



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

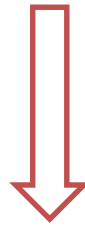
Principi di Anestesia e Analgesia

Noemi Romagnoli

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

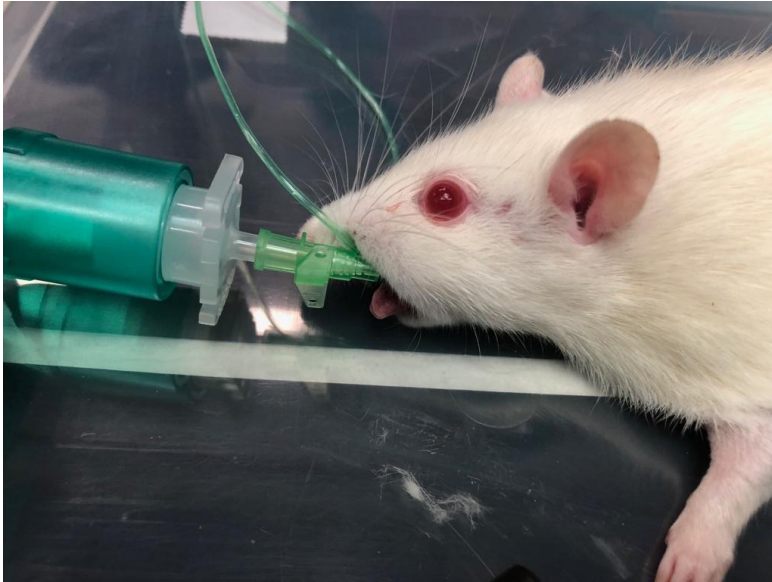
Reduce

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



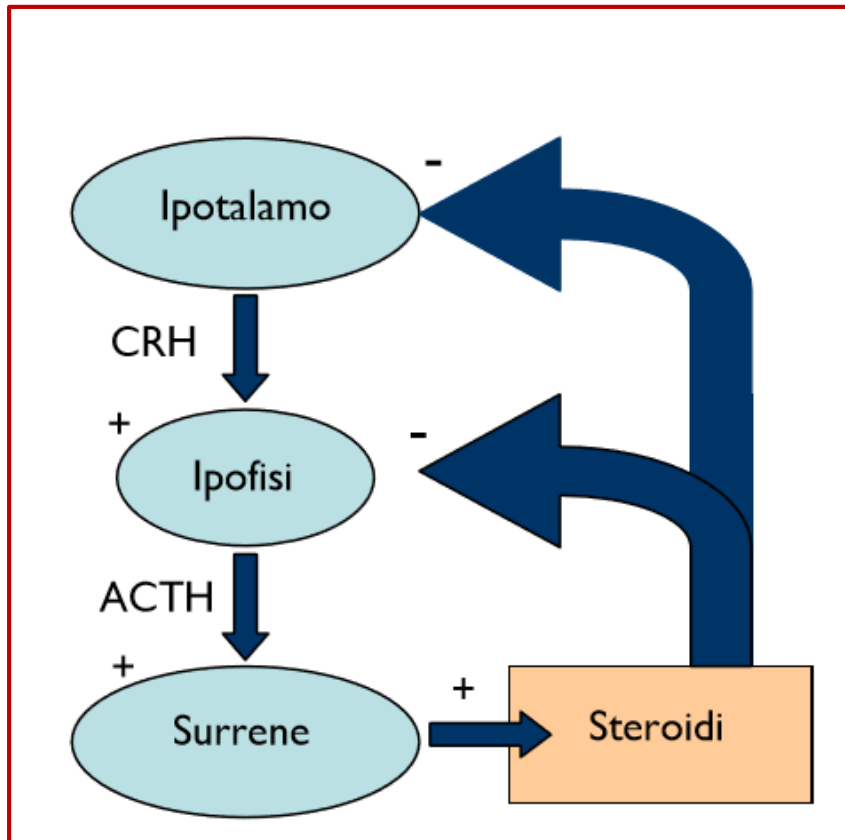
Dolore e Stress



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)



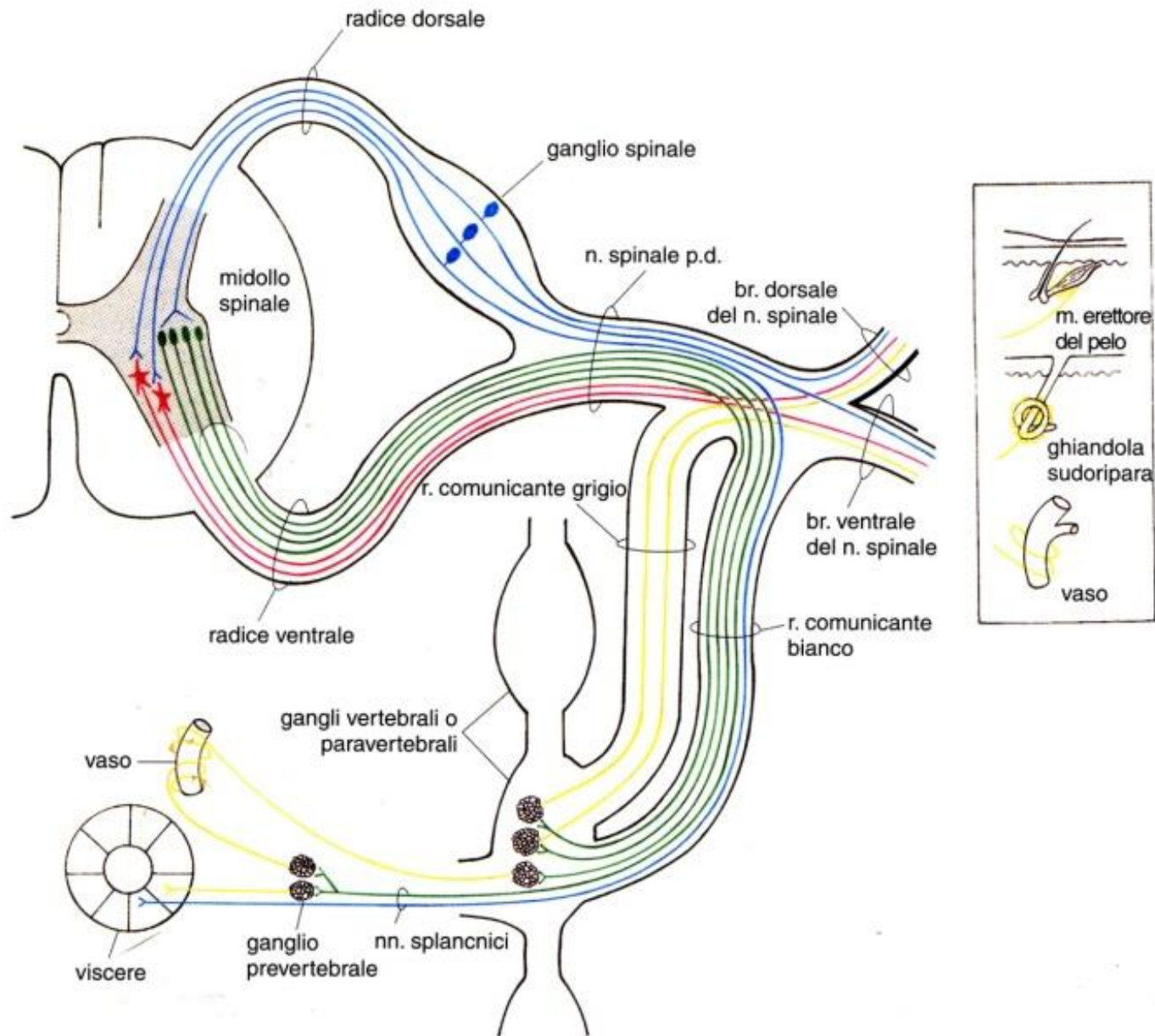
DOLORE

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

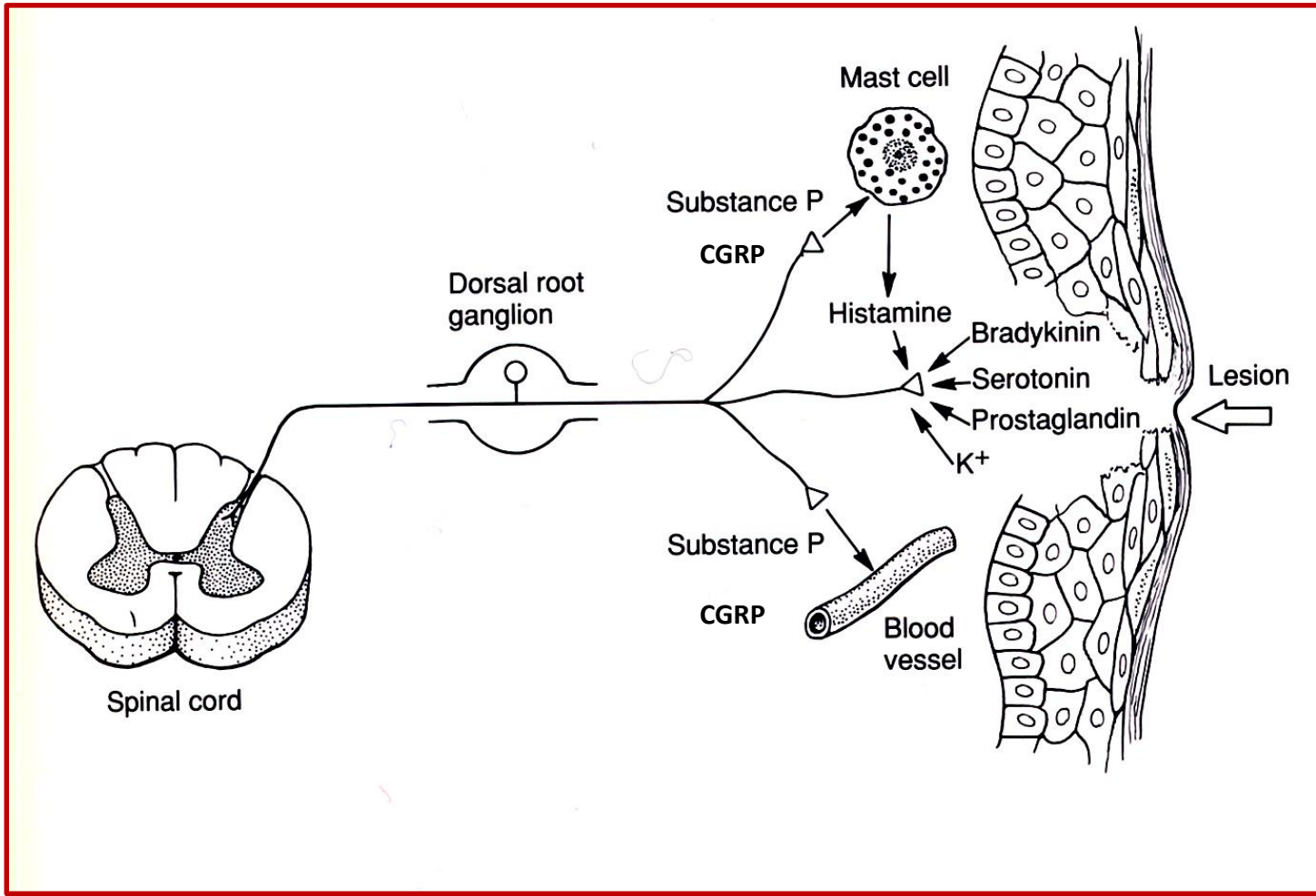
La nocicezione consiste nell'attivazione di un sottoinsieme di recettori, di fibre afferenti primarie (periferiche) e di vie nervose afferenti (centrali) per effetto di stimoli che possono indurre danno tessutale





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.

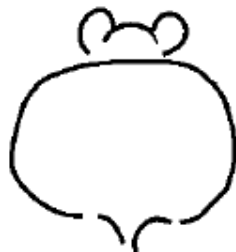
- *Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.*



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.*

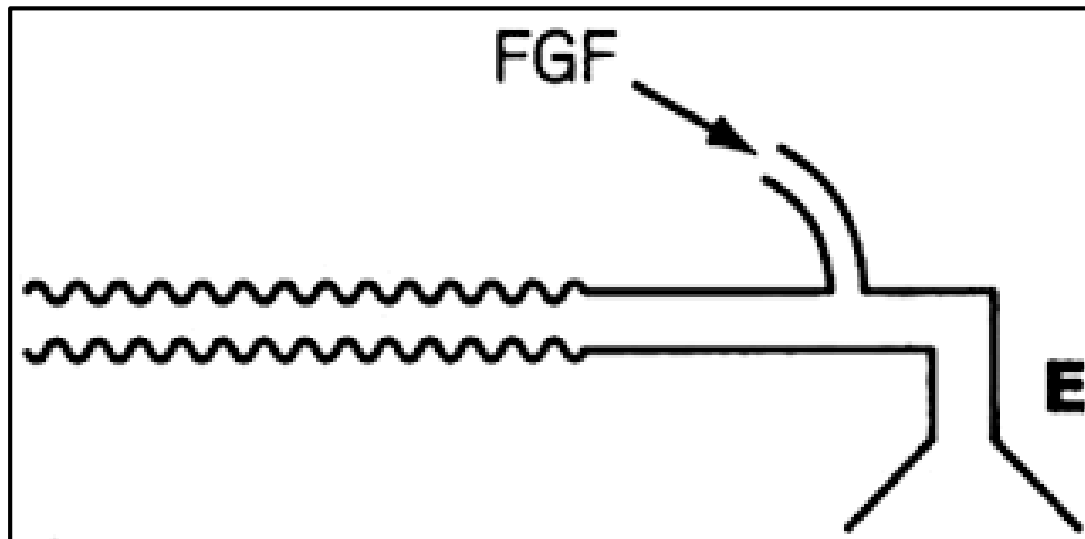
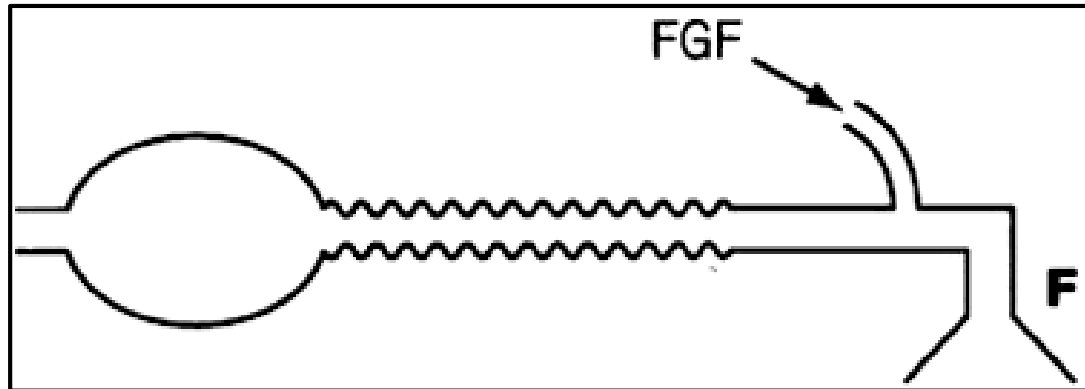
Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice

Mollie H. Ullman-Culleré^{1*} and Charmaine J. Foltz^{2†}



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EQUIPAGGIAMENTO



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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

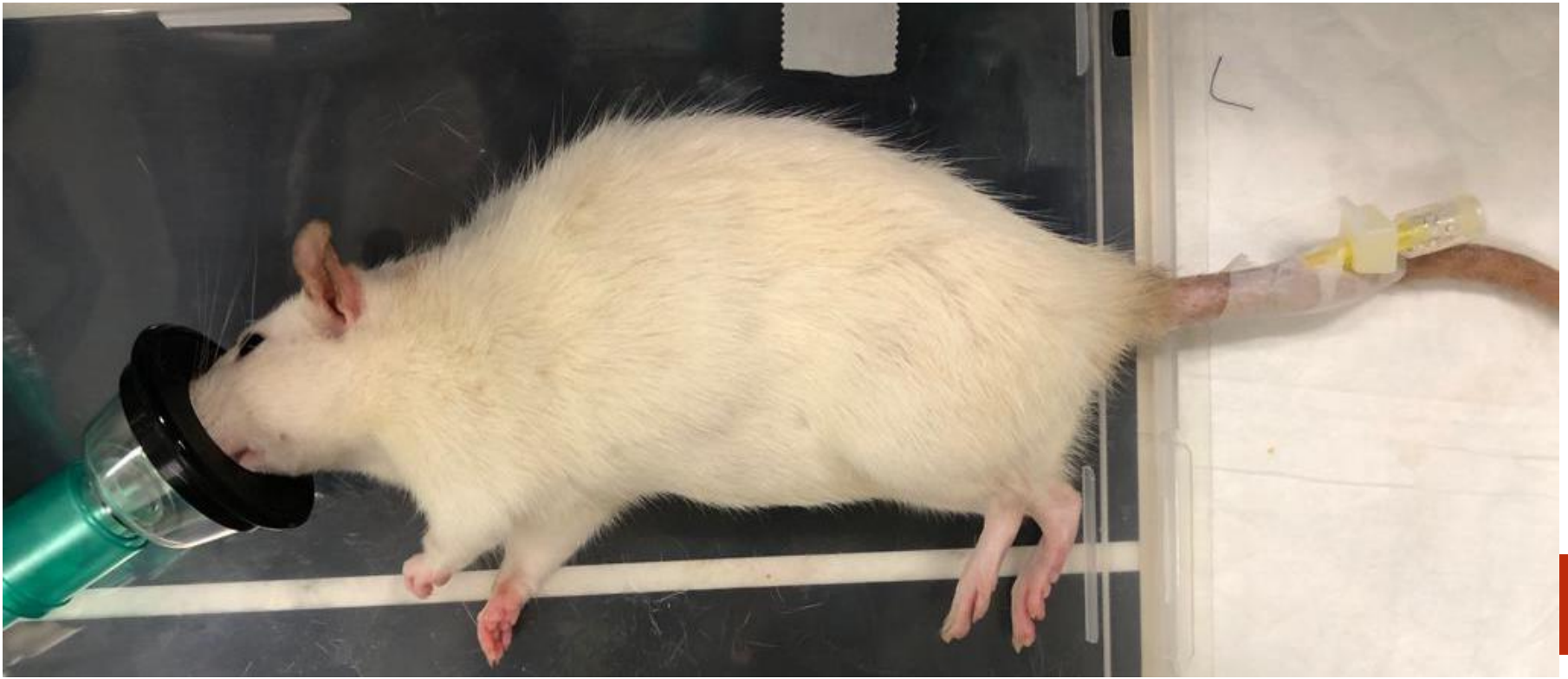
MANTENIMENTO

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

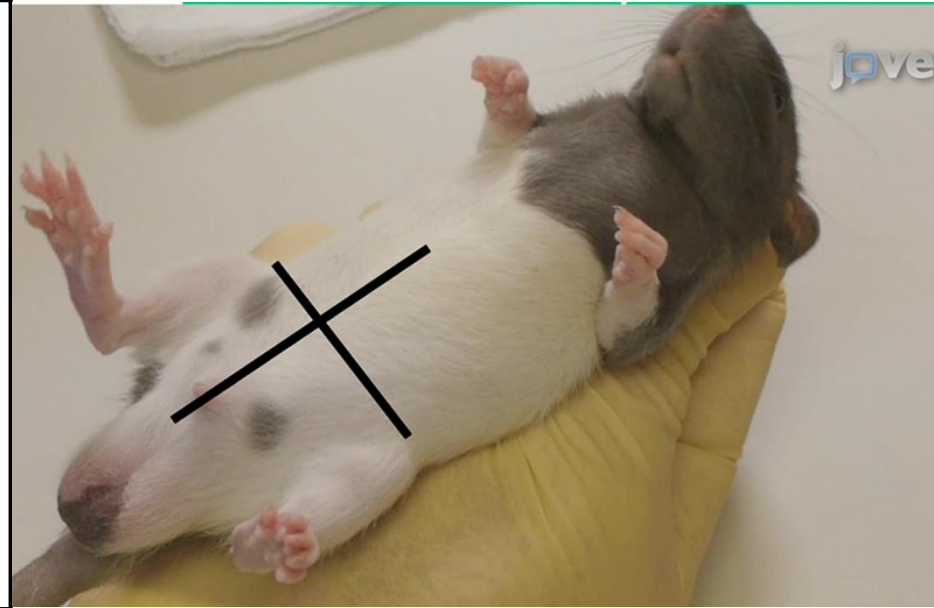
Alfaxalone

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

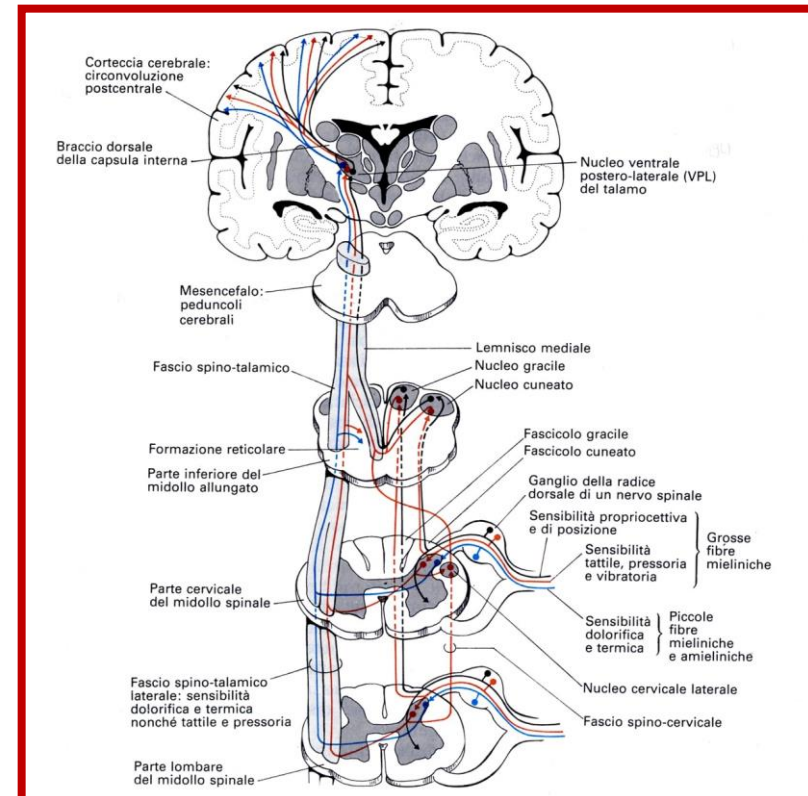
Ipertensione

Ipotensione

Riduzione della gittata cardiaca

Ostruzione uretrale nel topo

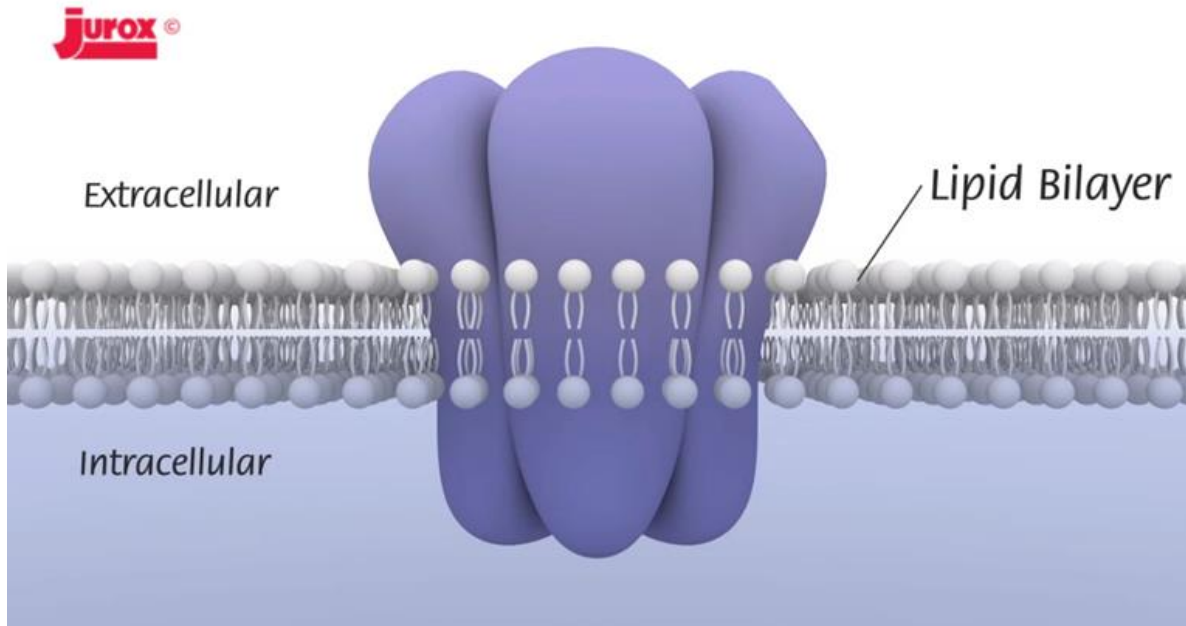
Possibile antagonizzare



BENZODIAZEPINE

Facilitano l'attivazione dei recettori GABA

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



OPPIOIDI

BUTORFANOLO

- ✓ Agonista recettori κ , antagonista recettori μ

FENTANYL

- ✓ Agonista dei recettori μ

BUPRENORFINA

- ✓ Agonista parziale recettori μ

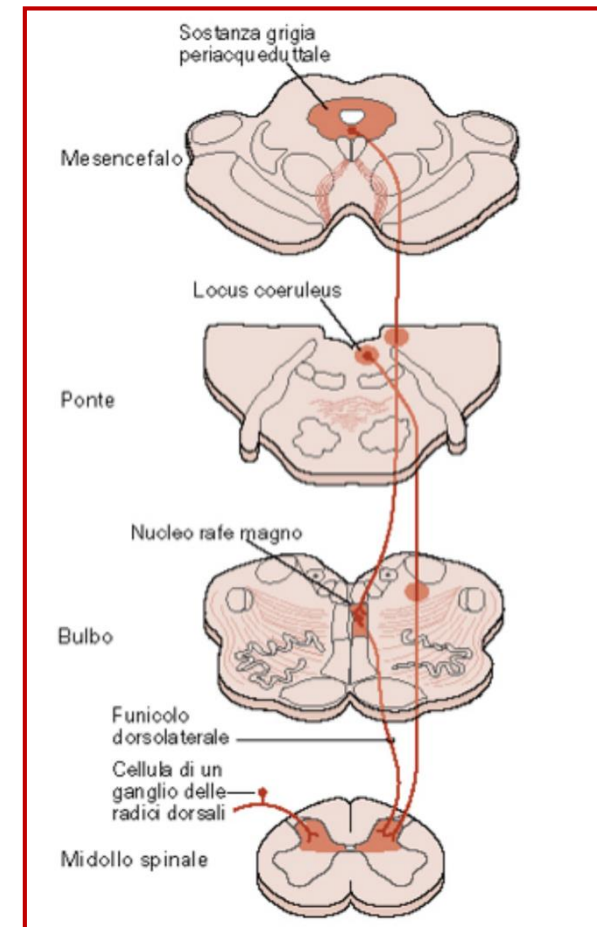
Somministrazione SC o IP

Potenziano le vie inibitorie discendenti

Lieve depressione della frequenza respiratoria

Potenziano effetto sedativo di altri farmaci

«Sparing effect»



KETAMINA

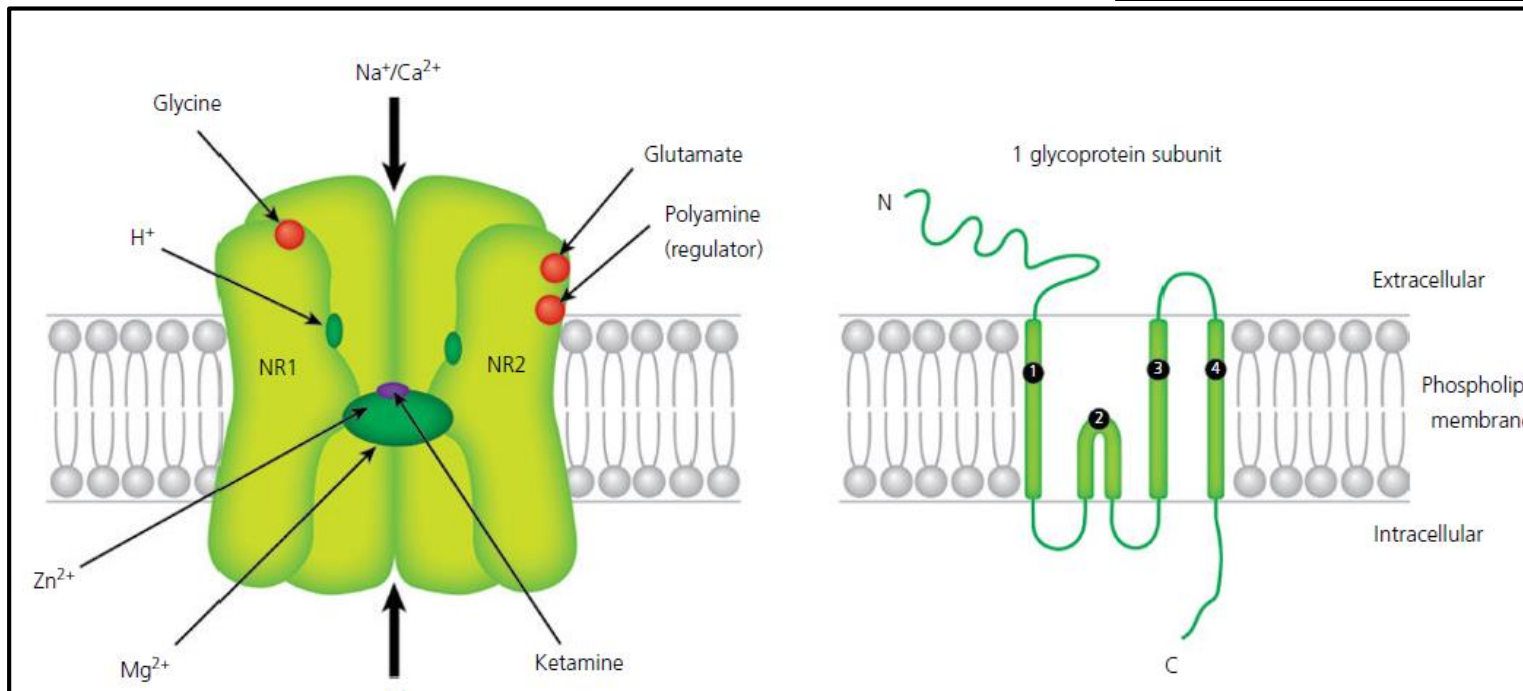
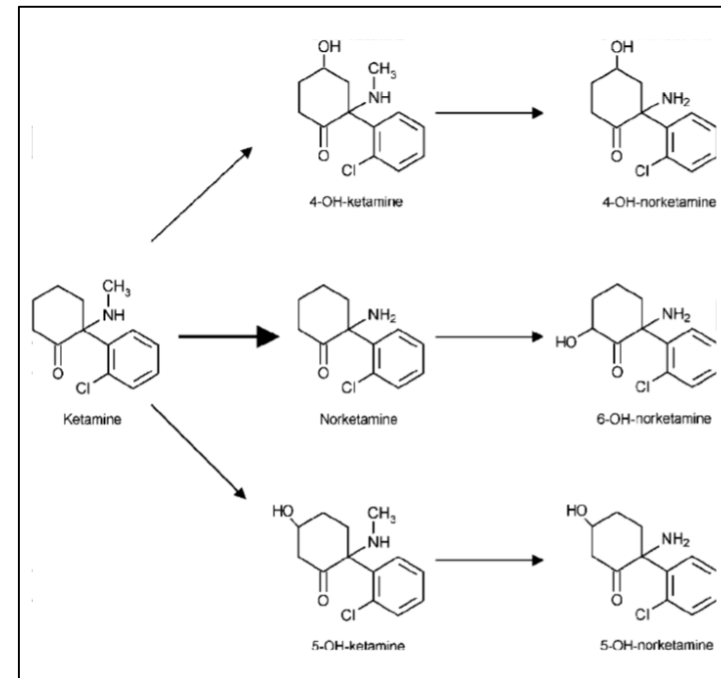
Farmaco dissociativo

Azione come antagonista sui recettori NMDA

Rigidità muscolare

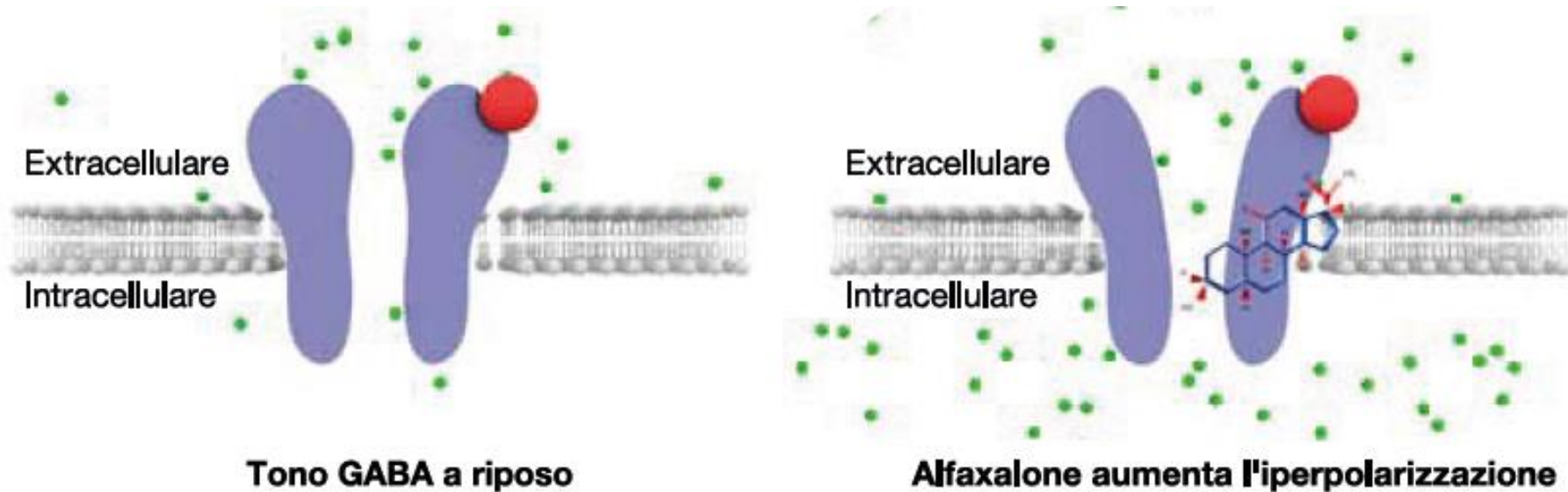
Analgesia

Risvegli disforici



ANESTETICI GENERALI

Thiopentale
Pentobarbitale
Alfaxalone
Propofolo



● Cl recettori

● GABA



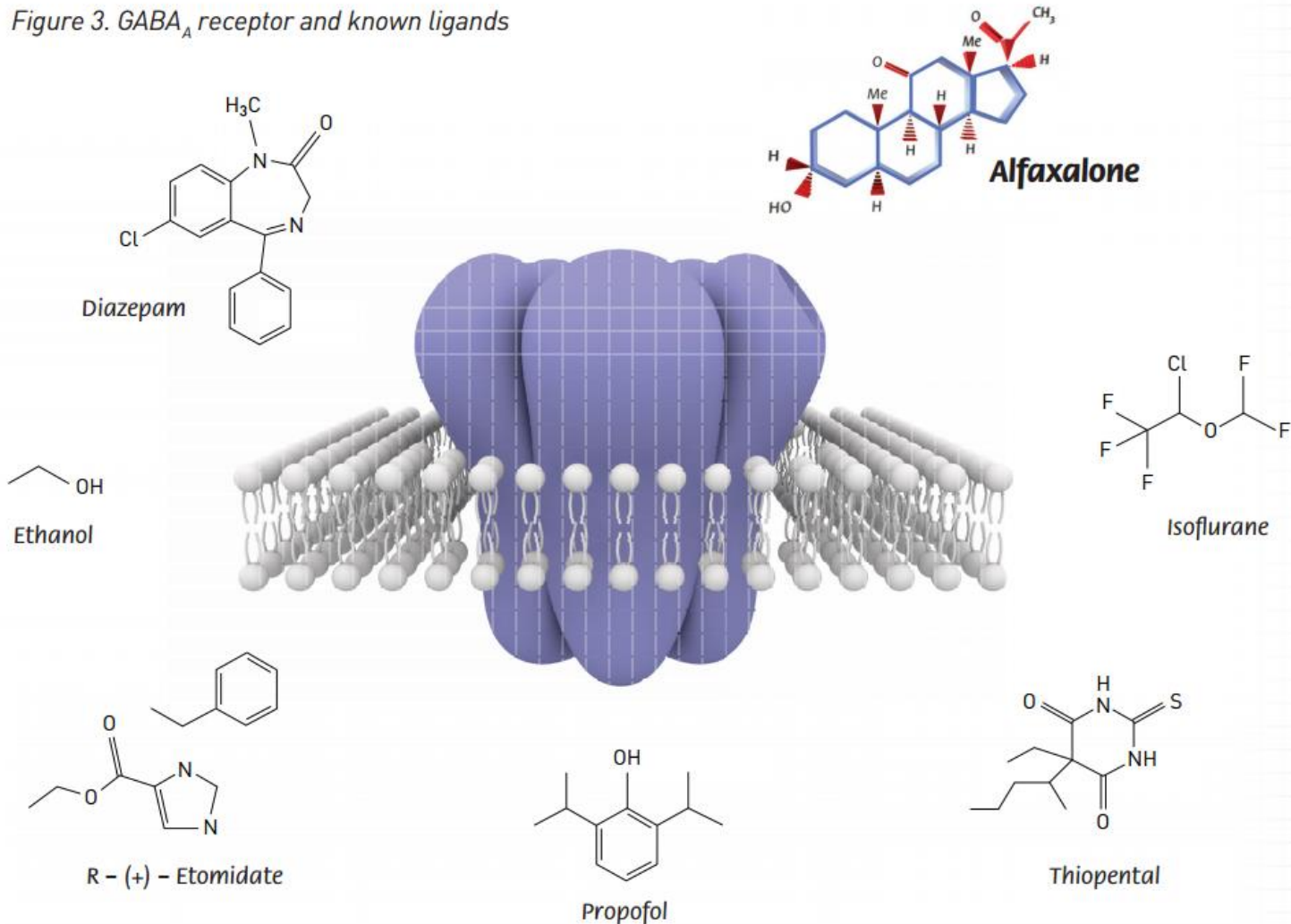
Alfaxalone



GABA_A

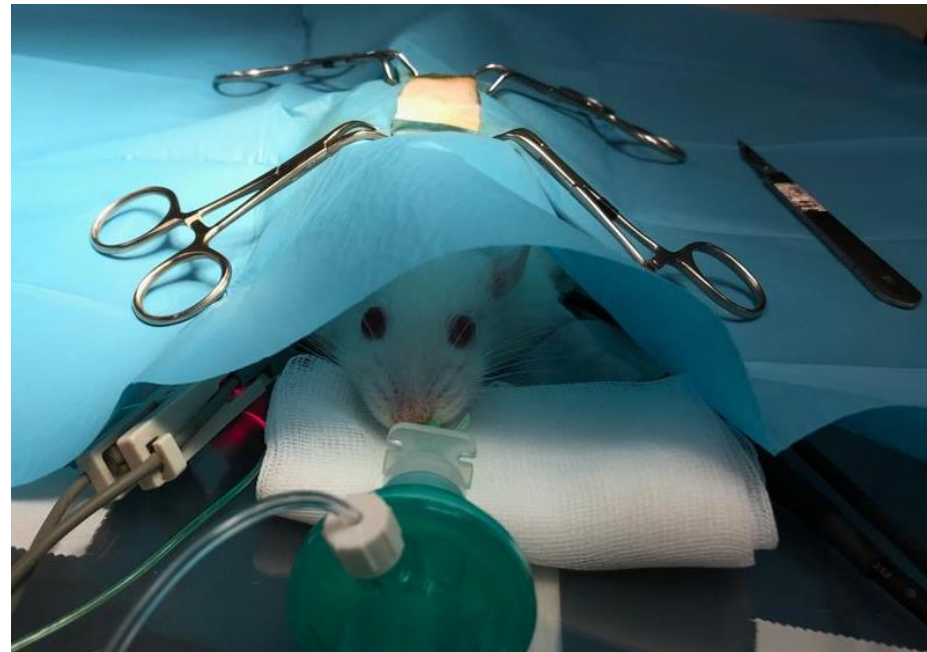
ANESTETICI GENERALI

Figure 3. GABA_A 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,¹ Lisa M. Franzi,¹ Steven E. Epstein,² Philip H. Kass,³
Ronald A. East,¹ and Nicholas J. Kovanos¹

	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

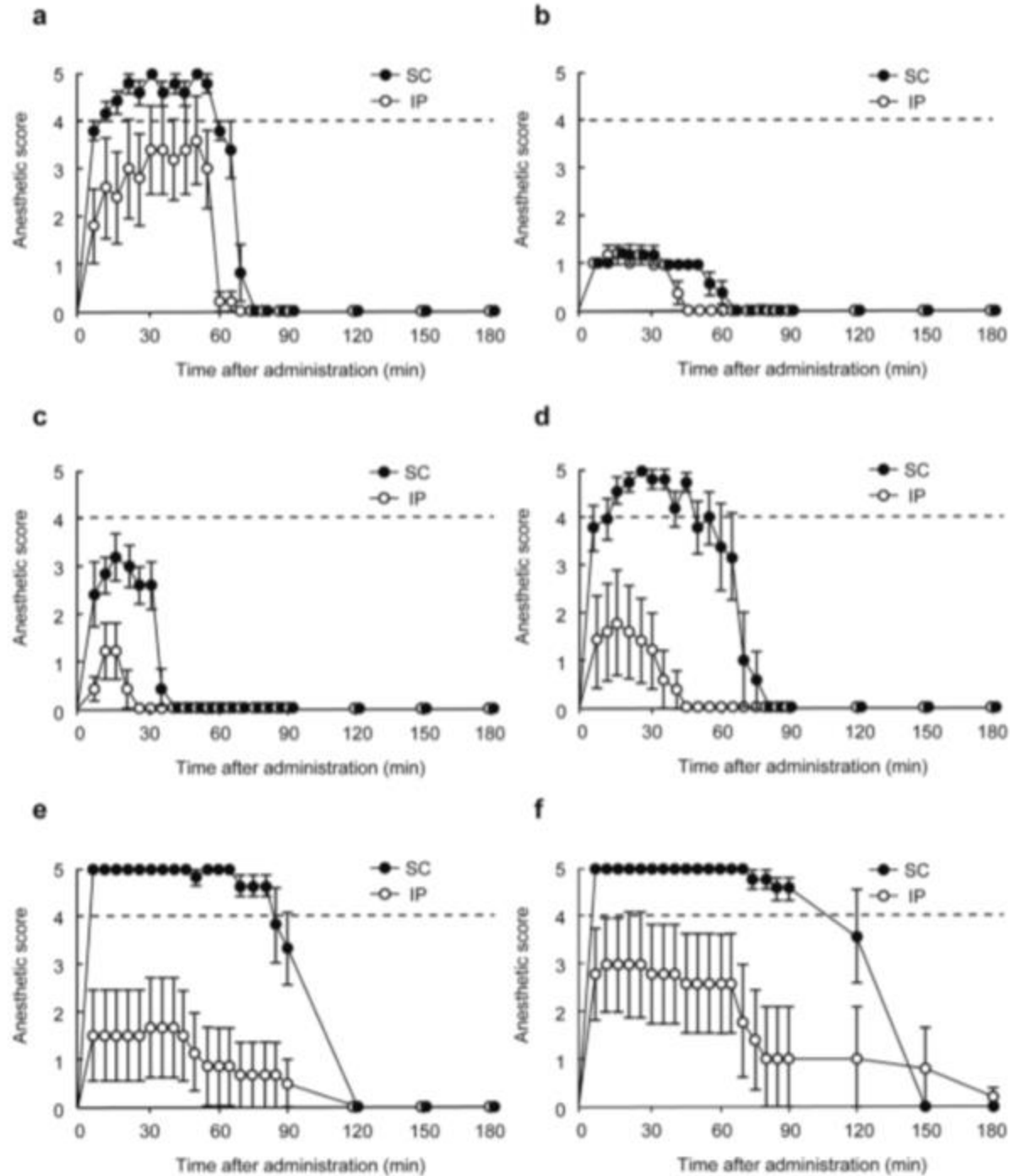
Table 1. Summary of the drugs and doses used in this study

Abbreviation	Dose (mg/kg)				Injection route ¹⁾	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

¹⁾IP, intraperitoneal; SC, subcutaneous.



ANESTESIA



ANESTESIA INIETTIVA RATTO

XYLAZINA+KETAMINA (XK)

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

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. Hedenqvist¹, J. V. Roughan² & P. A. Flecknell²

¹Karolinska Institute, Stockholm, Sweden and ²Comparative 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 ⁻¹)
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

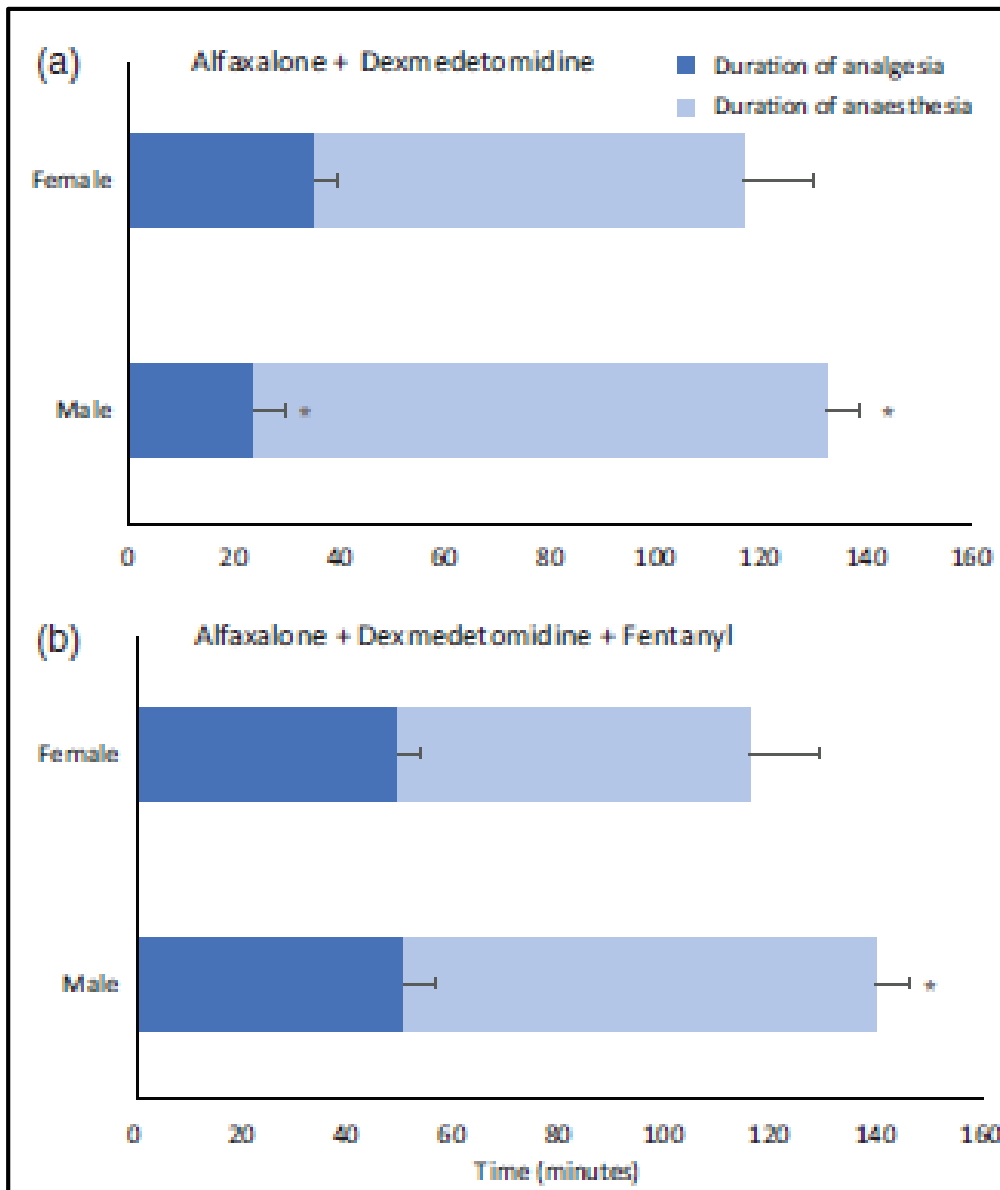


ALFAXALONE

Dose (mg kg ⁻¹)	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 ⁻¹)					
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 ⁻¹) + Fentanyl (0.1 mg kg ⁻¹)					
20	Females	8	6 ± 1	116 ± 8	50 ± 16
60	Males	8	4 ± 1	140 ± 15*	51 ± 14



ALFAXALONE



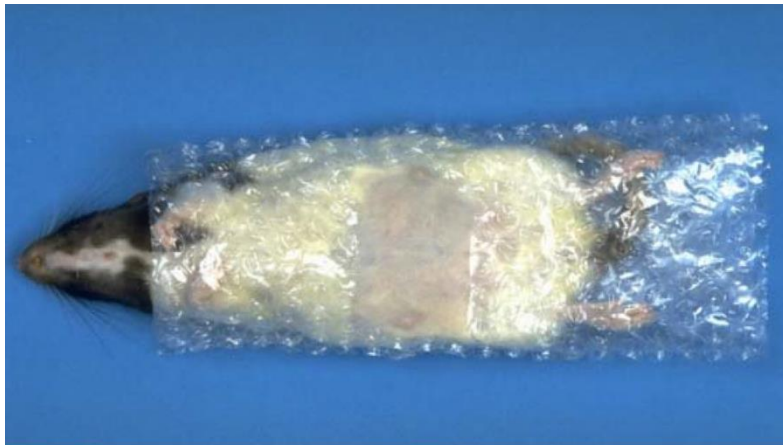
MONITORAGGIO INTRAOPERATORIO

Frequenza cardiaca

spO₂ periferico

Frequenza respiratoria

Temperatura



ANALGESIA POST-OPERATORIA

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



SANE





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