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Graded surgical stress reveals postoperative behavioral changes associated with systemic oxidative stress

Yuki Kinishi[1,2], Yoshihisa Koyama[2,3]✉, Ayako Takahashi[1], Takeshi Yoshida[1], Yuji Fujino[1] & Shoichi Shimada[2,3,4]

Postoperative delirium (POD) is a postoperative complication characterized by acute-onset attention deficits and cognitive impairment following surgery. Recently, oxidative stress has been increasingly recognized in its pathogenesis. This study investigated the relationship between postoperative behavioral changes and systemic oxidative stress responses under graded levels of surgical stress. Male C57BL/6J mice were divided into three groups: anesthesia alone, standard laparotomy, and open surgery with added localized thermal injury (OPT). The buried food test was performed 24 h after anesthesia induction to evaluate search latency. Serum was collected after the behavioral test to measure derivatives of reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), and the BAP/d-ROMs ratio. Search latency became more prolonged under conditions of increased surgical invasiveness. The d-ROMs values increased progressively with increasing surgical stress, whereas the BAP/d-ROMs ratio progressively decreased. Group-stratified and interaction analyses suggested that the relationship between postoperative behavioral changes and systemic oxidative stress responses differed according to the degree of surgical invasiveness, particularly regarding the BAP/d-ROMs ratio. These findings suggest that oxidative stress responses may contribute differently to postoperative behavioral changes under different levels of graded surgical stress.

Keywords Postoperative delirium, Oxidative stress, Buried food test, Surgical stress

Postoperative delirium (POD) is a brain dysfunction characterized by the acute onset of impaired attention and cognitive impairment following surgery and is one of the most common complications associated with general anesthesia and surgery[1,2]. The incidence of POD increases with the severity of surgical stress, with longer operative duration, greater procedural complexity, and higher invasiveness identified as major risk factors[3]. Moreover, POD is associated with prolonged hospitalization, an increased incidence of postoperative complications, and increased mortality[4], underscoring the clinical importance of elucidating its underlying pathophysiological mechanisms.

One prevailing hypothesis proposes that peripheral inflammatory responses induced by surgical stress reach the central nervous system via blood–brain barrier disruption, thereby triggering neuroinflammation[1,5]. In this process, inflammatory cytokines such as interleukin (IL)−1β, IL-6, and tumor necrosis factor-α promote glial activation and synaptic dysfunction, leading to cognitive impairment[5]. Recently, oxidative stress has gained attention as a factor that is associated with inflammation and contributes to blood–brain barrier dysfunction and neuroinflammation[6,7]. However, the relationship between systemic oxidative stress and postoperative behavioral changes across different levels of surgical invasiveness remains unclear.

Basic research aimed at elucidating the pathological basis of POD-associated postoperative behavioral changes has commonly employed laparotomy-based surgical stress models, although most studies have been limited to single, fixed levels of surgical insult [8]. Although models incorporating lipopolysaccharide administration to enhance inflammatory responses have been reported, such interventions differ fundamentally from the

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pathology driven by surgical stress itself[8]. Against this background, postoperative behavior in animal models of POD is typically assessed using multiple behavioral paradigms, including the open field, Y-maze, and fear conditioning tests, which assess learning, memory, and locomotor activity, respectively[9]. Many of these tasks rely on spatial memory, motor function, or prior learning and are therefore susceptible to the confounding effects of postoperative systemic illness and physical impairment[10]. In contrast, the buried food test (BFT) requires no prior training and primarily assesses delayed initiation of behavior and reduced exploratory motivation. Because it does not require prior learning or complex motor coordination, it may be less affected by postoperative physical impairment than cognitive tasks such as the Y-maze or fear conditioning. This feature makes the BFT suitable for detecting behavioral changes that emerge during the early postoperative period[10–12].

This study investigated the association between postoperative behavioral changes and systemic oxidative stress responses under surgically augmented conditions with localized thermal injury. Specifically, we focused on the behavioral changes that emerged following invasiveness-enhancing manipulation and examined their relationship with systemic oxidative stress.

Results

Pronounced changes in search latency following thermal injury–augmented laparotomy

To evaluate postoperative behavioral changes under enhanced surgical invasiveness, three groups were established: anesthesia alone (Sham), standard laparotomy (OP), and open surgery with localized thermal injury added (OPT) (Fig. 1a). Animals were fasted for 24 h after the induction of anesthesia, after which BFT was performed. Search latency, defined as the time to first contact with food buried in the bedding (Fig. 2a), was compared between the Sham ($n=18$), OP ($n=11$), and OPT ($n=26$) groups (Fig. 2b). The measurement was capped at 600 s. Median (Q1–Q3) search latency was 125 s (77.8–164.5) in the Sham, 73 s (57.0–129.0) in the OP, and 217.5 s (137.0–600.0) in the OPT group. Search latency was compared among groups using the Kruskal-Wallis test followed by Steel-Dwass post hoc test. Search latency was significantly prolonged in the OPT group compared with the Sham and OP groups ($p<0.01$), whereas no significant difference was observed between the Sham and OP groups ($p=0.186$). In the OPT group, several animals reached the upper limit of 600 s, and the interquartile range (Q1–Q3) was markedly wider than that in the Sham and OP groups, indicating greater variability in search latency.

To determine whether motor function influenced the behavioral changes observed in the BFT, we performed an exploratory open-field test in an independent cohort of mice. No significant differences were observed among the Sham, OP, and OPT groups in total distance traveled, mean speed, or mobile time, indicating no apparent differences in locomotor activity among the groups (Kruskal–Wallis test; Supplementary Fig. 1).

These results indicate that increasing surgical invasiveness produced a distinct distribution of search latency compared with less invasive conditions.

Surgical stress increased systemic oxidative stress, further enhanced by additional thermal injury

To assess systemic oxidative stress under enhanced surgical invasiveness, blood samples were analyzed using derivatives of reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) assays. The d-ROMs reflect circulating oxidant levels, BAP indicates antioxidant capacity, and the BAP/d-ROMs ratio serves as an index of systemic oxidative stress.

The d-ROMs values increased stepwise across the Sham (48.5 ± 2.8 U.Carr), OP (65.8 ± 3.8 U.Carr), and OPT (92.2 ± 4.4 U.Carr) groups (Fig. 3a). Group comparisons of oxidative stress markers were performed using the Kruskal–Wallis test followed by the Steel–Dwass post hoc test. The OP group showed significantly higher d-ROMs values than the Sham group ($p<0.01$), whereas the OPT group exhibited significantly higher d-ROM values than the OP ($p<0.01$) and Sham groups ($p<0.01$). The mean BAP values were 3315.7 ± 78.4, 3302.8 ± 87.1, and 3006.9 ± 61.3 µmol/L in the Sham, OP, and OPT groups, respectively (Fig. 3b). BAP values were significantly lower in the OPT group than in the Sham and OP groups ($p<0.05$), whereas no significant difference was observed between the Sham and OP groups ($p=0.978$). The BAP/d-ROMs ratio decreased progressively across the groups, with values of 72.3 ± 4.3 in the Sham, 51.4 ± 2.5 in the OP, and 34.1 ± 1.5 in the OPT group (Fig. 3c). The OP group showed a significantly lower ratio than the Sham group ($p<0.01$), and the OPT group showed significantly lower ratios than the OP and Sham groups ($p<0.01$).

These findings indicate that surgical stress increases systemic oxidative stress, and that this response is markedly amplified by localized thermal injury.

Association between search latency and systemic oxidative stress

We examined the association between search latency in the BFT and systemic oxidative stress markers, including d-ROMs, BAP, and the BAP/d-ROMs ratio. Associations between search latency and oxidative stress markers were evaluated using Spearman's rank correlation analysis. In the analysis of all subjects, search latency showed a significant positive correlation with d-ROMs values (Fig. 4a; $r=0.55$, $p<0.01$). A significant negative correlation was observed between search latency and BAP values (Fig. 4b; $r=-0.27$, $p<0.05$), whereas a significant negative correlation was observed between search latency and the BAP/d-ROMs ratio (Fig. 4c; $r=-0.58$, $p<0.01$). Group-stratified analyses suggested that associations between search latency and oxidative stress markers were more apparent under the OPT condition (Fig. 4d–f). In the OPT group, search latency showed a significant positive correlation with d-ROMs values ($r=0.52$, $p<0.01$) and a significant negative correlation with the BAP/d-ROMs ratio ($r=-0.49$, $p<0.05$). In contrast, no significant correlations were observed in the Sham or OP groups. No significant correlation was observed between search latency and BAP values in any group. However, because differences in sample size among groups may influence statistical significance, these within-group findings should be interpreted cautiously.

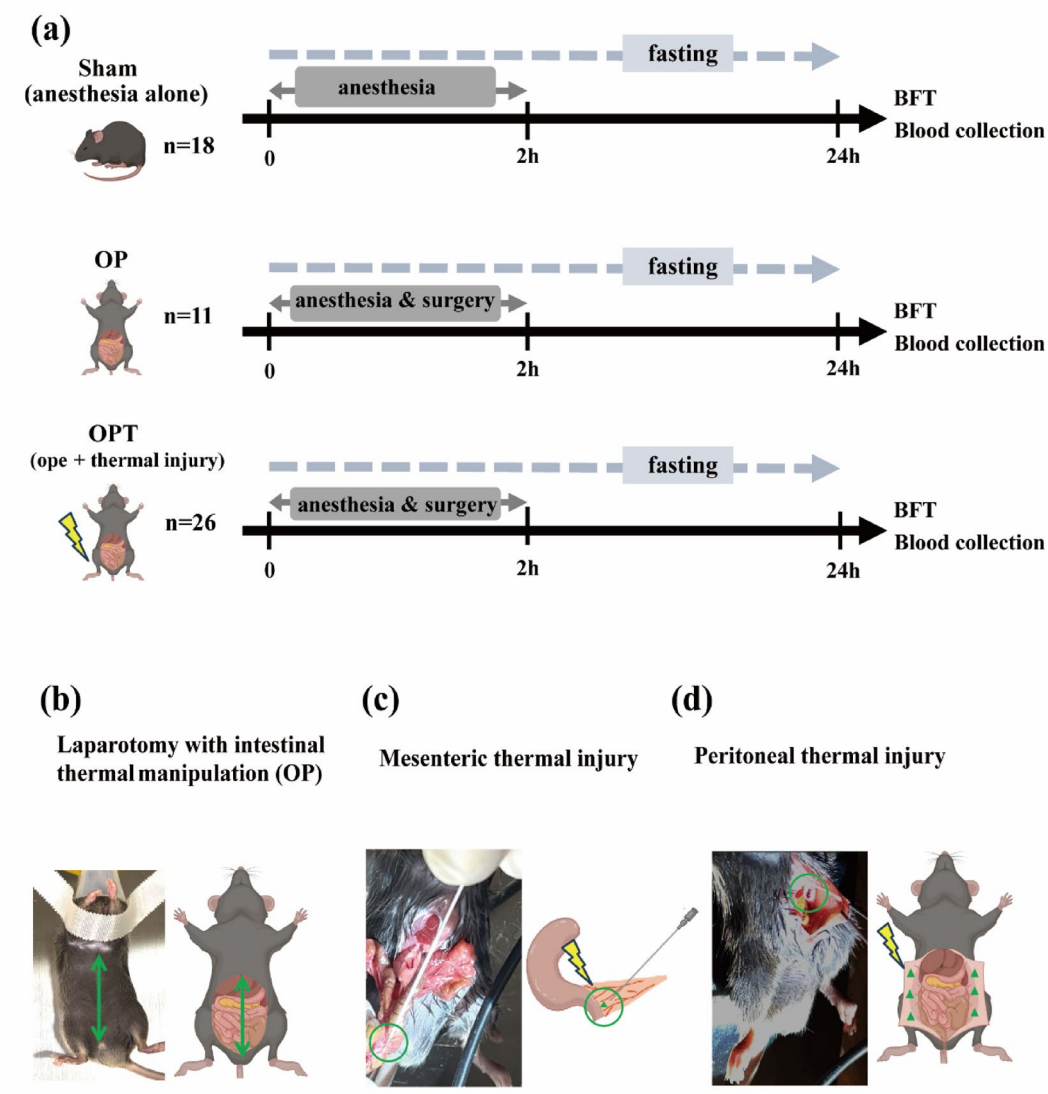


Fig. 1. (**a**) Experimental design. The experimental design and timeline included three groups: anesthesia alone (Sham), standard laparotomy (OP), and the group receiving open surgery with additional localized thermal injury (OPT). Anesthesia was initiated at time 0, and the total duration of anesthesia, including surgery or sham procedure, was fixed at 2 h. Fasting was maintained for 24 h from the start of anesthesia, and the buried food test (BFT) was performed at the end of this period. Blood samples were collected after completion of the BFT. (**b**-**d**) Schematic of thermal injury-enhanced abdominal surgery. (b) Laparotomy with intestinal thermal manipulation (OP): A midline incision was made from the xiphoid process to the proximal pubic symphysis (green arrow). The small intestine was exteriorized and gently manipulated for a fixed period to avoid excessive injury. (c) Mesenteric thermal injury: In the OPT group, localized thermal injury was applied to two mesenteric sites near the cecal root (green circle and triangle), in addition to the procedure described in (b). (d) Peritoneal thermal injury: In the OPT group, additional thermal injury was applied to six peritoneal sites (three sites on each side; green circle and triangles) during abdominal closure. Each procedure is shown using intraoperative photographs (left) and schematic diagrams (right).

To further examine whether the association between search latency and oxidative stress markers differed among surgical groups, interaction analyses were performed using regression models that included a group × oxidative stress interaction term. No significant interaction was observed for d-ROMs (Fig. 4d; p for interaction = 0.18) or BAP (Fig. 4e; p for interaction = 0.47), whereas a significant interaction was detected between the surgical group and the BAP/d-ROMs ratio (Fig. 4f; p for interaction = 0.004).

These results suggest that the association between oxidative stress balance, as reflected by the BAP/d-ROMs ratio, and postoperative behavioral changes may differ according to the degree of surgical invasiveness.

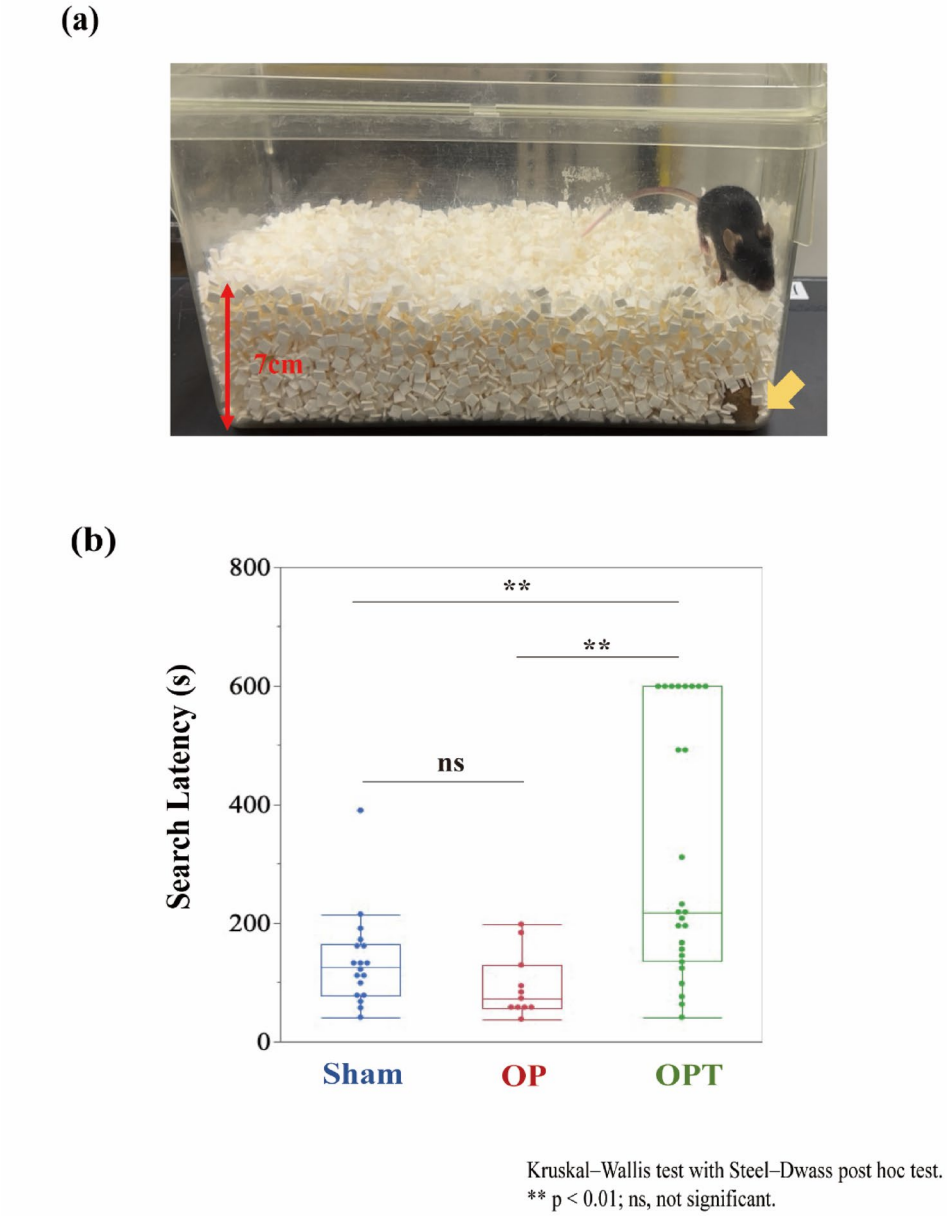


Fig. 2. (**a**) Representative photograph of the buried food test. Behavioral assessments were conducted in the cage, with the bedding depth maintained at approximately 7 cm (red arrow). The test food was completely buried in the bedding, allowing for the evaluation of exploratory behavior, primarily guided by olfactory cues. The yellow arrow indicates the location of the buried food. (**b**) Distribution of search latency in the buried food test. The search latencies for individual mice in the Sham (blue: $n = 18$), OP (red: $n = 11$), and OPT (green: $n = 26$) groups are shown. Boxplots indicate the median and interquartile range (Q1–Q3), and the y-axis represents search latency (s). Group differences were analyzed using the Kruskal–Wallis test, followed by the Steel–Dwass post-hoc test. $p < 0.01$ is indicated by ** and ns denotes no significant difference.

Discussion

This study employed a graded surgical stress design, in which the level of invasiveness was experimentally modulated by adding localized thermal injury, allowing evaluation of the relationship between surgical stress intensity, systemic oxidative stress, and postoperative behavioral changes. Although systemic oxidative stress increased with the degree of surgical stress, group-stratified and interaction analyses suggested that the relationship between postoperative behavioral changes and oxidative stress balance may differ according to the degree of surgical invasiveness. Consistent with these observations, correlation and interaction analyses indicated that the association between search latency and oxidative stress balance varied across surgical conditions. Taken together, the results suggest that the relationship between postoperative behavioral changes and systemic oxidative stress responses may differ depending on the degree of surgical invasiveness.

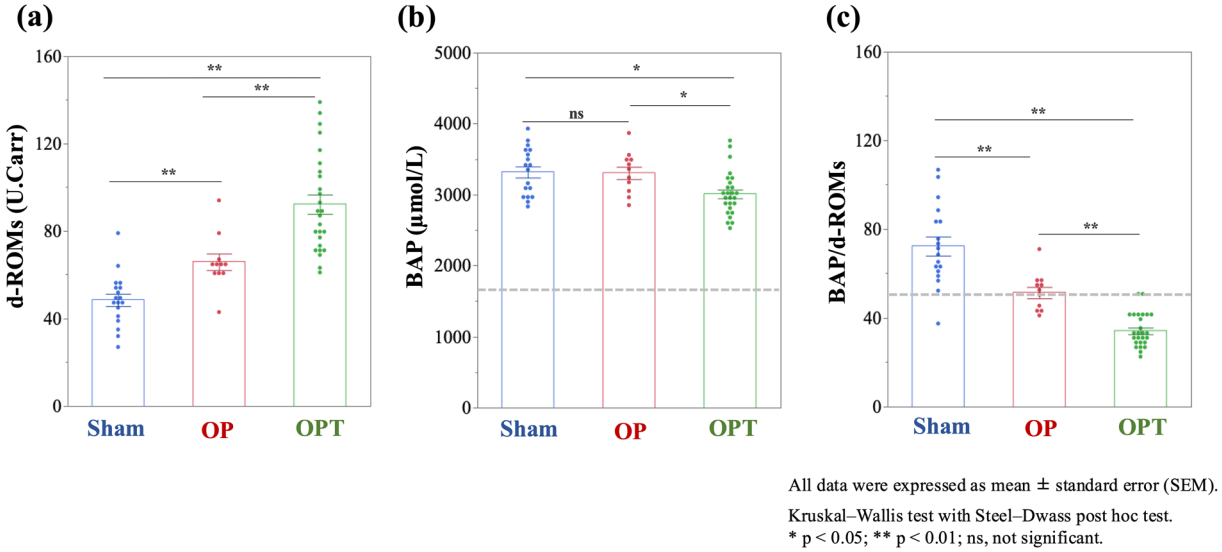


Fig. 3. Bar graphs showing mean d-ROM (**a**), BAP (**b**), and BAP/d-ROM (**c**) values in the Sham (blue), OP (red), and OPT (green) groups. Individual points represent individual values, and bars indicate the mean ± standard error of the mean (SEM). Dotted lines denote reference values for serum oxidative stress markers in age-matched healthy mice of the same strain, as reported by Nakatsuka et al. (d-ROMs: 61.5 U.Carr, BAP: 3650 μmol/L, BAP/d-ROMs: 59.4)[20]. Group differences were analyzed using the Kruskal–Wallis test, followed by the Steel–Dwass post-hoc test. $p < 0.05$ is indicated by * and $p < 0.01$ by **.

Oxidative stress has recently emerged as an important factor in the development of POD and postoperative cognitive dysfunction. Anesthesia and surgery increase reactive oxygen species (ROS) production and lipid peroxidation, and reduce antioxidant capacity in both the systemic circulation and central nervous system, changes that may promote delirium-like behaviors through blood–brain barrier disruption and enhanced neuroinflammation[11,13,14]. In this study, the relationship between postoperative behavioral changes and systemic oxidative stress responses varied with the degree of surgical invasiveness, suggesting that the intensity of surgical stress may modulate the relationship between oxidative stress balance and postoperative behavioral changes. Regarding search latency, no clear correlation was observed with BAP values. Although associations with d-ROMs values were observed in some analyses, the relationship was more consistently demonstrated for the BAP/d-ROMs ratio, particularly in the interaction analysis. These results suggest that postoperative behavioral changes may be more closely related to an increased systemic oxidative stress burden driven by enhanced ROS production and lipid peroxidation than to a reduced antioxidant capacity alone. Notably, the significant interaction observed for the BAP/d-ROMs ratio suggests that the balance between oxidant production and antioxidant capacity may be particularly relevant to postoperative behavioral changes under increased surgical stress. This finding is consistent with current hypotheses implicating oxidative stress in postoperative behavioral changes[11,13]. Furthermore, the stratified analysis shown in Supplementary Fig. 2 was performed as an exploratory auxiliary analysis using the mean search latency to evaluate within-group variability in the OPT group. Mice with prolonged search latency tended to show higher d-ROMs values and lower BAP/d-ROMs ratio, indicating a greater systemic oxidative stress burden. These findings support the possibility that variability in postoperative behavioral changes under enhanced invasive conditions may be associated with differences in systemic oxidative stress responses among mice. Because search latency showed a skewed distribution, the mean was used to represent overall trends within the group. Notably, the analysis of search latency within the OPT group revealed marked intragroup variability, with some mice showing pronounced prolongation and others exhibiting only mild changes (Fig. 2b). Similar variability has been reported in animal models of POD, where studies using the BFT and multiple behavioral tasks have demonstrated that delirium-like behaviors occur in only a subset of mice under identical anesthesia and surgical conditions [11,12,16]. Clinically, POD also shows substantial interindividual variability in susceptibility, influenced by factors such as age, underlying disease, and inflammatory or oxidative stress response [1–3]. Thus, the variability observed in this study may partially reflect the heterogeneous susceptibility to postoperative behavioral changes observed in clinical POD and supports the possibility that systemic oxidative stress responses contribute differently among mice subjected to greater surgical invasiveness. Because search latency included an upper observation limit (600 s), right-censored data may have masked an even greater degree of variability in the OPT group. Therefore, the observed variability may represent an underestimation of the true distribution of postoperative behavioral responses under enhanced surgical invasiveness. Nevertheless, the presence of a subset of animals with markedly prolonged latency may reflect the heterogeneous manifestation of postoperative behavioral changes.

Studies using the BFT after simple laparotomy have reported that search latency changes at 24 h are mild and not important [11]. The discrepancy in the present results may reflect differences in behavioral task design, in addition to evaluation timing. The BFT assesses sustained exploratory behavior and motivation driven by

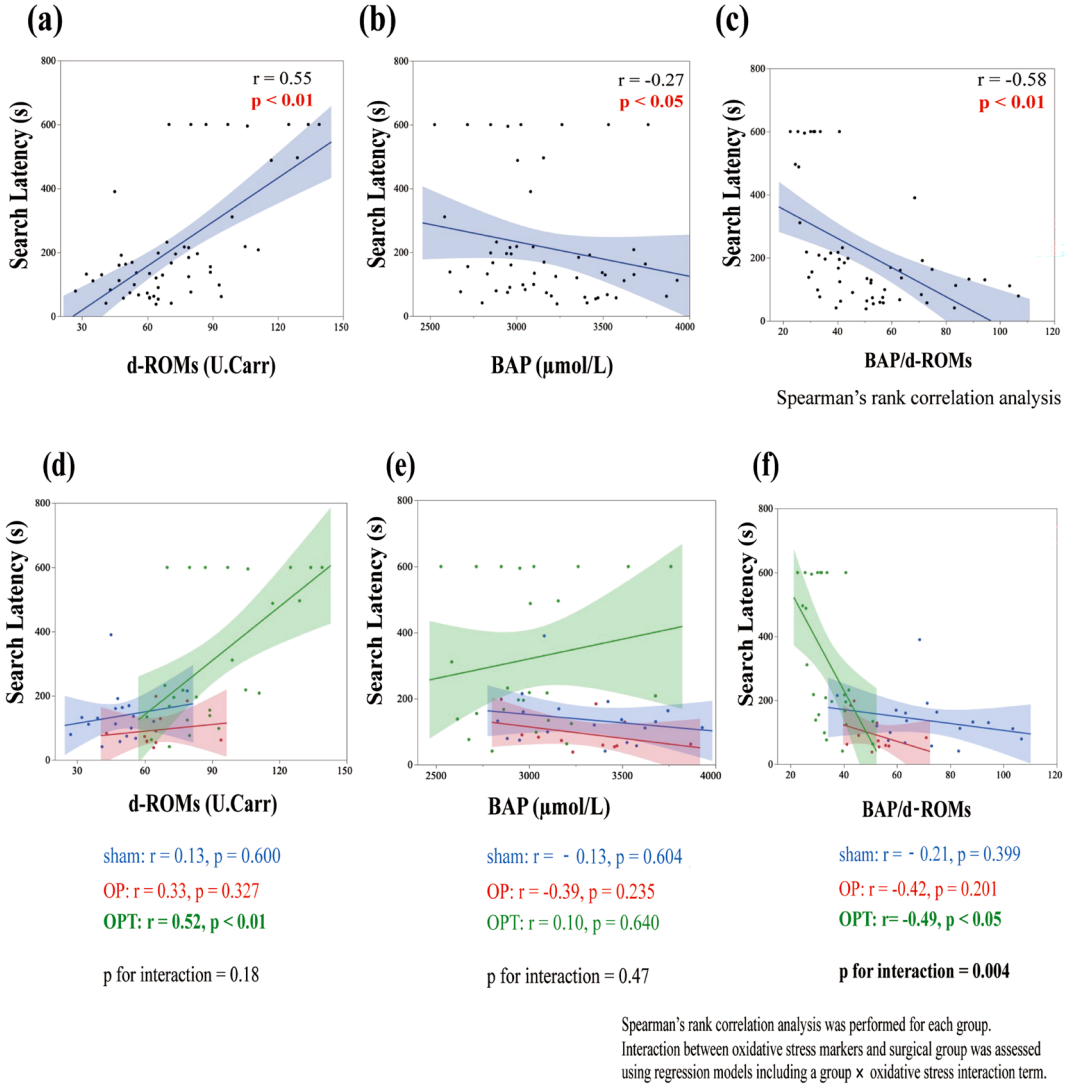


Fig. 4. (**a**-**c**) Scatter plot showing correlations between search latency and oxidative stress markers in all mice. Scatter plots illustrating the relationship between the buried food test (BFT) search latency and d-ROMs (a), BAP (b), and the BAP/d-ROMs ratio (c) in all mice. Each dot represents an individual mouse. Blue lines indicate regression lines, and shaded areas represent 95% confidence intervals. Spearman's rank correlation coefficients (r) and corresponding p-values are shown. d-f: Scatter plots showing group-specific associations between search latency and oxidative stress markers and their interactions with the surgical group. Scatter plots showing the association between search latency and d-ROMs (**d**), BAP (**e**), and the BAP/d-ROMs ratio (**f**) in the Sham (blue), OP (red), and OPT (green) groups. Colored lines represent group-specific regression lines, and shaded areas indicate 95% confidence intervals. Spearman's rank correlation coefficients and the corresponding p-values are shown for each group. The interaction between oxidative stress markers and the surgical group was assessed using regression models that included a group × oxidative stress interaction term.

olfactory cues, rather than explicit learning or memory [13]. In this study, a 24-h fasting period and complete burial of food under thick bedding likely required greater exploratory effort than a simple olfactory response. Accordingly, prolongation of search latency became more apparent under conditions of enhanced surgical invasiveness. These findings suggest that both assessment timing and task design influence sensitivity in detecting invasiveness-dependent postoperative behavioral changes. Therefore, the experimental framework used here may provide a useful conceptual model for examining relationships among surgical invasiveness, postoperative behavioral changes, and systemic oxidative stress.

This study had some limitations. First, the BFT does not directly assess POD-like behavioral phenotypes but instead serves as an exploratory behavioral assessment for detecting postoperative behavioral changes based on search activity[10,11]. Therefore, it remains unclear whether a prolonged search latency reflects the core clinical features of POD, such as cognitive impairment, attentional deficits, or disturbances in consciousness[1,2]. This study utilized young adult mice to reduce age-related differences and assess the connection between varying levels of surgical invasiveness and systemic oxidative stress responses under relatively stable baseline conditions.

However, because POD predominantly occurs in older patients[3] and aged mice are known to exhibit enhanced and more prolonged postoperative cognitive and behavioral impairments after similar surgical stress[9,13,14], caution is required when extrapolating the present findings directly to aged POD pathology. To more accurately reproduce clinically relevant conditions, future studies using aged models will be important. Because the present study was conducted as an exploratory experimental study, prospective statistical power analysis was not performed, and marked interindividual variability was observed within the OPT group. Although this variability may reflect the heterogeneous clinical features of POD, it may also reduce the statistical power and complicate interpretation of subgroup analyses. Furthermore, because search latency included an upper observation limit (600 s), the observed distribution may have been partially influenced by right-censored data. Future studies using staged invasiveness design and central nervous system oxidative stress markers may further clarify the pathophysiology of POD-like behaviors.

In summary, by analyzing postoperative behavioral changes in relation to systemic oxidative stress under conditions of enhanced surgical invasiveness, this study offers insights into how the relationship between postoperative behavioral changes and oxidative stress responses may vary according to the degree of surgical invasiveness. An experimental framework linking surgical invasiveness to systemic oxidative stress may provide a useful basis for investigating postoperative behavioral changes under graded surgical stress conditions.

Conclusion

This study demonstrated that postoperative behavioral changes assessed by search latency were associated with systemic oxidative stress markers under conditions of increased surgical stress. Although oxidative stress responses increased progressively with surgical stress, its association with postoperative behavioral changes differed across surgical conditions. These findings suggest that oxidative stress may relate differentially to postoperative behavioral changes under graded surgical stress conditions.

Materials and methods

Study approval

All animal experiments were approved by the Ethics Committee of the University of Osaka (Approval No.: 24–085-004) and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, as well as the ARRIVE and AVMA guidelines. A total of 63 male C57BL/6J mice aged 8–12 weeks (specific pathogen-free; Japan SLC, Shizuoka, Japan) were used in this study. All mice were housed three per cage under a 12-hour light-dark cycle in a temperature-controlled environment (23–25 °C). Prior to surgery, mice had free access to standard rodent chow and water. Every effort was made to minimize the number of animals used and their suffering. Humane endpoints were predefined, and mice showing signs of severe distress, including impaired feeding or drinking, respiratory distress, self-injury, or rapid body weight loss exceeding 20% within a few days, were promptly euthanized by intraperitoneal administration of pentobarbital (200 mg/kg).

Experimental design

This study used an experimental design that incorporated graded levels of surgical invasiveness. Specifically, three treatment groups were established: anesthesia alone (Sham group; $n = 18$), standard laparotomy (OP group; $n = 12$), and open surgery supplemented with localized thermal injury (OPT group; $n = 33$). Initially, the experimental design focused on comparing the Sham and OPT groups to evaluate the effects of increased surgical invasiveness. The OP group was subsequently added to provide comparison with a conventional laparotomy condition and to better assess the graded surgical stress design.

Because higher attrition was anticipated during model establishment under the more invasive OPT condition, more animals were initially allocated to this group to ensure sufficient evaluable data while minimizing unnecessary repetition of experiments. Animals were randomly assigned within each phase of the exploratory study. An overview of the experimental design and timeline for each group is presented in Fig. 1a. The number of animals included in the final analysis was 18, 11, and 26 in the Sham, OP, and OPT groups, respectively. Animals excluded from the final analysis met predefined exclusion criteria described below. In all experiments, inhalation anesthesia with isoflurane (isoflurane inhalation solution; Abbott Japan, Tokyo, Japan) was initiated at time 0, and the mice were awakened after a uniform total anesthesia duration of 2 h, including surgery or Sham treatment. Food was withheld for 24 h from the start of anesthesia, whereas water was freely available. The BFT was performed 24 h after the induction of anesthesia. Following the test, blood was collected from the right ventricle under deep anesthesia using a combination of anesthetic regimen (0.3 mg/kg medetomidine, 4.0 mg/kg midazolam, and 5.0 mg/kg butorphanol)[15].

Abdominal surgery procedures

Building upon the previously reported open abdominal surgery model[11,14,16], we established a more invasive condition by adding localized thermal injury to intestinal manipulation[16,17]. In all groups, anesthesia was maintained with 1.4% isoflurane at an oxygen flow rate of 1 L/min, and body temperature was maintained at 36–37 °C using a warming pad. After the induction of anesthesia, the mice were placed in the supine position, and all procedures were performed under aseptic conditions. Inhalation anesthesia was continued after the completion of either the surgery or sham procedures to standardize the total duration of anesthesia to 2 h[11]. An overview of the surgical procedure is shown in Fig. 1b-d.

For the laparotomy, a ~ 3 cm midline incision was made from the xiphoid process to the proximal pubic symphysis (Fig. 1b). The small intestine was exteriorized and subjected to gentle friction for 30 s[14], after which the bowel was returned to the abdominal cavity, and the incision was closed. In the OPT group, localized thermal injury was added to enhance invasiveness, based on a previously reported postoperative bowel adhesion model[17].

Thermal injury was applied for several seconds using a pulsed radiofrequency generator (JK3 Neuro Thermo; Abbott Medical Japan, Tokyo, Japan) until the mesenteric surface near the cecal root turned white and the tissue temperature reached ~ 80 °C (Fig. 1c). Additional thermal injury was applied to six sites (three per side) at closure (Fig. 1d). The OP group did not undergo thermal injury, whereas the Sham group received anesthesia alone without laparotomy.

BFT

The BFT was conducted as an exploratory behavioral assessment to evaluate attention and goal-directed search behavior, based on previously reported methods, with the aim of detecting postoperative behavioral changes[12]. The animals were fasted for 24 h before testing with free access to water. The test was performed in a plastic cage (30 × 16 × 14 cm) filled with bedding to a uniform depth of 7 cm (Fig. 2a). Before testing, each mouse was allowed to freely explore the cage for 10 min for acclimatization. Subsequently, one feed pellet was completely buried at the bottom of the cage, and the mice were returned. The latency to contact the pellet was recorded as search latency. A maximum cutoff of 600 s was applied, and trials exceeding this limit were assigned a value of 600 s. The BFT is typically performed with a predefined observation period, although the cutoff duration varies across studies[12]. In the present study, a 600 s upper limit was adopted to allow detection of delayed exploratory behavior under conditions of increased surgical stress.

Oxidative stress measurement

After behavioral testing, whole blood was collected from the right ventricle under deep anesthesia using a combination of anesthetics (0.3 mg/kg medetomidine, 4.0 mg/kg midazolam, and 5.0 mg/kg butorphanol)[15]. After centrifuging the samples (3000 rpm, 10 min, 4 °C), serum was used to assess systemic oxidative stress by measuring ROS metabolites and antioxidant capacity as indicators of blood oxidative stress. The d-ROMs and BAP values were measured using REDOXLIBLA (Wismerll Co., Ltd., Tokyo, Japan). The d-ROMs values were expressed in arbitrary units (U.Carr), where 1 U.Carr corresponds to 0.8 mg/L of hydrogen peroxide. The BAP value reflects blood reducing power, assessed by the reduction of Fe^{3+} to Fe^{2+}. The BAP/d-ROMs ratio was calculated as an integrated indicator of systemic oxidative stress balance[18,19].

Group allocation, blinding, and exclusion criteria

The mice were allocated to the Sham ($n = 18$), OP ($n = 12$), and OPT ($n = 33$) groups based on the exploratory experimental design, with random assignment performed within each experimental phase according to litter origin and body weight. Because full blinding was not feasible, behavioral assessments and biochemical analyses were performed with awareness of the group assignment. The exclusion criteria were defined a priori and included postoperative death, abdominal wall suture failure, suspected severe circulatory impairment (e.g., intestinal ischemia), and insufficient blood collection for serum analysis. Accordingly, one mouse from the OP group and seven mice from the OPT group were excluded. Most exclusions in the OPT group occurred during the early phase of model establishment and were primarily related to technical aspects of the invasiveness-enhancing procedures rather than outcome-based exclusions. All outcome measures were quantitative and predefined to minimize subjective bias.

Statistical analysis

Nonparametric statistical analyses were performed. Group comparisons were conducted using the Kruskal–Wallis test, followed by the Steel–Dwass post-hoc test when appropriate. The Wilcoxon rank-sum test was used for two-group comparisons. Associations between search latency and oxidative stress markers were assessed using Spearman's rank correlation coefficients. To evaluate whether the relationship between oxidative stress markers and search latency differed among the surgical groups, interaction analyses were performed using regression models that included a group × oxidative stress interaction term. The significance level was set at $p < 0.05$. All statistical tests were two-tailed. Search latency, which included truncation at the upper limit (600 s), is expressed as the median and interquartile range (Q1–Q3). In contrast, oxidative stress indicators were continuous variables without an upper limit and are presented as the mean ± standard error of the mean. All analyses were performed using the JMP Pro 17 software (JMP Statistical Discovery LLC, Cary, NC, USA).

Data availability

All relevant data are included within this paper.

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Author contributions

Y.ki. and Y.Ko. designed the study, analyzed the data, and wrote the paper. Y.Ki., Y.Ko., and A.T. performed measurements and quantifications. T.Y., Y.F. and S.S. supervised this study and provided intellectual directions. All authors discussed the findings and commented on this manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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