

hM3Dq CNO water bottles

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Paul injected VgatFlp;LepRbCre mice with a Flp+Cre dependent AAV expressing hM3Dq in the ventromedial hypothalamus (VMH). He then singly housed the mice and measured blood glucose, body weight, food intake, and water intake daily. After 3 days, he switched the water to a water containing CNO and continued measuring the same parameters. Also, some mice were on high-fat diet (HFD) and some were on chow (CHW). He also included a separate cohort (**Control**) that were not injected and were kept on chow, but got the same regimen of water bottles.

Paul sent me the raw data file (watr botles.xlsx). He said:

control water: day 0-> day 1 & day 1-> day2 CNO water : day 2->3, day 3->4, day 4->5 control water: day5->6 and day 6-> day 7

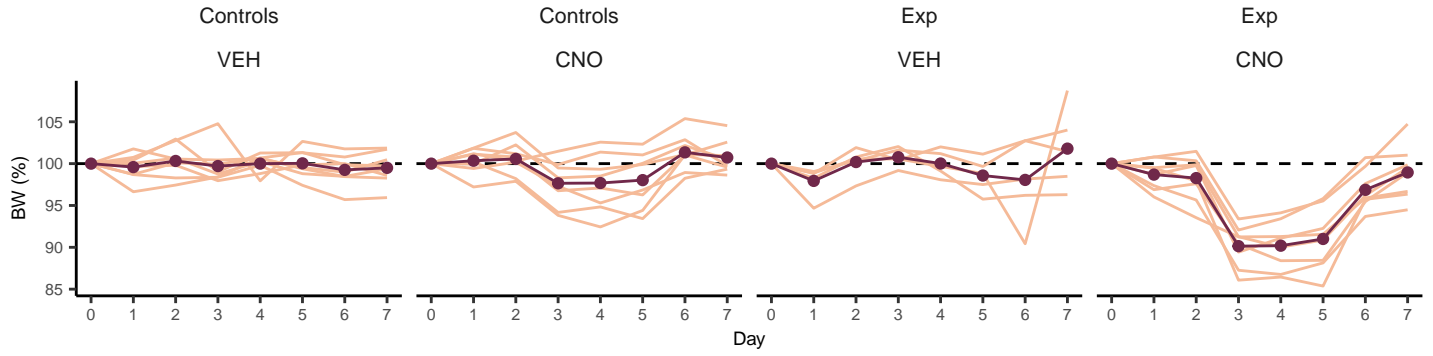
we had to change the CNO water with fresh stuff each day. so water mass 1 corresponded to the previous 24 hours, while water mass 2 was for the proceeding 24 hours

I'm going to use lme4 1.1.26 to run linear mixed models of measurements to look for effects of CNO. For all upcoming plots, the black lines with gray shading is the modeled data and the red line is the daily average for the raw data.

Table 1: Sample breakdown

Group	Treatment	0	1	2	3	4	5	6	7
Controls	VEH	8	8	8	8	8	8	8	8
Controls	CNO	8	8	8	8	8	8	8	8
Exp	VEH	5	5	5	5	5	5	5	5
Exp	CNO	8	8	8	8	8	8	8	8

Body weight



Model

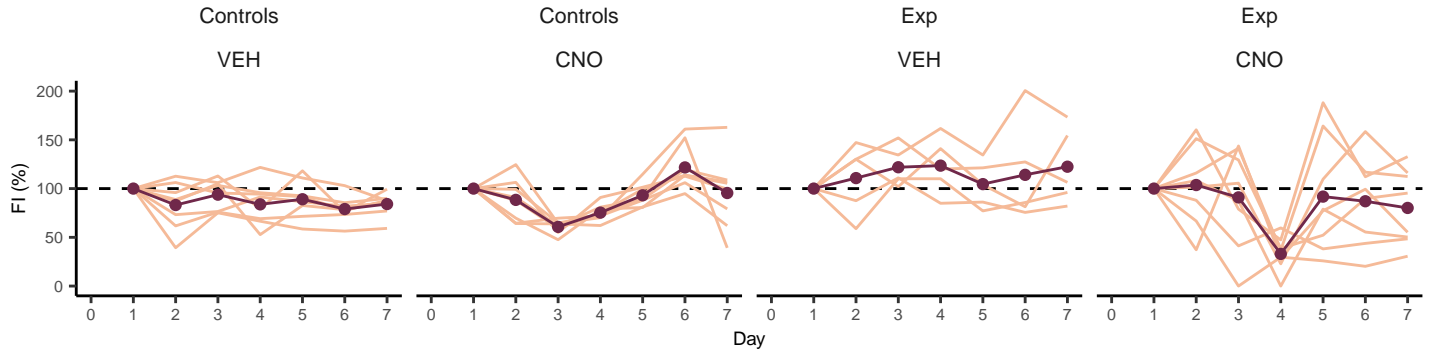
```
model <- aov(value ~ Treatment * Group * factor(Day), df$BW)
```

Comparing across regiments (Controls-CNO v. Controls-VEH and Exp-cNO v. Exp-VMH) for all days of treatment.

Table 2: Body weight results

contrast	Day	Group	estimate	p.value	stars
VEH - CNO	0	Controls	0.00	1.0e+00	
VEH - CNO	1	Controls	-0.76	5.2e-01	
VEH - CNO	2	Controls	-0.25	8.3e-01	
VEH - CNO	3	Controls	2.06	8.0e-02	
VEH - CNO	4	Controls	2.32	4.9e-02	*
VEH - CNO	5	Controls	2.00	8.9e-02	
VEH - CNO	6	Controls	-2.10	7.5e-02	
VEH - CNO	7	Controls	-1.24	2.9e-01	
VEH - CNO	0	Exp	0.00	1.0e+00	
VEH - CNO	1	Exp	-0.75	5.7e-01	
VEH - CNO	2	Exp	1.92	1.5e-01	
VEH - CNO	3	Exp	10.63	1.2e-13	***
VEH - CNO	4	Exp	9.82	4.6e-12	***
VEH - CNO	5	Exp	7.58	4.7e-08	***
VEH - CNO	6	Exp	1.18	3.8e-01	
VEH - CNO	7	Exp	2.84	3.4e-02	*

Food intake



Model

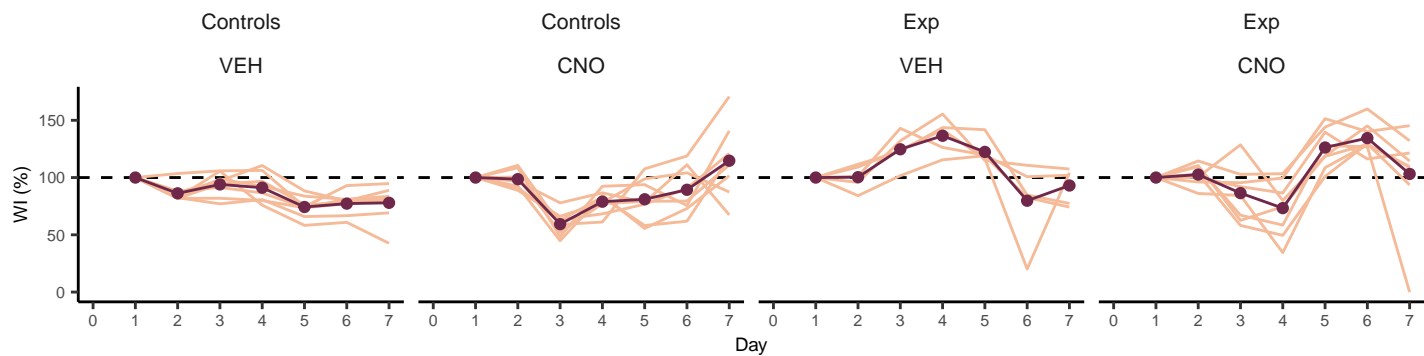
```
model <- aov(value ~ Treatment * Group * factor(Day), df$FI)
```

Comparing across regiments (Controls-CNO v. Controls-VEH and Exp-cNO v. Exp-VMH) for all days of treatment.

Table 3: Food intake results

contrast	Day	Group	estimate	p.value	stars
VEH - CNO	1	Controls	0.00	1.0e+00	
VEH - CNO	2	Controls	-5.29	7.1e-01	
VEH - CNO	3	Controls	33.07	2.1e-02	*
VEH - CNO	4	Controls	8.50	5.5e-01	
VEH - CNO	5	Controls	-4.06	7.8e-01	
VEH - CNO	6	Controls	-42.71	3.1e-03	**
VEH - CNO	7	Controls	-11.33	4.3e-01	
VEH - CNO	1	Exp	0.00	1.0e+00	
VEH - CNO	2	Exp	7.13	6.6e-01	
VEH - CNO	3	Exp	30.93	5.9e-02	
VEH - CNO	4	Exp	90.57	9.3e-08	***
VEH - CNO	5	Exp	12.81	4.3e-01	
VEH - CNO	6	Exp	27.10	9.7e-02	
VEH - CNO	7	Exp	42.31	1.0e-02	*

Water intake



Model

```
model <- aov(value ~ Treatment * Group * factor(Day), df$WI)
```

Comparing across regiments (Controls-CNO v. Controls-VEH and Exp-cNO v. Exp-VMH) for all days of treatment.

Table 4: Water intake results

contrast	Day	Group	estimate	p.value	stars
VEH - CNO	1	Controls	0.00	1.0e+00	
VEH - CNO	2	Controls	-12.37	1.6e-01	
VEH - CNO	3	Controls	34.68	1.2e-04	***
VEH - CNO	4	Controls	12.21	1.7e-01	
VEH - CNO	5	Controls	-6.74	4.5e-01	
VEH - CNO	6	Controls	-12.09	1.7e-01	
VEH - CNO	7	Controls	-36.75	4.8e-05	***
VEH - CNO	1	Exp	0.00	1.0e+00	
VEH - CNO	2	Exp	-2.25	8.2e-01	
VEH - CNO	3	Exp	38.31	1.9e-04	***
VEH - CNO	4	Exp	63.20	2.5e-09	***
VEH - CNO	5	Exp	-3.85	7.0e-01	
VEH - CNO	6	Exp	-54.68	1.8e-07	***
VEH - CNO	7	Exp	-10.10	3.2e-01	

Session info

```
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19041)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_United States.1252
## [2] LC_CTYPE=English_United States.1252
## [3] LC_MONETARY=English_United States.1252
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.1252
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods    base
##
## other attached packages:
## [1] emmeans_1.5.4    lme4_1.1-26      Matrix_1.2-18    readxl_1.3.1
## [5] forcats_0.5.0    stringr_1.4.0    dplyr_1.0.2      purrr_0.3.4
## [9] readr_1.4.0      tidyr_1.1.2      tibble_3.0.4     ggplot2_3.3.2
## [13] tidyverse_1.3.0  kableExtra_1.3.1 knitr_1.30
##
## loaded via a namespace (and not attached):
## [1] Rcpp_1.0.5        mvtnorm_1.1-1    lubridate_1.7.9.2 lattice_0.20-41
## [5] assertthat_0.2.1  digest_0.6.27    plyr_1.8.6       R6_2.5.0
## [9] cellranger_1.1.0  backports_1.2.0  reprex_0.3.0     evaluate_0.14
## [13] httr_1.4.2        pillar_1.4.7     rlang_0.4.9      rematch_1.0.1
## [17] rstudioapi_0.13   minqa_1.2.4      nloptr_1.2.2.2    rmarkdown_2.6
## [21] labeling_0.4.2    splines_4.0.3    webshot_0.5.2     statmod_1.4.35
## [25] munsell_0.5.0     broom_0.7.2      compiler_4.0.3    modelr_0.1.8
## [29] xfun_0.19         pkgconfig_2.0.3  htmltools_0.5.0   tidyselect_1.1.0
## [33] fansi_0.4.1       viridisLite_0.3.0 crayon_1.3.4      dbplyr_2.0.0
## [37] withr_2.3.0       MASS_7.3-53      grid_4.0.3        xtable_1.8-4
## [41] nlme_3.1-149      jsonlite_1.7.2    gtable_0.3.0      lifecycle_0.2.0
## [45] DBI_1.1.0         magrittr_2.0.1    scales_1.1.1      estimability_1.3
## [49] cli_2.2.0         stringi_1.5.3     farver_2.0.3      fs_1.5.0
## [53] xml2_1.3.2        ellipsis_0.3.1    generics_0.1.0    vctrs_0.3.5
## [57] boot_1.3-25       tools_4.0.3       glue_1.4.2        hms_0.5.3
## [61] yaml_2.2.1        colorspace_2.0-0  rvest_0.3.6       haven_2.3.1
```