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Characterization of pain behaviors in kittens following ovariohysterectomy using video assessment

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This study aimed to characterize the duration and/or frequency of pain behaviors in kittens following ovariohysterectomy using video assessment. A total of 229 videos comprising 18 h of recordings were obtained during a prospective, randomized, clinical trial using an opioid-free protocol with (multimodal group, MMG) or without (control group, CG) multimodal analgesia. Videos included behaviors of 36 kittens (≤ 6 months) before and after surgery, as well as pre/post rescue analgesia. A veterinary behaviorist blinded to treatments and timepoints performed the behavioral assessment using an ethogram. Statistical analyses were performed using linear models ($P < 0.05$). Duration (%) of 'no attention to surroundings' (5 ± 16 and 0.0 ± 0.7, $P = 0.02$), 'lowered head position' (4 ± 12 and 0.3 ± 2, $P = 0.009$) and 'eyes partially closed' (15 ± 29 and 5 ± 17, $P < 0.02$) was longer in kittens before than after analgesia, respectively. When compared with baseline, kittens in MMG had longer duration of playing (i.e. 'pawing', %) (35 ± 34) than CG (7 ± 12, $P = 0.001$) at 1 h postoperatively. This study identified behavioral differences between painful and non-painful kittens following ovariohysterectomy contributing to feline acute pain assessment.

Keywords Kittens, Behavior, Acute pain, Feline, Pain assessment

Feline early-age neutering is one solution of a major societal challenge[1,2]. Free-roaming cats can experience suboptimal welfare but also inflict high levels of predation on wildlife[3]. According to the American Association of Feline Practitioners (AAFP) and the Canadian Veterinary Medical Association (CVMA), the USA and Canada are home to an estimated 70 to 100 and 5 to 9 million free-roaming cats, respectively[1,4]. Moreover, ethical concerns arise from the euthanasia of healthy animals in case of overpopulated shelters. Several organizations including the International Society of Feline Medicine (ISFM) and the AAFP now support early-age neutering (≤ 4 months)[1,2]. Unfortunately, adequate pain management is not always provided during early-neutering[5]. This concern may be due to the lack of drug availability or training in pain assessment of young cats, early patient discharge or even neglecting that kittens feel pain after common procedures such as castration or ovariohysterectomy.

Pain is a sensory and affective-emotional individual experience, impacting health and welfare[5,6]. Human and animal studies report that exposure to pain in early life has long-lasting consequences for sensory perception, stress responsiveness, changes in brain development and cognition, and emotional health[7,8]. Historically, feline pain has been neglected and underestimated. Although feline pain assessment has improved with the development of validated pain scoring tools[9–14], existing studies have primarily focused on adult cats[9,11,13,15]. Recently, the Feline Grimace Scale© (FGS) has been validated in kittens providing an option for pain assessment in early-age spay-neuter programs[16].

Behavioral signs of pain may be unique to the individual cat[5,17]. The 2021 AAFP/ISFM life stage guidelines state that each life stage has its own physical, nutritional, and behavioral characteristics[18]. Therefore, kittens have special needs and knowledge on the subject should not be extrapolated from adult cats. It is currently unknown whether evoked pain behaviors differ between kittens and adult cats. Quantitative (i.e. duration and frequency) assessment of pain-induced behaviors may identify behavioral variability and characteristics that can distinguish painful from non-painful individuals.

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Analgesia	Dependent variables	Mean ± SD	Independent variable	Estimates	SE	t-value	*P*-value
Post	Lowered head position (D)	0.3 ± 2	Intercept	0.368	0.963	0.382	0.703
Pre		**4 ± 12**	Pre-rescue analgesia	**3.872**	**1.461**	**2.649**	**0.009**
Post	Oriented head/eyes/ears (D)	**100 ± 0.7**	Intercept	**9.901**	**1.573**	**6.471**	**< 0.001**
Pre		**94 ± 17**	Pre-rescue analgesia	**-5.127**	**1.986**	**-2.581**	**0.011**
Post	No attention to surroundings (D)	0 ± 0.7	Intercept	0.105	1.567	0.067	0.946
Pre		**5 ± 16**	Pre-rescue analgesia	**4.576**	**1.945**	**2.352**	**0.021**
Post	Eyes partially closed (D)	5 ± 17	Intercept	5.112	3.246	1.574	0.118
Pre		**15 ± 29**	Pre-rescue analgesia	**9.91**	**4.201**	**2.359**	**0.02**
Post	Eyes open (D)	**93 ± 20**	Intercept	**93.136**	**3.884**	**23.976**	**< 0.001**
Pre		**74 ± 37**	Pre-rescue analgesia	**-18.691**	**5.299**	**-3.527**	**< 0.001**

Table 1. Results of linear mixed models and mean and standard-deviation comparing normal and pain-related behaviors in kittens undergoing ovariohysterectomy using an opioid-free with (multimodal group, MMG) or without (control group, CG) multimodal analgesia[19] pre- and post-administration of rescue analgesia. Ethogram-based behaviors were considered dependent variables and rescue analgesia (i.e. pre- and post-) an independent variable. Post-rescue analgesia was considered the reference category. D, duration of a behavior (%); F, frequency of a behavior (times/min). Bold is highlighting $P < 0.05$.

Group	Dependent variables	Mean ± SD	Timepoint	Independent variable	Estimates	SE	t-value	*P*-value
CG	Back of the cage (D)	**28 ± 20**	Baseline	Intercept	**28.31**	**6.84**	**4.13**	**0.002**
MMG		**54 ± 33**	Baseline	MMG	**25.89**	**9.54**	**2.71**	**0.01**
CG	Elsewhere in the cage (D)	**71 ± 21**	Baseline	Intercept	**71.68**	**6.84**	**10.47**	**0.00**
MMG		**45 ± 33**	Baseline	MMG	**-25.88**	**9.54**	**-2.71**	**0.01**
CG	Moving (D)	**24 ± 24**	1 h postoperatively	Intercept	**26.45**	**4.27**	**6.19**	**0.001**
MMG		**5 ± 4**	1 h postoperatively	MMG	**-21.42**	**6.18**	**-3.46**	**0.001**
CG	Attention to wound (F)	1 ± 0.2	1 h postoperatively	Intercept	0.08	0.16	0.5	0.62
MMG		**0.5 ± 1**	1 h postoperatively	MMG	**0.5**	**0.24**	**2.07**	**0.04**
CG	Pawing ribbon (D)	7 ± 12	1 h postoperatively	Intercept	7.59	5.49	1.38	0.17
MMG		**35 ± 34**	1 h postoperatively	MMG	**28.02**	**8.09**	**3.45**	**0.001**

Table 2. Results of linear mixed models and mean and standard-deviation comparing normal and pain-related behaviors in kittens undergoing ovariohysterectomy using an opioid-free with (multimodal group, MMG) or without (control group, CG) multimodal analgesia.[19] Ethogram-based behaviors were considered dependent variables; group allocation, baseline and 1 h postoperatively as independent variables. CG was the reference category. D, duration of a behavior (%); F, frequency of a behavior (times/min). Bold is highlighting $P < 0.05$.

This study aimed to characterize the duration and/or frequency of specific behaviors in painful (i.e. control group) versus pain-free kittens (i.e. multimodal analgesia group) following ovariohysterectomy using video assessment and an ethogram of acute pain behaviors in cats. The hypothesis was that the duration and/or frequency of some behaviors would be significantly different between painful and pain-free kittens as well as before and after the administration of rescue analgesia.

Results

Real-time pain assessment reported a higher prevalence of rescue analgesia in kittens in the control group (CG; $n = 18/18$) than in the multimodal group (MMG; $n = 2/18$)[19]. To mitigate bias, post-rescue time points were excluded from statistical analysis. Linear mixed models are described in Tables 1, 2 and 3. Non-significant results and full models are presented as supplementary material (Tables S5-S9).

Before and after rescue analgesia

Duration (%) of 'no attention to surroundings' (5 ± 16 and 0 ± 0.7, $p = 0.02$), 'lowered head position' (4 ± 12 and 0.3 ± 2, $P = 0.009$) and 'eyes partially closed' (15 ± 29 and 5 ± 17, $P < 0.02$) was longer in kittens before than after the administration of rescue analgesia, respectively. Inversely, duration (%) of 'oriented head/eyes/ears' (94 ± 17 and 100 ± 0.7, $P = 0.01$) and 'eyes open' (74 ± 37 and 93 ± 20, $P < 0.001$) were shorter before than after the administration of rescue analgesia, respectively (Supplementary Video).

MMG versus CG

Kittens in the MMG had longer duration of 'positioned in the back of the cage' (54 ± 33) than CG (28 ± 20, $P = 0.01$) at baseline. Kittens in the MMG had longer duration of 'pawing ribbon' (i.e. playing with an object) (35 ± 34) than CG (7.5 ± 12, $P = 0.001$) at 1 h postoperatively. 'Attention to wound' (times/min) (0.5 ± 1 and

Group	Dependent variables	Timepoint	Mean ± SD	Independent variable	Estimates	SE	t-value	*P*-value
CG	Moving (D)	Baseline	**12 ± 10**	Intercept	**12.34**	**4.41**	**2.79**	**0.01**
		1 h postoperatively	**24 ± 24**	1 h postoperatively	**13.36**	**5.17**	**2.58**	**0.01**
	Withdrawing/hiding (F)	Baseline	**0.2 ± 0.5**	Intercept	**0.26**	**0.09**	**2.81**	**0.01**
		1 h postoperatively	**0 ± 0**	1 h postoperatively	**-0.26**	**0.12**	**-2.05**	**0.05**
	Pawing ribbon (D)	Baseline	**57 ± 39**	Intercept	**56.92**	**6.45**	**8.81**	**<0.001**
		1 h postoperatively	**7 ± 12**	1 h postoperatively	**-49.37**	**8.79**	**-5.61**	**<0.001**
	Not pawing (D)	Baseline	**43 ± 37**	Intercept	**42.98**	**8.58**	**5**	**<0.001**
		1 h postoperatively	**77 ± 35**	1 h postoperatively	**34.25**	**11.82**	**2.89**	**0.009**
	Grooming post feeding (D)	Baseline	**9 ± 14**	Intercept	**9.64**	**2.36**	**4.07**	**0.007**
		1 h postoperatively	**0.7 ± 2**	1 h postoperatively	**-8.91**	**3.26**	**-2.73**	**0.01**
MMG	Back of the cage (D)	Baseline	**54 ± 22**	Intercept	**53.17**	**8.05**	**6.6**	**<0.001**
		1 h postoperatively	**36 ± 35**	1 h postoperatively	**-18.89**	**7.76**	**-2.43**	**0.02**
	Elsewhere in the cage (D)	Baseline	**45 ± 33**	Intercept	**46.84**	**8.03**	**5.83**	**<0.001**
		1 h postoperatively	**63 ± 35**	1 h postoperatively	**18.76**	**7.6818**	**2.44**	**0.02**
	Moving (D)	Baseline	**14 ± 11**	Intercept	**14.17**	**2.02**	**6.98**	**<0.001**
		1 h postoperatively	**5 ± 4**	1 h postoperatively	**-8.99**	**2.88**	**-3.12**	**0.007**
	Restlessness (D)	Baseline	0 ± 0	Intercept	-0.03	3.97	-0.008	0.99
		1 h postoperatively	**14 ± 24**	1 h postoperatively	**14.37**	**5.59**	**2.56**	**0.02**
	Attention to wound (F)	Baseline	0 ± 0	Intercept	0.03	0.17	0.2	0.83
		1 h postoperatively	**0.5 ± 1.0**	1 h postoperatively	**0.54**	**0.24**	**2.25**	**0.03**
	Grooming (D)	Baseline	1 ± 3	Intercept	1.11	2.22	0.5	0.62
		1 h postoperatively	**8 ± 13**	1 h postoperatively	**6.14**	**2.73**	**2.25**	**0.03**
	Pawing ribbon (D)	Baseline	**61 ± 31**	Intercept	**61.75**	**7.71**	**8**	**<0.001**
		1 h postoperatively	**35 ± 31**	1 h postoperatively	**-27.29**	**9.65**	**-2.82**	**0.01**
	Not pawing (D)	Baseline	**38 ± 31**	Intercept	**37.27**	**7.76**	**4.79**	**<0.001**
		1 h postoperatively	**58 ± 36**	1 h postoperatively	**24.05**	**7.8**	**3.08**	**0.007**

Table 3. Results of within-group comparisons using linear mixed models results of normal and pain-related behaviors in kittens undergoing ovariohysterectomy using an opioid-free with (multimodal group, MMG) or without (control group, CG) multimodal analgesia.[19] behaviors were considered dependent variables and timepoint 1 h postoperatively as independent variables. Baseline timepoint was the reference category. D, duration of a behavior (%). F, frequency of a behavior (times/min). Bold is highlighting $P < 0.05$.

1 ± 0.2, $p = 0.04$) was less frequently observed and duration of 'moving' was shorter in the MMG (5 ± 4) than CG (24 ± 24, $P = 0.001$) at 1 h postoperatively.

Within-group comparisons

Kittens in CG presented less frequently 'withdrawing/hiding (times/min) (0.2 ± 0.5 and 0 ± 0, $P = 0.05$) and shorter duration of 'pawing ribbon' (57 ± 39 and 7 ± 12, $P < 0001$) at 1 h postoperatively than baseline. Duration of 'not pawing' (43 ± 37 and 77 ± 35, $P < 0.001$) was longer in CG at 1 h postoperatively than baseline. Duration of 'grooming post-feeding' (9 ± 14 and 0.7 ± 2, $P = 0.01$) was shorter and duration of 'moving' (12 ± 10 and 24 ± 24, $P = 0.01$) longer at 1 h postoperatively than baseline.

In MMG, duration of 'positioned in the back' (36 ± 35 and 54 ± 22, $P < 0.001$) was shorter and duration of 'elsewhere in the cage' (63 ± 35.0 and 45 ± 33, $P = 0.02$) longer at 1 h postoperatively than baseline. Duration of 'grooming' (1 ± 3 and 8 ± 14, $P = 0.03$) was longer at 1 h postoperatively than baseline. Duration of 'pawing ribbon' (61 ± 31 and 35 ± 31, $P < 0.001$) was shorter and duration of 'not pawing' (38 ± 31 and 58 ± 36, $P = 0.007$) longer at 1 h postoperatively than baseline. Duration of 'moving' (14 ± 11 and 5 ± 4, $P = 0.007$) was shorter and duration of restlessness (14 ± 24 and 0 ± 0, $P = 0.02$) longer at 1 h postoperatively than baseline.

Discussion

This study characterized normal and pain-related behaviors in duration and/or frequency at different time points in kittens following ovariohysterectomy using video assessment. The study identified behaviors that were significantly different between painful and non-painful kittens (i.e. treatment groups), before and after the administration of rescue analgesia, but also before and after surgery (i.e. 1 h postoperatively).

Pain-related behaviors that have been described in adult cats (e.g. 'lowered head position'[13], 'eyes partially closed'[13] and 'no attention to surroundings')[20] were also observed in painful kittens in this study. The administration of analgesia changed the duration and frequency of these behaviors and cats returned to their normal behaviors once pain was controlled. Additionally, kittens were more attentive to the surroundings (i.e. 'oriented head/eyes/ears') and with 'eyes open' after analgesia. These results are not surprising considering the role of changes in facial expressions during acute pain assessment[13]. Indeed, grimace scales have been studied and validated in

several species[21]. Orbital tightening, ear and head position are three of the action units involved in acute pain assessment using the FGS[13,16], whereas the short-form of the UNESP-Botucatu Feline Pain Scale includes an item related to orbital tightening (i.e. the cat's eyes are partially closed)[10]. The Glasgow Multidimensional Pain Scale – Feline[12] also includes two questions related to muzzle tension and ear position.

Demeanor may bias pain assessment as shy or fearful cats may present high pain scores that are not necessarily related to pain[22]. Our exclusion criteria included cats with shy or feral behavior in an attempt to minimize the effects of demeanor on pain assessment. Kittens were from the same shelter but their levels of socialization were unknown. In addition to their different personality traits, different behavioral responses to stressors associated with transportation, hospitalization and surgery even using cat friendly interactive techniques may have been expressed[23,24]. Kittens in the MMG spent more time at the back of the cage at baseline than in the CG, perhaps indicating that their inhibition and/or avoidance response to environmental stressors before surgery was different from those in the CG. On the other hand, kittens in MMG were more active, moving in the cage, at 1 h postoperatively. These post-surgery changes in behavioral expressions can be related, but are not limited to, disinhibition, acclimation, the administration of anesthetics and analgesics, and/or ketamine-induced hyperlocomotion in the absence of pain.

Some changes in behavior occurred independently of treatment groups. 'Attention to wound' was less frequently observed in MMG than in CG at 1 h postoperatively. 'Attention to wound' has been reported as an indicator of pain in behavioral-based scales including the Glasgow Multidimensional Pain Scale – Feline[12] and the UNESP-Botucatu Feline Pain Scale[10]. This behavior was initially reported in adult cats after ovariohysterectomy[15] and described in dogs[25], horses[26], and rabbits[27]. Attention to wound may be considered an evoked behavioral response to protect and restore homeostasis in response to real and/or perceived noxious stimuli[28]. On the other hand, this study showed that this behavior was unrelated to postoperative pain and at least in kittens following ovariohysterectomy, it may represent a healthy cat that, in the absence of pain, will demonstrate its natural grooming behavior. Attention to the wound may also occur in an attempt to remove antiseptics used during surgery around the abdominal area. Grooming is a species-specific behavior of all Felidae species and an indicator of health status[8]. Indeed, grooming was significantly more frequent in pain-free kittens and not often observed post-feeding in painful kittens.

Duration of 'no attention to surroundings' was significantly longer in painful kittens. This behavior is similarly described as 'indifferent', 'not attentive, the cat may face the back of the cage' in the UNESP Botucatu Feline Pain Scale[10] and as 'disinterested/quiet, dull or depressed/grumpy' in the Glasgow Multidimensional Pain Scale – Feline[12]. However, longer episodes of 'restlessness' were observed in MMG and 'moving' in CG kittens at 1 h postoperatively when compared with baseline. It is possible that residual effects of anesthetic drugs affected postoperative locomotor behaviors and ketamine may increase locomotor activity[29]. Therefore, these findings may be reflective of the injectable anesthetic protocol used in the study.

Playing behaviors have a crucial role in neurophysiological, motor and behavioral-cognitive skills, particularly at early ages[8]. The duration of playing was significantly lower postoperatively in painful compared to pain-free kittens (i.e. kittens with pain scores requiring rescue analgesia versus those without). Decreased playfulness can be considered a behavioral indicator of pain, and has been described in adult cats with oral pain after multiple dental extractions[20]. In adult cats, behavioral changes associated with distress were observed in the absence of playing[30]. Kittens are notorious for engaging in social, individual or object-directed play and/or spontaneous/ludic play when developing their predatory and social skills[31,32]. This study showed that playing is affected by pain in kittens and may represent a behavioral sign of positive welfare states in the hospital setting. One can infer that a playful kitten after surgery is likely not painful.

Previous studies described lower dynamic interactive visual analog scale and simple descriptive scale scores in kittens when compared with adult cats[33,34] and lower mechanical nociceptive thresholds 24 h hours after ovariohysterectomy[33,34]. These pain scoring systems have not been validated for acute pain assessment in cats but may demonstrate changes in the expression of pain related to aging. Another study reported that kittens had a lower prevalence of rescue analgesia when compared with adult cats when using an opioid-free injectable protocol for ovariohysterectomy[35]. It is not known if these differences are related to the previous lack of validated pain scoring tools in kittens[16], pharmacokinetic-pharmacodynamic differences of analgesics related to aging[36], the lack of training in kitten pain assessment or the anecdotal idea that young cats may feel less pain than adult ones. Future studies may characterize pain-induced behaviors between adult cats and kittens using identical settings for comparison.

This study has limitations. Although video recordings were standardized, video trimming for blinding resulted in length variations. As a result, some behaviors may have been under- or overestimated. Behavioral coding was performed by a single board-certified observer; therefore, it is not known how the results would vary if individuals with different training performed coding. Assessing inter- and intra-rater reliability assessment (e.g. to evaluate whether the behavioral assessment remains consistent across different observers and over time) would have enhanced the robustness of the findings and reduced biases. This study included kittens between 10 weeks and 6 months of age and some behavioral differences were therefore possible in these kittens due to age. The authors used the ISFM life stage guidelines to define kitten aging[18] and the aim of the study was to characterize pain-induced behavioral changes in this population, instead of a subpopulation of kittens of the same age. Moreover, the results are from a prospective, randomized, clinical trial design involving kittens following an elective procedure using an injectable anesthetic protocol. It is unknown how different surgical procedures, anesthetic or analgesic protocols or coding would modify results of the study. Finally, coding of 'depressed' behavior was not conducted in a singular dimension due to its complexity and subjective nature of interpretation. Thus, qualitative behavioral assessment, as reported in other species[37,38], may be an adequate alternative in addition to quantitative measurements, if the goal is a deep understanding of affective-emotional states intertwined with pain-related behaviors.

This study identified behavioral differences between painful and non-painful kittens following ovariohysterectomy contributing to feline acute pain assessment and promoting feline welfare.

Materials and methods

Ethical statement

This study was approved by the Institutional Animal Care and Use Committee of the Université de Montréal (21-Rech-2132) and conducted at the veterinary teaching hospital (Centre Hospitalier Universitaire Vétérinaire - CHUV) of the Faculty of Veterinary Medicine, Université de Montréal, June to August 2021. This study is performed according to the Canadian Council on Animal Care (CCAC) and the Animal Research Report of In Vivo Experiment (ARRIVE) guidelines[39,40]. Kittens enrolled in the study were part of a larger experiment[16,19], which contributes to one of the 3 R's of animal experimentation (i.e. reduce)[39].

Study design

This was a prospective, blinded, randomized clinical trial. The study was designed to compare an opioid-free injectable anesthesia with or without multimodal analgesia in kittens following ovariohysterectomy[19]. Video recordings occurred simultaneously to validate the Feline Grimace Scale© (FGS)[16] and to characterize pain behaviors in kittens (i.e. current methodology).

Animals

Thirty-six domestic short-haired cats from shelter facilities were included. Inclusion criteria involved healthy kittens of any breed ranging from 10 weeks to 6 months of age. Kittens were considered healthy based on history and physical examination using feline-friendly interactive techniques[23]. Written informed consent for each kitten enrolled in the study was obtained and signed by a representative of the responsible shelters[19]. At the end of the study, kittens were returned to the shelter for adoption[19].

Anesthesia and pain management

Anesthesia was performed using an injection of ketamine-dexmedetomidine-midazolam [4 mg/kg, 40 µg/kg and 0.25 mg/kg, respectively] mixed in the same syringe and administered into the lumbar epaxial muscles. In the MMG group ($n = 18$), kittens received meloxicam [0.1 mg/kg SC] and intraperitoneal bupivacaine 0.25% [2 mg/kg], whereas the same volume of saline was administered in the CG group ($n = 18$). Atipamezole [0.4 mg/kg IM) was given 15 min postoperatively. Rescue analgesia (buprenorphine 0.02 mg/kg IM in MMG/CG and meloxicam 0.1 mg/kg SC in CG) was administered if pain scores were ≥ 4/12 on the short-form of the UNESP-Botucatu Feline Pain Scale (i.e. pain assessment was performed before surgery and at 1, 2, 4, 6, 8, 12 and 24 h postoperatively)[10,19]. In the previous study, no significant differences were found between treatment groups regarding surgery and anesthesia variables. In CG and MMG, mean onset of anesthesia was 105 and 101 s, respectively. The mean duration of surgery and anesthesia were approximately 14 and 40 min, respectively. Time to head lift occurred at 17 (CG) and 19 min (MMG), and time to sternal recumbency at 18 (CG) and 19 min (MMG). The studies are reported in detail elsewhere[16,19].

Video recording

Upon admission and complete physical examination, kittens were housed (i.e. from arrival until discharge) in individual cages containing water, food bowl, a card-board box, litter box, and blankets for approximately 16 h before elective ovariohysterectomy. The cage walls were covered with a light-colored card-board to ensure background homogeneity. Pain scoring and video recordings of six to nine minutes were performed at six time-points (e.g. baseline, 1 h, 2 h, 4 h, 8 h and 24 h). Two wide-angle glass lenses high-definition cameras (GoPro Hero5 and GoPro Hero9; Riverside, CA, USA) were used and each was attached to the cage bars for video recording. Kittens were allowed to acclimate to the camera's position for 10 min. For baseline, and 1 h, 8 h and 24 h after surgery, the cage was emptied (e.g. aforementioned items were removed; however, the kittens remained in their respective cages) and video-recordings were performed in the following order: (a) general behavior without any interaction with observers during three minutes; (b) playing behavior using a ribbon toy during two minutes, (c) feeding behavior immediately after offering soft food for two minutes, (d) post-feeding behaviors immediately after removing soft food during two minutes. For time points 2 h and 4 h after surgery, video recordings were performed for general behaviors during six minutes. Additional pain scoring and video recordings were also performed 30 min after the administration of rescue analgesia.

Video editing

Videos were edited using Movavi Video Editor 2021 (www.movavi.com) by one observer (SM) blinded to treatment groups. Any information related to the kitten and time point was deleted. At the end of the study, there was a total of one video per time point for each kitten and two extra videos for painful kittens (before and after rescue analgesia; six to eight videos in total for each kitten). Six videos were deleted due to technical issues. Following video editing (trimming), a total of 229 videos were randomized (http://www.randomization.com) and labeled into consecutive numbers.

Video analysis

A total of 18 h of video material were analyzed. Videos were assessed by a board-certified behaviorist (DF) of the American College of Veterinary Behaviorists, blinded to treatments groups. The % duration (i.e. duration of each behavior/video length x 100) and frequency (i.e. number of times for an event/minute or total number of each behavior during the video/video length) of each behavior were characterized using a software (The Observer XT, Noldus Information Technology, VA, USA) and based on an ethogram of acute pain behaviors in cats[41]. This

software converts duration of behavior (i.e. state events; the proportion of time a behavior is present within the observed period) into percentages based on video length, while transforming behavioral counts into frequencies (i.e. point events), ensuring consistency and continuous data representation despite variations in video length. The ethogram consisted of a repertoire of twenty-four pain-related and normal behaviors of cats and were used during behavioral coding[41].

Coding

Four ethogram-based schemes were used for behavioral coding (supplementary material, Table S1-S4). In addition to the behaviors described in the ethogram, position in the cage (back of the cage versus elsewhere), exploratory behaviors (no attention to surroundings versus oriented head/eyes/ears), posture & body position (crouched/hunched up position, lying dorsoventrally with limb extended/contracted, lying dorsoventrally or laterally, not lying) were added into the coding. For the category 'Activity', 'moving [i.e. *free-roaming normally in the cage*]' and 'immobile not hunched/crouched' [i.e. *immobile in addition* to *changes in position from sitting to lying or vice-versa*] were included to differentiate from 'restlessness' and 'crouched/hunched', respectively. Head position was assessed as 'normal', 'lowered' or 'not visible'. 'Lowered head position' was only considered when the head was below the shoulder line or angled down with the chin moving towards the chest and the cat was inactive. Coding for eyes included 'eyes open' [i.e. *round-shaped eyes*, *completely open*], 'eyes partially closed' [i.e. *around 50% of eyelid closure*, *visible sustained orbital tightening*], feigned sleep [i.e. *eyes partially closed*], 'eye squinting' [i.e. *eyelid closure tightly and briefly*], 'blepharospasm' [i.e. *one eye eyelid closure*], and 'blinking' [i.e. *eyelid closure completely and open eyelids rapidly; no tension*].

Statistical analysis

Statistical analyses were conducted using R software (https://www.r-project.org/; version 4.2.3). Repeated measures linear mixed effect models were used to compare within-group behaviors pre- and post-surgery (i.e. baseline vs. 1 h), pre- and post-rescue analgesia, and between CG and MMG at baseline and 1 h after surgery. Individual kittens were considered as random effect and behaviors as dependent variables. '*Surgery*' (e.g. baseline and 1 h postoperatively), '*analgesia*' (e.g. pre- and post-), and '*groups*' (e.g. CG or MMG) were considered as independent variables. Significance was considered at 5% ($p < 0.05$). Feeding behavior was not included in the statistical analysis since food intake between groups was previously compared and reported elsewhere[19]. Behaviors that were coded less than five times[20] and/or not related to abdominal pain (e.g. abnormal gait, non-weight bearing, blepharospasm, difficult grasping or holding food and head shaking during feeding) were not included in the analysis.

Conference presentation

An abstract of this study was part of an oral presentation at the International Society of Feline Medicine Congress in Malta (June 2024). This manuscript represents a portion of a Ph.D degree thesis of the first author to the Université de Montréal.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

All authors participated in the scientific discussion. S.M. and P.V.S. conceived the research. S.M performed video edition, randomization and database organization. D.F performed behavioral coding. S.M and P.V.S. wrote the manuscript. All authors revised and commented on the manuscript. P.V.S. supervised and was the principal investigator related to funding.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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