifici e del grooming
- isolamento e riduzione della gestione del nido
- secrezione di porfirina (lacrime rosse) nel ratto segno generico di distress
- posture anormali

Ratto



University of Michigan
ULAM

Pain Assessment in Rats

What if you see …?

6/22/2015

Analgesics must be administered as outlined in the UCUCA-approved protocol. When "as needed" analgesics are specified, animals must be monitored for signs of pain, and treated accordingly.

Listed below are easily identifiable indicators of pain in rats. This is neither a comprehensive nor specific list, and as such, other observations should be taken into account when assessing pain status in rats. **Please contact ULAM veterinary staff** for additional assistance with identification and/or treatment of pain.

Appearance

(A) Interpretation of **facial expression** can be used to qualify pain. Below are images of rats who show varying levels of pain.

Orbital Tightening
Rats in pain display a narrowing of the orbital area, manifesting as either (partial or complete) eye closure or eye "squeezing"[1]

Nose/Cheek Flattening
Rats in pain display successively less bulging of the nose and cheek, with eventual absence of the crease between the cheek and whisker pads[1]

Ear changes
The ears of rats in pain tend to fold, curl and angle forwards or outwards, resulting in a pointed shape. The space between the ears may appear wider[1]

Whisker change
The whiskers of rats in pain move forward (away from the face) from the baseline position, and tend to bunch, giving the appearance of whiskers standing on end[1]

(B) Rough hair coat and **porphyrin staining** are indicators of **lack of grooming**, which is indicative of pain and/or stress.

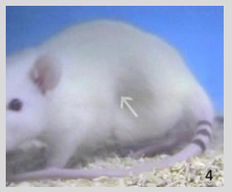
Porphyrin staining
Porphyrin (a red-brown pigment) is a normal secretion produced by the tear gland around rat eyes. When the animal is not grooming, the pigment builds up around the eyes, nose, and on the fur.

Behavior

(C) When rats are experiencing abdominal pain, they may demonstrate the following **abnormal behaviors**:

Cat-like Back Arching
The animal arches its back upwards; this looks like normal cat stretching, but is abnormal in rodents.

Writhing
Lateral contortion of the flank abdominal muscles[2]. It may look like the animal is "sucking in" its stomach.



Twitching
A short-lived involuntary muscular contraction of any body part[2].

No image available

References

1. The Rat Grimace Scale images and accompanying descriptions are borrowed verbatim from: Sotocinal SG, Sorge RE, Zaloum A, et al. 2011. The Rat Grimace Scale: A Partially automated method for quantifying pain in the laboratory rat via facial expressions. *Molecular Pain* **7**: 55.

2. Behavioral indicators from: Roughan JV, and Flecknell PA. 2001. Behavioural effects of laparotomy and analgesic effects of ketoprofen and carprofen in rats. *Pain* **90** (1): 65-74.

Roughan JV, and Flecknell PA. 2005. Training in behavior-based post-operative pain scoring in rats – An evaluation based on improved recognition of analgesic requirements. *Applied Animal Behaviour Science* **96** (3-4): 327-342.

3. Porphyrin staining image from: Humane Endpoints in laboratory animal experimentation: Eye crusting ('Red tears') (rat): www.humane-endpoints.info

4. The behavior images are borrowed directly from videos on: Newcastle University. 2014. Assessing the Health and Welfare of Laboratory Animals. "An Introduction: Recognising Post-Operative Pain in Animals:" http://www.ahwla.org.uk/site/tutorials/RP/RP01-Title.html

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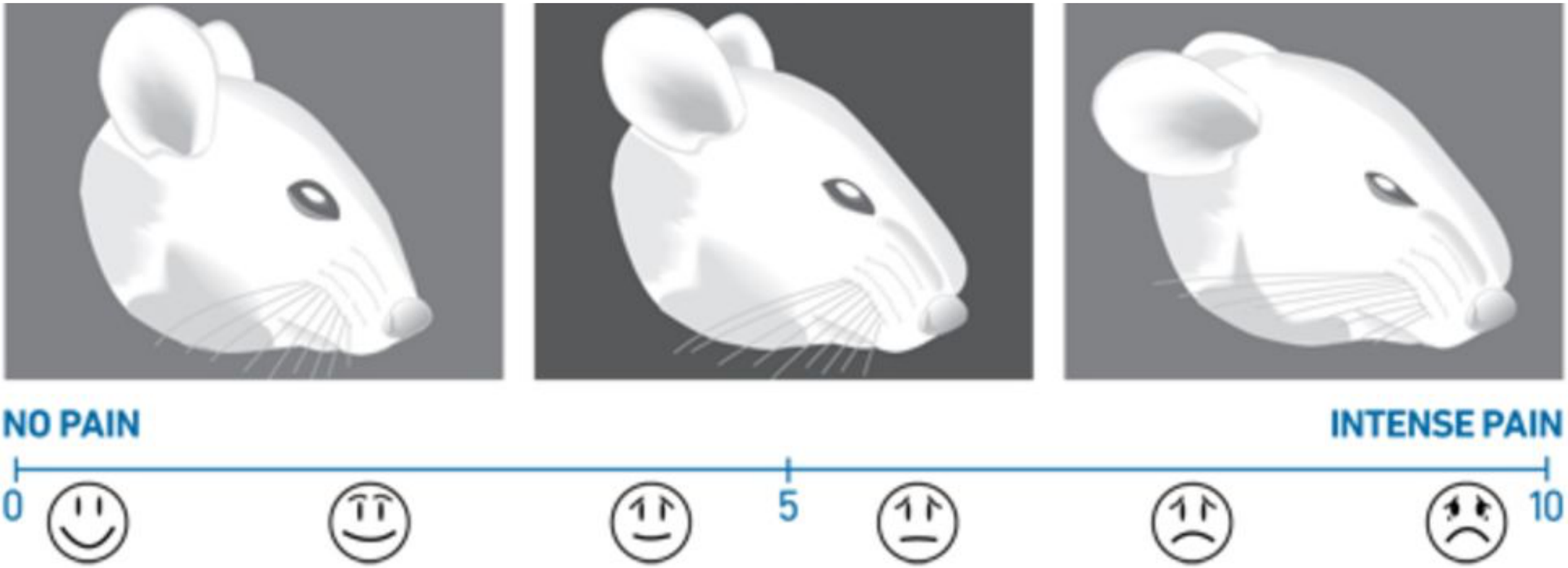
Ratto

Ratto

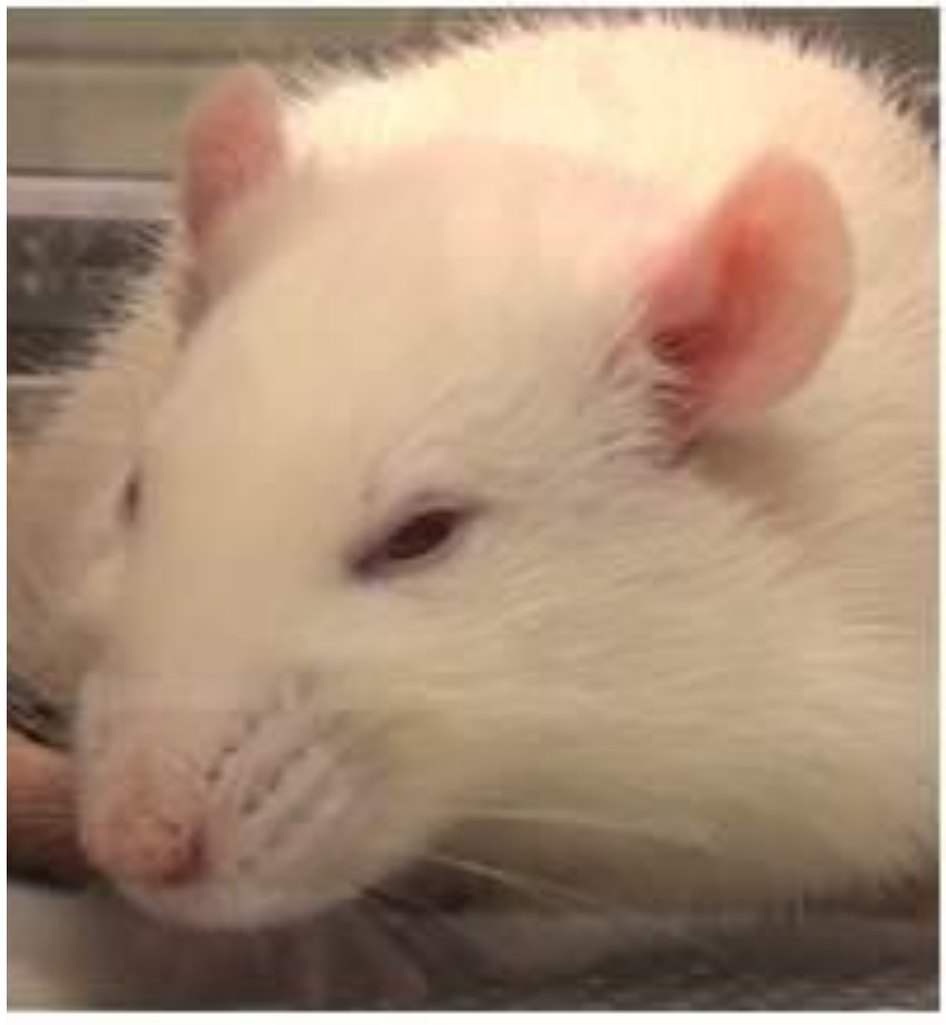
Valutazione del dolore: Rat Grimace Scale (RGS)



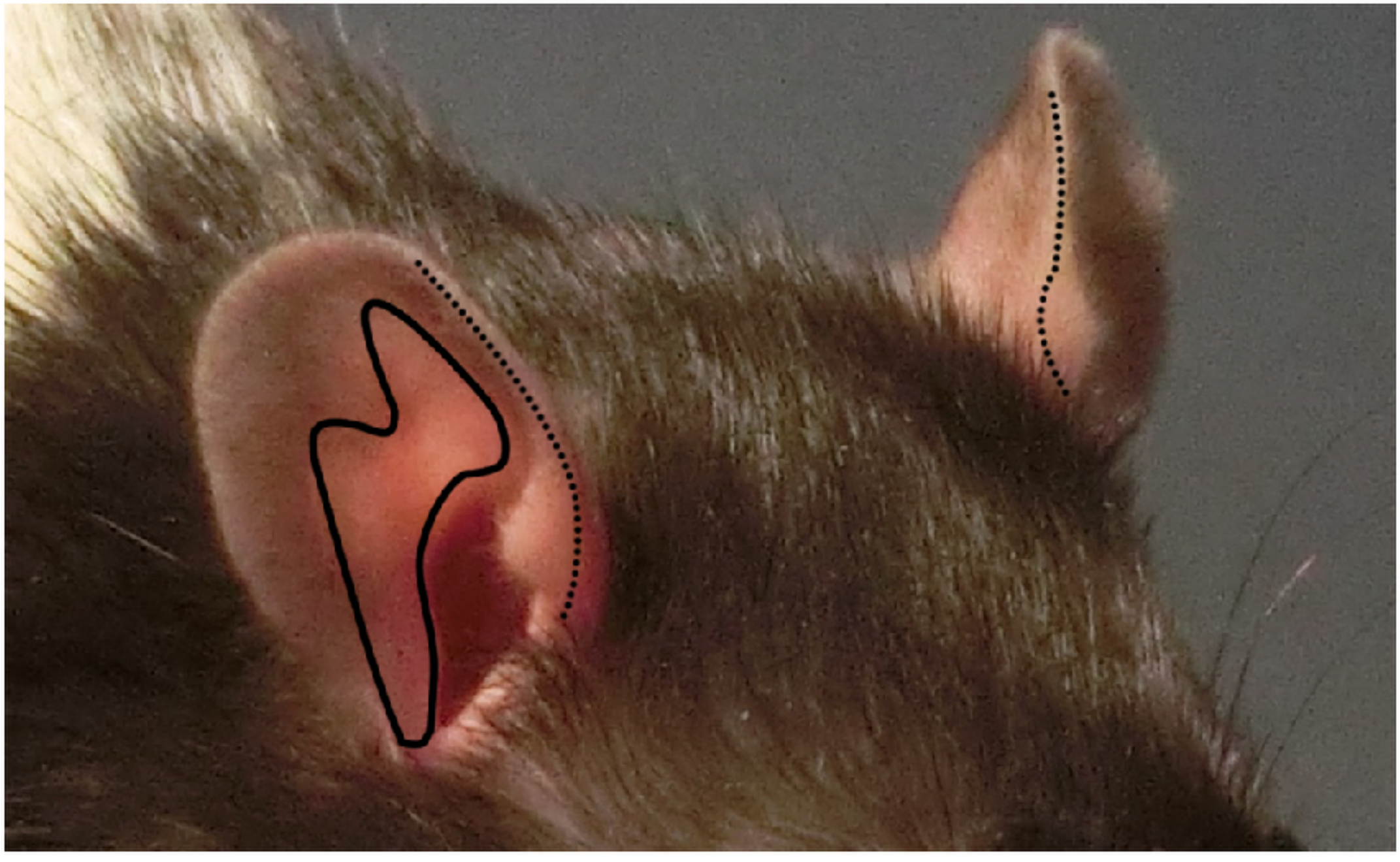
- orbital
- nose/cheek
- ear
- wiskers

Ratto

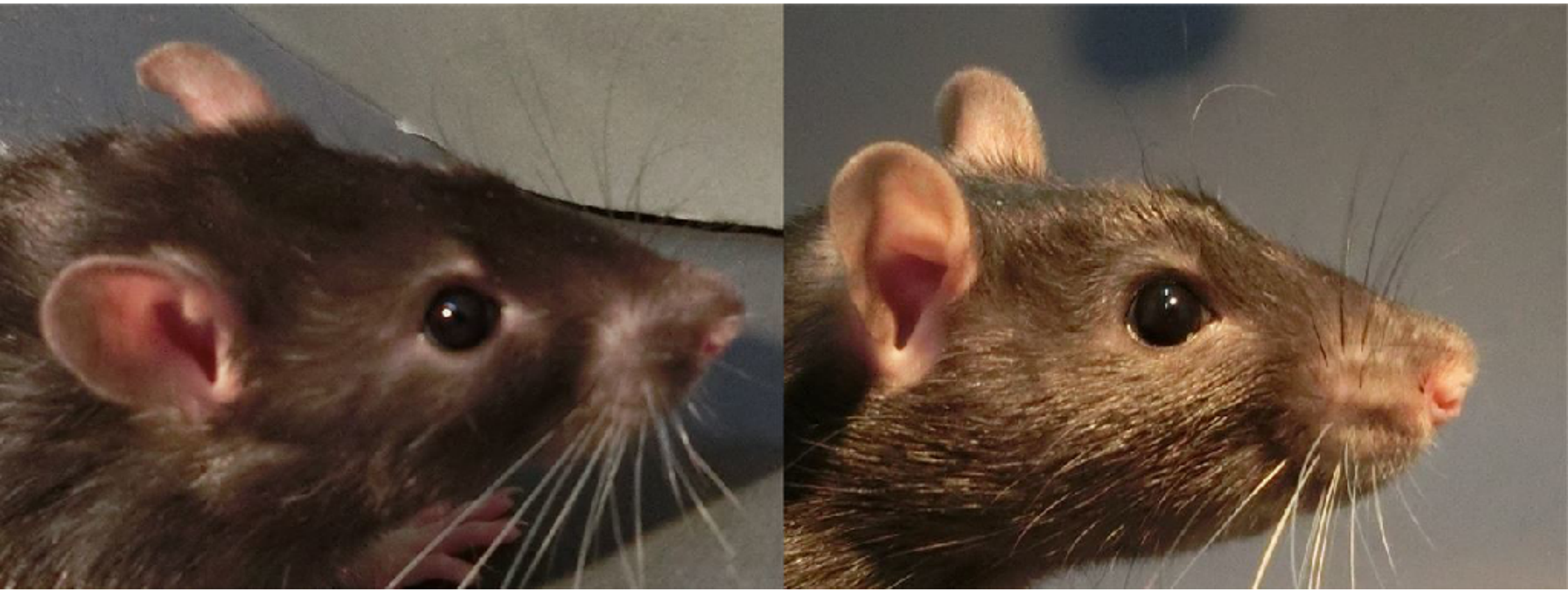
VS



Ratto: ride con le orecchie!!!



Ratto: ride con le orecchie!!!



Ratto: no valutazione mentre dorme

Topo

- Perdita del “gusto” per il dolce: il topo tende a prediligere una soluzione zuccherina, in condizioni di stress non fa nessuna differenza

- Tende a raggomitolarsi e cercare posti con meno luce

- Perdita del comportamento interattivo

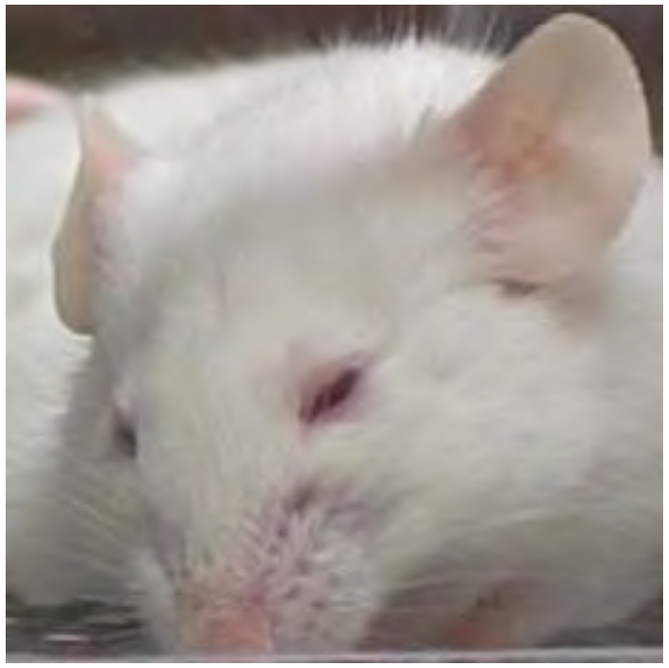
- Riduzione della capacità di reazione: ***“tail suspension test”***

Topo

Valutazione del dolore: Mouse Grimace Scale (MGS)

"0"

"1"



"2"

Segni Clinici: Coniglio

- perdita appetito, ansioso, inattivo
- può reagire in maniera esagerata alle manipolazioni
- automutilazione
- schiena inarcata, contrazioni addominali
- ileo paralitico e costipazione

Rabbit Grimace Scale

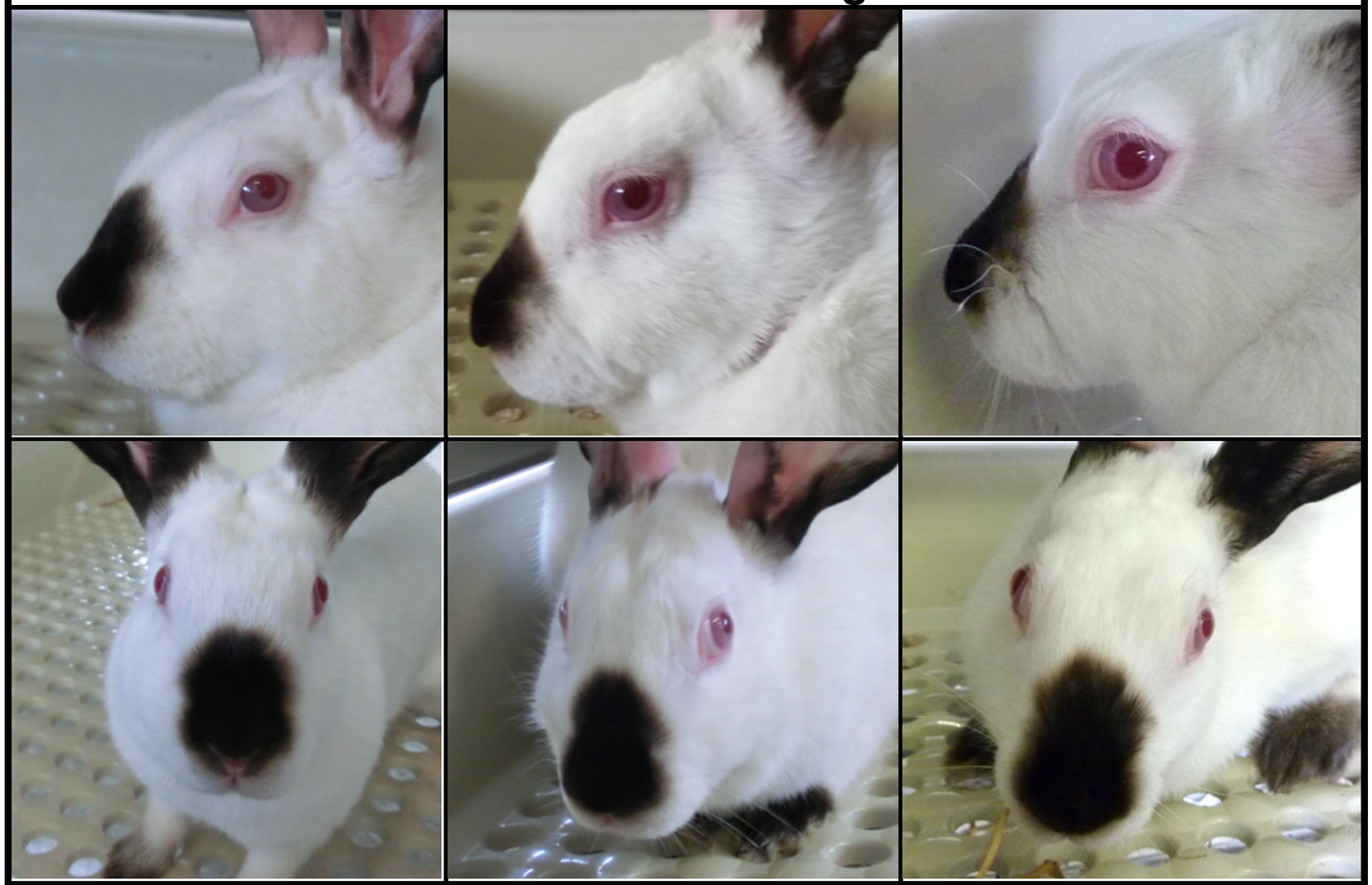
Orbital Tightening

0	1	2
Not Present	Moderately Present	Obviously Present

- Closing of the eyelid (narrowing of orbital area)
- A wrinkle may be visible around the eye

Rabbit Grimace Scale

Cheek Flattening

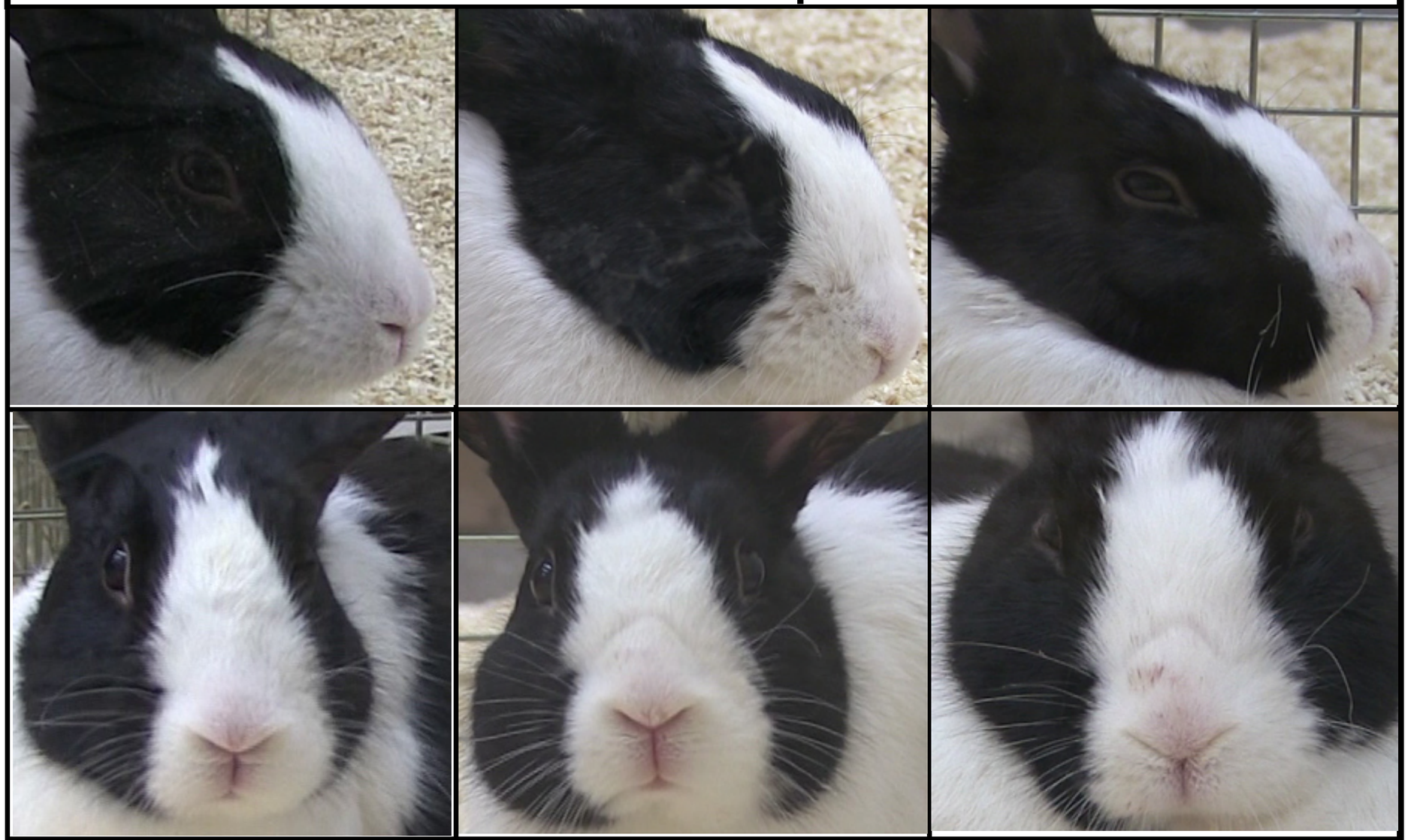


0	1	2
Not Present	Moderately Present	Obviously Present

- Flattening of the cheeks. When ‘obviously present’, cheeks have a sunken look.
- The face becomes more angular and less rounded

Rabbit Grimace Scale

Nostril shape



0	1	2
Not Present	Moderately Present	Obviously Present

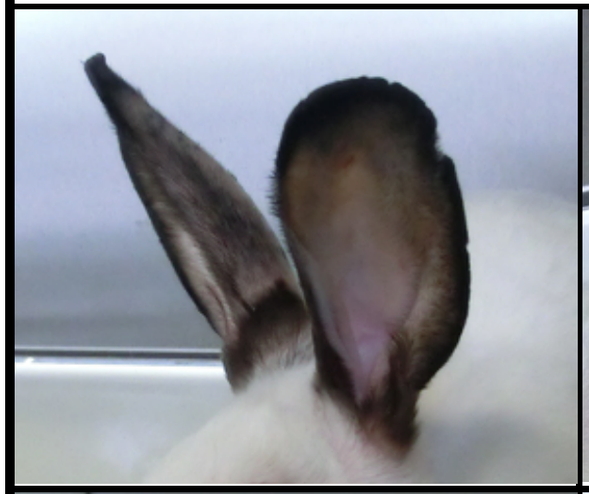
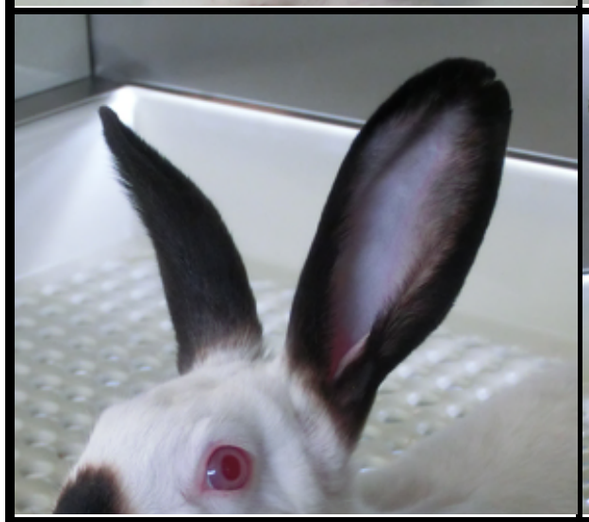
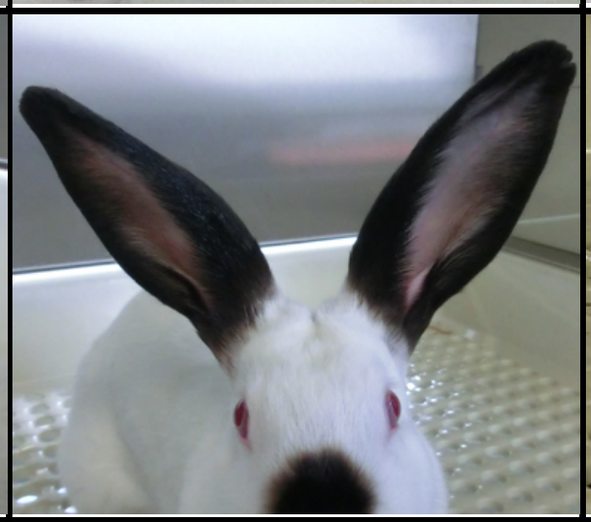
- Nostrils (nares) are drawn vertically forming a ‘V’ rather than ‘U’ shape
- Nose tip is moved down towards the chin

Rabbit Grimace Scale

Whisker Change & Position		
		
0	1	2
Not Present	Moderately Present	Obviously Present

- Whiskers are pushed away from the face to ‘stand on end’
- Whiskers stiffen and lose their natural, downward curve
- Whiskers increasingly point in the same direction. When ‘obviously present’, whiskers move downwards

Rabbit Grimace Scale

Ear Shape & Position		
		
		
0	1	2
Not Present	Moderately Present	Obviously Present

- Ears become more tightly folded / curled (more cylindrical) in shape
- Ears rotate from facing towards the source of sound to facing towards the hindquarters
- Ears may be held closer to the back or sides of the body

Valutazione dolore coniglio

OPEN ACCESS Freely available online

PLoS one

Are We Looking in the Wrong Place? Implications for Behavioural-Based Pain Assessment in Rabbits (*Oryctolagus cuniculi*) and Beyond?

Matthew C. Leach*, Claire A. Coulter, Claire A. Richardson, Paul A. Flecknell

Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, United Kingdom

Conclusions: In conclusion, irrespective of experience and gender, observers focused on the face when using behaviour to assess pain and were unable to effectively identify rabbits in pain. Focusing on the face is unlikely to be effective when using behavioural indicators of pain since they involve other body areas. Alternatively, if animals exhibit pain-related facial expressions, then it could improve our ability to assess pain. In addition, these results have potential implications for the use of behaviour to assess how rabbits and potentially other species feel.

Table 1. Pain severity classification and description of each of the video sequences observed.

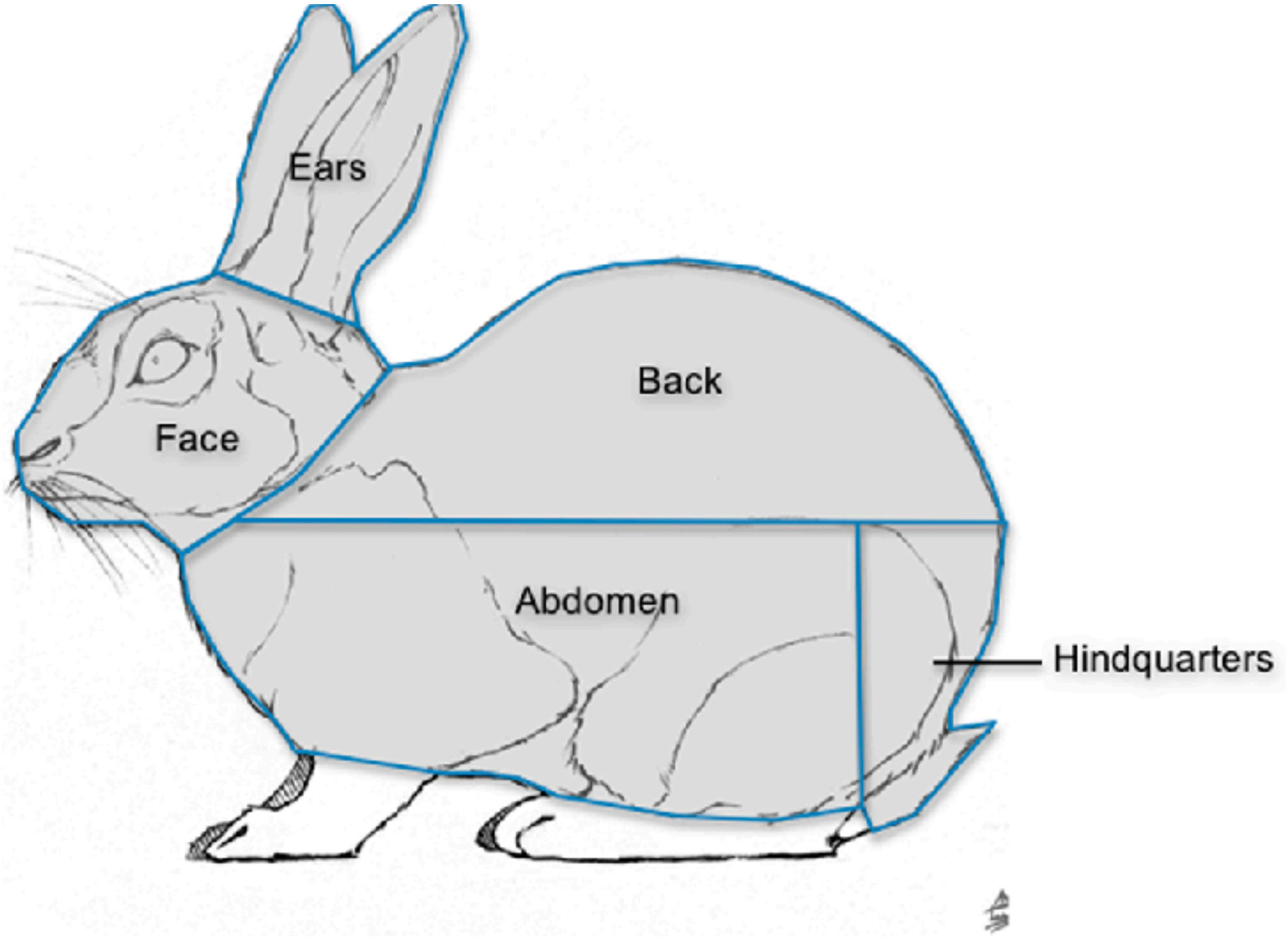
Sequence	Severity	Description
1	Normal	Exhibiting no pain related behaviour or postures
2	Mild	Exhibiting less than 2 pain related behaviour or postures
3	Moderate	Exhibiting between 3–5 pain related behaviour or postures
4	Severe	Exhibiting greater than 6 pain related behaviour or postures

Valutazione dolore coniglio

Behaviour	Description
Twitch	Rapid movement of fur on back
Flinch	Body jerks upwards for no apparent reason.
Wince	Rapid movement of the backwards in a rocking motion accompanied by eye closing and swallowing action
Stagger	Partial loss of balance
Fall	Complete loss of balance when moving
Press	Abdomen pushed towards floor, usually before walking
Arch	Full arching of the back upwards
Writhe	Contraction of the oblique flank muscles
Shuffle	Walking at a very slow pace
Quiver	Slow rhythmic side-to-side movement

Behavioural and postural indicators of rabbit pain following ovariohysterectomy [1].
doi:10.1371/journal.pone.0013347.t002

Valutazione dolore coniglio



Valutazione dolore coniglio

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Segni Clinici: Maiale

- perdita appetito, ansioso, inattivo
- può reagire alle manipolazioni e urla se toccato nella zona dolente
- perdita del sonno e della preparazione del “letto”
- alterazioni della postura e della camminata

Maiale

Ear Position

Absent (0)

Moderately present (1)

Obviously present (2)

When the animal is in pain, the ears are drawn back from forward (baseline) position

Cheek Tightening/Nose Bulge

Absent (0)

Moderately present (1)

Obviously present (2)

When the animal is in pain, a bulge of skin is apparent on the snout in response to cheek tightening

Orbital Tightening

Absent (0)

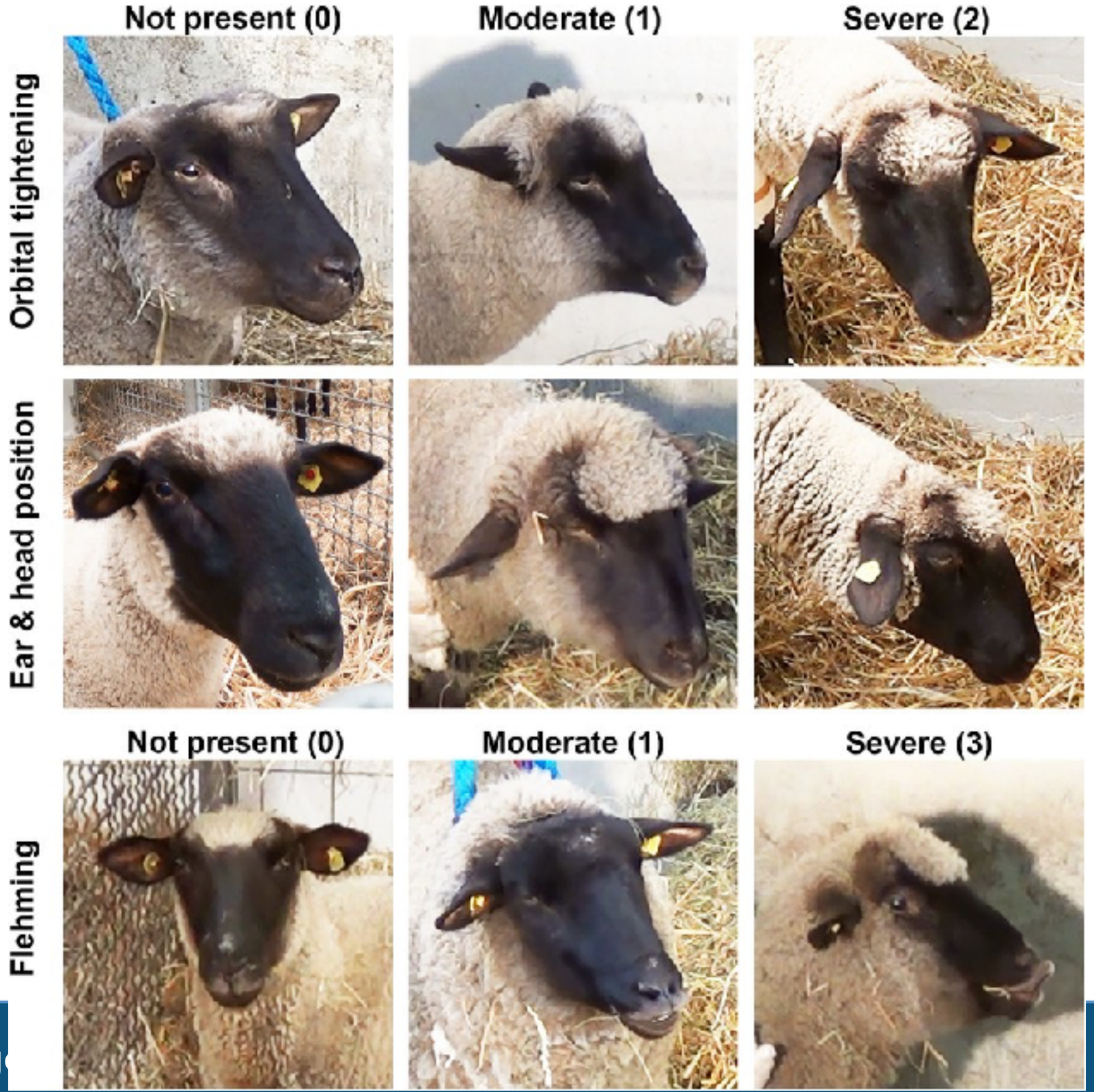
Present (1)

When the animal is in pain, the orbital area is narrowed as the eyelids are squeezed together (scored on a two-point scale)

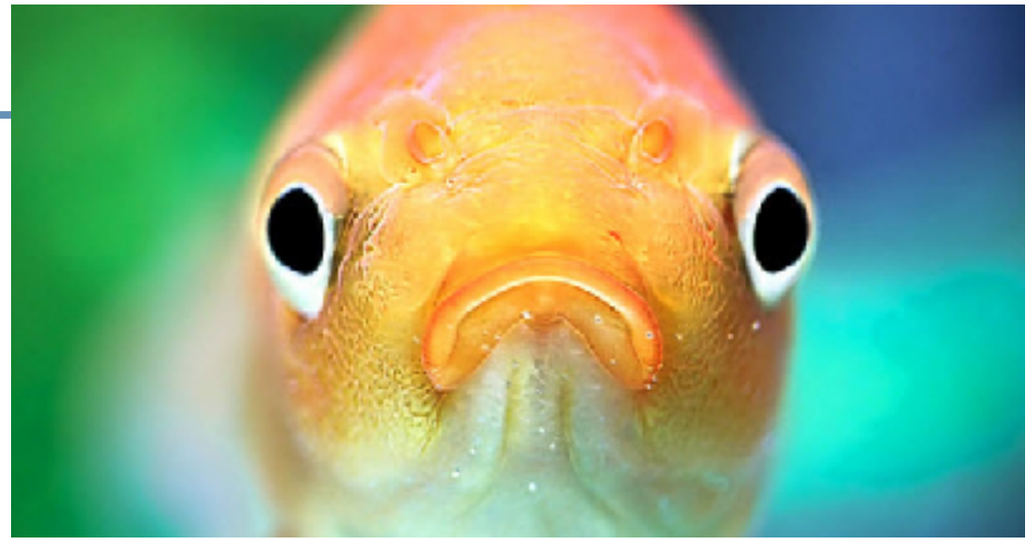
Segni Clinici: Pecora

- perdita appetito, cessazione della ruminazione
- aumento del movimento, dello stato di ansia
- digrignano i denti, cambiamenti frequenti della postura e appaiono agitate
- vocalizzazioni

Pecora


Not present (0)
Moderate (1)
Severe (2)
Orbital tightening
Ear & head position
Not present (0)
Moderate (1)
Severe (3)
Flehming

Pesci



- movimenti violenti, tendono a grattarsi contro i sassi o il fondale
- comportamento di apprendimento di evitamento del dolore

Utilizzo pratico della valutazione del dolore

- valutare lo stato di benessere degli animali: prima e dopo il trattamento

- valutare l'efficacia del trattamento analgesico (evitare "buchi analgesici")

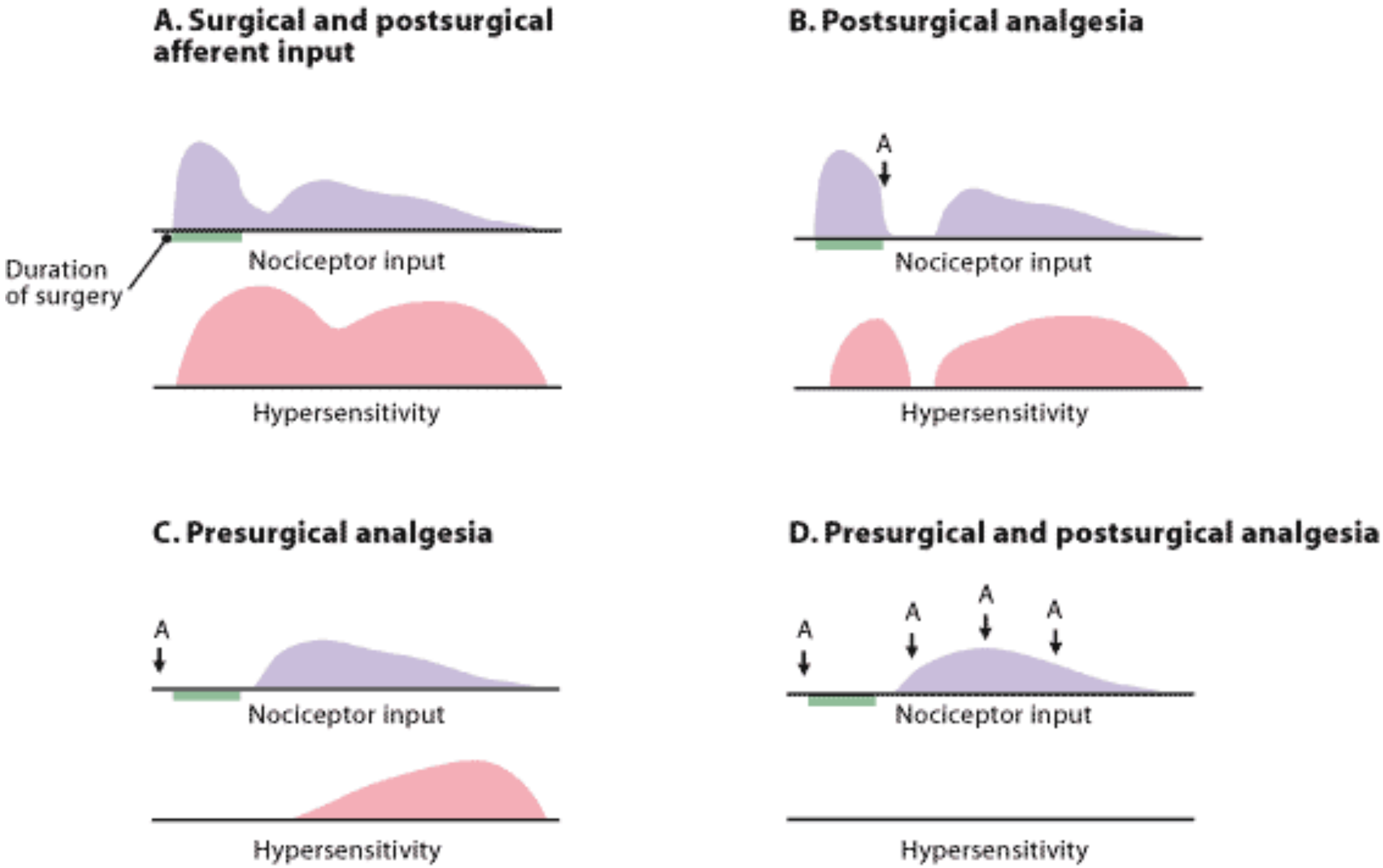
- valutare le tempistiche di riduzione/sospensione del trattamento analgesico (evitare effetti collaterali dei farmaci analgesici)

- approcciare la gestione de dolore in maniera sistematica: check list e protocollo rescue

Utilizzo pratico della valutazione del dolore

- migliorare e approfondire interazione uomo/animale
- riconoscere precocemente stati di distress
- intraprendere rapidamente trattamenti o modifiche per migliorare il benessere
- ridurre o stress correlato alla sperimentazione

Interventi terapeutici



Interventi terapeutici

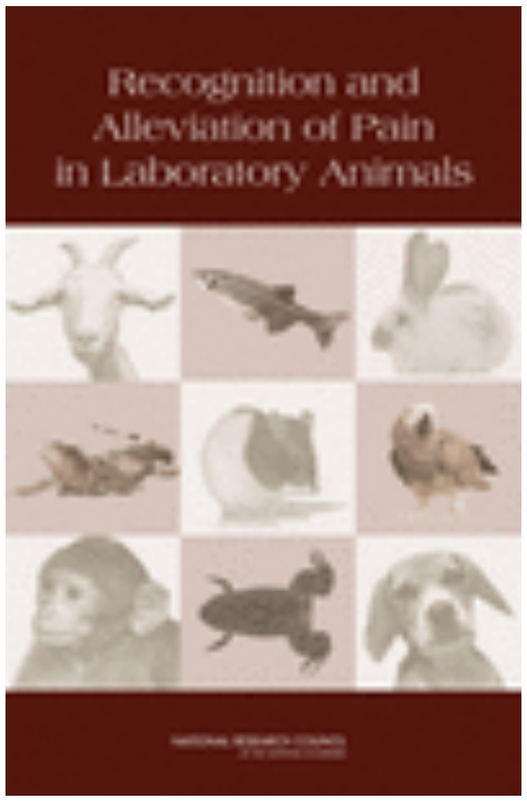
- oppioidi
- alfa-2-agonisti
- ketamina
- FANS

- Grazie per l’attenzione!!!!

BOX 3-1
Pain Assessment Protocol

The following approach can be helpful for assessing pain in particular animal models:

- Prepare a checklist of the examinations to be undertaken, allow space for a general comment, and perhaps include an overall assessment tool (e.g., a visual analogue scale (VAS) score sheet). Familiarize all staff who will be involved in the assessment with this checklist and any other assessment tools that will be used. Whenever possible, the same staff member should conduct each assessment of the same animal. Specific training must be provided for new or inexperienced staff.
- Begin by observing the animal without disturbing it. If the animal's behavior changes markedly in the presence of an observer (e.g., as is the case with nonhuman primates, rabbits, and guinea pigs) it may be more practical to assess postoperative or postprocedural behavior by setting up a video camera or viewing panel.
- Assess the animal's response to the observer (the technician who routinely cares for the animal may be best able to assess this).
- Examine the animal and assess its response to gentle palpation or handling of any presumed painful areas (e.g., the site of surgery, the site of a lesion) when practicable.
- Weigh the animal, record its food and water consumption if possible, and examine the cage or pen for signs of normal or abnormal urination or defecation.
- Administer analgesic treatment if necessary, and repeat the assessment outlined above 30-60 minutes after treatment to determine whether the drug and the dose administered have been effective. In the absence of certainty about the presence of pain, assessing the response to an analgesic can be helpful.
- Review these protocols regularly.
- Remember that:
  - the signs described here can be caused by conditions other than pain,
  - the signs may vary between animals of the same species, even after the same procedure, and
  - the signs will vary between different strains and breeds.



Recognition and Alleviation of Pain in Laboratory Animals

Committee on Recognition and Alleviation of Pain in Laboratory Animals, National Research Council

ISBN: 0-309-12835-8, 196 pages, 6 x 9, (2009)