BDP2-448 Lovasz

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2019-02-13

# Import data

getwd()

## [1] "C:/Users/Ben/Google Drive/Work/BDP2-448-Lovasz/scripts"

f <- "../data/raw/Becky-Bedding Exposure Study Data for Stats.2.xlsx"

Import data file *../data/raw/Becky-Bedding Exposure Study Data for Stats.2.xlsx*

df <- f %>% read\_excel()

## readxl works best with a newer version of the tibble package.  
## You currently have tibble v1.4.2.  
## Falling back to column name repair from tibble <= v1.4.2.  
## Message displays once per session.

oldnames <- names(df)  
newnames <- c("id",  
 "sex",  
 "time",  
 "bedding",  
 "order",  
 "room",  
 "foodIntakeNormalized",  
 "orts",  
 "foodIntakeRaw",  
 "cumulativeFoodIntakeNormalized")  
names(df) <- newnames  
data.frame(oldnames, newnames) %>% kable()

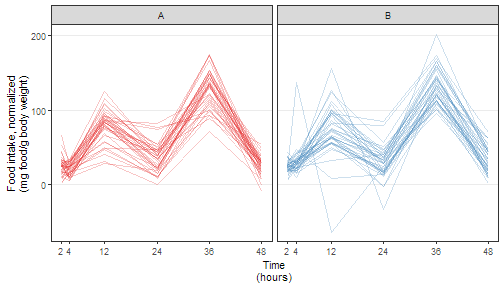
|  |  |
| --- | --- |
| oldnames | newnames |
| Mouse ID | id |
| Sex | sex |
| Time (hr) | time |
| Bedding | bedding |
| Order | order |
| Housing Room | room |
| Normalized food intake (mg food/g BW) | foodIntakeNormalized |
| Orts (g) | orts |
| Raw food intake (g) | foodIntakeRaw |
| Normalized cumulative food intake (mg food/g BW) | cumulativeFoodIntakeNormalized |

Plot data.

G <-  
 df %>%  
 ggplot() +  
 aes(x = time, y = foodIntakeNormalized, group = id, color = bedding) +  
 geom\_line(alpha = 1/4) +  
 facet\_wrap(~ bedding) +  
 scale\_color\_brewer(palette = "Set1") +  
 scale\_x\_continuous("Time\n(hours)", breaks = c(2, 4, 12, 24, 36, 48)) +  
 scale\_y\_continuous("Food intake, normalized\n(mg food/g body weight)") +  
 theme\_bw() +  
 theme(legend.position = "none",  
 panel.grid.major.x = element\_blank(),  
 panel.grid.minor = element\_blank())  
ggsave("../figures/lineplot.png", width = 7, height = 4, dpi = 300)  
ggsave("../figures/lineplot.svg", width = 7, height = 4, dpi = 300)

## Warning: package 'gdtools' was built under R version 3.5.2

G



plot of chunk lineplot

# Setup data

t <- 36

Reshape data to mouse-level analytic data set. Data point at t = 36 is used to evaluate difference between A and B bedding types.

Outcome variable is diff, which is the difference between normalized food intake at t = 36 under A-B bedding types.

* Positive values indicate the mouse ate more at t = 36 with bedding type A
* Negative values indicate the mouse ate more at t = 36 with bedding type B

dfA <-  
 df %>%  
 filter(time == t) %>%  
 filter(bedding == "A") %>%  
 select(id, foodIntakeNormalized) %>%  
 rename(A = foodIntakeNormalized)

## Warning: package 'bindrcpp' was built under R version 3.5.2

dfB <-  
 df %>%  
 filter(time == t) %>%  
 filter(bedding == "B") %>%  
 select(id, foodIntakeNormalized) %>%  
 rename(B = foodIntakeNormalized)  
dfDiff <-  
 merge(dfA, dfB, by = "id") %>%  
 mutate(diff = A - B)  
covariates <-  
 df %>%  
 filter(order == 1) %>%  
 select(id, sex, order, bedding, room) %>%  
 unique() %>%  
 mutate(isBeddingAFirst = order == 1 & bedding == "A")  
dfDiff <-  
 merge(dfDiff, covariates, by = "id") %>%  
 select(-c(order, bedding))

Exploratory comparisons.

dfDiff %>%  
 group\_by(sex) %>%  
 summarize(n = n(), mean = mean(diff), sd = sd(diff)) %>%  
 kable(digits = 1)

|  |  |  |  |
| --- | --- | --- | --- |
| sex | n | mean | sd |
| F | 20 | -9.0 | 41.3 |
| M | 20 | 2.3 | 28.8 |

dfDiff %>%  
 group\_by(room) %>%  
 summarize(n = n(), mean = mean(diff), sd = sd(diff)) %>%  
 kable(digits = 1)

|  |  |  |  |
| --- | --- | --- | --- |
| room | n | mean | sd |
| 0543 | 15 | 2.0 | 29.0 |
| 0545C | 25 | -6.6 | 39.3 |

dfDiff %>%  
 group\_by(isBeddingAFirst) %>%  
 summarize(n = n(), mean = mean(diff), sd = sd(diff)) %>%  
 kable(digits = 1)

|  |  |  |  |
| --- | --- | --- | --- |
| isBeddingAFirst | n | mean | sd |
| FALSE | 20 | -27.9 | 31.4 |
| TRUE | 20 | 21.2 | 18.7 |

# Model A-B difference at t = 36

M <- lm(diff ~ 1, data = dfDiff)   
M %>% tidy() %>% kable(digits = 3)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | p.value |
| (Intercept) | -3.376 | 5.635 | -0.599 | 0.553 |

There appears to be **no** significant difference in the amount of food intake at t = 36. Normalized food intake is higher with bedding type B by 3.38 mg/g (p-value = 0.55).

lm(diff ~ sex, data = dfDiff) %>% tidy() %>% kable(digits = 3)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | p.value |
| (Intercept) | -9.044 | 7.967 | -1.135 | 0.263 |
| sexM | 11.336 | 11.267 | 1.006 | 0.321 |

lm(diff ~ room, data = dfDiff) %>% tidy() %>% kable(digits = 3)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | p.value |
| (Intercept) | 1.960 | 9.257 | 0.212 | 0.833 |
| room0545C | -8.537 | 11.709 | -0.729 | 0.470 |

The difference is still not significant after adjusting for sex or room.

M <- lm(diff ~ isBeddingAFirst, data = dfDiff)  
M %>% tidy() %>% kable(digits = 3)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| term | estimate | std.error | statistic | p.value |
| (Intercept) | -27.929 | 5.783 | -4.830 | 0 |
| isBeddingAFirstTRUE | 49.106 | 8.178 | 6.005 | 0 |

However, the difference appears to depend on which bedding type the mouse experiences first. Mice that experience bedding type A first have higher food intakes at t = 36 with bedding type A: 21.2 mg/g (p-value = 0.00076) Mice that experience bedding type B first have higher food intakes at t = 36 with bedding type B: 27.9 mg/g (p-value = 2.3e-05)

This suggests that a crossover design might not be the most optimal study design for this research question.

An alternative analysis is to assume a model that ignores the cross-sectional study design. Instead, the model includes order as a fixed effect and an interaction between bedding and order. The model also includes a random intercept effect for mouse.

# Model normalized food intake at t = 36

This model is a longitudinal model where each mouse contributes 2 data points, one for each bedding type (A, B). The model includes an order effect and an interaction between bedding and order.

dfRanEff <-  
 df %>%  
 filter(time == t) %>%  
 select(id, foodIntakeNormalized, order, bedding, sex, room) %>%  
 mutate(order = factor(order))  
dfRanEff %>%  
 group\_by(order, bedding) %>%  
 summarize(n = n(), mean = mean(foodIntakeNormalized), sd = sd(foodIntakeNormalized)) %>%  
 kable(digits = 1)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| order | bedding | n | mean | sd |
| 1 | A | 20 | 143.9 | 18.3 |
| 1 | B | 20 | 143.2 | 23.6 |
| 2 | A | 20 | 115.3 | 21.8 |
| 2 | B | 20 | 122.7 | 18.8 |

A t = 36, normalized food intake for the mice under bedding type A is almost identical to the mice under bedding type B at the start of the experiment (order = 1). After crossover (order = 2) there is a slightly lower normalized food intake among the mice under bedding type A compared to bedding type B.

dfRanEff <-  
 dfRanEff %>%  
 mutate(orderRev = factor(order, levels = c(2, 1)))  
M <-  
 lmer(foodIntakeNormalized ~ bedding + orderRev + bedding \* orderRev + (1 | id),   
 data = dfRanEff)  
M %>%   
 tidy(conf.int = TRUE, effects = "fixed") %>%   
 mutate(pvalue = pt(statistic, df = 1, lower.tail = FALSE)) %>%   
 kable(digits = 3)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| effect | term | estimate | std.error | statistic | conf.low | conf.high | pvalue |
| fixed | (Intercept) | 115.292 | 4.638 | 24.857 | 106.201 | 124.383 | 0.013 |
| fixed | beddingB | 7.411 | 6.559 | 1.130 | -5.445 | 20.268 | 0.231 |
| fixed | orderRev1 | 28.588 | 6.559 | 4.358 | 15.732 | 41.445 | 0.072 |
| fixed | beddingB:orderRev1 | -8.071 | 10.258 | -0.787 | -28.176 | 12.035 | 0.712 |

After crossover, mice under bedding type B had higher normalized food intake compared to bedding type A: 7.41 mg/g (p-value = 0.23)

Before crossover, there was virtually no difference between normalized food intake by bedding type: -0.659 mg/g (p-value = 0.53)

# R session information

For debugging purposes.

## [1] "Run time: 2019-02-13 22:34:46"

## R version 3.5.1 (2018-07-02)  
## Platform: x86\_64-w64-mingw32/x64 (64-bit)  
## Running under: Windows 10 x64 (build 17134)  
##   
## Matrix products: default  
##   
## attached base packages:  
## [1] stats graphics grDevices utils datasets methods base   
##   
## other attached packages:  
## [1] bindrcpp\_0.2.2 gdtools\_0.1.7 broom.mixed\_0.2.3  
## [4] lme4\_1.1-19 Matrix\_1.2-14 svglite\_1.2.1   
## [7] broom\_0.5.1 readxl\_1.2.0 forcats\_0.3.0   
## [10] stringr\_1.3.1 dplyr\_0.7.8 purrr\_0.2.5   
## [13] readr\_1.3.1 tidyr\_0.8.2 tibble\_1.4.2   
## [16] ggplot2\_3.1.0 tidyverse\_1.2.1 magrittr\_1.5   
## [19] checkpoint\_0.4.5 rmarkdown\_1.11 knitr\_1.21   
##   
## loaded via a namespace (and not attached):  
## [1] TMB\_1.7.15 tidyselect\_0.2.5 xfun\_0.4   
## [4] reshape2\_1.4.3 glmmTMB\_0.2.2.0 splines\_3.5.1   
## [7] haven\_2.0.0 lattice\_0.20-35 colorspace\_1.3-2   
## [10] generics\_0.0.2 htmltools\_0.3.6 rlang\_0.3.0.1   
## [13] nloptr\_1.2.1 pillar\_1.3.1 glue\_1.3.0   
## [16] withr\_2.1.2 RColorBrewer\_1.1-2 modelr\_0.1.2   
## [19] bindr\_0.1.1 plyr\_1.8.4 munsell\_0.5.0   
## [22] gtable\_0.2.0 cellranger\_1.1.0 rvest\_0.3.2   
## [25] coda\_0.19-2 evaluate\_0.12 labeling\_0.3   
## [28] highr\_0.7 Rcpp\_0.12.19 scales\_1.0.0   
## [31] backports\_1.1.3 jsonlite\_1.6 hms\_0.4.2   
## [34] digest\_0.6.18 stringi\_1.2.4 grid\_3.5.1   
## [37] cli\_1.0.1 tools\_3.5.1 lazyeval\_0.2.1   
## [40] crayon\_1.3.4 pkgconfig\_2.0.2 MASS\_7.3-50   
## [43] xml2\_1.2.0 lubridate\_1.7.4 minqa\_1.2.4   
## [46] assertthat\_0.2.0 httr\_1.4.0 rstudioapi\_0.8   
## [49] R6\_2.3.0 nlme\_3.1-137 compiler\_3.5.1