

# Antidepressant effects of esketamine in adolescents with major depressive disorder and suicidal ideation: a case series

Reproducible analysis code

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# 1 Note on reproducibility

This R Markdown file will exactly reproduce our data tidying, transformation, analysis and graphing processes in the R software and produce an illustrative PDF file. Code-only scripts are also available in the Open Science Framework repository for this study, at <https://osf.io/2ujqn/>. The reproduction of this R Markdown file requires that the dataset available in the OSF repository is located in the same directory of this file, and that the user has the necessary dependencies to create a PDF using the `{bookdown}` package. Further information is available at the package website: <https://bookdown.org>. Please refer to the repository for a detailed description of each variable contained herein. All files in the repository, including this one, are licensed under CC-BY-4.0.

The `{renv}` package was used to help with reproducibility. The `renv.lock` file is available and should be imported to the same location on your computer as this script. For further information on how to use these files visit the package website: <https://rstudio.github.io/renv/articles/renv.html>.

## 2 Load the required packages

```
# Required Packages
library(tidyverse)
library(haven)
library(lsr)
library(rstatix)
library(boot)
library(kableExtra)
library(patchwork)
library(RColorBrewer)
library(ggsci)
library(car)
library(purrr)
library(broom)
library(ggpubr)
```

## 3 Load the dataset

The first step is to load the dataset into R. You can load the dataset directly from the GitHub repository.

```
set.seed(2509)
## Load from GitHub repository

urlRemote <- "https://raw.githubusercontent.com/brenosouzamarques/"
pathGithub <- "LANP_Esketamine_Adolescents/master/"
fileName <- "Dataset.csv"

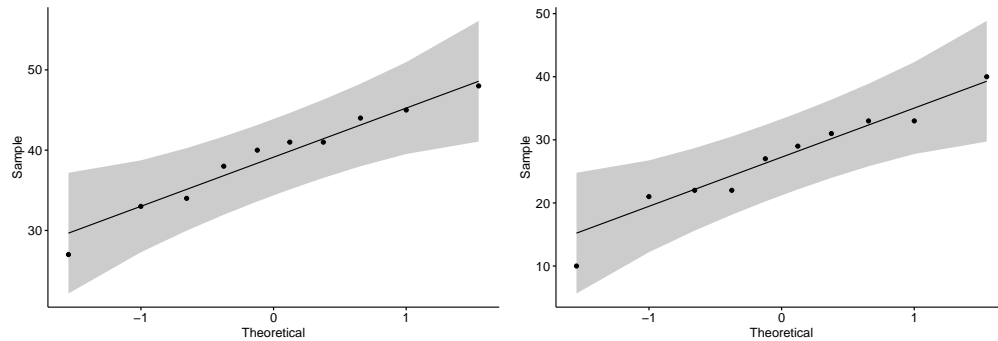
df <- paste0(urlRemote, pathGithub, fileName) %>%
  read.csv()
```

## 4 Assess normality of outcomes

Q-Q Plots are used to assess the symmetry of dependent variables, that is, **MADRS total score** and **MADRS item 10** at baseline and 24-h post-infusion.

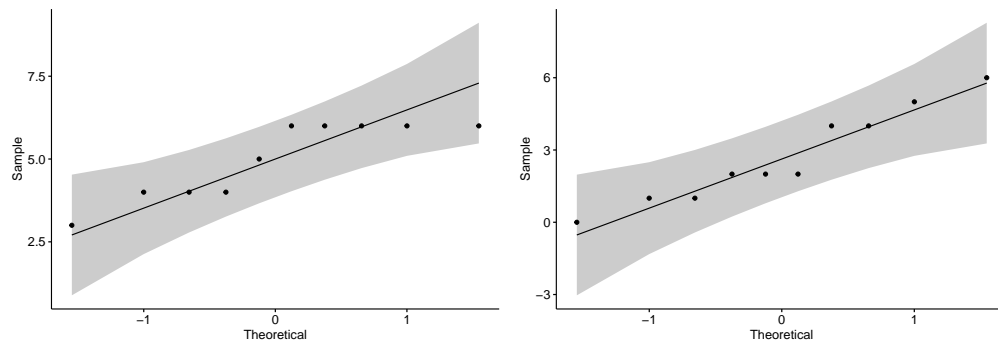
### 4.1 MADRS total score at baseline and 24h post-treatment

```
ggqqplot(df$MADRS_Baseline)
ggqqplot(df$MADRS_Post)
```



### 4.2 MADRS item 10 score at baseline and 24h post-treatment

```
ggqqplot(df$MADRS_Suicide_Baseline)
ggqqplot(df$MADRS_Suicide_Post)
```



## 5 Descriptives

Total depressive symptoms and suicidal ideation scores are presented as means and standard deviations.

### 5.1 MADRS total scores

```
madsr_total <- c(df$MADRS_Baseline, df$MADRS_Post)
time <- rep(c("baseline", "post"), each = 10)
data_madsr_total <- tibble(time, madsr_total)
kbl(data_madsr_total %>% group_by(time) %>%
  summarise(
    Mean = mean(madsr_total),
    "Std dev" = sd(madsr_total),
    Maximum = max(madsr_total),
    Minimum = min(madsr_total),
    Median = median(madsr_total)),
  caption = "MADRS total score",
  booktabs = T) %>% kable_styling(latex_options = c("hold_position"))
```

Table 1: MADRS total score

time	Mean	Std dev	Maximum	Minimum	Median
baseline	39.1	6.297266	48	27	40.5
post	26.8	8.403703	40	10	28.0

### 5.2 MADRS item 10 scores

```
item_10_total <- c(df$MADRS_Suicide_Baseline, df$MADRS_Suicide_Post)
time <- rep(c("baseline", "post"), each = 10)
data_madsr_total <- tibble(time, item_10_total)
kbl(
  data_madsr_total %>%
  group_by(time) %>%
  summarise(
    Mean = mean(item_10_total),
    "Std dev" = sd(item_10_total),
    Maximum = max(item_10_total),
    Minimum = min(item_10_total),
    Median = median(item_10_total)),
  caption = "MADRS item 10 score",
  booktabs = T) %>% kable_styling(latex_options = c("hold_position"))
```

Table 2: MADRS item 10 score

time	Mean	Std dev	Maximum	Minimum	Median
baseline	5.0	1.154700	6	3	5.5
post	2.7	1.946507	6	0	2.0

## 6 T-tests

We aimed to determine if a single 0.5mg/kg dose of esketamine can reduce depressive symptoms and suicidal ideation in adolescents. We test this hypothesis with a paired-samples t-test.

```
### T-test - MADRS Total Scores
```

```
ttest_total_madrs <- t.test(x = df$MADRS_Baseline,
                           y = df$MADRS_Post,
                           paired = T,
                           alternative = "two.sided")

ttest_item10_madrs <- t.test(x = df$MADRS_Suicide_Baseline,
                             y = df$MADRS_Suicide_Post,
                             paired = T,
                             alternative = "two.sided")

table_madrs <- map_df(list(ttest_total_madrs, ttest_item10_madrs), tidy)

table_madrs$'Outcome' <- c("MADRS total score",
                           "MADRS item 10")

table_madrs <- table_madrs %>% rename(
  "Mean Difference" = estimate,
  "T statistic" = statistic,
  "DF" = parameter,
  "95% CI lower" = conf.high,
  "95% CI higher" = conf.low,
  "P-value" = p.value)

kbl(table_madrs[c(9, 4, 2, 3, 1, 5, 6)],
     caption = "T-tests", booktabs = T) %>%
  kable_styling(latex_options = c("hold_position"))
```

Table 3: T-tests

Outcome	DF	T statistic	P-value	Mean Difference	95% CI higher	95% CI lower
MADRS total score	9	4.221349	0.0022350	12.3	5.708616	18.891384
MADRS item 10	9	3.146129	0.0118109	2.3	0.646234	3.953766

## 6.1 Effect sizes

Effect sizes allow further interpretation of changes from baseline to 24 hours post-ketamine treatment.

```
effectsize_madrs <- lsr::cohensD(df$MADRS_Baseline,
                                df$MADRS_Post,
                                method = "paired")

effectsize_item10 <- lsr::cohensD(df$MADRS_Suicide_Baseline,
                                  df$MADRS_Suicide_Post,
                                  method = "paired")

kbl(tribble(~Outcome, ~"Effect Size (Cohen's D)",
            "MADRS total score", effectsize_madrs,
            "MADRS item 10", effectsize_item10),
     caption = "Effect sizes",
     booktabs = T) %>% kable_styling(latex_options = c("hold_position"))
```

Table 4: Effect sizes

Outcome	Effect Size (Cohen's D)
MADRS total score	1.3349078
MADRS item 10	0.9948934

## 7 Bootstrapped 95% confidence intervals

### 7.1 Create a bootstrap function

This functions creates a main element, the `data_dens` data frame. It stores the bootstrapped samples of means as they are calculated using the bias-corrected and accelerated (Bca) method. These are later used to plot density graphs that compare bootstrapped distributions of pre-and post-treatment symptoms.

```
boot_funs <- function(df, f1, f2){
  a <- df[[f1]]
  b <- df[[f2]]
  boot_a <- boot(a,
    function(x, j) mean(x[j], na.rm = T), #Boot baseline variable
    R = 10000)
  boot_b <- boot(b,
    function(x, j) mean(x[j], na.rm = T), #Boot 7 days variable
    R = 10000)
  boot_ci_a <- boot.ci(boot.out = boot_a, #Calculate baseline CI
    conf = 0.95,
    type = "bca")
  boot_ci_b <- boot.ci(boot.out = boot_b, #Calculate post-treatment CI
    conf = 0.95,
    type = "bca")
  print(boot_ci_a) # Return baseline bootstrapped CI
  print(boot_ci_b) # Return post-treatment bootstrapped CI

  ## Code to extract bootstrapped sample means
  densitiy_a <- boot_a$t
  densitiy_b <- boot_b$t
  dens <- c(densitiy_a, densitiy_b)
  time <- rep(c("Baseline", "Post-Treatment"), each = 10000)
  data_dens <-< data.frame(dens, time)
  data_dens$time <- factor(data_dens$time)
}
```

## 7.2 Run bootstraps and create ggplot graph objects

### 7.2.1 Bootstrap - MADRS total score

```
### Bootstrap CI - MADRS total score
boot_funs(df, "MADRS_Baseline", "MADRS_Post")

## BOOTSTRAP CONFIDENCE INTERVAL CALCULATIONS
## Based on 10000 bootstrap replicates
##
## CALL :
## boot.ci(boot.out = boot_a, conf = 0.95, type = "bca")
##
## Intervals :
## Level      BCa
## 95%      (34.9, 42.4 )
## Calculations and Intervals on Original Scale
## BOOTSTRAP CONFIDENCE INTERVAL CALCULATIONS
## Based on 10000 bootstrap replicates
##
## CALL :
## boot.ci(boot.out = boot_b, conf = 0.95, type = "bca")
##
## Intervals :
## Level      BCa
## 95%      (21.2, 31.1 )
## Calculations and Intervals on Original Scale

plot_madrs <- ggplot(data_dens)+
  geom_density(aes(x = dens, fill = time),
               color = "white",
               alpha = 0.9)+
  labs(x = "Total MADRS scores")+
  scale_fill_grey(name = "Time of assessment:")+
  theme_classic()+
  theme(text = element_text(family = "serif"),
        axis.text.x = element_text(size = 14),
        axis.title.x = element_text(size = 14),
        axis.text.y = element_text(size = 14),
        axis.title.y = element_text(size = 14),
        legend.text = element_text(size = 13),
        legend.title = element_text(size = 13)) ##ggplot2 graph
```



## 7.2.2 Bootstrap - MADRS item 10

### Bootstrap CI - MADRS item 10

```
boot_funs(df, "MADRS_Suicide_Baseline", "MADRS_Suicide_Post")
```

```
## BOOTSTRAP CONFIDENCE INTERVAL CALCULATIONS
```

```
## Based on 10000 bootstrap replicates
```

```
##
```

```
## CALL :
```

```
## boot.ci(boot.out = boot_a, conf = 0.95, type = "bca")
```

```
##
```

```
## Intervals :
```

```
## Level      BCa
```

```
## 95%   ( 4.1,  5.5 )
```

```
## Calculations and Intervals on Original Scale
```

```
## BOOTSTRAP CONFIDENCE INTERVAL CALCULATIONS
```

```
## Based on 10000 bootstrap replicates
```

```
##
```

```
## CALL :
```

```
## boot.ci(boot.out = boot_b, conf = 0.95, type = "bca")
```

```
##
```

```
## Intervals :
```

```
## Level      BCa
```

```
## 95%   ( 1.5,  3.8 )
```

```
## Calculations and Intervals on Original Scale
```

```
plot_madrs10 <- ggplot(data_dens)+  
  geom_density(aes(x = dens, fill = factor(time)),  
               color = "white",  
               alpha = 0.9)+  
  labs(x = "Item 10 MADRS scores",  
       y = element_blank())+  
  scale_fill_grey(name = "Time of assessment:")+  
  theme_classic()+  
  theme(text = element_text(family = "serif"),  
        axis.text.x = element_text(size = 14),  
        axis.title.x = element_text(size = 14),  
        axis.text.y = element_text(size = 14),  
        axis.title.y = element_text(size = 14),  
        legend.text = element_text(size = 13),  
        legend.title = element_text(size = 13)) ##ggplot2 graph
```

## 8 Graphics

### 8.1 Density plots of bootstrapped sample means

Creates an annotated plot using the {ggplot2} density plots for the bootstrapped sample means and the {patchwork} package.

```
## Density plots of bootstrapped sample means

## Creates patchwork object
graficos <- plot_madrs + plot_madrs10 &
  theme(legend.position = "bottom",
        text = element_text(family = "serif"),
        title = element_text(size = 12),
        legend.title = element_text(size = 12))

## Creates guided patchwork object
graficos_f <- graficos + plot_layout(guides = "collect")

## Edits and annotates patchwork object
plota <- graficos_f + plot_annotation(
  title = 'Supp. Fig. 1 - Bootstrapped sample means for total and item 10 MADRS scores',
  subtitle = 'Density plots of results at baseline and 24 hours post-treatment')

## Saves plot in high resolution
ggsave("density_plot.png", plota, width=25, height=15, units="cm", dpi=450)
```

## 8.2 Main plots: pre-and post-treatment effects for depressive symptoms and suicidal ideation

This next part uses a long formatted dataset to plot pre-and post-infusion scores for each patient.

```
## Create dataset for total MADRS scores
madr_total <- c(df$MADRS_Baseline, df$MADRS_Post)
time <- rep(c("baseline", "post"), each = 10)
id <- rep(1:10, 2)
id <- factor(id)
madr_data_plot <- tibble(id, time, madrs_total)

## plot total MADRS scores
madr_plot <- madrs_data_plot %>% ggplot()+
  geom_boxplot(aes(x = time, y = madrs_total),
    width = .3,
    alpha = .3,
    color = "grey")+
  scale_y_continuous(breaks = seq(from = 10,
    to = 50,
    by = 5))+
  scale_x_discrete(labels = c("Baseline", "24 hours \npost-treatment"))+
  geom_point(aes(x = time, y = madrs_total,
    group = id),
    size = 1.5)+
  geom_line(aes(x = time, y = madrs_total,
    group = id,
    alpha = .4),
    size = 1.5)+
  scale_color_grey()+
  theme_classic()+
  guides(alpha = "none")+
  labs(x = "Time of assessment",
    y = "Total MADRS scores",
    color = "Patient ID")+
  theme(axis.title.x = element_text(size = 14,
    face = "bold"),
    axis.text.x = element_text(size = 13),
    axis.title.y = element_text(size = 15,
    face = "bold"),
    axis.text.y = element_text(size = 13),
    legend.position = "bottom",
    legend.text = element_text(size = 13,
    face = "bold"))

## Create dataset for MADRS Item 10
si_pre <- df$MADRS_Suicide_Baseline
si_post <- df$MADRS_Suicide_Post

madr_item10 <- c(si_pre, si_post)
time <- rep(c("baseline", "post"), each = 10)
id <- rep(1:10, 2)
id <- factor(id)
madr_item_plot <- tibble(id, time, madrs_item10)
```

```

item10_plot <- madsr_item_plot %>% ggplot()+
  geom_boxplot(aes(x = time, y = madsr_item10),
    width = .3,
    alpha = .3,
    color = "grey")+
  scale_y_continuous(breaks = c(0, 1, 2, 3, 4, 5, 6))+
  scale_x_discrete(labels = c("Baseline", "24 hours \npost-treatment"))+
  geom_point(aes(x = time, y = madsr_item10,
    group = id),
    size = 1.5,
    position = position_dodge(0.08))+
  geom_line(aes(x = time, y = madsr_item10,
    group = id,
    alpha = .4),
    size = 1.5,
    position = position_dodge(0.08))+
  scale_color_grey()+
  theme_classic()+
  guides(alpha = "none")+
  labs(x = "Time of assessment",
    y = "MADRS item 10 scores")+
  theme(axis.title.x = element_text(size = 14,
    face = "bold"),
    axis.text.x = element_text(size = 13),
    axis.title.y = element_text(size = 15,
    face = "bold"),
    axis.text.y = element_text(size = 13),
    legend.position = "bottom",
    legend.text = element_text(size = 13,
    face = "bold"),
    text = element_text(family = "serif"))

```

### 8.2.1 Code to join plots and produce the final image using {patchwork}.

```

## Creates patchwork object
graficos_i10 <- (madsr_plot +
  plot_layout(guides = "collect") &
  theme(legend.position = "right",
    text = element_text(family = "serif"))) +
  (item10_plot +
  theme(legend.position = "none"))

## Edits and annotates patchwork object
graficos_i10 <- graficos_i10 + plot_annotation(
  title = 'Total and item 10 MADRS scores at baseline and 24h post-treatment',
  subtitle = 'Scores are given for each patient') &
  theme(plot.title = element_text(size = 12),
    plot.subtitle = element_text(size = 11,
    face = "italic"))

## Saves plot in high resolution
ggsave("MADRS_plot.png", graficos_i10, width=23, height=12, units="cm", dpi=650)
ggsave("MADRS_plot.tiff", graficos_i10, width=23, height=12, units="cm", dpi=450)

```

```
ggsave("MADRS_plot.pdf", graficos_i10, width=23, height=12, units="cm", dpi=1200)
```