Effects of Environmental Enrichment on Behavior and the Microbiota in Mice

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Background

- Reproducibility is a principle of the scientific method – increasing it would have profound effects on all fields of research.
- Environmental enrichment is shown to decrease stress levels in mice.
- Gut microbiota (GM) varies with several environmental factors.
- This study sought to evaluate the effects of environmental enrichment on GM and anxiety-related behaviors.

Hypotheses

- Differing forms of enrichment will affect GM composition with sunflower seeds and handling having the most significant changes.
- The most dramatic change in GM composition will occur between day 0 and day 7 of treatment.
- The handled mice will have lower anxiety-like behaviors, such as increased exploration of open arms in the elevated plus maze, along with increased GM richness and diversity.

Methods
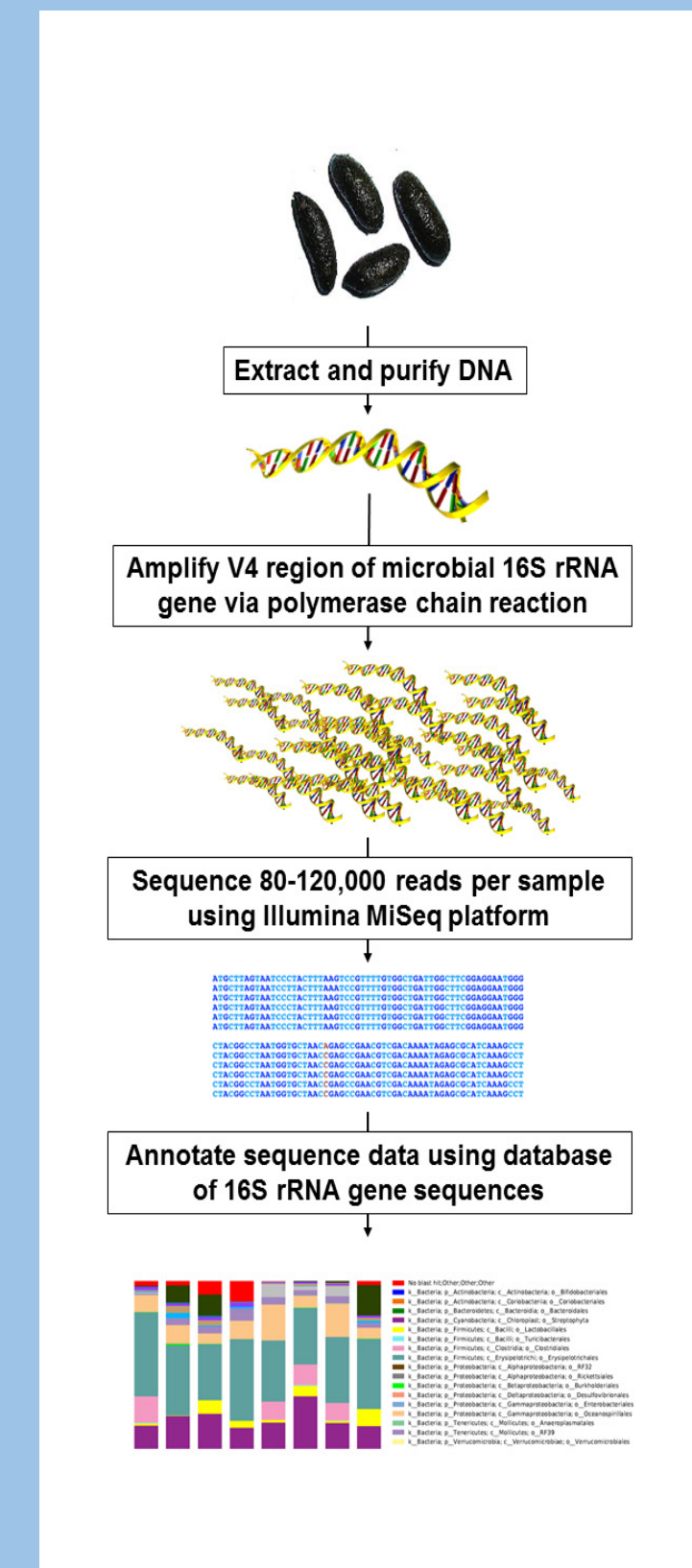
- Two strains of mice were used, BALB/c (anxiogenic behavior phenotype) and C57BL/6 (B6; anxiolytic behavior phenotype) (n=40 per strain). Each strain was divided into five treatment groups (n=8) as follows:
  - **Control**: standard husbandry, which included a nestlet.
  - **Sunflower Seeds**: standard husbandry plus ~20-30 sunflower seeds per day per cage.
  - **Igloo**: standard husbandry plus an igloo hut.
  - **Handled**: standard husbandry plus 3 minutes of handling per mouse per day.
  - **Paper**: standard husbandry plus Enviro-Dri bedding (Shepherd Specialty Paper, Watertown, TN).
- Treatments lasted 42 days, with fecal samples taken for analysis on days 0, 7, and 42. GM was analyzed using 16s rRNA sequencing techniques. Behavior tests were run at the end of the 42 days for evaluation of anxiety-like behaviors. Due to self injurious activity, behavioral testing was not performed on one B6 mouse from the paper group.
- Differences between groups in richness and alpha-diversity were tested using a one-way analysis of variance (ANOVA) with Dunnett's post-hoc analysis.
- Beta-diversity differences were analyzed by one-way permutational multivariate analysis of variance (PERMANOVA) of Jaccard and Bray-Curtis distances using Past 3.15.

Acknowledgements

Conclusions

- Daily handling of B6 mice reduced anxiety-like behaviors as evidenced by decreased activity when placed in open field chambers. In contrast, daily handling of BALB/c mice resulted in increased anxiety-like behaviors as evidenced by greater activity in open field and light/dark transition test.
- Analysis of B6 GM composition at day 42 showed significant differences in bacterial communities in the igloo, sunflower seed, and paper groups when compared to controls. Analysis of BALB/c GM composition at day 42 showed significant differences in the handled, igloo, and sunflower seed groups when compared to controls.
- Higher p-values and F-values were noted when using Jaccard distances (as compared to Bray Curtis distance), suggesting that composition changes resulted more from presence/absence of OTUs as opposed to evenness of OTUs present.

Ongoing Studies

- Comparison of GM composition of in fecal samples from days 0 to 7 to assess acute changes.

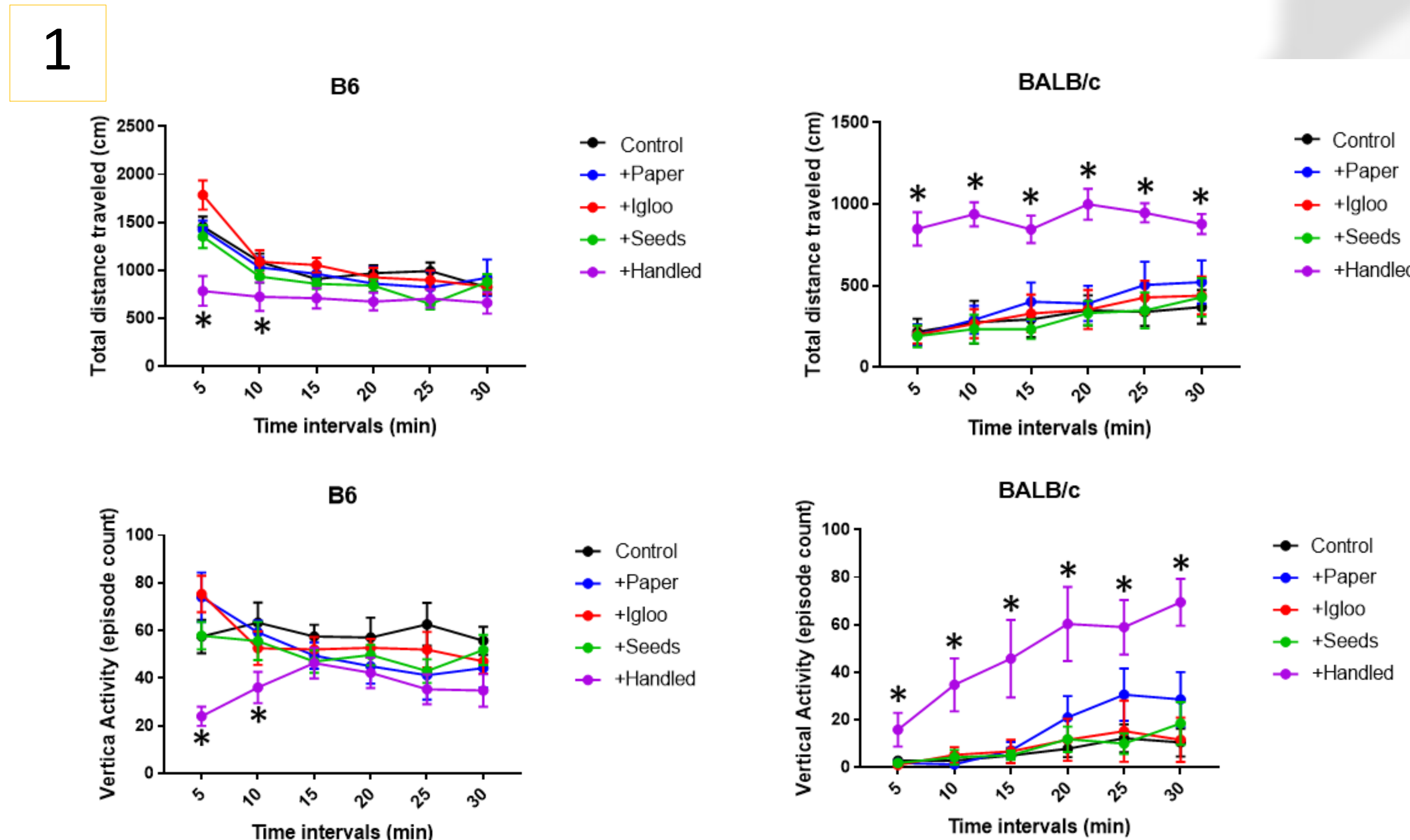## Handling Modified Behavior Differently in Anxiogenic and Anxiolytic Strains

Figure 1. Data from Open Field test on all subjects at the end of experimentation. Open Field test was run for 30 minutes per mouse. Total distance traveled, vertical activity, and margin time were evaluated using one-way ANOVA with Dunnett's post-hoc analysis. There were no significant differences among groups in the margin time test. Handled B6 mice showed statistically lower distance traveled and vertical activity when compared to the control group at the 5 and 10 minute time points, suggesting a more rapid acclimation to the open field chamber. Handled BALB/c mice showed significant elevations in vertical and horizontal activity suggesting that handling increased anxiety-like behaviors in these mice.

Use of Igloos Decreased Anxiety-Like Behaviors in Anxiolytic Strains
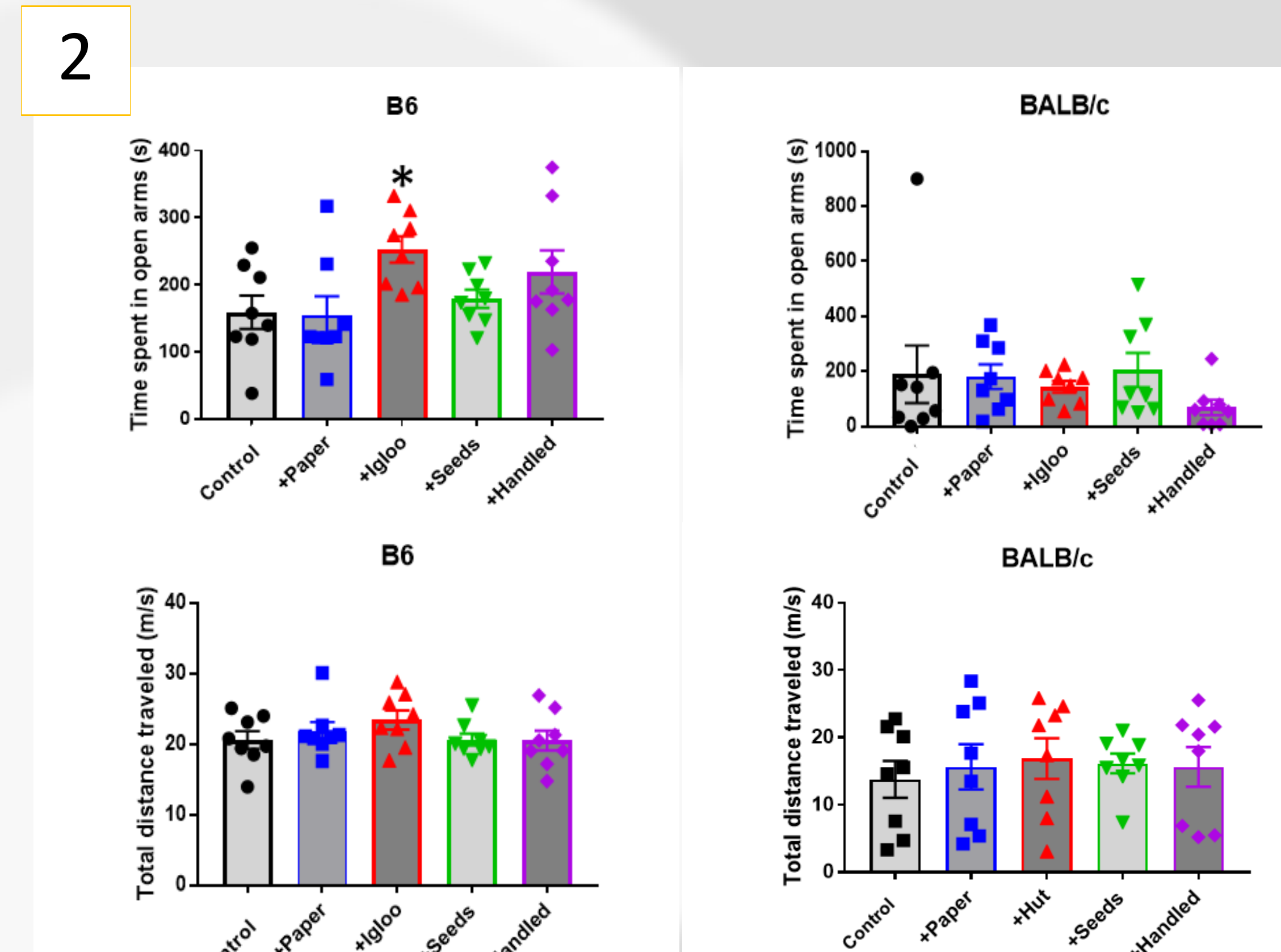

Figure 2. Data from Elevated Plus Maze for all subjects at the end of experimentation. Elevated Plus Maze test was run for 15 minutes per mouse. Time spent in open arms and total distance traveled were evaluated using one-way ANOVA with Dunnett's post-hoc analysis. B6 mice that received igloos showed significant increases in the amount of time spent in open arms of the maze, suggesting decreased anxiety-like behaviors in this group. BALB/c mice showed no significant differences compared to the control group in this test.

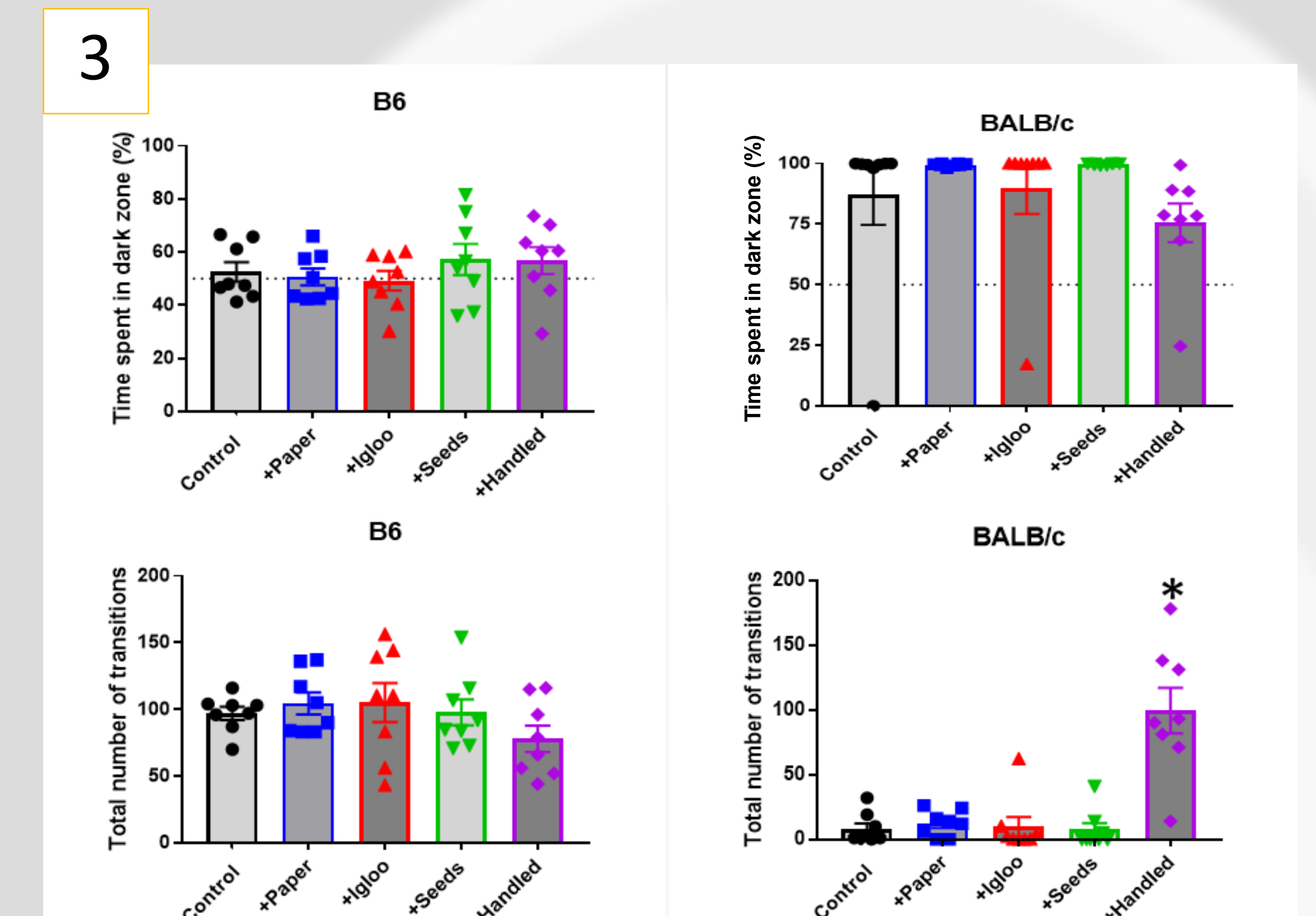## Handling Increased Anxiety-Like Behaviors in Anxiogenic Strains

Figure 3. Data from Light/Dark transition test for all subjects at the end of experimentation. The light/dark test was run for 10 minutes per mouse. Time spent in the dark zone and total number of transitions were evaluated using one-way ANOVA with Dunnett's post-hoc analysis. Handled BALB/c mice showed significant increases in total number of transitions, suggesting heightened anxiety-like behaviors in this group. B6 mice showed no significant differences compared to the control group in this test.

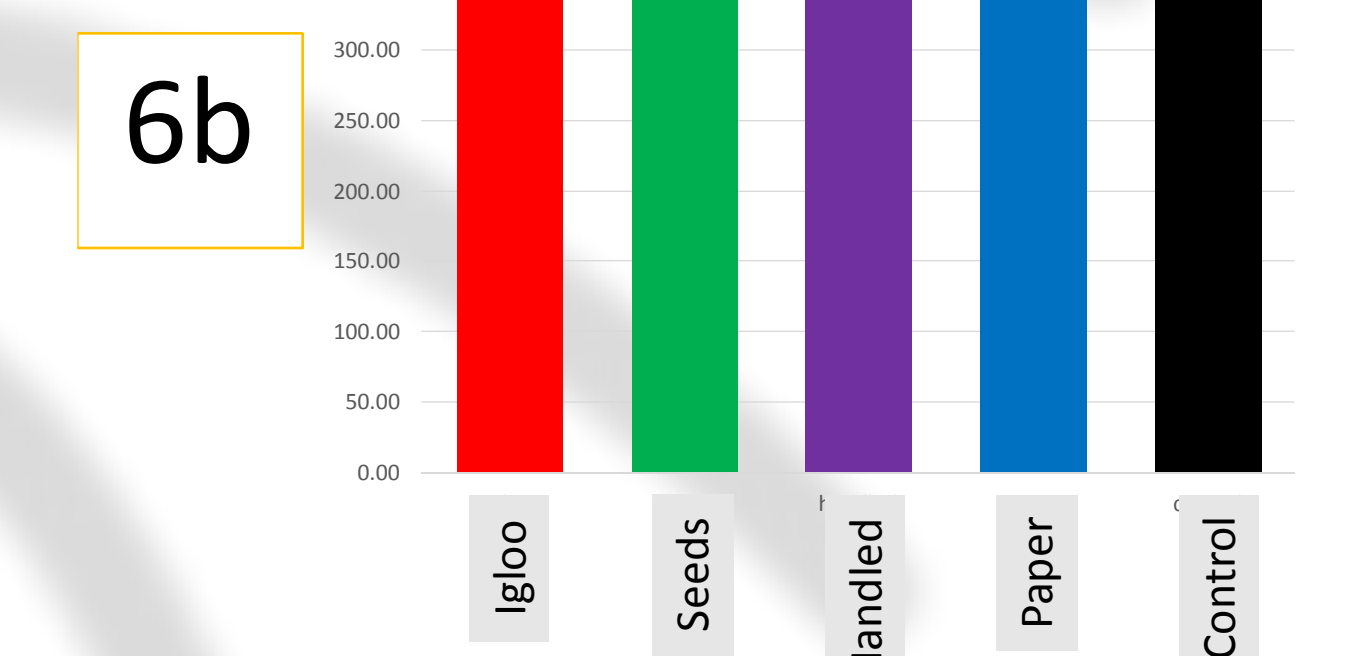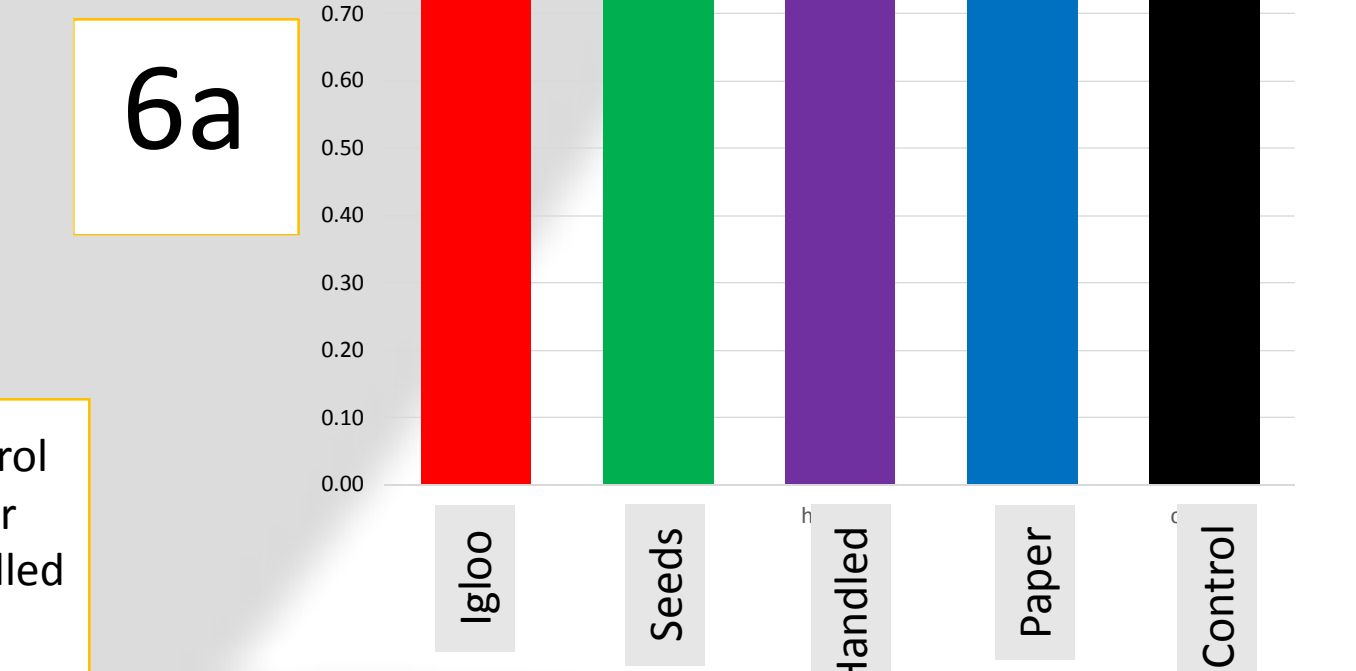## Significant Changes in Diversity of GM in B6 (4a, 5a) and BALB/c (4b, 5b) Mice Among Treatment Groups
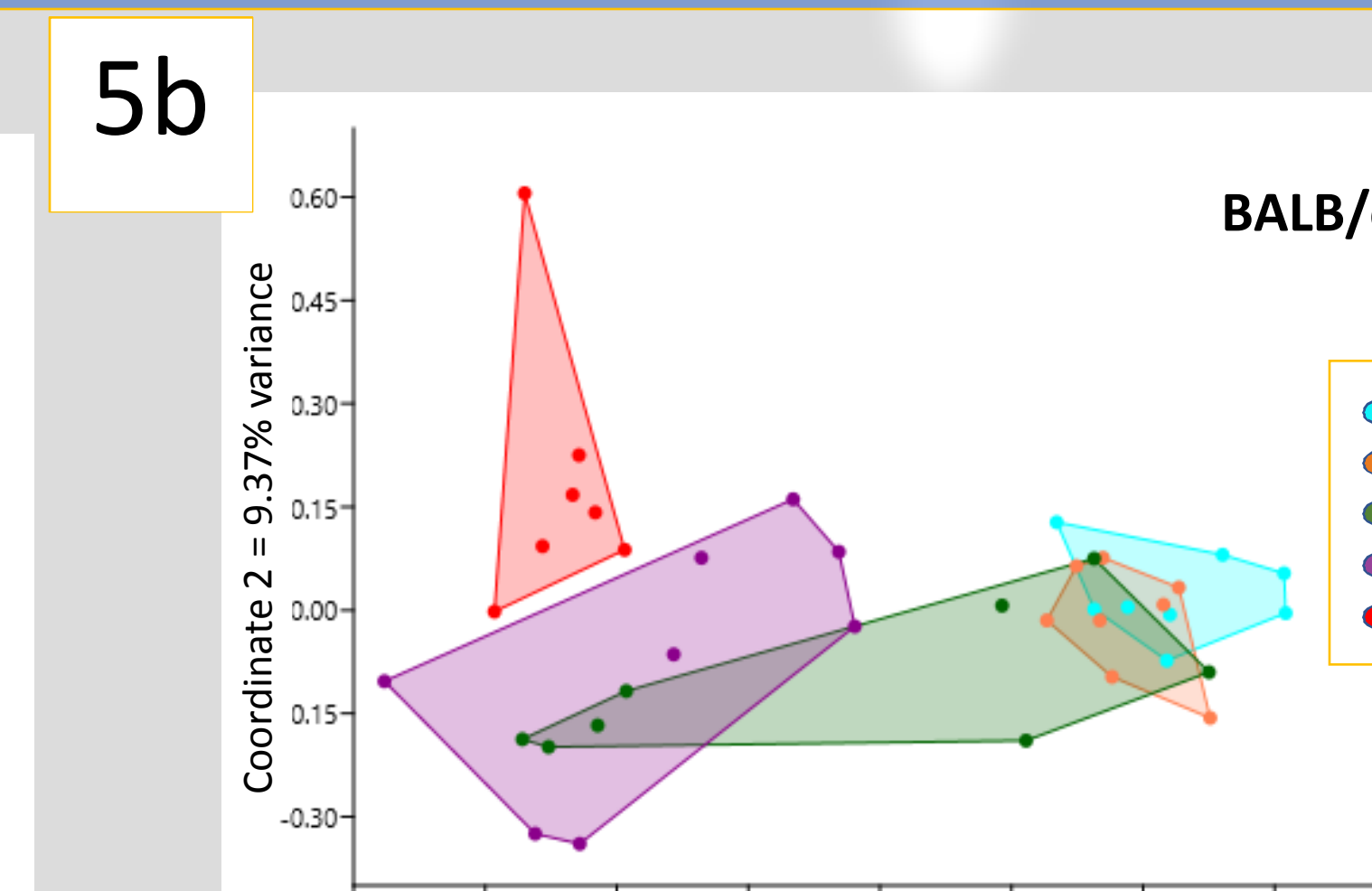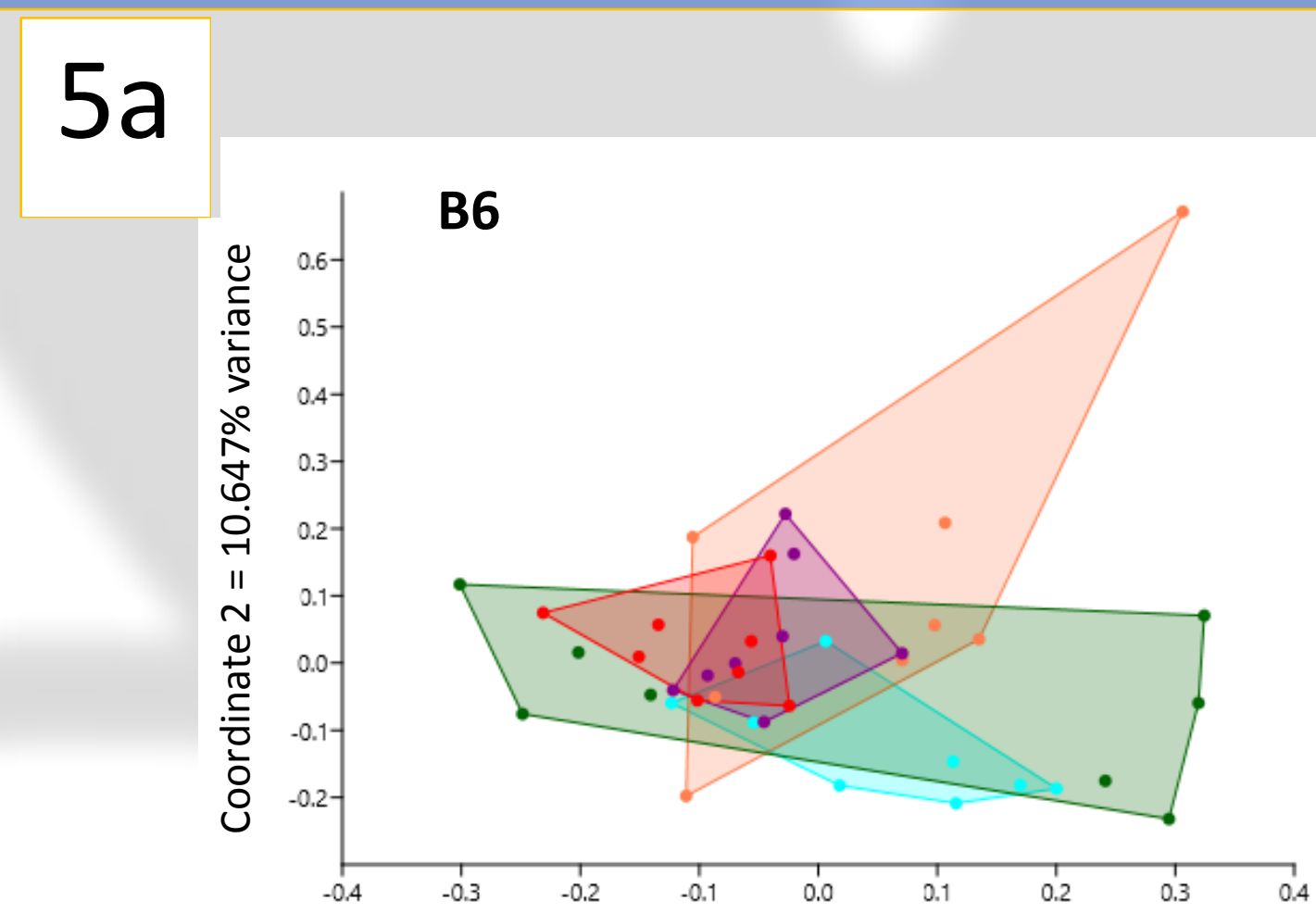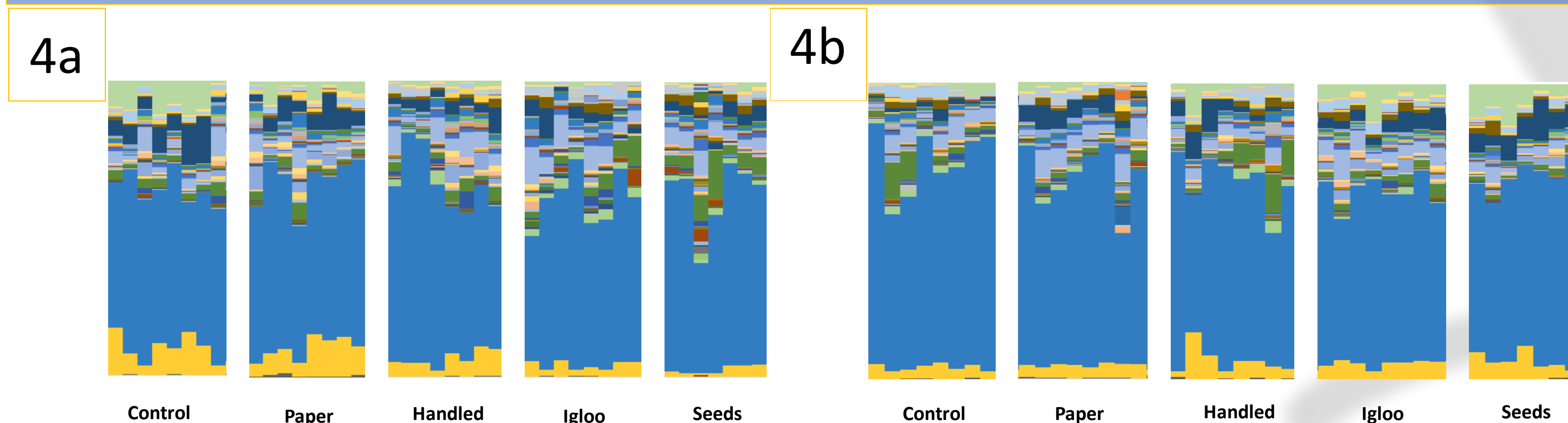

Figure 4a and 4b. Stacked bar charts of fecal microbiota composition in BALB/c and B6 mice, respectively, on day 42 of experimentation. Data represents OTU level analysis. **Figure 5a and 5b. Principal Coordinate Analyses (PCoA) of day 42 GM samples in B6 and BALB/c mice, respectively.** Both Jaccard distances and Bray-Curtis dissimilarities were calculated, with the Jaccard distances pictured here. For B6 mice, Jaccard distances showed significant differences in the paper, igloo, and sunflower seed when group were compared to the controls. For BALB/c mice, Jaccard distances showed significant differences in the handled, the sunflower seed, and the igloo groups when compared to the control group. **Figure 6a. Bar chart showing GM diversity in BALB/c mice at day 42 using the Simpson diversity index.** Significant differences in GM diversity were seen in the igloo, the sunflower seed, and handled groups. **Figure 6b. Bar chart showing GM diversity in BALB/c mice at day 42 using the Chao diversity index.** Significant differences in GM diversity were seen in the igloo and sunflower seed groups.