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Posted Date: 5 March 2024

doi: 10.20944/preprints202403.0234.v1

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Comparison of Intra-Operative Analgesia and Peri-Operative Behaviour and Gastrointestinal Motility in Rabbits Receiving a Brachial Plexus Block or Lidocaine Constant Rate Infusion for Orthopaedic Surgery

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Simple Summary: Local anaesthetic nerve blocks can be used to provide pain relief during and after surgery. While specific local anaesthetic techniques are commonplace in humans, and some species, it is a relatively new field of research in rabbits. Rabbits are prey species that hide pain well but may express changes in behaviour, food intake and production of faeces as a result of pain, making them challenging to study. This study aimed to investigate a specific local anaesthetic technique in rabbits undergoing orthopaedic surgery on a front leg. Its effectiveness was investigated by comparing the requirement for extra pain relief during and after surgery, and comparing changes food intake, faeces production and behaviour after surgery. Both remote filming and direct observation were used. The rabbits who received the block required no additional pain relief during surgery, whereas every rabbit who received intravenous pain relief did require additional pain relief. However after surgery, the severity of pain and requirement for extra pain relief was the same and there was no difference in behaviour between the groups. In conclusion, this local anaesthetic nerve block was easy to administer and provided effective pain relief during surgery, reducing the need for additional drug therapy.

Abstract: Locoregional anaesthetic techniques are invaluable for providing multimodal analgesia for painful surgical procedures. This prospective, randomised study describes a nerve stimulator-guided brachial plexus blockade (BPB) in rabbits undergoing orthopaedic surgery in comparison to systemic lidocaine. Premedication was provided with intramuscular (IM) medetomidine, fentanyl and midazolam. Anaesthesia was induced (propofol IV) and maintained with isoflurane. Nine rabbits received a lidocaine BPB (2%; 0.3 ml kg^{-1}), and eight received lidocaine constant rate infusion (CRI) (2 mg kg^{-1} IV, followed by 100 µg kg^{-1} $minute^{-1}$). Rescue analgesia was provided with Fentanyl IV. Carprofen was administered at the end of surgery. Post-operative pain was determined using the Rabbit Grimace Scale (RGS) and a composite pain scale. Buprenorphine was administered according to pain score for two hours after extubation. Rabbits were filmed during the first two hours to measure distance travelled and behaviours. Food intake and faeces output were compared. Every rabbit in CRI required intraoperative rescue analgesia compared to no rabbits in BPB. However, rabbits in both groups had similar pain scores and there was no difference in administration of postoperative analgesia. There were no significant differences in food intake or faeces production over 18 hours, and no significant differences in distance travelled, or behaviours examined during the first two hours. BPB seems superior for intraoperative analgesia. Postoperatively both groups were comparable.

 doi:10.20944/preprints202403.0234.v1

Keywords: locoregional anaesthesia; multimodal analgesia; faecal output; nerve stimulator; pain score; activity; behaviour; video processing

1. Introduction

In accordance with the orthopaedic surgery literature, surgical procedures are often associated with moderate to severe pain, meaning that suitable intraoperative and postoperative analgesia regimens are of paramount importance [1,2]. Pain and stress in the postoperative period are associated with several physiological changes including sympathetic nervous system activation and endocrine changes[3–5]. These lead to protein catabolism and hyperglycaemia, causing impaired wound healing, weight loss and infection[3,4]. Therefore, adequate perioperative pain management is mandatory for painful procedures and, therefore, requires regular pain assessment. With rabbits commonly used as animal model in experimental orthopaedic surgeries, accurate pain assessment remains particularly challenging due to their tendency to suppress pain behaviours in light of being prey animals. This further emphasizes the importance of recognising and managing pain using balanced anaesthesia protocols.

The use of locoregional anaesthesia techniques for various surgical procedures, now commonplace in dogs and cats, has been reported in other species, including sheep[6], calves[7], goats[8,9] and pigs[10,11]. Local nerve blocks may be performed blind or guided by electrical nerve stimulation or ultrasound. Electrical nerve stimulation facilitates more precise localisation of the nerves than using anatomical landmarks alone. Ultrasound-guided local blocks have been associated with increased block success, faster onset of effect, increased duration and reduced risk of vascular puncture and reduced volume of drug required[12,13]. A variety of ultrasound guided local nerve blocks have recently been described in rabbits[14,15].

Both an ultrasound guided approach to the brachial plexus block and combined ultrasound and peripheral nerve stimulator guided approach have been described in rabbits[16,17], both of which concluded that the techniques are feasible, reproducible and safe, providing adequate analgesia for rabbit thoracic limb surgery.

The aim of this study was to assess the efficacy of a solely nerve stimulator guided axillary approach to the brachial plexus block for rabbits undergoing thoracic limb orthopaedic surgery. In this study the brachial plexus block with lidocaine was compared to a lidocaine constant rate infusion (CRI), which has previously been demonstrated to maintain gastrointestinal motility and provide analgesia for soft tissue surgical procedures[18].

We hypothesised that the nerve stimulator guided brachial plexus block would provide effective intraoperative analgesia without negative impact on food intake and faecal output. In addition, we hypothesised that an intraoperative lidocaine CRI would increase faecal output postoperatively.

2. Materials and Methods

This prospective randomized study was conducted as a spin-off study of a larger project assessing the efficacy of bone scaffolds for correcting critical size bone defects in rabbits. For the current study, 19 rabbits undergoing experimental thoracic limb surgery in general anaesthesia with isoflurane where either subjected to a brachial plexus block with lidocaine (group BPB) or to an intraoperative lidocaine CRI (group CRI). Rabbits were evenly allocated to either group via lot prior to the surgery. Perioperative rescue analgesia and post-operative food intake, faecal output and behaviour was assessed at 30, 60, 90 and 120 minutes, and 10 and 18 hours post recovery and compared between the groups.

Experimental protocols were approved by the United Kingdom Home Office as governed by the UK law under the Animals (Scientific Procedures) Act 1986, project license number PP1153947, and abide by the ARRIVE guidelines. The work was conducted at University of Cambridge biomedical services research animal facilities. Nineteen female New Zealand white rabbits (15 purchased from

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ENVIGO RMS LTD- Loughborough, UK; four from Charles River UK Limited- Kent, UK), aged 12 weeks, were acclimatised to their housing for 4-6 weeks prior to starting the study.

Animals and Housing

The rabbits were housed in pairs in floor pens with overall dimensions of 150 cm width, 150 cm depth and no ceiling. Each cage contained an elevated resting platform 25 cm high which also served as a shelter. After surgery the rabbits were housed individually, with the original floor pens divided in half, each containing an elevated resting platform. They continued to have visual and tactile contact with their previous companion. All animals had ad libitum access to food and water during the experimental period. The food consisted of hay and a commercial dry pellet food formulated for rabbits. As environmental enrichment, a selection of dried herbs and vegetables or fresh carrot was supplied once daily, though these were withheld for 24 hours following surgery. Each pen also contained a large cardboard tube which provided environmental enrichment and shelter. Water was supplied in water bottles which were refreshed once daily.

Anaesthesia and Monitoring

Prior to each surgery, a clinical examination of each animal was performed. One rabbit was excluded from the study at this stage due to persistent tachypnoea. Premedication consisted of medetomidine 100 μg kg^{-1} (Sedator, Dechra), fentanyl 5 μg kg^{-1} (Fentadon, Dechra) and midazolam 0.5 mg kg^{-1} (Hypnovel) administered intramuscularly. After onset of sedation (15 minutes later) an intravenous (IV) cannula was placed in the left marginal auricular vein and anaesthesia was induced with propofol, 1 – 3 mg kg^{-1}, administered to effect. The rabbits were intubated using a capnograph-guided technique. If there were three unsuccessful attempts to intubate, a supraglottic airway device (V-gel®, Docsinnovent) was placed. The airway device was connected via a heat and moisture exchanger (HME) to non-rebreathing system. Anaesthesia was maintained with isoflurane (Isofane, Covetrus) to maintain sufficient anaesthetic level to facilitate the procedure, in 100% oxygen. All anaesthetics were performed by two experienced anaesthetists (SM, CG) and adjustments to level of anaesthesia were based on reaction to surgical stimulus: increased heart rate (HR) or respiratory rate (*f*R) , breathing against ventilator or movement. Routine intraoperative monitoring included sidestream capnography, fraction expired inhalant (FE'ISO %), oesophageal temperature, pulse oximetry and oscillometric blood pressure with a Mindray Beneview T8 multiparameter monitor. Physiological parameters were monitored continuously and recorded every five minutes. Recordings of HR, assessed by pulse oximetry and manual pulse palpation, were averaged in each rabbit for "HR surgical prep" and "HR during surgical stimulation". The HR change was calculated by the difference between these two periods.

Intermittent positive pressure ventilation (IPPV) was used to maintain end-tidal carbon dioxide partial pressure at 35 – 50 mmHg using a mechanical thumb ventilator. Hypotension (MAP < 65 mmHg) or bradycardia (HR < 140 bpm) were treated at the discretion of the anaesthetist. Lactated Ringers solution (Aqupharm 11, Animalcare) was administered at 10 ml kg^{-1} hr^{-1} during the anaesthesia. No intravenous fluids were administered postoperatively. An electric heat mat was used to maintain normothermia (38 – 39.9°C) throughout the anaesthesia.

The left thoracic limb was clipped and aseptically prepared for radial ostectomy according to the guidelines of the main orthopaedic study. After aseptic preparation of the limb, the rabbits were administered either a brachial plexus block with lidocaine (Hameln Pharma, UK) (group BPB) or an IV bolus of lidocaine followed by a CRI (group CRI).

Rabbits were administered rescue analgesia (fentanyl 5 μg kg^{-1}) if the HR or fR increased by 20% or more from the individual baseline during the surgery. Baseline values were recorded immediately prior to surgical start time. Rabbits receiving rescue analgesia remained within the study. Carprofen 4 mg kg^{-1} (Rimadyl, Pfizer) subcutaneously (SC) was administered at the end of surgery and once every 24 hours for five days thereafter.

 doi:10.20944/preprints202403.0234.v1

Brachial Plexus Block

Ten rabbits received a peripheral nerve stimulator-guided brachial plexus block. After intubation, rabbits were placed in right lateral recumbency, allowing access to the left thoracic limb. The cranial aspect of the shoulder and ventral neck was clipped and aseptically prepared. The positive electrode of the nerve stimulator was positioned on the lateral aspect of the left elbow. The landmarks used were the acromion, the cranial border of the greater tubercle of the humerus and the cranial border of the first rib. The insulated needle was inserted cranial to the acromion, immediately dorsal to the clavicle and advance in a ventral and caudal direction, parallel to the longitudinal access of the vertebral column and thoracic wall. The needle was advanced slowly with an initial current of 2mA and monitored for nerve stimulation corresponding to radial nerve stimulation: extension of the elbow, carpus and digits. Once the appropriate response had been elicited, the current was reduced incrementally to 0.5 mA to ensure proximity to the nerve. Once position was confirmed, the current was reduced to 0.2 mA to rule out intraneural placement of the needle tip. Intravascular placement was excluded by aspiration prior to 0.1 ml kg^{-1} of lidocaine 2%. The needle was then withdrawn approximately 0.5 cm, and a further 0.1 ml kg^{-1} of lidocaine 2% injected following aspiration. This was repeated once more, to give a total volume of 0.3 ml kg^{-1} of lidocaine 2%, equating to a total dose of 6 mg kg^{-1}.

Lidocaine CRI

Eight rabbits received lidocaine 2 mg kg^{-1} IV over 5 minutes during surgical preparation of the limb, followed by an infusion of 100 µg kg^{-1} min^{-1} for the duration of the surgical procedure. The infusion was delivered using a calibrated syringe driver (BD Alaris Syringe Pump) and was stopped at the same time as the isoflurane.

Surgery

During surgery, the rabbits were positioned in left lateral recumbency to allow a medial approach to the left antebrachium. A titanium K wire was positioned in the radius in a mediolateral orientation as a marker for postoperative radiographic analysis. A 15 mm full thickness defect was created in the radius diaphysis by two osteotomies and then cutting the interosseus ligament between the radius and the ulna. The defect was then filled with bone scaffold as part of the primary orthopaedic study. No further stabilisation of the bone was provided. The surgical site was then closed and radiographs were acquired prior to recovery.

Recovery and postoperative assessment

Rabbits were extubated when a swallow reflex was present and were returned to individual enclosures with clean bedding once they were able to support their head and maintain sternal recumbency. The time from turning the isoflurane off to extubation was recorded as the recovery time.

The rabbits were filmed in their enclosures for the first two hours immediately following their return using a GoPro camera. This was fixed at a height of 140 cm above the ground to the centre of one end of the enclosure, angled to include the entire enclosure. The camera position was consistent for each rabbit to allow for post hoc video processing.

The rabbits were pain scored by either appointed observer using the Rabbit Grimace Scale and the Bristol Rabbit Composite Pain Scale 30, 60, 90 and 120 minutes, and 10- and 18-hours post recovery. During the first 90 minutes, buprenorphine 0.05 mg kg^{-1} SC (Buprecare, Animalcare) was administered as rescue analgesia if the RGS score was greater than 5/10. If no buprenorphine was administered beforehand one dose was given at T120 to ensure adequate pain relief following the orthopaedic surgery. In all rabbits, another dose of buprenorphine 0.05 mg kg^{-1} SC was administered at T10h and T18h.

To assess food intake, the food pellets were weighed upon return of the rabbits to their enclosures and then weighed at the above time points. Rabbits had continuous access to hay as it was

 doi:10.20944/preprints202403.0234.v1

fed loose, but intake amount could not be assessed. Instead, hay consumption was classified as "eating", "showing interest in food" or "hay undisturbed" based on the animal behaviour at the predetermined observation points (30, 60, 90 and 120 minutes, and 10- and 18-hours post-surgery). As a substitute for gastrointestinal motility, faecal output was assessed by collecting the faeces produced by each rabbit at each time point. The morphology was subjectively assessed each time as "normal" or "abnormal". "Normal" faeces were defined as uniform, round and smooth. "Abnormal" faeces were irregular in size and shape, sticky or crumbly. The rabbits were weighed daily for three days after surgery.

Post Hoc Video Processing

Videos obtained from each rabbit during the immediate recovery period (up to T120), were automatically processed afterwards. For this purpose, a computer programme was designed using the Python (Python Software Foundation, version 3.10.5) coding language to detect whole body movement of the rabbits, such as hopping or crawling. It automatically analysed each video and was designed to detect movement of the white rabbit against the contrasting background, allowing automatic measurement of the distance travelled by each rabbit during the first two hours after recovery. The videos were also used to manually assess the presence or absence of the following behaviours with the first two hours postoperatively in each rabbit: eating hay or pellets, generalised grooming, grooming the affected limb, hopping, sprawling, and interacting with their environment. Interacting with their environment included hopping through or chewing their cardboard tube or interacting with their bedding material.

Statistics

Data were assessed for normality by inspection of QQ plots. Bartlett's and Levene's tests were used to assess equality of variance. Data were reported as median (first quartile, third quartile) when not normally distributed or as mean (standard deviation) when normally distributed. The Mann Whitney-U test and descriptive statistics were used to compare demographic and surgical data. Fisher's Exact test was used to compare the requirement for rescue analgesia intraoperatively and postoperatively and to compare the number of rabbits who produced faces in the first two hours postoperatively. Pain scores between groups BPB and CRI were examined using descriptive statistics. Data were compared using commercially available software (R: R Studio, version 4.2.1; JASP, version 0.17.3) and significance interpreted at $P < 0.05$ where applicable.

3. Results

Of the 19 initially included rabbits, one rabbit (allocated to group CRI) had to be excluded from the study due to persistent pre-operative tachycardia. From the remaining 18 rabbits, which initially entered the study, one animal (allocated to group BPB) was retrospectively excluded due to surgical complications. The remaining 17 rabbits completed the surgery and had an uneventful recovery, with nine rabbits in group BPB and eight animals in group CRI for data analysis. There was no difference in preoperative rabbit weight between the groups (Table 1). The general anaesthesia time was 115 +/- 14.1 minutes in group BPB and 117 +/- 20.9 minutes in group CRI, showing that the brachial plexus block did not significantly increase total anaesthesia time. There were no differences in surgery or recovery time (Table 2) and all rabbits were returned to their pens within 15 minutes of extubation.

Table 1. The bodyweight of the rabbits receiving either a brachial plexus block (BPB, n = 9) or a lidocaine CRI (CRI, n = 8) preoperatively and the bodyweight change three days postoperatively. Data are given as mean (SD).

Variable	Group		Significance
	BPB	CRI	
Weight (kg)	3.4 (3.22 – 3.62)	3.4 (3.35 – 3.62)	P = 0.63

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Weight Change 3 Days Post-surgery	-0.075 (0.046)	-0.091 (0.042)	P = 0.18

Table 2. Descriptive data of anaesthesia monitoring in rabbits undergoing experimental thoracic limb surgery with either brachial plexus block (BPB, n = 9) or intraoperative lidocaine (CRI, n = 8). Data are given as mean (SD).

Variable		Group		Significance
		BPB	CRI	
General anaesthesia time (minutes)		115 (14.1)	117 (20.9)	P = 0.63
Surgical time (minutes)		51 (6.4)	53 (10.5)	P = 0.84
Recovery time (minutes)		9.9 (4.1)	10.3 (3.9)	P = 0.81
Induction dose of propofol (mg kg^{-1})		3.55 (1.5)	2.56 (2.21)	P = 0.28
FE'ISO (%)		1.51 (0.17)	1.71 (0.04)	P = 0.01*
Heart rate	Surgical Prep	175.2 (16.6)	190.2 (11.4)	P = 0.036*
	Surgery	163.7 (16.2)	190.4 (19.9)	P = 0.011*
	Change	-11.5 (3.8)	0.2 (4.0)	P = 0.18

* Significant result; P < 0.05.

The total dose of propofol required for intubation was similar in all rabbits with 3.55 ± 1.5 mg kg^{-1} and 2.56 ± 2.21 mg kg^{-1} in groups BPB and CRI, respectively (Table 2). All of the rabbits in the CRI group were intubated successfully with a size 3.5mm internal diameter PVC endotracheal tube, whereas 5/9 in the BPB group were intubated, with supraglottic airway devices placed in the remaining four rabbits. In the latter cases, laryngeal masks were placed after three unsuccessful attempts to orotracheally intubate.

Baseline values for HR obtained prior to start surgical stimulation were significantly lower in group BPB compared to group CRI (Table 2). In addition, HR during surgery was lower in group BPB than in group CRI. However, the change in HR between baseline and during surgery was not significant different between the groups.

Mean FEISO (%) during surgical stimulation was significantly lower in group BPB than group CRI (Table 1).

The oscillometric blood pressure proved to be unreliable, providing inconsistent readings at multiple time points in many of the rabbits. Hence these data were excluded from any analysis.

All of the rabbits in group CRI required at least one bolus (range: 1 – 3 boluses) of rescue analgesia during surgery whereas no rabbit in group BPB required rescue analgesia (Table 3; $p < 0.0001$). However, in the first two hours post-operatively, there was no difference in the number of rabbits in each group receiving rescue analgesia (Table 3; $p = 0.49$).

Table 3. The frequency of intraoperative and postoperative rescue analgesia administration in group BPB and group CRI. No rabbits in group BPB required rescue analgesia compared to all rabbits in group CRI. Fisher exact tests were used to compare frequencies of rescue analgesia administration.

Rescue Analgesia	Group		Significance
	BPB	CRI	
Intraoperative administration	0/9	8/8	P < 0.0001*
Postoperative administration	2/9	3/8	P = 0.49

* Significant result; P < 0.05.

All rabbits had a temperature of at least 37°C at the time of recovery, measured by oesophageal and rectal thermometers.

 doi:10.20944/preprints202403.0234.v1

During the postoperative observation period, faecal production was similarly between group BPB and group CRI. Within 120 minutes, six out of nine rabbits in group BPB and four out of eight rabbits in group CRI produced faeces with normal morphology, with the remaining rabbits producing faeces of normal morphology within the first ten hours (Table 4). All rabbits were observed eating hay or pellets during the first two hours.

Post-operative behavioural assessment showed a similar occurrence of grooming of the operated front leg during the first 2 hours (Table 4). All rabbits showed hopping, grooming themselves in a generalised manner, and interacting with their environment (Table 4).

Table 4. The frequency of eating, normal faeces production and of recorded behaviours in group BPB and group CRI. Fisher exact tests were used to compare frequencies of the behaviours in each group.

Behaviour	Group		Significance
	BPB	CRI	
Normal faeces in first two hours	6/9	4/8	P = 0.63
Eating hay and/or pellets	9/9	8/8	P = 1
Grooming the Affected Limb	8/9	4/8	P = 0.13
Generalised grooming	9/9	8/8	P = 1
Hopping	9/9	8/8	P = 1
Sprawling	6/9	4/8	P = 0.63
Interacting with Environment	9/9	8/8	P = 1

Post-hoc analysis showed a similar activity budget for the first 2 hours with similar distances travelled in group BPB (mean = 17.4 +/- 10.77 m) and group CRI (mean 16.14 +/- 7.97 m); p = 0.89).

With regard to postoperative pain scores, a similar trend with decrease in score points over time was seen for both groups (Tables 5 and 6).

Table 5. Postoperative pain scores using the Rabbit Grimace Scale in rabbits undergoing experimental thoracic limb surgery with either brachial plexus block (BPB, n = 9) or intraoperative lidocaine (CRI, n = 8). Data are expressed as median (IQR).

Timepoint	Group	
	BPB	CRI
T0	4 (3 – 4)	4 (4 – 4)
T30	4 (3 – 4)	4 (3 – 4)
T60	3 (2 – 4)	4 (3 – 5)
T90	3 (2 – 4)	4 (4 – 6)
T120	3 (2 – 4)	4 (3 – 5)
T10h	2 (1 – 3)	2 (1 – 3)
T18h	2 (1 – 3)	2 (1 – 4)

Table 6. Postoperative pain scores using the Bristol Rabbit Pain Scale in rabbits undergoing experimental thoracic limb surgery with either brachial plexus block (BPB, n = 9) or intraoperative lidocaine (CRI, n = 8). Data are expressed as median (IQR).

Timepoint	Group	
	BPB	CRI
T0	8 (6 – 10)	10 (8 – 11)
T30	9 (7 – 11)	10 (8 – 10)
T60	6 (5 – 10)	9 (8 – 10)
T90	7 (4 – 9)	8 (7 – 10)
T120	5 (4 – 8)	8 (6 – 9)

 doi:10.20944/preprints202403.0234.v1

T10h	4 (3 – 6)	6 (3 – 7)
T18h	3 (2 – 5)	3 (2 – 4)

Three days postoperatively, all rabbits except one had lost weight. There was no significant difference in weight loss between the groups (Table 1) and no weight loss was of clinical concern.

4. Discussion

In this prospective study the peripheral nerve stimulator-guided brachial plexus block with lidocaine provided sufficient intra-operative analgesia but the lidocaine CRI did not, as evidenced by the unanimous requirement for rescue analgesia in the CRI group. This finding is in line with studies investigating the efficacy of brachial plexus blocks[16,19,20] . Successful block was assumed in all cases by consistent lack of response to surgical stimulation. All BPB rabbits exhibited incomplete motor function of the thoracic limb during the first two hours of recovery, but it was difficult to assess return of normal motor function due to the nervous temperaments of the rabbits and lameness associated with the surgical procedure. Potential complications associated with brachial plexus blocks include intraneural or intravascular injection, pneumothorax and, rarely, hemi-diaphragmatic paralysis following phrenic nerve anaesthesia. There were no complications associated with the brachial plexus block noted in this study. Two rabbits, one from each group, were euthanised seven days after surgery due to necrosis of the paw of the left thoracic limb, distal to the surgical site. This was unrelated to the brachial plexus block.

There are two previous studies describing brachial plexus blockade in rabbits, one using an ultrasound-guided axillary approach[16] and the other describing a combined ultrasound and nerve stimulator-guided axillary approach[17]. One reported advantage of using ultrasound guidance is a smaller volume of local anaesthetic required to achieve effective nerve block, however the total volume used in this study, 0.3 ml kg^{-1}, was smaller (0.7 - 0.8 ml kg^{-1}[16]) or comparable (0.8 +/- 0.3 ml in rabbits with a mean weight of 2.5kg[17]) to previous studies. In this study, adequate coverage of all nerves within the brachial plexus was not visually confirmed but efficacy of the block was assumed in all cases by a lack of expected response to surgical stimulus and no requirement for intraoperative rescue analgesia.

Although lidocaine CRIs have previously been found effective in managing pain associated with soft tissue surgical pain in rabbits[21] , it was not sufficient for orthopaedic surgery, which could be due more severe pain associated with orthopaedic surgeries. Intravenous lidocaine is associated with reduced postoperative pain and decreased recovery times in humans[22] and dogs[23] and has dose dependent minimum alveolar concentration (MAC) sparing effects in many species. In rabbits[24] a lidocaine infusions of 50 μg kg^{-1} min^{-1} and 100 μg kg^{-1} min^{-1} reduced the MAC of isoflurane by 12% and 21.7% respectively. In contrast, in the current study the mean end-tidal isoflurane concentration required to maintain stable anaesthesia was lower in group BPB, which could be due to insufficient analgesia in group CRI. Lidocaine overdose can result in adverse effects such as tremors, convulsions and arrhythmias, including bradycardia and prolonged PR and QRS intervals[25,26]. Rabbits have a high LD50 of 20 mg kg^{-1}[26], making lidocaine infusions relatively safe compared to other domestic species, and no adverse effects were observed during this study. The short duration of infusion during this study reduced accumulation of the drug and this reduces the risk of adverse effects, despite the high dose and loading dose used. The same infusion rate of 100 μg kg^{-1} min^{-1} has been used for two days without complications observed in rabbits following ovariohysterectomy[21].

The intra-operative differences in rescue analgesia were not reflected in the post-operative period in this study, during which there were no differences in pain scores or requirement for rescue analgesia. This could be due to a limited duration of action of lidocaine block in group BPB of 1-2 hours into the postoperative period. However, only five out of 17 rabbits required rescue analgesia in the two hours immediately post-op. One reason for this may have been limits in the ability to accurately detect pain effectively. Due the difficulty in assessing pain in prey species such as rabbits, multiple methods were used in this study. Both the Rabbit Grimace Scale[27] and the Bristol Rabbit Pain Scale[28] were used. The Rabbit Grimace Scale uses facial expression to evaluate pain. Based on

 doi:10.20944/preprints202403.0234.v1

the mouse and rat grimace scales[29,30], it uses similar facial action units which include orbital tightening, cheek flattening, nostril shape, whisker shape and position, and ear shape and position. It was found to be more reliable than behavioural markers of pain in rabbits undergoing ear tattooing. There are no pain scales validated for orthopaedic pain in rabbits. The Bristol Rabbit Pain Scale is a composite pain scale designed to aid pain assessment in rabbits experiencing acute pain. Since data collection, this scale has been validated for acute pain associated with ovariohysterectomy and orchiectomy[31]. This scale was used as a secondary indicator of pain, after the rabbit grimace scale to improve the ability to detect pain following surgery in these rabbits. The pain scales exhibited progressively stronger correlation with time. Neither scale is appropriate for use in sedated patients and the scores in the first two hours following surgery may have been affected by residual sedation. All of the rabbits were observed hopping, eating and interacting with their environment during this time but it is impossible to exclude the effect of anaesthesia on the pain scores during this period.

Food intake, faecal output and weight loss were assessed in this study because changes in both parameters have been associated with pain in laboratory animals[32–34]. Both pain and a change in food intake are risk factors for gastrointestinal stasis and are indicated by a reduction in food intake and change to number and morphology of faecal pellets. No control observations were made prior to surgical intervention but faecal output was subjectively reduced compared to the author's experience of normal faecal output in rabbits. However, none required intervention for gastrointestinal stasis or management of excessive weight loss. A single dose of buprenorphine (100 $\mu g\ kg^{-1}$ IM)[35] does not appear to reduce gastrointestinal motility in rabbits that have not been anaesthetised but general anaesthesia followed by buprenorphine (30 $\mu g\ kg^{-1}$) TID did increase gastrointestinal transit time and reduce faecal output[36]. Any prokinetic effects of the intraoperative lidocaine administration may have been reduced by the postoperative administration of buprenorphine.

The rabbits' activity and behaviour were also monitored to gain a thorough assessment of comfort and welfare. Ethograms, nesting behaviour and burrowing behaviour have all been used extensively to assess pain in laboratory rodents[37,38] but have been the subject of very limited investigation in rabbits. The object tracker reliably detected movement of the rabbits after the parameters were manually adjusted to filter out isolated head movement. Movement parameters have been included in some behaviour-based pain scales but none are validated[39]. This study found a very large variation in distance travelled by the rabbits in each group, with no significance difference between groups, suggesting that it is not a useful indicator of pain. However, the post-operative pain scores were also similar between groups so the value of this information in this study is limited. Pain may be expressed through general behaviour changes[32], or specific changes in response to the painful region. In this study twice as many rabbits in group BPB were observed grooming the affected leg during the first two hours than group CRI, which was unexpected. The return of normal sensation in the blocked limb may cause discomfort that caused the rabbits to groom more than those who retained normal sensation throughout. Though this difference was statistically insignificant, this study was underpowered in this regard and specific behavioural changes in response to pain and regional anaesthesia warrant further investigation in rabbits.

In an attempt to monitor pain as thoroughly as possible, both direct observation and videography were used to assess different parameters. Distant monitoring via video allows us to better assess natural behaviour and increases the possibility of objective measures such as distance travelled and duration spent performing behaviours of interest. However, direct observation allows for closer examination of the demeanour, alertness and facial signs of pain. It also allows for quantitative measurement of food intake and faecal output. A combined approach may provide the most effective method of assessing pain in rabbits who are less accustomed to human contact.

There were a number of limitations in this study. The sample size was controlled by primary orthopaedic study and limited the power to detect differences in postoperative pain score and behaviour between the groups, such as grooming the affected leg. The observers were not blinded to the groups which may have introduced bias into the pain scores. Many of the other parameters measured were objective measures recorded as either 'present' or 'absent' or were measured by the

 doi:10.20944/preprints202403.0234.v1

computer program, which reduce the potential to introduce bias to the results. The rabbits were acclimatised to receiving loose hay which was difficult to weigh accurately. Feeding only pellets for the duration of the study would have allowed for accurate measurement of food intake but this diet change may have increased the risk of gastrointestinal disturbance and ileus. As result, we simply recorded whether the rabbits had eaten or not, but food intake could be better quantified in future studies. After the first two hours, the frequency of pain scoring decreased and limited the observer's ability to detect difference between the groups. Between the study timepoints the rabbits were observed by trained staff at the facility and the study team notified if any individuals were exhibiting abnormal behaviour or signs of pain but these were not included in the study in order to reduce the number of observers and inter-rater variation. No concerns were raised between the study time points.

5. Conclusions

In conclusion, the axillary approach to the brachial plexus block, guided by a peripheral nerve stimulator was straightforward to administer and effective in providing analgesia for thoracic limb surgery in rabbits. No complications were reported in this study, suggesting that it is a safe technique. Pain remains challenging to assess in rabbits but the Bristol Rabbit Pain Scale is an exciting addition to the set of tools at clinicians' disposal, proving easy to use in this study.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Python code used to process the videos recorded during recovery.

Author Contributions: Conceptualization and methodology, SM and CG; formal analysis and computation, SM; investigation SM and CG.; resources, MA and SHE; data curation, SM and CG.; writing—original draft preparation, SM; writing—review and editing, CG; supervision, CG.; project administration, MA and SHE.; funding acquisition (main study), MA and SHE. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Experimental protocols were approved by the United Kingdom Home Office as governed by the UK law under the Animals (Scientific Procedures) Act 1986, project license number PP1153947, and abide by the ARRIVE guidelines. The work was conducted at University of Cambridge biomedical services research animal facilities.

Data Availability Statement: The data presented in this study are available on request from the corresponding author (accurately indicate status).

Acknowledgments: Thank you to the team at the University of Cambridge biomedical services research animal facilities for their care of the rabbits and for providing facilities and resources for the study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. McGuire L, Heffner K, Glaser R, Needleman B, Malarkey W, Dickinson S, Lemeshow S, Cook C, Muscarella P, Melvin WS, Ellison EC, Kiecolt-Glaser JK. 2006. Pain and wound healing in surgical patients. Ann Behav Med 31:165–172. https://doi. org/10.1207/s15324796abm3102_8.
2. Morrison RS, Magaziner J, McLaughlin MA, Orosz G, Silberzweig SB, Koval KJ, Siu AL. 2003. The impact of post- operative pain on outcomes following hip fracture. Pain 103:303– 311. https://doi.org/10.1016/S0304-3959(02)00458-X.
3. Hernández-Avalos I, Flores-Gasca E, Mota-Rojas D, Casas-Alvarado A, Miranda-Cortés AE, Domínguez-Oliva A. Neurobiology of anesthetic-surgical stress and induced behavioral changes in dogs and cats: A review. Vet World. 2021;14(2):393-404. doi:10.14202/vetworld.2021.393-404
4. Desborough J.P. The stress response to trauma and surgery. Br. J. Anaesth. 2000;85(1):109–117.
5. I. Chaniotakis, E. Antoniou, N. Kostomitsopoulos, S. Karapsias, P. Mirilas & C. Salakos (2018) Stress response to ovariohysterectomy in rabbits: role of anaesthesia and surgery, Journal of Obstetrics and Gynaecology, 38:5, 697-701, DOI: 10.1080/01443615.2017.1400523
6. Stenger V, Zeiter S, Buchholz T, et al. Is a Block of the Femoral and Sciatic Nerves an Alternative to Epidural Analgesia in Sheep Undergoing Orthopaedic Hind Limb Surgery? A Prospective, Randomized, Double

Blinded Experimental Trial. Animals (Basel). 2021;11(9):2567. Published 2021 Aug 31. doi:10.3390/ani11092567

7. Re, M., Blanco-Murcia, J., Villaescusa Fernández, A., De Gaspar Simón, I., & Gómez de Segura, I. A. (2014). Ultrasound-guided anaesthetic blockade of the pelvic limb in calves. Veterinary journal (London, England : 1997), 200(3), 434–439. https://doi.org/10.1016/j.tvjl.2014.04.010
8. Koehler, P., Romano, M., & Portela, D. A. (2022). Ultrasound-guided and nerve stimulator-assisted sciatic and saphenous nerve blocks in a goat undergoing tarsal fracture repair. Veterinary anaesthesia and analgesia, 49(6), 676–677. https://doi.org/10.1016/j.vaa.2022.09.001
9. Mohmmad Abrar Gayas, Mujeeb R. Fazili, Nida Handoo, Abrar A. Malik, Raja Aijaz, Syed Tahreem, Haris R. Beigh, Aamir S. Teeli, Riaz A. Shah, Distal paravertebral nerve block in goats undergoing reproductive laparoscopy: Are 1% and 2% lidocaine hydrochloride equally effective?, Small Ruminant Research, Volume 200, 2021, 106395, ISSN 0921-4488, https://doi.org/10.1016/j.smallrumres.2021.106395.
10. Trujanovic R, Otero PE, Marhofer P, Auer U, Kau S. Proximal Perineural Femoral Nerve Injection in Pigs Using an Ultrasound-Guided Lateral Subiliac Approach-A Cadaveric Study. Animals (Basel). 2021;11(6):1759. Published 2021 Jun 12. doi:10.3390/ani11061759
11. Lee MG, Choi SU, Lim JK, et al. Ultrasound-guided sciatic nerve block at the midthigh level in a porcine model: A descriptive study. Vet Med Sci. 2020;6(3):543-549. doi:10.1002/vms3.265
12. Marhofer, P., Greher, M. & Kapral, S. Ultrasound guidance in regional anaesthesia. Br. J. Anaesth. 94, 7–17 (2005).
13. Marhofer, P. et al. Ultrasonographic guidance reduces the amount of local anesthetic for 3-in-1 blocks. Reg. Anesth. Pain Med. 23, 584–588 (1998).
14. Torres Cantó L, Felisberto R, Economou A, Flaherty D, Moreno Aguado B, Tayari H. Ultrasound-Guided Dorsolateral Approach for Quadratus Lumborum Block in Rabbits (Oryctolagus cuniculus): A Prospective, Randomized, Blinded, Cadaveric Study Comparing Four Different Injectate Volumes. Animals (Basel). 2023;13(16):2559. Published 2023 Aug 8. doi:10.3390/ani13162559
15. Felisberto R, Flaherty D, Tayari H. Ultrasound-Guided Saphenous Nerve Block in Rabbits (Oryctolagus cuniculus): A Cadaveric Study Comparing Two Injectate Volumes [published correction appears in Animals (Basel). 2022 Jun 14;12(12):]. Animals (Basel). 2022;12(5):624. Published 2022 Mar 1. doi:10.3390/ani12050624
16. Fonseca C, Server A, Esteves M, Barastegui D, Rosal M, Fontecha CG, Soldado F. An ultrasound-guided technique for axillary brachial plexus nerve block in rabbits. Lab Anim (NY). 2015 May;44(5):179-84. doi: 10.1038/laban.732. PMID: 25897939.
17. Mencalha R, Sousa CA, Costa O, Abidu-Figueiredo M. Ultrasound and gross anatomy of the brachial plexus and major nerves of the forelimb. An anesthetic approach using the domestic rabbit (Oyctolagus cuniculus) as an experimental model1. Acta Cir Bras. 2016 Apr;31(4):218-26. doi: 10.1590/S0102-865020160040000001. PMID: 27168533.
18. Schnellbacher RW, Divers SJ, Comolli JR, et al. Effects of intravenous administration of lidocaine and buprenorphine on gastrointestinal tract motility and signs of pain in New Zealand White rabbits after ovariohysterectomy. Am J Vet Res. 2017;78(12):1359-1371. doi:10.2460/ajvr.78.12.1359
19. Portela DA, Verdier N, Otero PE. Regional anesthetic techniques for the thoracic limb and thorax in small animals: A review of the literature and technique description. Vet J. 2018;241:8-19. doi:10.1016/j.tvjl.2018.09.006
20. Ghadirian S, Vesal N, Maghsoudi B, Akhlagh SH. Comparison of lidocaine, lidocaine-morphine, lidocaine-tramadol or bupivacaine for neural blockade of the brachial plexus in fat-tailed lambs. Vet Anaesth Analg. 2016;43(1):109-116. doi:10.1111/vaa.12280
21. Schnellbacher RW, Divers SJ, Comolli JR, Beaufrère H, Maglaras CH, Andrade N, Barbur LA, Rosselli DD, Stejskal M, Barletta M, Mayer J, Rodriguez P, Quandt JE. Effects of intravenous administration of lidocaine and buprenorphine on gastrointestinal tract motility and signs of pain in New Zealand White rabbits after ovariohysterectomy. Am J Vet Res. 2017 Dec;78(12):1359-1371. doi: 10.2460/ajvr.78.12.1359. PMID: 29182394.
22. Groudine SB, Fisher HAG, Kaufman RP, et al. Intravenous lidocaine speeds the return of bowel function, decreases postoperative pain, and shortens hospital stay in patients undergoing radical retropubic prostatectomy. Anesth Analg 1998; 86:235–239.
23. Ortega M, Cruz I. Evaluation of a constant rate infusion of lidocaine for balanced anesthesia in dogs undergoing surgery. Can Vet J 2011; 52:856–860.
24. Schnellbacher RW, Carpenter JW, Mason DE, et al. Effects of lidocaine administration via continuous rate infusion on the minimum alveolar concentration of isoflurane in New Zealand White rabbits (Oryctolagus cuniculus). Am J Vet Res 2013; 74:1377–1384.
25. Liu PL, Feldman HS, Giasi R, et al. Comparative CNS toxicity of lidocaine, etidocaine, bupivacaine, and tetracaine in awake dogs following rapid intravenous administration. Anesth Analg 1983;62:375–379.
26. Plumb DC. Lidocaine. Plumb's Veterinary Drugs.

27. Keating SCJ et al. (2012) Evaluation of EMLA cream for preventing pain during tattooing of rabbits: Changes in physiological, behavioural and facial expression responses. PLOS ONE 7(9): e44437.
28. Benato L, Murrell J, Knowles TG, Rooney NJ. Development of the Bristol Rabbit Pain Scale (BRPS): A multidimensional composite pain scale specific to rabbits (Oryctolagus cuniculus). PLoS One. 2021 Jun 11;16(6):e0252417. doi: 10.1371/journal.pone.0252417. PMID: 34115781; PMCID: PMC8195426.
29. Sotocina SG, Sorge RE, Zaloum A, et al. The Rat Grimace Scale: A Partially Automated Method for Quantifying Pain in the Laboratory Rat via Facial Expressions. Molecular Pain. 2011;7. doi:10.1186/1744-8069-7-55
30. Matsumiya LC, Sorge RE, Sotocinal SG, et al. Using the Mouse Grimace Scale to reevaluate the efficacy of postoperative analgesics in laboratory mice. J Am Assoc Lab Anim Sci. 2012;51(1):42-49.
31. Benato, L., Murrell, J. & Rooney, N. Bristol Rabbit Pain Scale (BRPS): clinical utility, validity and reliability. BMC Vet Res 18, 341 (2022). https://doi.org/10.1186/s12917-022-03434-x
32. Leach MC, Allweiler S, Richardson C, Roughan JV, Narbe R, et al. (2009) Behavioural effects of ovariohysterectomy and oral administration of meloxicam in laboratory housed rabbits. Res Vet Sci 87: 336–347.
33. Weaver LA, Blaze CA, Linder DE, et al. A model for clinical evaluation of perioperative analgesia in rabbits (Oryctolagus cuniculus). J Am Assoc Lab Anim Sci 2010; 49:845–851.
34. Shavit Y, Fish G, Wolf G, et al. The effects of perioperative pain management techniques on food consumption and body weight after laparotomy in rats. Anesth Analg 2005; 101:1112–1116.
35. Deflers H, Gandar F, Bolen G, Farnir F, Marlier D. Influence of a single dose of buprenorphine on rabbit (Oryctolagus cuniculus) gastrointestinal motility. Vet Anaesth Analg. 2018 Jul;45(4):510-519. doi: 10.1016/j.vaa.2018.01.011. Epub 2018 Mar 29. PMID: 29880277.
36. Feldman ER, Singh B, Mishkin NG, Lachenauer ER, Martin-Flores M, Daugherity EK. Effects of Cisapride, Buprenorphine, and Their Combination on Gastrointestinal Transit in New Zealand White Rabbits. J Am Assoc Lab Anim Sci. 2021 Mar 1;60(2):221-228. doi: 10.30802/AALAS-JAALAS-20-000064. Epub 2021 Feb 25. PMID: 33632373; PMCID: PMC7974813.
37. Tappe-Theodor A, King T, Morgan MM. Pros and Cons of Clinically Relevant Methods to Assess Pain in Rodents. Neurosci Biobehav Rev. 2019 May;100:335-343. doi: 10.1016/j.neubiorev.2019.03.009. Epub 2019 Mar 15. PMID: 30885811; PMCID: PMC6528820.
38. Jirkof P. Burrowing and nest building behavior as indicators of well-being in mice. J Neurosci Methods. 2014 Aug 30;234:139-46. doi: 10.1016/j.jneumeth.2014.02.001. Epub 2014 Feb 10. PMID: 24525328.
39. Miller AL, Leach MC. Pain Recognition in Rabbits. Vet Clin North Am Exot Anim Pract. 2023 Jan;26(1):187-199. doi: 10.1016/j.cvex.2022.07.007. PMID: 36402481.