Guideline – Rodent Analgesia (Procedure Specific)

I. OBJECTIVE

This guideline aims to establish standard expectations for the management of pain in rodents undergoing potentially painful procedures at UQ[1]. The analgesia protocols described in this guideline may be varied, however, the AEC[2] will expect strong justification with specific details of any proposed variations. It is strongly recommended that variations are only submitted to the AEC for their review following prior consultation with a UQ Biological Resources (UQBR) veterinarian.

II. DEFINITIONS

"Assessed for pain" - Pain scoring: astute observation of animal behaviour in conjunction with assessment criteria to judge whether pain is being experienced. Use of the standard mouse or rat score sheets, available via the "monitoring animals" UQ webpage interface, are sufficient for pain scoring (particularly those criteria related to activity and responsiveness, body position, and facial grimace.

Competent - "the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments." (as per, Australian code for the care and use of animals for scientific purposes, 2013)

Laparotomy (major) - Where the peritoneal space is accessed surgically, and intervention is then performed which is likely to cause anything greater than minimal intra-abdominal trauma. For example, there is manual manipulation of organs to access the site of interest, internal ligaments or connective tissue are ruptured to access the site of interest, there is resection of tissue (e.g. splenectomy, nephrectomy), or the surgical time is prolonged (e.g. >15 minutes).

Laparotomy (minor) - Where the peritoneal space is accessed surgically, however, there is minimal risk of further trauma. For example, there is no manual manipulation of organs, tissue is not resected, and the surgical time is short (e.g. <15 minutes).

III. COMMENTS / RECOMMENDATIONS

- Analgesia should routinely be administered pre-emptively (i.e. given before the painful procedure) and then continued for the total duration of days in which pain can reasonably be expected to be experienced by the animal. This approach results in a "smoother" anaesthesia and recovery, less post operative complications, and more consistent physiological and behavioural outcomes. Reduced patient variability is experienced when pain is controlled largely because there is less potential for pain sensitisation e.g. hypersensitisation and allodynia. This point is exemplified within Image 1.
- Please note: although an excellent and "safe" general anaesthetic option, isoflurane alone provides little analgesia.
- Buprenorphine (an opiate analgesic agent) administered in conjunction with injectable anaesthetics (especially xylazine), has been reported to increase the risk in anaesthetic mortality (likely due to respiratory depression). For this reason, buprenorphine may be administered during the anaesthetic recovery period, i.e. following the surgical procedure, but this should only be done after having sought project-specific advise from a veterinarian.
- Following completion of any analgesia protocol if an animal continues to show signs of pain, analgesia should be continued (as appropriate). This should be done promptly, and wherever possible in line with veterinary advise. Such circumstance may be considered an unexpected adverse event.
- It is a standard expectation that analgesia is administered into the subcutaneous (SC), as compared to intraperitoneal (IP) space. IP injections increase the risk of mis-injection (e.g. into the GI tract) resulting in variable efficacy.
- Ad libitum oral (PO) analgesia (e.g. buprenorphine-impregnated jelly or Nutella on the cage floor) may be a useful delivery mechanism, however, careful planning is required to manage the risk of suboptimal delivery and consumption. As examples: rodents should be habituated to any new supplements (including supplemented water) in the days prior to surgery (to manage their innate neophobia), and some medications do not reliably dissolve or suspend within water. As such, prior advise from a veterinarian is strongly recommended.
- Ad libitum PO analgesia can be used in combination with parentally administered analgesia on day 0 (i.e. SC administered analgesia), however, it cannot be used in place of parentally administered analgesia on day 0 (see table 1C, for example). This is because ad libitum PO administration is generally less reliable, especially in rodents post operative, as they will have less of an appetite for consumption (food and water) than usual, and thus will not self-medicate reliably.

[1] "Pain and distress may be difficult to evaluate in animals. Unless there is evidence to the contrary, it must be assumed that procedures and conditions that would cause pain and distress in humans cause pain and distress in animals." (NHMRC, 2013)

[2] AEC – Animal Ethics Committee, relative to your application (UQ has multiple institutional AECs).

Table 1A | Analgesia Expectations for Mice and Rats (with consideration of associated monitoring) [for abbreviations (SID, BID, TID) see footnotes]

Classification #	1	2	3
Pain level	**Mild**	**Moderate**	**Severe**
Examples	• Intra-femoral injection (surgical access); • Subcutaneous pocket (e.g. mini-pump or xenograph); • <2cm skin wounding (simple, clean cut)	• Laparotomy (simple); • Ovariectomy; • Embryo transfer; • Simple soft tissue surgery (e.g. cut down for vessel cannulation); • Intraperitoneal mini-pump placement*; • Craniotomy**;	• Thoracotomy; • Laparotomy (major); • Orthopaedic fractures; • Caecal ligation and puncture; • Spinal or nerve injury;
Pre-operative analgesia: ideally analgesia is administered BEFORE surgery commences. [ONLY following veterinary consultation, the first dose of buprenorphine may be administered post operative (i.e. upon anaesthetic recovery). This is usually only appropriate where injectable anaesthesia is being used (e.g. ketamine/xylazine)]			
Perioperative analgesia (i.e. Day 0)	**LOCAL ANESTHESIA (LA)** via local infiltration into the tissue at the surgical site. This is done prior to making the first cut and is generally recommended for use with all surgical procedures. [NB: ENSURE THE DOSE USED IS ACCURATE; refer to table 2 for details of maximum safe dosages]		
	Day 0: Non-steroidal anti-inflammatory drug (**NSAID**)◊ SID and/or **BUPRENORPHINE** (once off injection; no repeat dosing routinely required)	Day 0: **NSAID** SID and **BUPRENORPHINE** (once off injection; no repeat dosing routinely required)	Day 0: **NSAID** SID and **BUPRENORPHINE**† BID-TID
Post-operative analgesia: frequency of analgesia administration is based on the known duration of the drug's effect. The total duration of analgesia administration is based on the expected duration of pain experience. This duration must be extended if signs of pain persist beyond that foreshadowed in your ethics application. In such cases specific veterinary advise should be sought.			
Post-operative analgesia	Day 1: routinely none is required. Ensure animals are assessed for pain	Day 1: continue **NSAID** SID Day 2: continue **NSAID** SID Day 3: routinely none is required. Ensure animals are assessed for pain	Day 1: continue **NSAID** SID and **BUPRENORPHINE**† BID-TID Day 2: continue **NSAID** SID and **BUPRENORPHINE**† BID-TID Day 3: routinely none is required. Ensure animals are assessed for pain
Post-operative monitoring: For each of these timepoints a monitoring record should be made following the standard mice/rat score sheet (or approved alternative) to affect pain scoring for each individual animal. If signs of pain persist, monitoring must continue at the frequency detailed below, as approved by the AEC, or as prescribed by a facility veterinarian. This describes the acute post operative monitoring expectations only. There are ongoing routine monitoring requirements, which should be guided by the animals' current and expected future condition (e.g. expected development or progression of symptoms), the details of which should be outlined in your approved ethics application.			
# of days (including day 0);	3 days	3 days	5 days
# times per day	1-2 times per day	2 times per day	2-3 times per day

Abbreviations: SID – once daily (i.e. every 24 hours); BID – twice daily (i.e. every 12 hours); TID – three times daily (i.e. every 8 hours)

* Intraperitoneal mini-pump placement – This procedure may require 24-72 hours of analgesia dependent upon the pump size and placement procedure (seek veterinary advise for individual models).

** Craniotomy – simple craniotomy = "moderate"; major craniotomy surgery (e.g. invasive cranial windows) = "severe"; if considered "severe", then NSAIDs as well as buprenorphine for 72 hours is indicated (as per the details within this table). Seek veterinary advise for individual models e.g. for guidance to identify if your craniotomy surgery should be considered "moderate" or "severe".

◊ Non-steroidal anti-inflammatory drugs (NSAIDs) – These are a category of anti-inflammatory drugs, some examples are provided in table 2. Except for paracetamol, these drugs should not be administered in combination (i.e. do not administered both meloxicam and carprofen to the same animal at the same time). These drugs have the potential to confound some models, see table 1C for further details.

† Buprenorphine - Following initial high-end dosages administered on day 0, if no signs of pain are observed, analgesia dosages may be reduced to the lower end of the therapeutic range provided in table 2. i.e. the initial dose = 0.1mg/kg, subsequent doses = 0.05mg/kg, so long as the animal does not show obvious pain scoring/ pain appears to be well controlled (this is demonstrated in table 1B & 1C).

† Buprenorphine - On days 1 and 2 (post-operative), buprenorphine may be provided ad-libitum per os (e.g. via buprenorphine-impregnated jelly or Nutella on the cage floor) instead of parental administration. Where buprenorphine is required on day 0, however, it must be provided parentally, even if this is occurring in conjunction with ad-libitum oral provisions.

Table 1B | Examples of analgesia application (and associated monitoring) in mice, as per table 1A. [for abbreviations see footnotes]

Classification #	1	2	3
Pain level	**Mild**	**Moderate**	**Severe**
Example surgery performed:	• Subcutaneous placement of neoplastic patient-derived xenograft (~1cm incision)	• Craniotomy (1mm diameter craniotomy to affect simple, low volume intra-cerebral injection)	• Nephrectomy, via laparotomy
Perioperative analgesia (i.e. Day 0)	**T = 0 hrs** (i.e. on Day 0) • Anaesthetic induction (isoflurane)*, • Buprenorphine 0.1mg/kg SC + carprofen 5mg/kg SC, then commence surgery once appropriately anaesthetised **T = 0.5 hrs** • Surgical completion, anaesthetic recovery	**T = 0 hrs** • Anaesthetic induction (ketamine/xylazine)*, • Lignocaine 6mg/kg SC at the incision site, • Once appropriately anaesthetised, commence surgery **T = 1 hrs** • Surgical completion, anaesthetic reversal agent (atipamezole 0.2mg/kg SC), shortly followed by buprenorphine† 0.1mg/kg SC + meloxicam◊ 5mg/kg SC	**T = 0 hrs** • Anaesthetic induction (isoflurane), • Buprenorphine 0.1mg/kg SC, • Lignocaine 6mg/kg SC at the incision site, • Once appropriately anaesthetised, commence surgery **T = 1 hrs** • Surgical completion, anaesthetic recovery • Meloxicam◊ 5mg/kg SC **T = 12hrs** • Buprenorphine 0.1mg/kg SC
Post-operative analgesia (i.e. after day 0)	Nil	**T = 24 hrs** (i.e. Day 1) • Meloxicam 5mg/kg SC **T = 48 hrs** (i.e. Day 2) • Meloxicam 5mg/kg SC	**T = 24 hrs** (i.e. Day 1) • Buprenorphine 0.05mg/kg SC + meloxicam 5mg/kg SC **T = 36 hrs** (i.e. Day 1) • Buprenorphine 0.05mg/kg SC **T = 48 hrs** (i.e. Day 2) • Buprenorphine 0.05mg/kg SC + meloxicam 5mg/kg SC **T = 60 hrs** (i.e. Day 2) • Buprenorphine 0.05mg/kg SC
Monitoring	Day 0: recheck the mice 4-6hrs post operative Day 1: monitoring record in the morning Days 2 and 3: monitoring record once daily	Day 0: recheck the mice 4-6hrs post operative Days 1 to 3: monitoring record morning and afternoon	Day 0: recheck the mice 4-6hrs post operative Days 1 to 5: monitoring record morning and afternoon

Abbreviations: T – time (in hours, relative to the time of anaesthetic induction); NSAID - Non-Steroidal Anti-Inflammatory Drug (e.g. carprofen and meloxicam, see table 2 for other examples)

* Inhalational anaesthesia (e.g. isoflurane via a precision vaporiser) vs. injectable (e.g. ketamine/xylazine) – although providing far less analgesia, inhalational anaesthesia is generally considered "safer", relative to injectable anaesthesia in rodents. For this reason, any surgery or procedure of a "mild" pain level (or less), should by default be performed via inhalational anaesthesia (i.e. isoflurane), unless specific justification can be provided, and this justification is considered ethically acceptable by the reviewing AEC.

† Buprenorphine – in the second scenario (craniotomy surgery) buprenorphine is administered within the surgical recovery period (i.e. buprenorphine is not administered pre-emptively). This would only be performed following project-specific veterinary advise, and due to concerns surrounding potential anaesthetic mortality (i.e. xylazine + buprenorphine associated cardio-respiratory depression).

◊ Meloxicam (NSAID analgesic agent) – in the second scenario (craniotomy) and third scenario (nephrectomy) meloxicam is administered within the surgical recovery period (i.e. meloxicam is not administered pre-emptively). This would only be performed following project-specific veterinary advise, and due to concerns surrounding NSAID associated side effects (reduced tissue perfusion, and potential for associated acute tissue injury, notably gastrointestinal and renal injury).

Table 1C | Examples of analgesia application (and associated monitoring) in mice, relative to table 1A, but where NSIADs must be avoided. [for abbreviations see footnotes]

In some animal models it is believed that NSAIDs have the potential to confound results. Examples may include where inflammatory mechanisms of the immune system are being studied or where novel anti-inflammatory therapeutics are being explored and developed. **If specific drugs must be avoided in your model, for example you intend to use one of the protocols below, THESE DETAILS MUST BE IDENTIFIED, AND JUSTIFICATION PROVIDED, IN YOUR ETHICS APPLICATION for the AEC to review and determine if the protocol is considered ethically acceptable.**

Classification #	1	2	3
Pain level	**Mild**	**Moderate**	**Severe**
Example surgery performed:	• Subcutaneous placement of neoplastic patient-derived xenograft (~1cm incision)	• Craniotomy (1mm diameter craniotomy to affect simple, low volume intra-cerebral injection)	• Nephrectomy, via laparotomy
Perioperative analgesia (i.e. Day 0)	T = 0 hrs (i.e. on Day 0) • Anaesthetic induction (isoflurane)*, • Buprenorphine 0.1mg/kg SC, • Lignocaine 6mg/kg SC at the incision site, then commence surgery once appropriately anaesthetised T = 0.5 hrs • Surgical completion, anaesthetic recovery T = 8 to 12 hrs • Buprenorphine 0.05mg/kg SC	T= (-)72 hrs • Buprenorphine impregnated jellies provided ad lib. PO, remaining in cage for ~6 days. T = 0 hrs • Anaesthetic induction (ketamine/xylazine)*, • Lignocaine 6mg/kg SC at the incision site, • Once appropriately anaesthetised, commence surgery T = 1 hrs • Surgical completion, anaesthetic reversal agent (atipamezole 0.2mg/kg SC), shortly followed by buprenorphine† 0.1mg/kg SC T = 6 to 12hrs** • Buprenorphine 0.05mg/kg SC + oral jelly ad lib. PO	T = 0 hrs • Anaesthetic induction (isoflurane), • Buprenorphine 0.1mg/kg SC, • Lignocaine 6mg/kg SC at the incision site, • Once appropriately anaesthetised, commence surgery T = 1 hrs • Surgical completion, anaesthetic recovery T = 6 to 12hrs** • Buprenorphine 0.1mg/kg SC
Post-operative analgesia (i.e. after day 0)	Nil	T = 24 hrs (i.e. Day 1)** • Buprenorphine 0.05mg/kg SC + oral jelly ad lib. PO T = 36 hrs (i.e. Day 1) • Buprenorphine 0.05mg/kg SC + oral jelly ad lib. PO T = 48 hrs (i.e. Day 2) • Buprenorphine oral jelly ad lib. PO T = 60 hrs (i.e. Day 2) • Buprenorphine oral jelly ad lib. PO	T = 24 hrs (i.e. Day 1)** • Buprenorphine 0.05mg/kg SC T = 36 hrs (i.e. Day 1) • Buprenorphine 0.05mg/kg SC T = 48 hrs (i.e. Day 2) • Buprenorphine 0.05mg/kg SC T = 60 hrs (i.e. Day 2) • Buprenorphine 0.05mg/kg SC
Monitoring	Day 0: recheck the mice 4-6hrs post operative Day 1: monitoring record in the morning Days 2 and 3: monitoring record sometime in the day	Day 0: recheck the mice 4-6hrs post operative, and then Days 1 to 3: monitoring record morning and afternoon	Day 0: recheck the mice 4-6hrs post operative Days 1 to 5: monitoring record morning and afternoon

Abbreviations: T – time (in hours, relative to the time of anaesthetic induction); ad lib. – ad libitum (refers to voluntary consumption); PO – per os; NSAID - Non-Steroidal Anti-Inflammatory Drug (see table 2)

* Inhalational anaesthesia (e.g. isoflurane via a precision vaporiser) vs. injectable (e.g. ketamine/xylazine) – see the relative foot note from table 1B.

† Buprenorphine – see the relative foot note from table 1B.

◊ Meloxicam (NSAID analgesic agent) – see the relative foot note from table 1B.

** Buprenorphine (repeat dosing) – in the second scenario (craniotomy) and third scenario (nephrectomy) buprenorphine is administered at T = 6-12 hrs, which is less than the routine retreatment interval of 8-12 hrs (see table 2). This should be performed following project-specific veterinary advise, and due to concerns surrounding the potential for pain in the absence of supplementary NSAID analgesia. Where shorter retreatment intervals are used, care must be taken to ensure subsequent scheduled doses do not occur at intervals >12 hrs apart (this would mean the routine dose at T = 24hr dose may need to be "brought forward" and administered at T = 18hrs if the previous dose was administered at T = 6hrs).

Table 2 | Shorthand Analgesia Formulary for Mice and Rats

Abbreviations: SID – once daily (i.e. every 24 hours); BID – twice daily (i.e. every 12 hours); TID – three times daily (i.e. every 8 hours). SC – subcutaneous; PO – per os.

Drug	Mice	Rat
Non-Steroidal Anti-Inflammatory Drugs (NSAIDs):		
Meloxicam [a]	1-5 mg/kg SC, PO 24-hourly	1 mg/kg SC, PO 24-hourly
Carprofen [a]	5 mg/kg SC, PO 24-hourly	5 mg/kg SC, PO 12–24-hourly
Ketoprofen [a]	5 mg/kg SC 24-hourly	5 mg/kg SC 24-hourly
Paracetamol	200 mg/kg PO 24-hourly	200 mg/kg PO 24-hourly
Opiates:		
Buprenorphine	0.05–0.10 mg/kg SC 8–12-hourly [b]	0.01–0.05 mg/kg SC 8–12-hourly [b] 0.10–0.25+ mg/kg PO [c] 8–12-hourly
Butorphanol (not recommended) [d]	1.0–2.0 mg/kg SC 4-hourly	
Local anaesthetics (LA):		
Lignocaine [e]	<7mg/kg SC 4-hourly	<7mg/kg SC 4-hourly
Bupivacaine [e]	<7mg/kg SC 4-hourly	<7mg/kg SC 4-hourly

[a] Generally should not be administered ad libitum PO (note: some exemptions apply, seek veterinary advise)

[b] If buprenorphine is being administered as the sole analgesic (e.g. where NSAIDS must be avoided), to appropriately manage their pain the first follow up dose may need to be administered sooner than the routine 8-12 hour interval (see table 1C for details).

[c] When administered PO, sublingual buprenorphine tablets are generally preferred over injectable buprenorphine, given injectable buprenorphine is often too bitter to inspire voluntary consumption. As per NHMRC (2008):

> "*Volker et al (2000) has described the following method for oral administration of buprenorphine in rats using jelly; acclimatise the rats to consumption of jelly over several days or weeks. Dissolve 85 g of jelly crystals in 250 mL of boiling water. Place aliquots of 4 mL of jelly liquid in ice-block moulds for refrigeration. Rats will accept berry, orange, lime and strawberry flavours.*
>
> *When analgesia is required, 3 buprenorphine sublingual tablets (Temgesic, Reckitts and Coleman, 0.2 mg/tablet) are crushed into the base of each ice-block mould and moistened with 0.5 mL warm water, prior to the addition of 3.5 mL of warm jelly (total 4 mL). The jelly disks are set at 4–8°C. For acute pain, the number of disks given to each animal is calculated on a dose rate of 2 mg/kg.*"

[d] Not recommended due to its relatively short retreatment interval, and only mild to moderate analgesia it provides.

[e] The toxicity of local anaesthetics is not unique to rodents, however, their small size means that they are at particular risk of inadvertent overdose. To avoid this, ensure you calculate the appropriate safe dose and prepare this volume for injection in advance of the procedure.

IV. REFERENCE INFORMATION

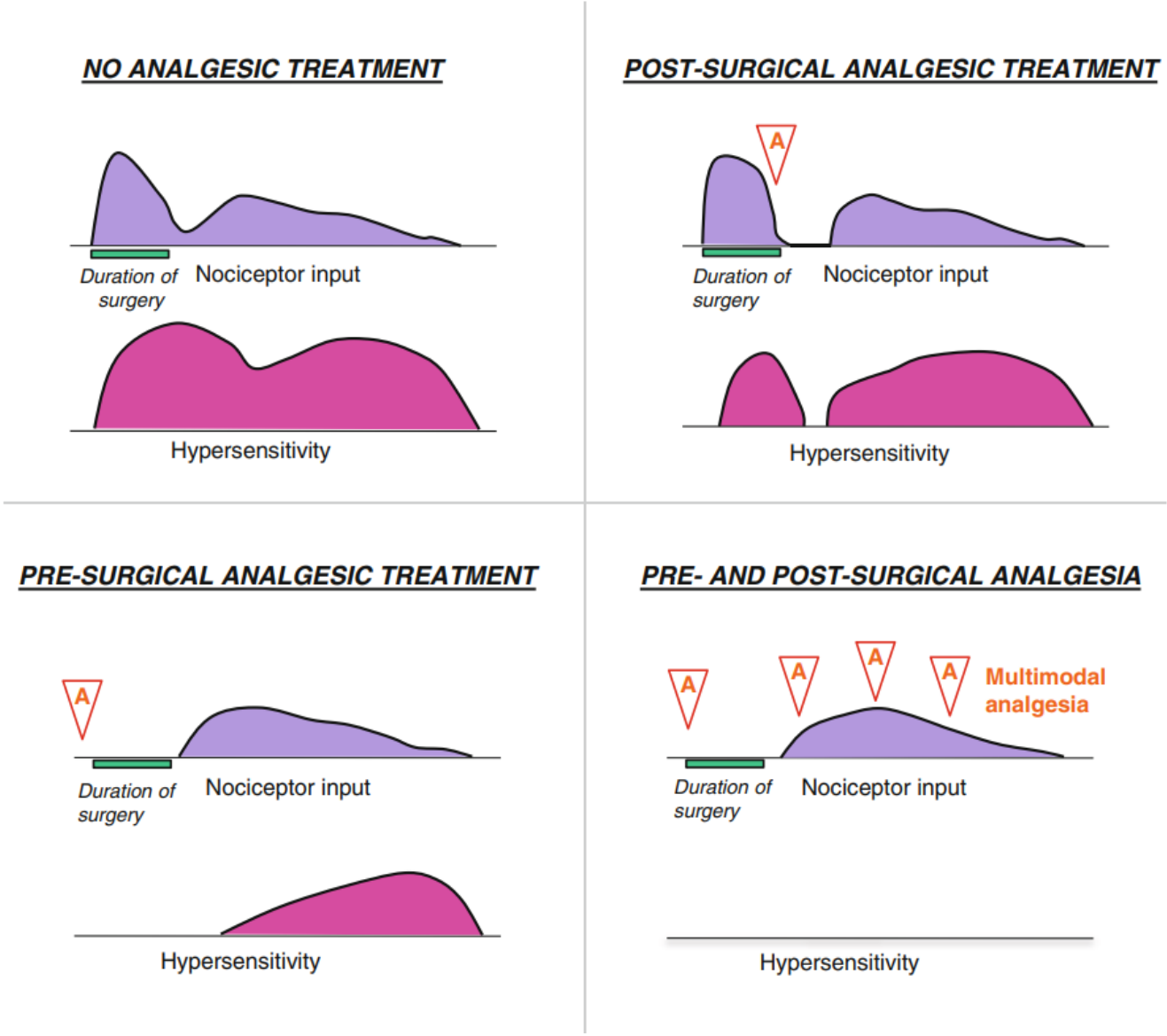

Image 1 | A generalised schematic representation of the effects effective analgesia has managing pain and hypersensitisation associated with a routine surgery (image modified from: Ferry, B., Gervasoni, D., Vogt, C., 2014).

Analgesic drug administration ( ) is depicted in three of the four scenarios.
TOP LEFT: No analgesia. Pain from the initial surgery (nociception, shown in purple) and subsequent hypersensitivity (pink) are both pronounced.
TOP RIGHT: Analgesia administered only once post operative, after initial sensitization. This is useful in the immediate post operative management, however, failure to continue analgesia means the intervention amounts to little long-term benefit.
BOTTOM LEFT: Analgesia administered before surgery only. This limits pain in the peracute period and helps to reduce the overall potential for hypersensitisation. Hypersensitivity will however still develop if nociception persists post operative.
BOTTOM RIGHT: analgesia administered both pre and post operatively as required for the expected duration of nociception. This protocol is preferred as it most effectively manages nociception and mitigates the risk of hypersensitivity.

V. BIBLIOGRAPHY

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Version #	Reviewing AECs	AEC Review Date	Approval To Date
1	HS, ABS, LMB, MBS	21/07/2022	21/07/2025