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Implementation of a Functional Observation Battery for the Assessment of Postoperative Well-being in Rats Subjected to Fimbria-Fornix Transection

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Abstract. *The postoperative well-being of Wistar rats subjected to fimbria-fornix transections was assessed using a functional observational battery (FOB), including observations of relative body weight change, general condition, fur quality, body posture and movement, appetite, and pica behavior. Fimbria-fornix transected animals (FF), sham-operated animals (Sham), and two non-operated control groups with and without administration of buprenorphine (+BUP and −BUP, respectively) were observed twice daily for seven days after surgery. Buprenorphine (0.4 mg/kg) mixed in a nut paste for voluntary ingestion was supplied twice daily for 84 h to all groups except the −BUP control group starting on the day of surgery. Body weight was slightly decreased postoperatively in both surgical groups (FF and Sham) compared to control groups. The +BUP control group lost weight starting at day four after discontinuation of buprenorphine. Furthermore, the FF group exhibited significantly reduced general condition one day after surgery, with significantly affected body posture and movement for two days after surgery. In addition, mild pica behavior was observed in the FF group during the first postsurgical day. In conclusion, the FOB implemented in the present study appears to be a sensitive and accurate protocol for assessing animal well-being in the experimental setup applied. It is apparent that the FF transection is an invasive procedure that causes mildly adverse postoperative effects on the rats' well-being. We therefore recommend that this FOB is applied as a routine welfare monitoring protocol in experiments using mechanical central nervous system injury models, such as FF transection.*

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Accurate monitoring of postoperative recovery in laboratory animals is essential in order to ensure the highest possible animal welfare and well-being. Postoperative pain and stress can alter physiology, behavior and endocrine data and thereby bias experimental data (1, 2). This requires the use of welfare monitoring protocols that are predictive of animal well-being and that are feasible for the person undertaking the monitoring (3-5). In the present study, we investigated changes in well-being and body weight of the laboratory rat after fimbria-fornix (FF) transection.

The FF transection paradigm has been used as a model of anterograde, retrograde and transneuronal degeneration (6-9). In the adult mammalian brain, the FF conveys afferent and efferent projections between the basal forebrain and the hippocampal formation (10). Fimbria-fornix damage significantly reduces hippocampal cholinergic and γ-amino-buturic acidergic innervation (11, 12), and causes long-term impairment of spatial learning and memory (13-16).

In the central nervous system, acute and chronic stress, for example caused by pain, may initiate structural and neurophysiological changes in hippocampus, amygdala, prefrontal cortex, and the paraventricular nucleus of the hypothalamus [for review, see (17)], which can result in biased data obtained with animal models. Furthermore, animals may differ in their pain and stress responses, thereby increasing data variation (17), and consequently increasing the number of animals needed to obtain sufficient statistical power. Thus, there is a need for thorough and accurate postoperative monitoring of laboratory animals when using mechanical brain injury techniques such as the FF transection in order to ensure animal welfare and optimize data validity and reliability. We, therefore, implemented an adapted functional observational battery (FOB) comprising

parameters and methods well-recognized in the laboratory animal literature [see *e.g.* (18)]. These included the animals' postoperative body weight changes, general condition, fur quality, body posture and movement, appetite, and pica behavior (the consumption of non-nutritive material).

The surgical procedure undertaken in the present study requires peri- and postoperative analgesic treatment. Buprenorphine is one of the most widely used drugs for postoperative pain alleviation in laboratory rodents (19). It is most commonly injected subcutaneously but a number of studies have shown that oral administration of the drug mixed in a palatable food item (for example in the nut paste Nutella®) for voluntarily ingestion is not only an effective analgesic strategy but also reduces stress and corticosterone levels in the blood and feces of laboratory rodents (20-26). Furthermore, voluntarily ingested buprenorphine resulted in a higher and prolonged serum buprenorphine concentration when compared to traditional parenteral administration regimens (22, 26). Based on this knowledge and in addition to our own experience that non-steroid anti-inflammatory drugs are unsuitable due to risk of postoperative intracranial bleeding, the abovementioned analgesic regimen of voluntary ingestion of buprenorphine was applied in the present study. However, studies have indicated adverse effects of buprenorphine treatment on food consumption and body weight of laboratory animals (27, 28). In addition, studies have shown that the use of high doses of buprenorphine is associated with pica behavior and abdominal distension (27, 29). Therefore, an additional control group neither undergoing surgery nor receiving buprenorphine was included in the present study in order to investigate possible effects on body weight and pica behavior due to the buprenorphine treatment itself.

Thus, the aim of the present study was to investigate recovery after a FF transection by implementing an FOB as well as a buprenorphine analgesic regimen. We hypothesized that the FF transection surgery would inflict minor welfare issues in comparison to sham–operated animals and non-operated animals and that this would be detected by the chosen FOB. Furthermore, it was hypothesized that the chosen analgesic treatment in itself would have little or no adverse effects on the animals' well-being.

Materials and Methods

Subjects. Forty-eight experimentally naïve, male Wistar rats (Taconic A/S, Borup, Denmark), aged 8-10 weeks and with a body weight of approximately 250-300 g at the beginning of the experimental procedures, were pair-housed in makrolon type 3 cages with elevated lids allowing rearing in the cage, under controlled temperature (22±2˚C) and humidity (50±5%). The diurnal rhythm was regulated through a 12 h light/12 h dark cycle (lights on at 19:00.). Food (commercial rat chow) and water were provided *ad libitum*. The experiments were carried out in accordance with the guidelines of the Danish Animal Experimentation Act and the European Directive of 22 September 2010 (2010/63/EU). The animals were randomly divided into four groups: i. Non-operated control group, not subjected to analgesic buprenorphine treatment (–BUP, n=9). ii. Non-operated group subjected to analgesic buprenorphine treatment (+BUP, n=11). iii. Sham-operated group subjected to peri- and postoperative analgesic buprenorphine treatment (Sham, n=11). iv. Fimbria-fornix-transected group subjected to peri- and postoperative analgesic buprenorphine treatment (FF, n=17).

Surgery. Surgery, that lasted approximately 30 min per animal, was performed with the aid of a surgical microscope under clean conditions. All experimental animals were anaesthetized by intraperitoneal injection of medetomidine ('Dexdomitor', 0.05 mg/kg) and ketamine ('Ketaminol', 75 mg/kg). Additionally, every animal was intraperitoneally administered 1% atropine sulphate (0.9 mg/kg). The scalp was swabbed with iodine and a subcutaneous injection of lidocaine was administered prior to making a midline incision to expose the skull. Bilateral transections of the fimbria-fornix were performed stereotaxically using a wire-knife. Detailed descriptions of the surgical procedures have been published previously (16, 30, 31). Surgical procedures for the sham-opened group were similar to those of the surgical group with the exception that no damage was inflicted to the skull and the brain tissue. The +BUP and –BUP groups were subjected to the anesthetic procedures without induction of any trauma to the skin, scalp or brain tissue.

Analgesia. On the first day of the experiment, three days prior to surgery, Nutella® (2 g/kg body weight/day; Ferrero, Pino Torinese, Italy) was introduced as part of the diet. Analgesia was given in accordance with the method used by Abelson *et al*. (32). Briefly, animals received 0.4 mg/kg buprenorphine ('Temgesic') ground to a fine powder and mixed in Nutella® at a concentration of 0.2 mg/g. During treatment animals were separated for one hour to ensure consumption of the correct dosage. The analgesic treatment was given twice daily (at 8 a.m. and 4 p.m.) and continued for 84 hours postoperatively.

Postoperative monitoring. All animals were weighed once daily for seven days (at 8:00). Additionally, the animals were observed for seven days, twice daily, at 8:00 and 16:00. An adapted functional observational battery comprising parameters and methods well-recognized in laboratory animal literature was applied [see *e.g.* Moser 1989 (18)]. These included the general condition of the animal, fur quality, body posture and movement, appetite, pica behavior as well as an overall behavior-based clinical assessment. All items were scored from 0 to 2 (0=normal state, 1=mild changes from the normal state, 2=pronounced changes from the normal state).

Statistical analysis. Data was analyzed by using SPSS version 20 (SPSS, Chicago, IL, USA). Differences in relative body weight, general condition of the animal, fur quality, body posture and movement, appetite and pica behavior were analyzed separately by utilizing repeated-measures analysis of variance (ANOVA). In cases of violated sphericity, Greenhouse-Geisser correction was applied. If the analysis of variance revealed significant differences, simple effect analyses and Bonferroni adjusted *post-hoc* pairwise comparisons were conducted to examine differences between the individual groups. Significance level was defined as $p<0.05$.

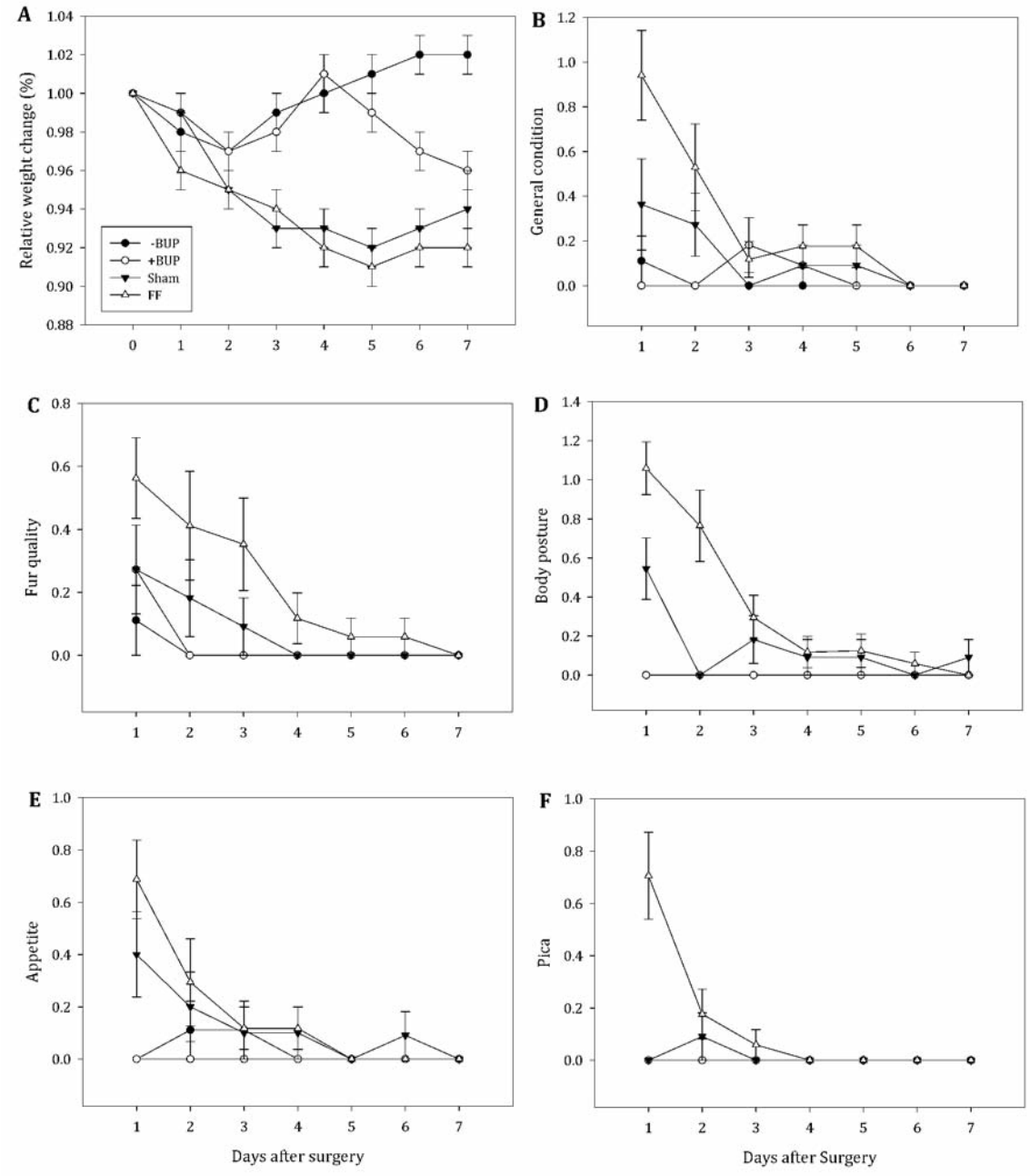

Figure 1. *Relative change in body weight from body weight before surgery (A), general condition (B), fur quality (C), body posture and movement (D) appetite (E) and pica behaviour (F). Bars display means±SEM. −BUP: Non-operated control group, not subjected to analgesic buprenorphine treatment (n=9); +BUP: Non-operated group subjected to analgesic buprenorphine treatment (n=11); Sham: Sham-operated group subjected to peri- and postoperative analgesic buprenorphine treatment (n=11); FF: Fimbria-fornix-transected group subjected to peri- and postoperative analgesic buprenorphine treatment (n=17).*

Results

Body weight. Changes in relative body weight from the preoperative weight are shown in Figure 1a. Repeated-measures ANOVA showed a significant effect of day [$F(3,513)=26.511$, $p<0.001$, $\eta_p^2=0.376$] and surgical group [$F(3,44)=16.324$, $p<0.001$, $\eta_p^2=0.525$] with the Sham as well as the FF group being significantly different from both control groups ($p<0.001$). No significant difference was obtained between the Sham and FF groups ($p=1$) or between

the two control groups (p=0.798). However, when only looking at data after discontinuation of buprenorphine treatment, analysis yielded a significant effect of surgical group [F(3,44)=20.938, p<0.05, η_p^2=0.588], in addition to a significant difference between the −BUP and +BUP groups (p<0.05). Overall, relative weight change was lowest for −BUP (M=0.9995, SE=0.008) followed by +BUP (M=0.983, SE=0.007) and Sham (M=0.948, SE=0.007) groups and highest for the FF group (M=0.940, SE=0.006). Furthermore, the interaction between day and surgical group was significant [F(10,538)=15.1, p<0.001, η_p^2=0.507].

General condition. Repeated-measures ANOVA yielded a significant effect of day, [F(6,264)=7.261, p<0.001, η_p^2=0.148], with decreasing values from postsurgical day 1 to 7, and surgical group [F(3,44)=4.00, p<0.05, η_p^2=0.214], and a significant interaction of day and surgical group [F(18,264)=3.936, p<0.001, η_p^2=0.212). The effect of surgical group was characterized by the FF group being significantly different from both non-operated groups (p<0.05), but not the Sham group (p=0.391). No significant difference was observed between the Sham and the non-operated groups (both p=1). Overall, values were lowest for –BUP (M=0.016, SE=0.073) followed by +BUP (M=0.039, SE=0.066) and Sham (M=0.117, SE=0.066) and highest for FF (M=0.277, SE=0.053).

Fur quality. Repeated-measures ANOVA yielded a significant effect of day [F(6,258)=7.932, p<0.001, η_p^2=0.156] with decreasing values from day 1 to 7, and group [F(3,43)=3.489, p<0.05, η_p^2=0.196]. However, pairwise comparisons showed no significant differences between groups (all p >0.05).

Body posture and movement. Repeated-measures ANOVA yielded a significant effect of day [F(3,91)=13.529, p<0.001, η_p^2=0.248), with decreasing values from day 1 to 7 and group (F(3,41)=8.741, p<0.001, η_p^2=0.39). Pairwise comparisons yielded a significant difference between FF (M=0.357, SE=0.051) and −BUP (M=0.00, SE=0.069) as well as +BUP (M=0.000, SE=0.065, both p<0.01). No difference was found between Sham (M=0.157, SE=0.065) and any of the other groups (all p>0.05). Furthermore, the interaction between day and surgical group was significant [F(11,729)=8.853, p<0.001, η_p^2=0.393].

Appetite. Repeated-measures ANOVA yielded a significant effect of day [F(6,234)=5.249, p<0.001, η_p^2=0.119], with decreasing values from day 1 to 7 and group [F(3,39)=3.022, p<0.05, η_p^2=0.189]. However, pairwise comparisons showed no significant differences between groups (all p>0.05). The interaction between days and group also proved to be significant [F(18,234)=2.712, p<0.001, η_p^2=0.173].

Pica behavior. Repeated-measures ANOVA yielded a significant effect of day [F(6,264)=5.335, p<0.001, η_p^2=0.108], with decreasing values from day 1 to 7 and group, F(3,44)=9.667, p<0.001, η_p^2=0.397. Pairwise comparisons showed significant differences between FF (M=0,134, SE=0.019) and all other groups (−BUP: M=0.00, SE=0.027; +BUP: M=0.00, SE=0.024; Sham: M=0.013, SE=0.019; all p<0.01). All other comparisons were non-significant (p=1). Furthermore, the interaction between day and surgical group was significant [F(18,234)=6.673, p<0.001, η_p^2=0.313].

Discussion

In the present study, we assessed changes of body weight and well-being in the laboratory rat after FF transections. We analyzed postoperative weight loss and well-being (*i.e.* the general condition of the animals, fur quality, body posture and movement, appetite, and pica behavior) with the aid of an FOB. We included four experimental groups: A cage control, not subjected to surgery or analgesic treatment; a second group not subjected to surgery but given buprenorphine analgesia; a third group subjected to sham-surgery and postoperative analgesic treatment; and a fourth group subjected to surgery in the form of FF transection and given postoperative analgesia.

Analysis of the FOB data showed expected changes in postoperative well-being in the FF and the sham-operated group, with a slightly greater effect in the FF group. Decrease in food intake and weight loss are considered common adverse side-effects of buprenorphine and are attributed to a loss of appetite, metabolic changes (33) or pica (27). However, the weight loss seen in the present study is not pathological (34) and can be considered to be transient.

No initial weight change difference was observed between the non-operated groups. However, the post-anesthetic weight of the buprenorphine-treated control animals progressed in an intriguing way. To begin with, the +BUP control animals followed the same course of initial weight loss after anesthesia and subsequent weight gain as seen in the group not given buprenorphine. After discontinuation of buprenorphine treatment, however, animals started losing weight again, leading to a weight loss of greater magnitude than the initial weight loss after anesthesia. A study by Liles and Flecknell suggested that analgesic treatment with buprenorphine might modify food intake and behavior; this effect seemed to be dose-dependent (28). In a study by Jablonski, Howden and Baxter, investigating effects of buprenorphine treatment after laparotomy causing mild pain, it was shown that buprenorphine injections (0.01 mg/kg or 0.05 mg/kg) had suppressing effects on food intake, resulting in weight loss after postoperative day one (35). The same

effect was seen in a study by Kalliokoski *et al*. Here, animals treated with voluntarily ingested buprenorphine (0.6 mg/kg) exhibited significantly lower body weight between post-surgical day four and seven than did non-treated animals (26). It is conceivable that this food intake-suppressing effect of buprenorphine might also have had an influence on the data presented in our study, since rats that were not in pain were treated with a relatively high dose of orally provided buprenorphine for a relatively long time. However, weight changes in the +BUP compared to the −BUP group set in only after buprenorphine was discontinued, rendering this explanation unlike. Furthermore, Jacobson, who likewise observed reduced weight gain in a buprenorphine-treated group, ascribed those weight changes to ingestion of non-nutritive material (*e.g*. bedding material) likely caused by the buprenorphine (27). This so-called pica behavior, however, was not observed in buprenorphine-treated naïve rats in our study, rebutting this explanation for the deviation in weight change over the course of the experiment in the buprenorphine-treated group.

Another possible explanation might be found in the attributes of buprenorphine as an addictive drug. Even though buprenorphine has generally been stated to be a drug with low abuse potential and been indicated as a therapeutic agent in the management of opiate addicts (36), buprenorphine abuse among opiate addicts seems to be increasing, and withdrawal from buprenorphine resembles opiate withdrawal (37). One possible explanation of a change in weight loss in the +BUP control animals might be caused by habituation of the animals to buprenorphine. This habituation might have been accelerated by the fact that these animals were never in any pain to begin with. It is possible that the weight loss after the discontinuation of buprenorphine might be an indicator of withdrawal; however, more studies are needed to further elucidate this finding.

In contrast to the non-operated control and Sham groups, the FF-transected animals exhibited pronounced pica behavior during the first postoperative days. Rats lack the emetic reflex but have been reported to engage in pica behavior in reaction to nausea (38). No pica behavior was observed in the sham- or non-operated groups. Furthermore, the observed ingestion of bedding and tissue decreased in synchrony with improvement in the general condition, fur quality, body posture and movement, as well as appetite, of the animals, indicating that pica might have been caused by brain surgery itself. However, it should be of little or no consequence to data collection later on.

In summary, the FOB implemented in the present study seems to be a sensitive and accurate protocol for assessing animal well-being in the applied experimental setup. It is apparent that FF transection has minor yet significant adverse effects on the postoperative well-being of the rats. We therefore recommend that this FOB is applied as a routine welfare monitoring protocol when mechanical central nervous system injury (*e.g*. FF transection) is used as a brain injury model. Furthermore, although buprenorphine had some effect on postoperative body weight in itself, the analgesic regimen is most likely beneficial to the operated animals and should not affect the outcome of the experiments. However, further research is needed to elucidate the actual analgesic effect of buprenorphine, as well as the possible effects on animal model-based experiments in which this drug is used. Such research will optimize both the quality of the data collected when using these injury models and promote the well-being of the laboratory animals.

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