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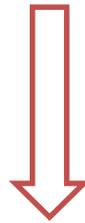
# Principi di Anestesia e Analgesia

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Dipartimento di Scienze Mediche Veterinarie

# SCOPO

Descrivere nel modo più dettagliato i metodi e lo strumentario utili per l'esecuzione dell'anestesia generale in ratti e topi



Personale che esegue anestesia su topi o ratti per fini didattici o scientifici



# BACKGROUND

## 3R

### Replace

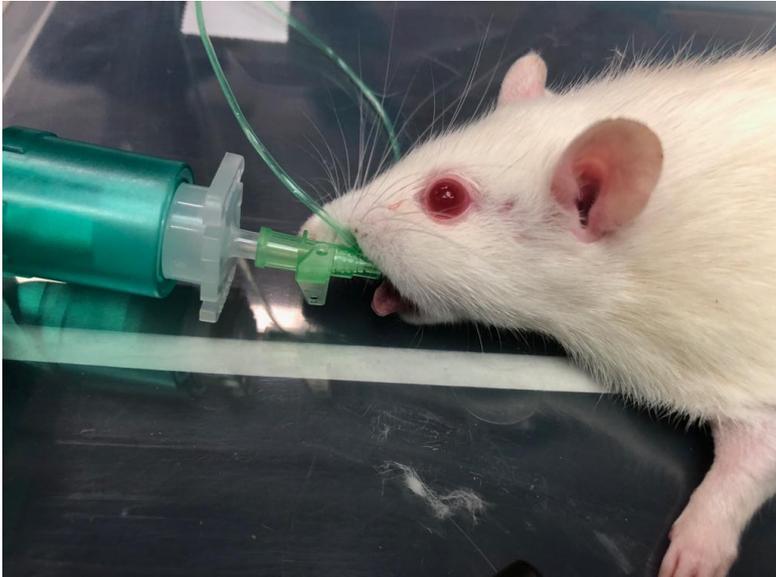
### Reduce

**Refine:** the way experiments are carried out, to make sure animals suffer as little as possible. This includes better housing and improvements to procedures which minimize pain and suffering and/or improve animal welfare.



# INTRODUZIONE

1. Dolore
2. Materiali e equipaggiamenti
3. Farmaci
4. Considerazioni generali



# ANESTESIA NEI RODITORI



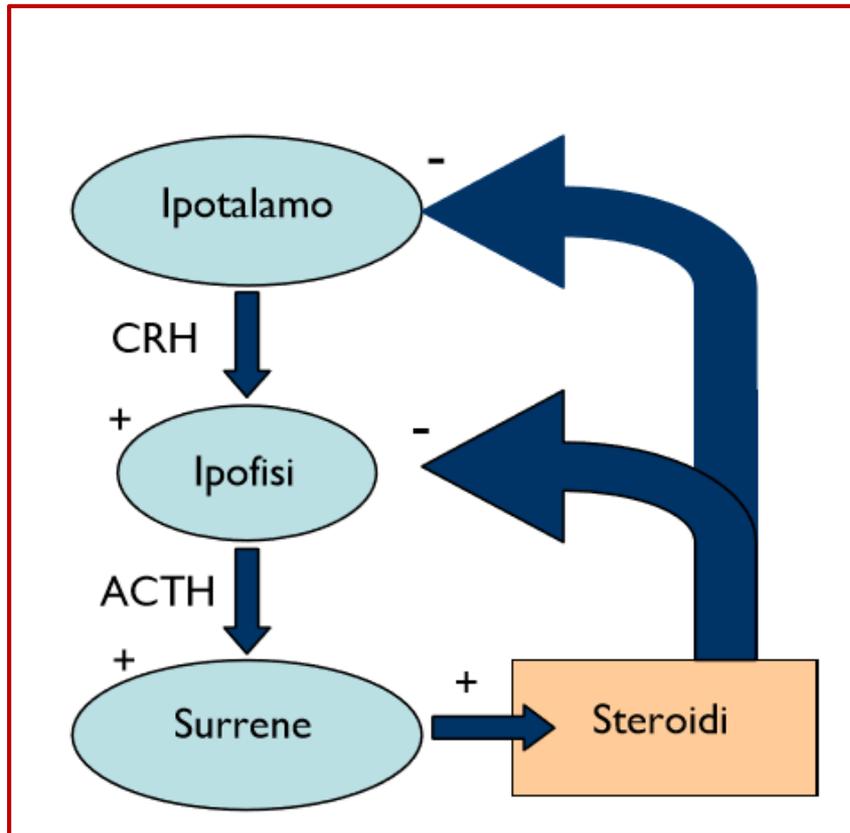
Dolore e Stress



# STRESS

«E' una risposta aspecifica dell'organismo a qualsiasi fattore che travolge o che minaccia di travolgere le proprie capacità compensatorie di mantenere l'omeostasi»

corticotropina



corticosterone (CTS)



# STRESS (porfirina)



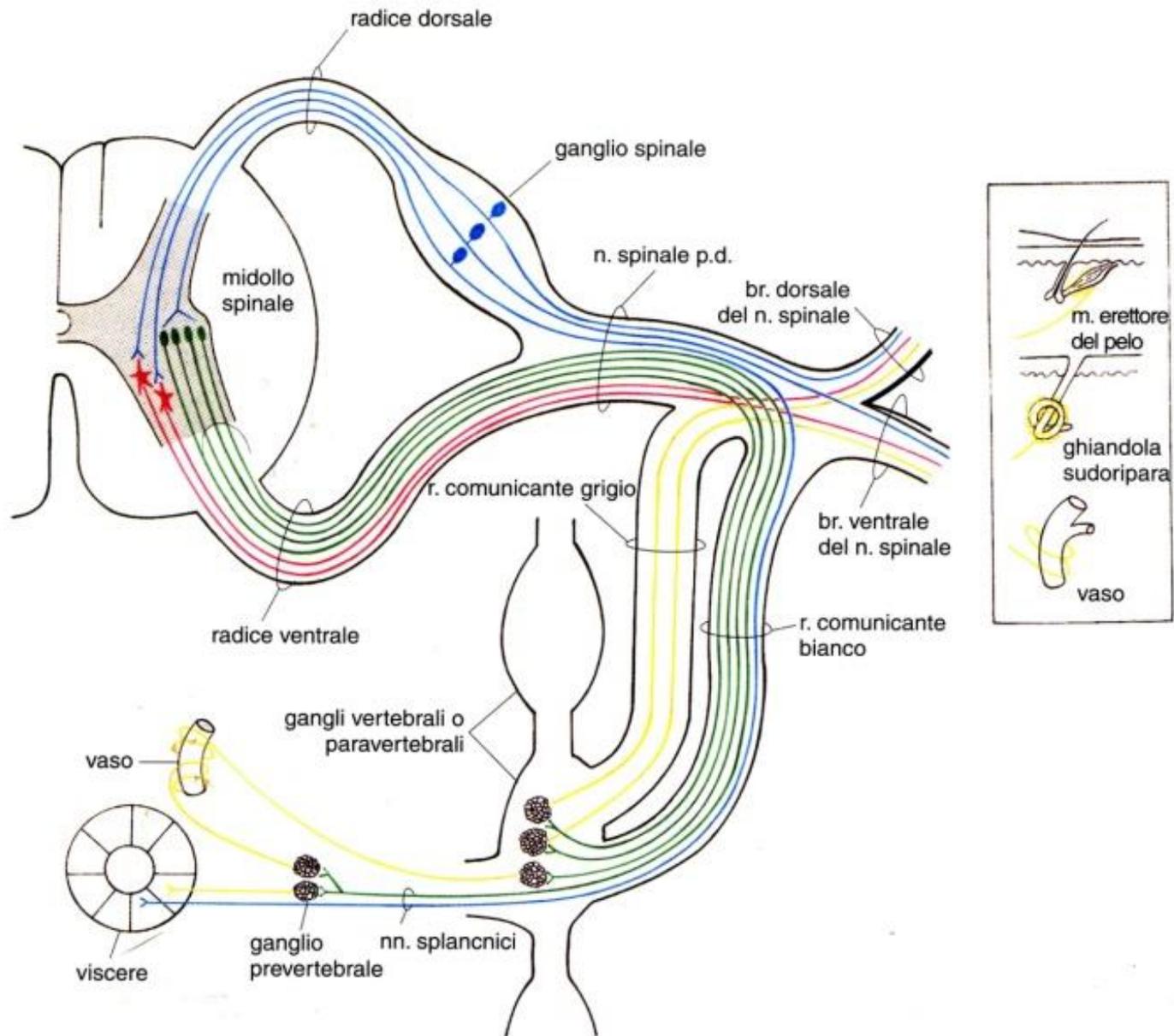
# DOLORE

Il dolore è un sistema di allarme che informa l'organismo della presenza di uno stimolo nocivo

Il dolore è una esperienza sensitiva ed emozionale spiacevole associata ad una condizione di danno reale o potenziale dei tessuti

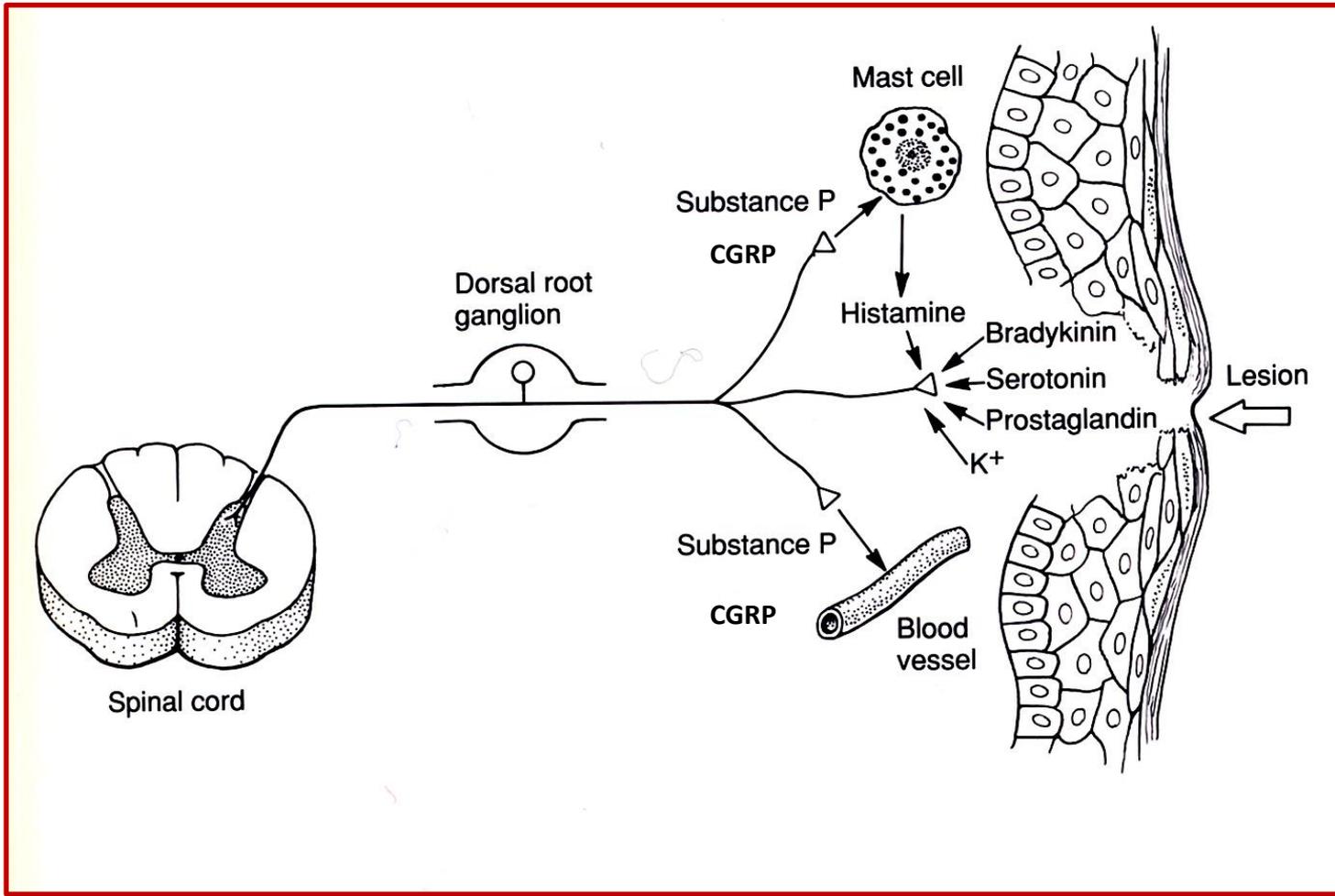
La nocicezione consiste nell'attivazione di un sottoinsieme di recettori, di fibre afferenti primarie (periferiche) e di vie nervose afferenti (centrali) per effetto di stimoli che possono indurre danno tessutale





Schema dell'organizzazione dei nervi spinali. In rosso = fibre motrici; in blu = fibre sensitive somatiche e viscerali; in verde = fibre simpatiche pregangliari; in giallo = fibre simpatiche postgangliari.

# ATTIVAZIONE DEI NOCICETTORI



# ANESTESIA NEI RODITORI



# VISITA PRE-ANESTETICA

Osservazione degli animali nel loro ambiente ( ratto e topo)

Valutazione apparato cardiocircolatorio

Valutazione dell'apparato respiratorio

## **Via di somministrazione farmaci**

Endovenoso (tecnicamente complesso)

Intramuscolare

Intra-peritoneale

Sottocute

Orale





### BC 1

Mouse is emaciated.

- *Skeletal structure extremely prominent; little or no flesh cover.*
- *Vertebrae distinctly segmented.*



### BC 2

Mouse is underconditioned.

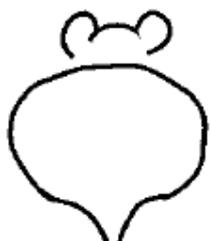
- *Segmentation of vertebral column evident.*
- *Dorsal pelvic bones are readily palpable.*



### BC 3

Mouse is well-conditioned.

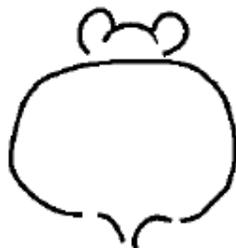
- *Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.*



### BC 4

Mouse is overconditioned.

- *Spine is a continuous column.*
- *Vertebrae palpable only with firm pressure.*



### BC 5

Mouse is obese.

- *Mouse is smooth and bulky.*
- *Bone structure disappears under flesh and subcutaneous fat.*

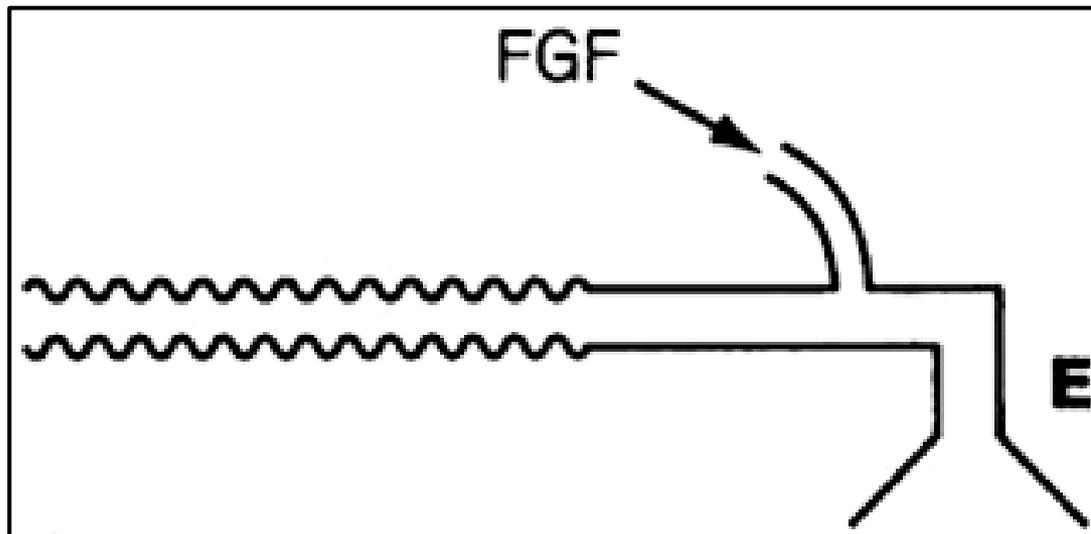
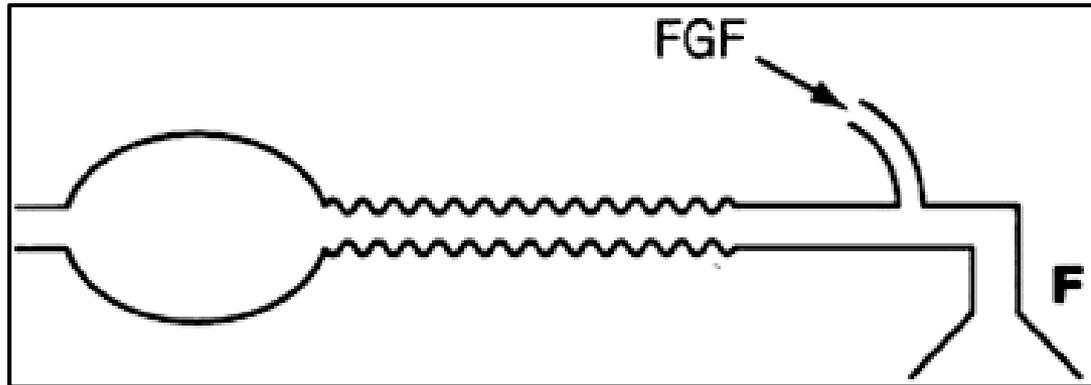
## Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice

Mollie H. Ullman-Culleré<sup>1\*</sup> and Charmaine J. Foltz<sup>2†</sup>



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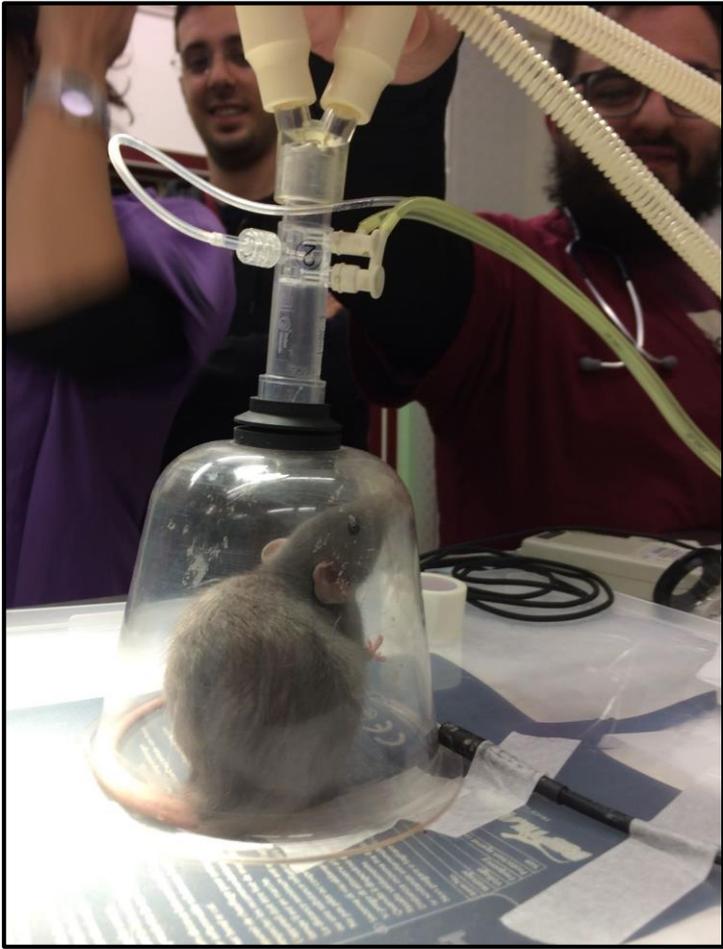
# EQUIPAGGIAMENTO



# EQUIPAGGIAMENTO



# EQUIPAGGIAMENTO



# EQUIPAGGIAMENTO



# ALOGENATI

- ✓ ISOFLURANO
- ✓ SEVOFLURANO

Induzione rapida  
Piano anestesilogico variabile  
Induzione poco stressante  
Risveglio rapido

Odore pungente (ISO)

Vasodilatazione

No analgesia

Rischio di pollution

Ipotermia



# PROTOCOLLO ANESTESIOLOGICO

Ossigeno al 100%: 2-4 L/min

Isoflurano: 5% per circa 5 minuti

Sevoflurano: 8% per circa 2-4 minuti

Rat

-

Inhalation Anaesthesia

Paul Flecknell and Hannah Orr  
University of Newcastle

# PROTOCOLLO ANESTESIOLOGICO

Ossigeno al 100%: 2-4 L/min

Isoflurano: 5% per circa 5 minuti

Sevoflurano: 8% per circa 2-4 minuti

Topo

-

Anestesia Inalatoria

Paul Flecknell and Hannah Orr  
University of Newcastle

# SOVRADOSAGGIO ISO

## Topo - Sovradosaggio di Isoflurano

Paul Flecknell and Hannah Orr  
University of Newcastle

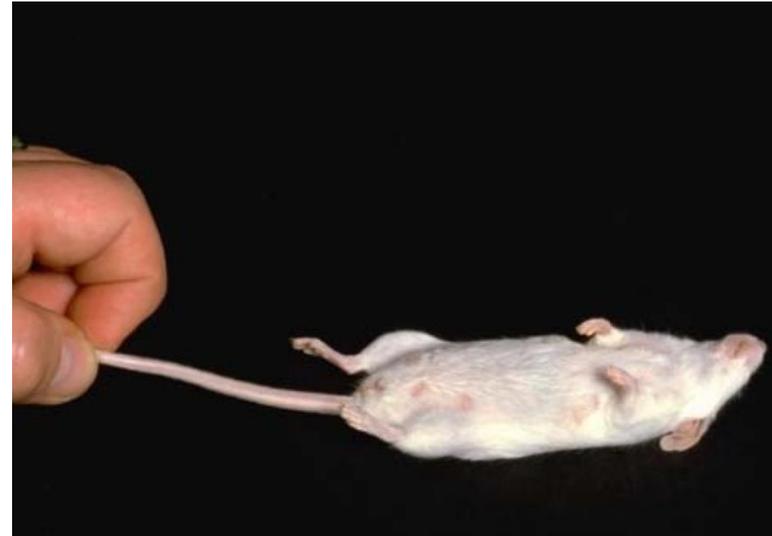


# ANESTESIA CON ALOGENATI

## COME VALUTARE IL PIANO ANESTESIOLOGICO?????

Attraverso la valutazione dei riflessi

- ✓ «Tail pinch»
- ✓ Riflesso dello stimolo podale
- ✓ Capacità di raddrizzamento
- ✓ Riflesso palpebrale



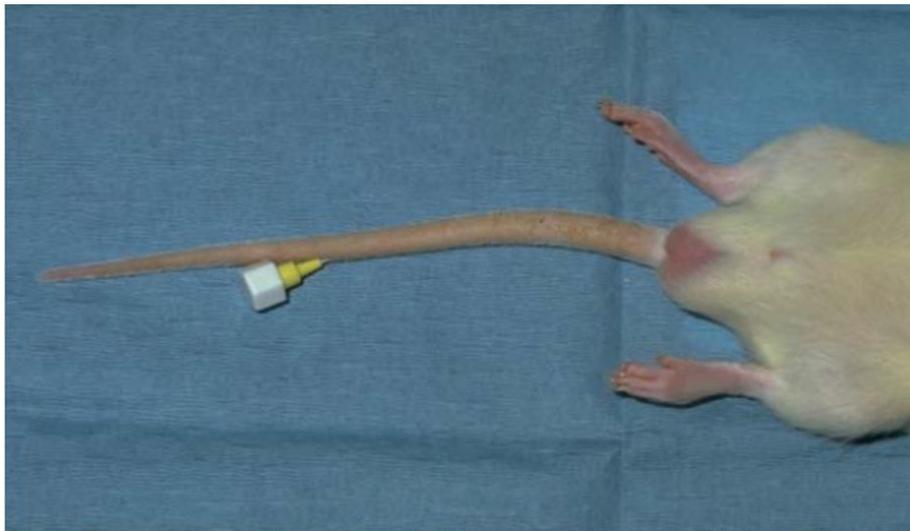
# ANESTESIA CON ALOGENATI



# Abstract

Over 234,000 rats were used in regulated procedures in the UK in 2014, many of which may have resulted in some degree of pain. When using animals in research, there is an ethical and legal responsibility to alleviate or at least reduce pain to an absolute minimum. To do this, we must be able to effectively assess pain in an accurate and timely manner. The Rat Grimace Scale (RGS) is a pain assessment tool, which is suggested to be both accurate and rapid in pain assessment. Many procedures involve the use of general anaesthesia. To date, the effects of anaesthesia on the RGS have not been assessed, limiting its potential utility for assessing pain following anaesthesia. Forty-eight Lister hooded rats were used in this study (24 in part A and 24 in a separate part B). Rats were randomly assigned to one of two treatment groups in part A; short duration isoflurane exposure, short duration control exposure (air) and one of two treatment groups in part B; surgical duration isoflurane exposure or surgical duration control exposure (oxygen). Rats were placed into an anaesthetic induction chamber and isoflurane, or control gas piped into the chamber for either 4 (short duration exposure) or 12 minutes (surgical duration exposure). Following recovery, photographs of the rats' faces were taken and then scored blindly using the RGS. **Short duration isoflurane anaesthesia had no effect on RGS scores.** However, when rats are anaesthetised for a longer duration, akin to a simple routine surgical procedure, **the RGS score increases significantly and this increase remains on repeated exposure to this duration of anaesthesia over a 4-day period.** This should be accounted for when using the RGS to assess pain in rats in the immediate time period following procedures involving the use of isoflurane anaesthesia.

# POSIZIONAMENTO CATETERE ENDOVENOSO



# ANESTESIA CON ALOGENATI

## **MANTENIMENTO**

Ossigeno 1-2 L/min

Isoflurano: 1.5-2.0 %

Oppure

Sevoflurano: 2.5-3 %



# ANESTESIA INNIETTIVA

- SEDAZIONE/TRANQUILLIZZAZIONE
- ANESTESIA GENERALE



NEUROLEPTOANALGESIA

Alfa2 agonisti → Xylazina, Medetomidina, Dexmedetomidina

Benzodiazepine → Midazolam, Diazepam

Oppioidi → Butorfanolo, Fentanyl, Buprenorfina

Farmaci Dissociativi → Ketamina, tiletamina/zolazepam

Barbiturici (ultra breve) → Thiopentale sodico

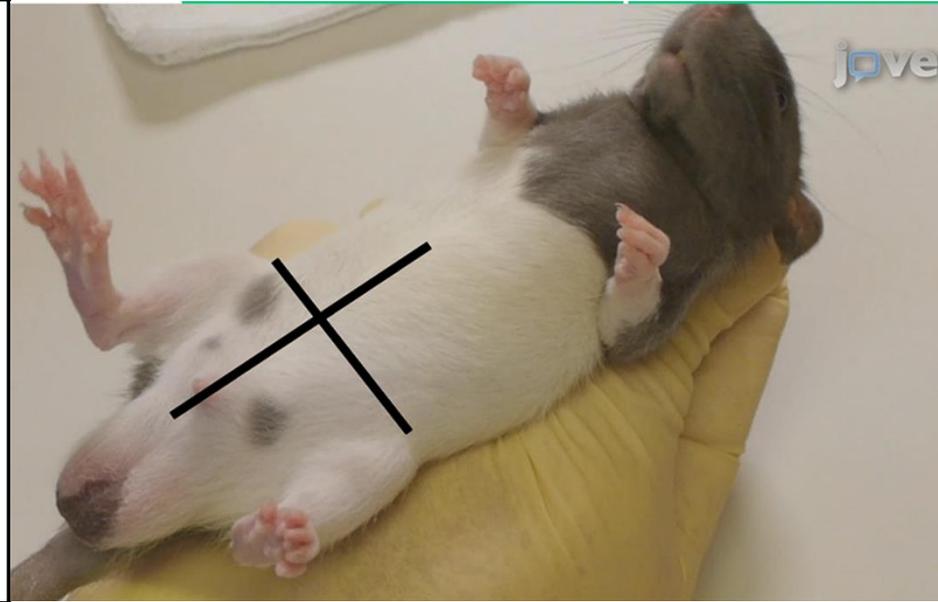
Alfaxalone

Propofolo



# VIA DI SOMMINISTRAZIONE

**Intraperitoneal  
(IP)**



**Subcutaneous  
(SC, SQ)**



# FARMACI INIETTIVI

Piano anestesilogico stabile

Induzione tranquilla

Non necessario equipaggiamento specifico

Analgesia in relazione ai farmaci utilizzati

Induzione/risveglio lento: varia a seconda dell'associazione

Depressione cardiocircolatorio: Ipotensione, bradicardia

Ipotermia



# ALFA2AGONISTI

## Azione a livello recettori alfa-adrenergici

- ✓ Effetto sedativo
- ✓ Miorilassamento
- ✓ Effetto analgesico

## Effetti avversi

Bradycardia

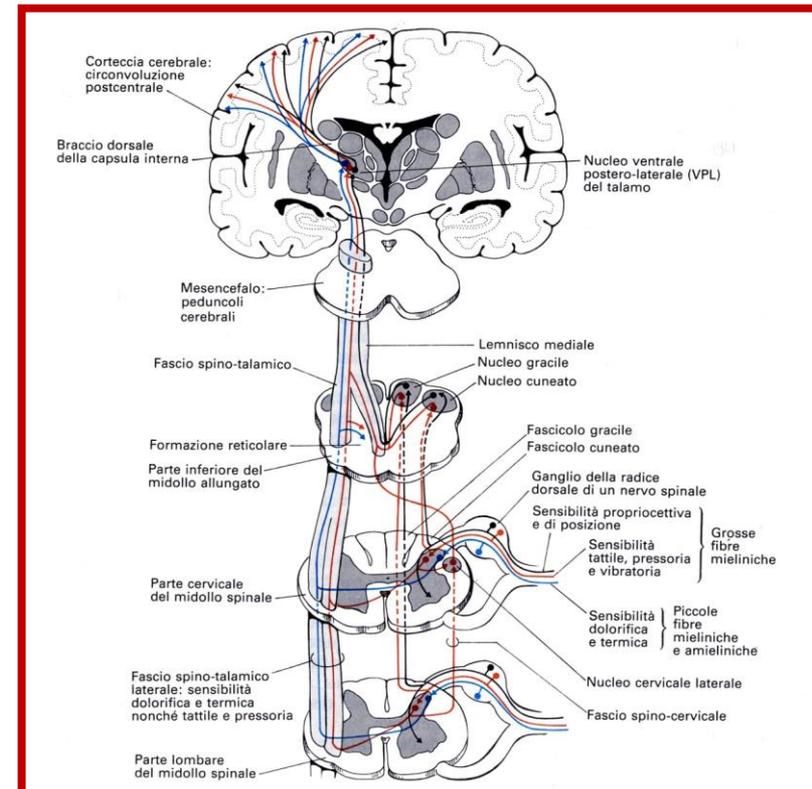
Ipertensione

Ipotensione

Riduzione della gittata cardiaca

Ostruzione uretrale nel topo

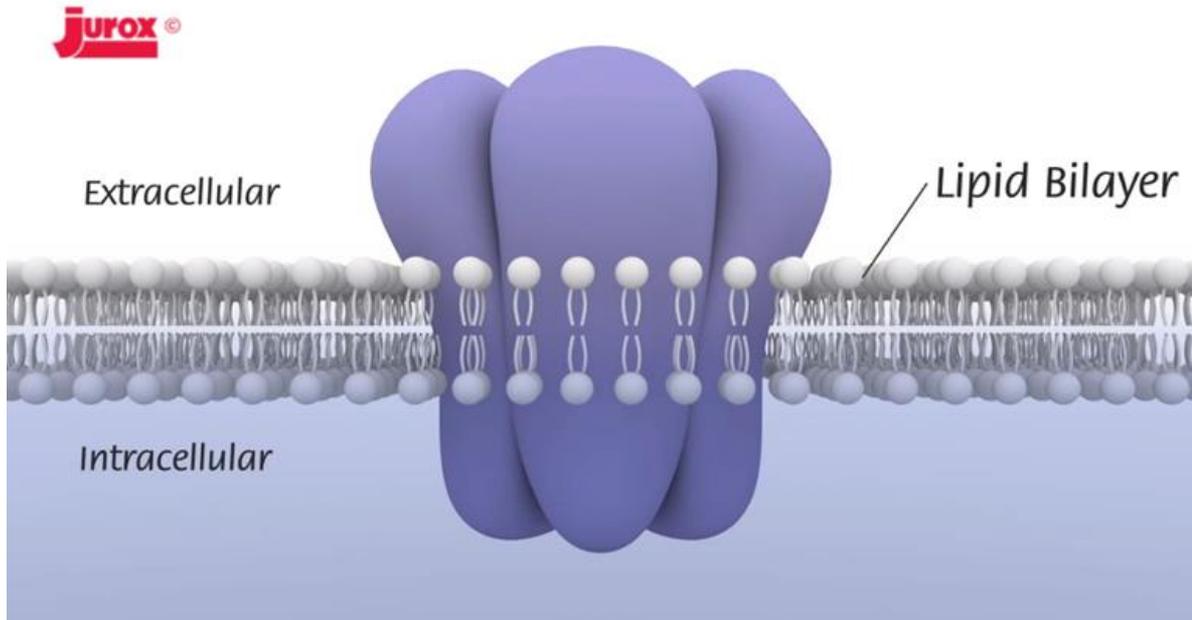
**Possibile antagonizzare**



# BENZODIAZEPINE

Facilitano l'attivazione dei recettori GABA

- ✓ Effetto sedativo
- ✓ Effetto miorilassante
- ✓ No effetti cardiovascolari



# OPPIOIDI

## BUTORFANOLO

- ✓ Agonista recettori  $\kappa$ , antagonista recettori  $\mu$

## FENTANYL

- ✓ Agonista dei recettori  $\mu$

## BUPRENORFINA

- ✓ Agonista parziale recettori  $\mu$

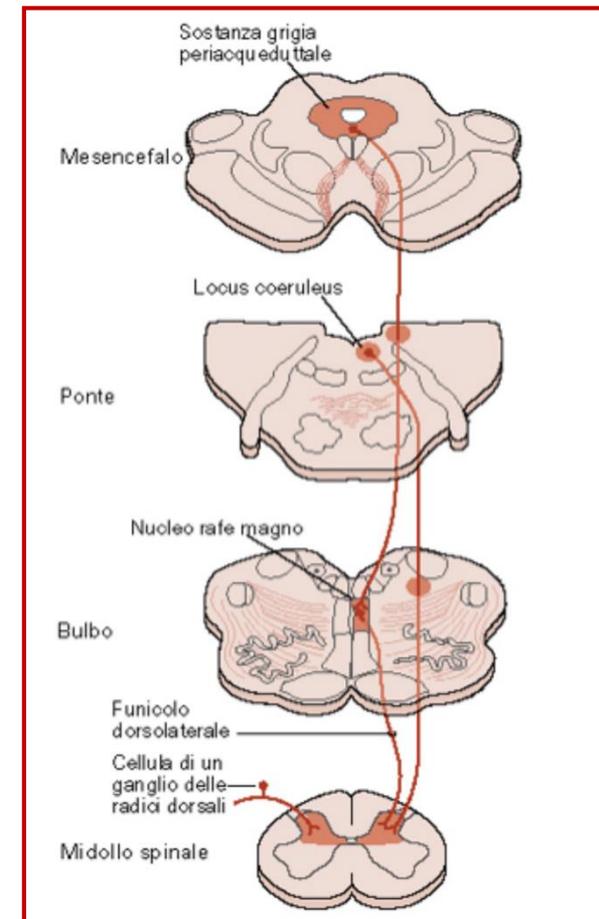
Somministrazione SC o IP

Potenziano le vie inibitorie discendenti

Lieve depressione della frequenza respiratoria

Potenziano effetto sedativo di altri farmaci

«Sparing effect»



# KETAMINA

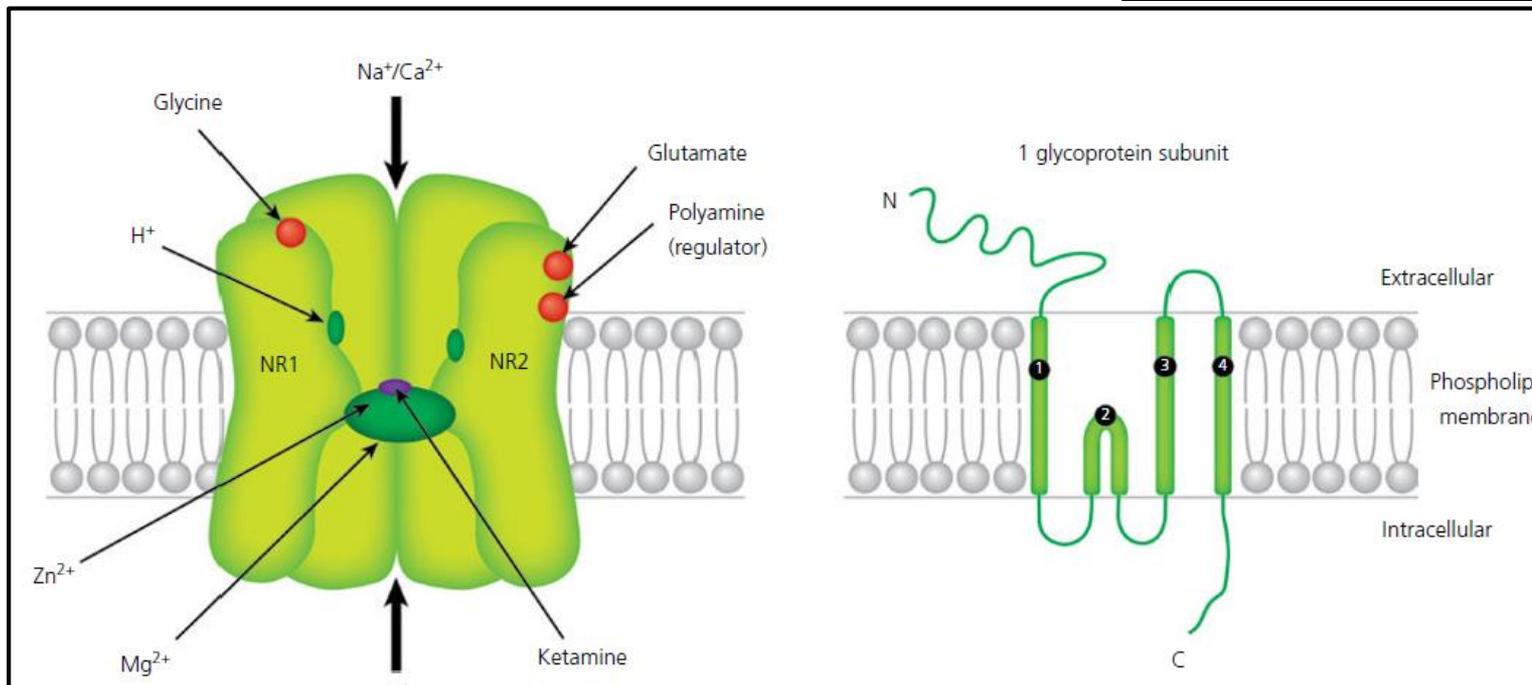
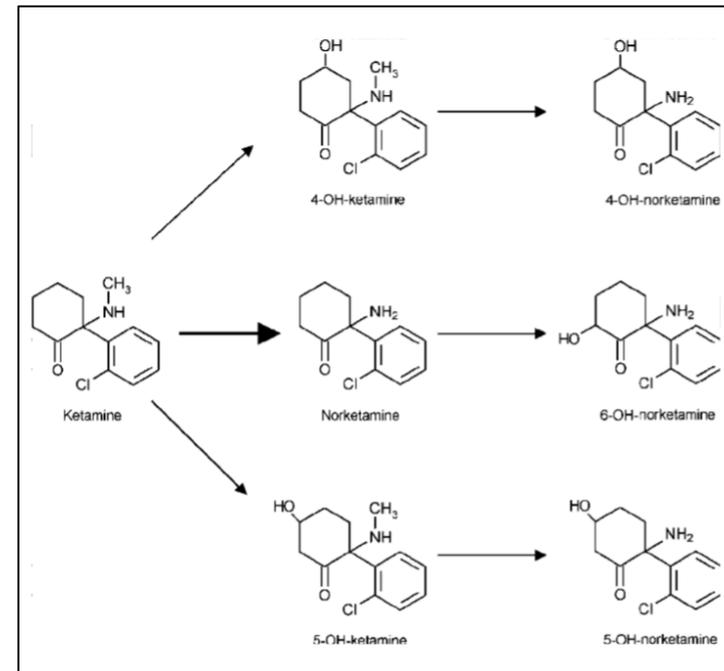
Farmaco dissociativo

Azione come antagonista sui recettori NMDA

Rigidità muscolare

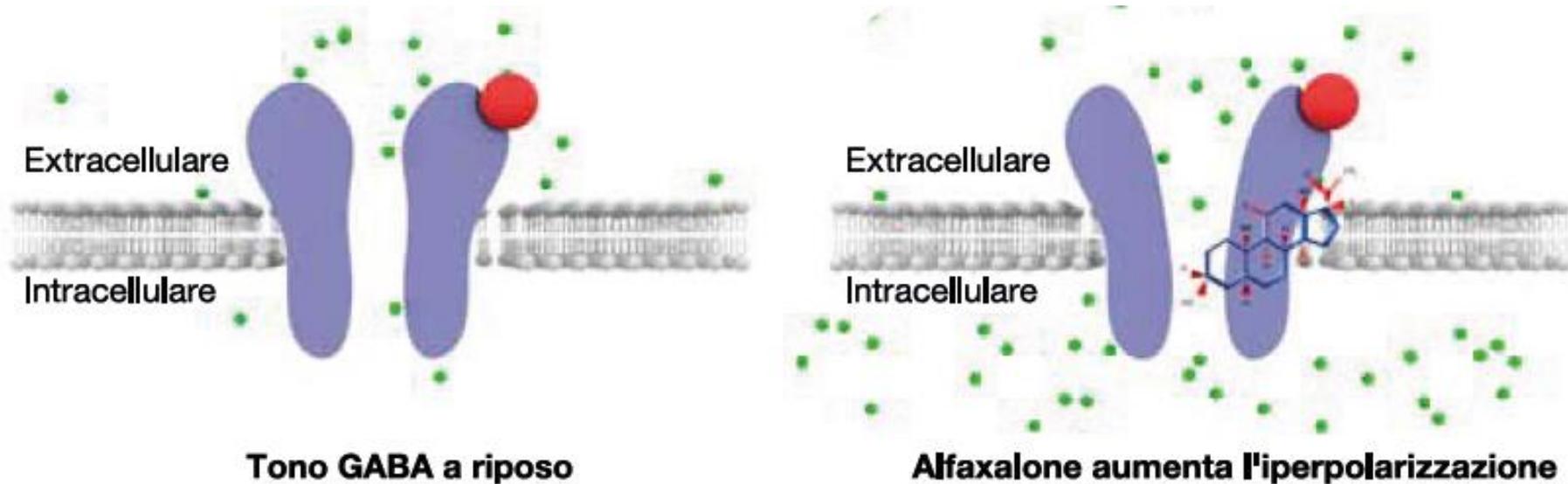
Analgesia

Risvegli disforici



# ANESTETICI GENERALI

Thiopentale  
Pentobarbitale  
Alfaxalone  
Propofolo



● Cl recettori

● GABA



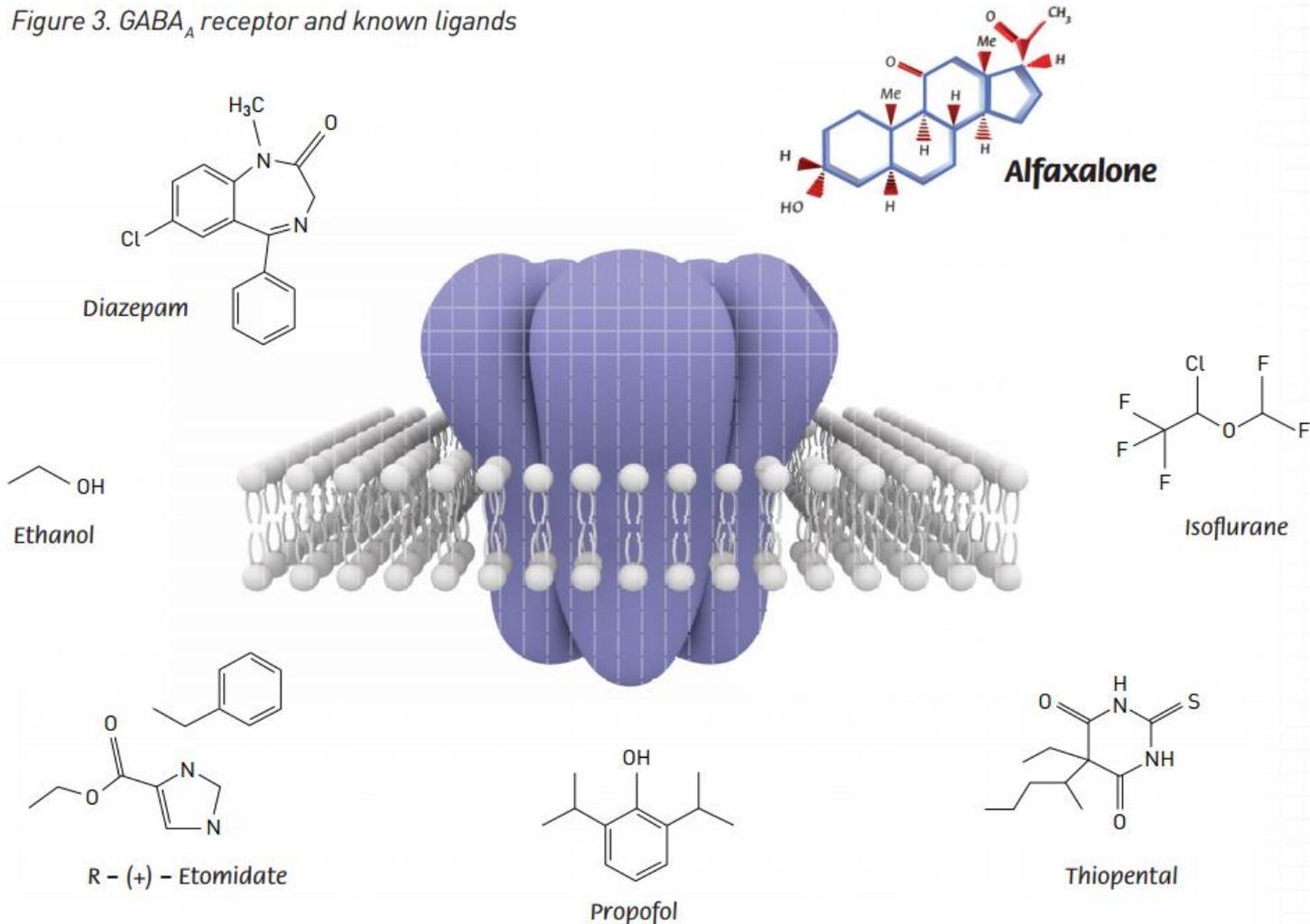
Alfaxalone



GABA<sub>A</sub>

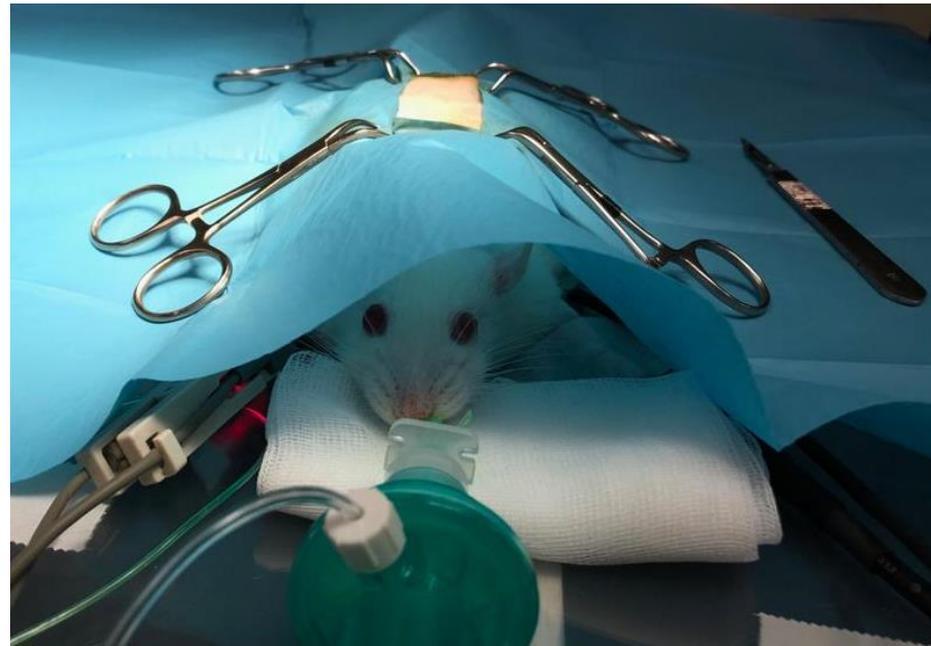
# ANESTETICI GENERALI

Figure 3. GABA<sub>A</sub> receptor and known ligands





# PROTOCOLLI



# ANESTESIA INIETTIVA TOPO

XYLAZINA+KETAMINA (XK)

X 5-10 mg/kg

K 80-100 mg/kg

IP

Modica depressione del respiro

Depressione del cardiocircolatorio

Ipotermia



# ANESTESIA INIETTIVA TOPO

Hindawi  
Anesthesiology Research and Practice  
Volume 2017, Article ID 9161040, 7 pages  
<https://doi.org/10.1155/2017/9161040>



Research Article

## Injectable Anesthesia for Mice: Combined Effects of Dexmedetomidine, Tiletamine-Zolazepam, and Butorphanol

Laura A. Cagle,<sup>1</sup> Lisa M. Franzi,<sup>1</sup> Steven E. Epstein,<sup>2</sup> Philip H. Kass,<sup>3</sup>  
Ronald A. East,<sup>1</sup> and Nicholas J. Kovanos<sup>1</sup>

	Anesthetic duration (minutes)	Recovery time (minutes)	Anesthesia achieved (%)	Heart rate (bpm)	Respiratory rate (bpm)
Dexmedetomidine 0.4 mg/kg					
Tiletamine-zolazepam					
20 mg/kg	63 (±22)	279 (±25)	50% (3/6)	187 (±14)	128 (±3)
40 mg/kg	67 (±22)	240 (±25)	33% (2/6)	222 (±14)	136 (±3)
60 mg/kg	77 (±22)	240 (±25)	33% (1/3)	227 (±20)	135 (±4)
Dexmedetomidine 0.6 mg/kg					
Tiletamine-zolazepam					
20 mg/kg	23 (±16)	222 (±17)	67% (4/6)	229 (±14)	134 (±3)
40 mg/kg	73 (±13)	265 (±16)	100% (6/6)	209 (±14)	132 (±3)
60 mg/kg	47 (±22)	243 (±25)	67% (2/3)	193 (±20)	130 (±4)
Dexmedetomidine 0.8 mg/kg					
Tiletamine-zolazepam					
20 mg/kg	58 (±18)	253 (±20)	50% (3/6)	254 (±14)	145 (±3)
40 mg/kg	48 (±18)	286 (±35)	40% (2/5)	207 (±16)	130 (±3)
60 mg/kg	175 (±31)	404 (±35)	50% (1/2)	233 (±25)	125 (±5)

# ANESTESIA INIETTIVA TOPO

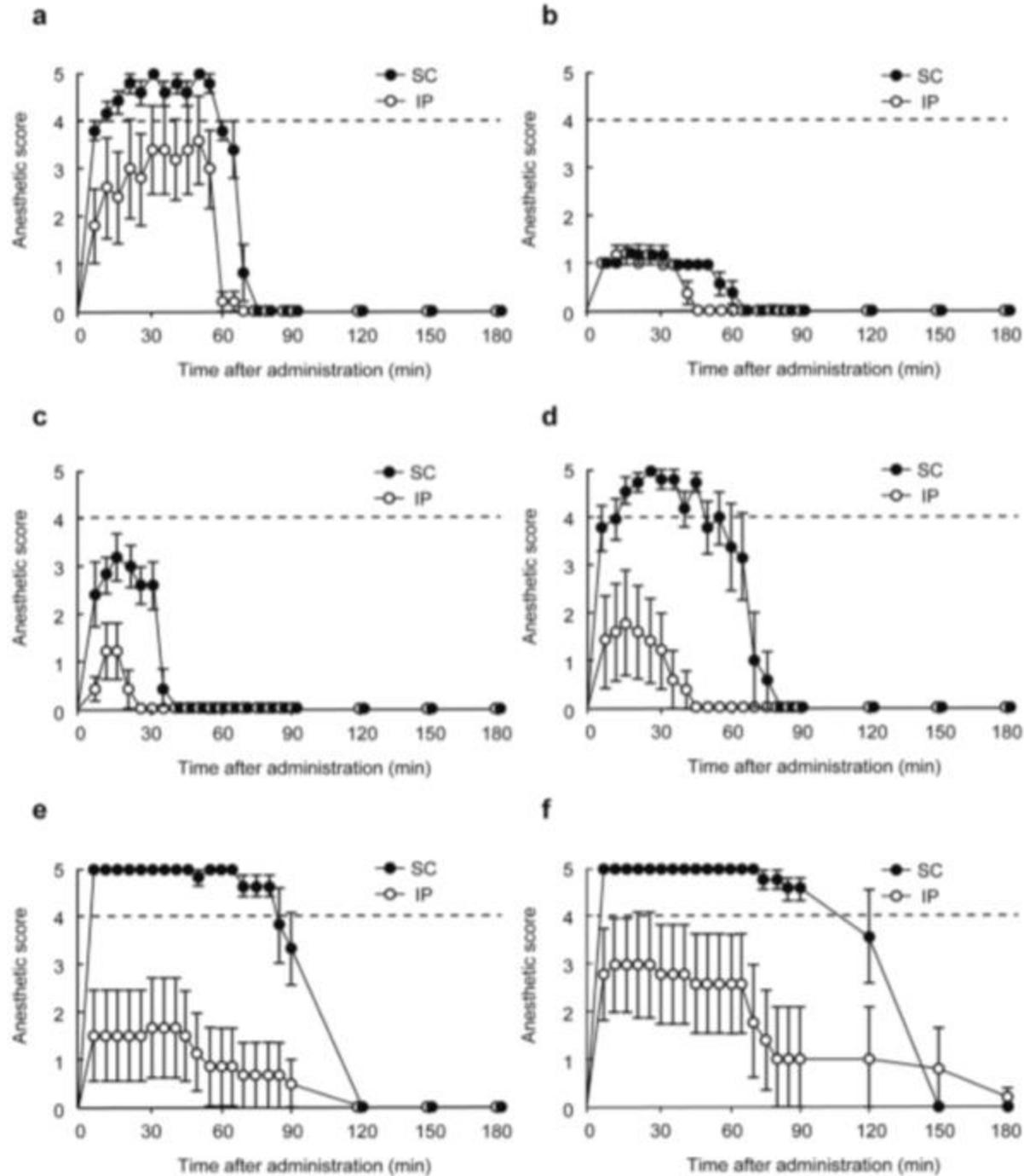
**Table 1. Summary of the drugs and doses used in this study**

Abbreviation	Dose (mg/kg)				Injection route <sup>1)</sup>	No. of mice
	Medetomidine	Midazolam	Butorphanol	Alfaxalone		
IP M/M/B	0.3	4	5	–	IP	5
IP ALFX	–	–	–	100	IP	5
IP M/B/A20	0.3	–	5	20	IP	5
IP M/B/A40	0.3	–	5	40	IP	5
IP M/B/A60	0.3	–	5	60	IP	6
IP M/B/A80	0.3	–	5	80	IP	5
SC M/M/B	0.3	4	5	–	SC	5
SC ALFX	–	–	–	100	SC	5
SC M/B/A20	0.3	–	5	20	SC	5
SC M/B/A40	0.3	–	5	40	SC	5
SC M/B/A60	0.3	–	5	60	SC	6
SC M/B/A80	0.3	–	5	80	SC	5

<sup>1)</sup>IP, intraperitoneal; SC, subcutaneous.



# ANESTESIA



# ANESTESIA INIETTIVA RATTO

XYLAZINA+KETAMINA (XK)

X 5-10 mg/kg  
K 80-100 mg/kg } IP

Modica depressione del respiro

Severa depressione del cardiocircolatorio

Ipotermia

Elevata mortalità



# ANESTESIA INIETTIVA RATTO

**Effects of repeated anaesthesia with ketamine/medetomidine and of pre-anaesthetic administration of buprenorphine in rats**

**P. Hedenqvist<sup>1</sup>, J. V. Roughan<sup>2</sup> & P. A. Flecknell<sup>2</sup>**

<sup>1</sup>Karolinska Institute, Stockholm, Sweden and <sup>2</sup>Comparative Biology Centre, Medical School, Framlington Place, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, UK

BUPRENORFINA+MEDETOMIDINA+KETAMINA (BMK)

B 0.05 mg/kg  
M 0,3-0,4 mg/kg  
K 45-60 mg/kg

} IP

Marcata depressione del respiro

Severa depressione del cardiocircolatorio

Ipotermia

Elevata mortalità



# ANESTESIA INIETTIVA RATTO

Original Article

## Anaesthetic effects of alfaxalone administered intraperitoneally alone or combined with dexmedetomidine and fentanyl in the rat

Mario Arenillas and Ignacio A Gomez de Segura 

Drugs	Dose (mg kg <sup>-1</sup> )
Study 1 ( <i>n</i> = 8; 4 females, 4 males)	
Alfaxalone	25, 35, 45
Study 2 ( <i>n</i> = 16; 8 females, 8 males)	
Alfaxalone	25 (females), 75 (males)
Dexmedetomidine	0.05
Study 3 ( <i>n</i> = 16; 8 females, 8 males)	
Alfaxalone	20 (females), 60 (males)
Dexmedetomidine	0.05
Fentanyl	0.1

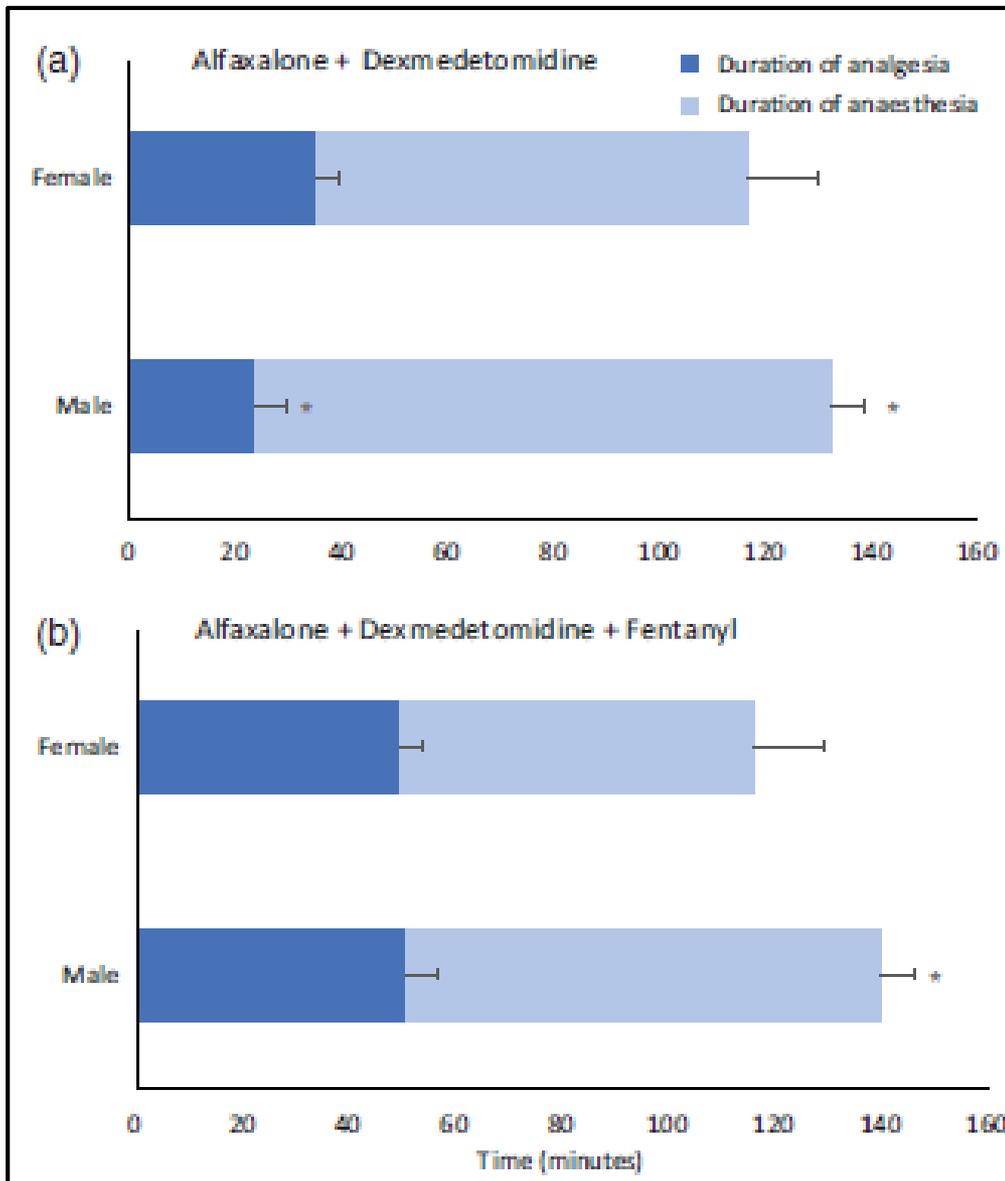


# ALFAXALONE

Dose (mg kg <sup>-1</sup> )	Sex	<i>n value</i>	Times (min)		
			Onset of anaesthesia	Duration of anaesthesia	Duration of analgesia
Study 1. Alfaxalone					
25	Females	4	4 ± 10	73 ± 10	–
	Males	4	6 ± 1	7 ± 4*	–
35	Females	4	3 ± 1	83 ± 9	–
	Males	4	5 ± 2*	39 ± 13*	–
45	Females	4	3 ± 1	117 ± 14	–
	Males	4	5 ± 2	40 ± 24*	–
Study 2. Alfaxalone (below) + dexmedetomidine (0.05 mg kg <sup>-1</sup> )					
25	Females	8	6 ± 2	117 ± 13	36 ± 4
75	Males	8	5 ± 1	133 ± 6*	24 ± 6
Study 3. Alfaxalone (below) + Dexmedetomidine (0.05 mg kg <sup>-1</sup> ) + Fentanyl (0.1 mg kg <sup>-1</sup> )					
20	Females	8	6 ± 1	116 ± 8	50 ± 16
60	Males	8	4 ± 1	140 ± 15*	51 ± 14



# ALFAXALONE



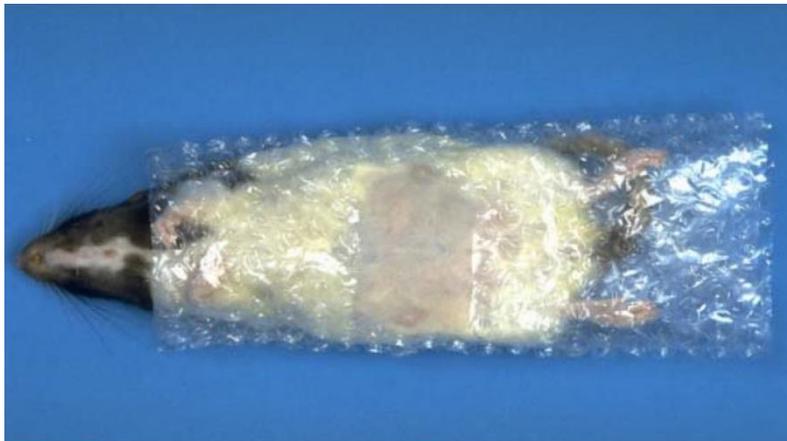
# MONITORAGGIO INTRAOPERATORIO

Frequenza cardiaca

spO<sub>2</sub> periferico

Frequenza respiratoria

Temperatura



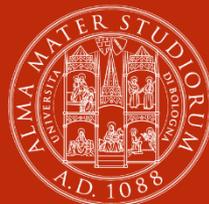
# ANALGESIA POST-OPERATORIA

	Mice	Rats
<b>Buprenorphine</b>	0.1 mg/kg IP, SC, q6–8 h	0.05 mg/kg SC, IP q6–8 h
<b>Butorphanol</b>	1–2 mg/kg IP, SC, q2 h	1–2 mg/kg IP, SC, q2 h
<b>Carprofen</b>	5 mg/kg SC or orally q12 h	5 mg/kg SC or orally q12 h
<b>Meloxicam</b>	5 mg/kg SC q24 h	0.5–1 mg/kg IP, SC or orally q24 h
<b>Morphine</b>	2–5 mg/kg IP, SC, q2–4 h	2–5 mg/kg IP, SC, q2–4 h
<b>Tramadol</b>	5–10 mg/kg IP, SC Q6–12 h	5–10 mg/kg IP, SC q6–12 h



# SANE





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