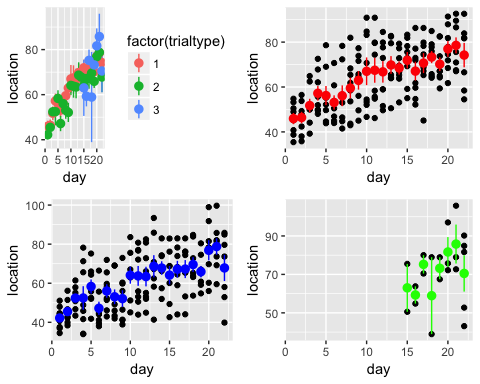
Tennant et al. 2018

This Notebook contains statistical analysis for the experiments described in Tennant et al. 2018. The analysis is organised according to the figures in the paper. All of the figure legends are trancribed. Where the legend refers to a summar plot or statistical analysis the plot and tests results are shown with their corresponding code.

## Figure 1. Mice learn to estimate location using a path integration strategy.

1. Schematic of the virtual track used on beaconed trials (upper) or non-beaconed and probe trials (lower). The reward location is indicated by visual cues from stripes on the floor and walls of the track only on the beaconed trials.
2. Configuration of trial types.
3. Examples of raster plots of stopping locations as a function of track position, separated according to trial type, on day 1 (upper left) and on day 17 (upper right), and corresponding mean number of stops / 10 second bin (lower plots). Stopping locations on the raster plots are indicated by dots, which are red for locations that triggered a reward, and otherwise are black. The mean numbers of stops are indicated by solid lines and shuffled means by dashed lines. The shaded bands around the means indicate the standard error of the mean.
4. Average probability of the first stop on each trial as a function of binned track location for all mice (N = 8 mice) across days 1-5 (blue lines) and days 18-22 of training (red lines) separated according to trial type. Shaded regions indicate standard error of the mean. Bin width is 10 cm.

#import data  
first <- read.csv("Figure1\_E\_0100.csv", header=TRUE)  
colnames(first) <- c("mouse","day","trialtype","location")  
first$trialtype <- factor(first$trialtype)  
  
# first stop data for trial types  
first\_b <- filter(first, trialtype == 1)  
first\_nb <- filter(first, trialtype == 2)  
first\_p <- filter(first, trialtype == 3)  
  
# Plot all data  
g1 <- ggplot(data=first,aes(x = day,y = location)) +  
 aes(colour = factor(trialtype)) +  
 stat\_summary(fun.data = "mean\_se")  
  
# Plot each trial type  
g2 <- ggplot(data=first\_b,aes(x = day,y = location)) +  
 geom\_point() +  
 stat\_summary(fun.data = "mean\_se", colour="red")  
g3 <- ggplot(data=first\_nb,aes(x = day,y = location)) +  
 geom\_point() +  
 stat\_summary(fun.data = "mean\_se", colour="blue")  
g4 <- ggplot(data=first\_p,aes(x = day,y = location)) +  
 geom\_point() +  
 stat\_summary(fun.data = "mean\_se", colour="green")  
  
grid.arrange(g1, g2, g3, g4, ncol=2, nrow=2)

 Average first stop location as a function of training day for each trial type.

am\_b\_model <- lmer(location ~ day + (1|mouse), data=first\_b)  
am\_b\_model\_null <- lmer(location ~ 1 + (1|mouse), data=first\_b)  
anova(am\_b\_model,am\_b\_model\_null)

## Data: first\_b  
## Models:  
## am\_b\_model\_null: location ~ 1 + (1 | mouse)  
## am\_b\_model: location ~ day + (1 | mouse)  
## Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)  
## am\_b\_model\_null 3 1404.9 1414.4 -699.45 1398.9   
## am\_b\_model 4 1287.5 1300.2 -639.76 1279.5 119.39 1 < 2.2e-16  
##   
## am\_b\_model\_null   
## am\_b\_model \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

am\_nb\_model <- lmer(location ~ day + (1|mouse), data=first\_nb)  
am\_nb\_model\_null <- lmer(location ~ 1 + (1|mouse), data=first\_nb)  
anova(am\_nb\_model,am\_nb\_model\_null)

## Data: first\_nb  
## Models:  
## am\_nb\_model\_null: location ~ 1 + (1 | mouse)  
## am\_nb\_model: location ~ day + (1 | mouse)  
## Df AIC BIC logLik deviance Chisq Chi Df  
## am\_nb\_model\_null 3 1430.2 1439.7 -712.12 1424.2   
## am\_nb\_model 4 1339.8 1352.5 -665.91 1331.8 92.417 1  
## Pr(>Chisq)   
## am\_nb\_model\_null   
## am\_nb\_model < 2.2e-16 \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

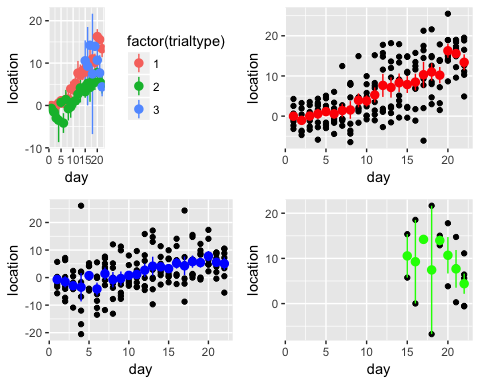
The location of the first stop varied as a function of day for beaconed (p < 2.2 x10-16, χ(1)2 = 119.4 , likelihood ratio test) and non-beaconed trials (p = 2.2 x 10-16, χ(1)2 = 92.4).

first\_groups <- subset(first, day > 17)  
am\_fg\_2 <- aov(location ~ trialtype + Error(mouse/(trialtype)), data=first\_groups)  
summary(am\_fg\_2)

##   
## Error: mouse  
## Df Sum Sq Mean Sq  
## trialtype 1 3038 3038  
##   
## Error: mouse:trialtype  
## Df Sum Sq Mean Sq  
## trialtype 2 389.8 194.9  
##   
## Error: Within  
## Df Sum Sq Mean Sq F value Pr(>F)  
## trialtype 2 396 198.2 1.48 0.233  
## Residuals 87 11652 133.9

There was no significant difference between the three trial types on days 18-22 (p = 0.23, F(2,87) = 1.48), 1-way repeated measures ANOVA). Error bars are standard error of the mean (N = 8 mice for beaconed and non-beaconed trials and N = 6 mice for probe trials).  
(F) Mean z-scored probability of stopping as a function of binned track location for all mice (N = 8) across days 1-5 (black lines) and days 18-22 of training (blue lines) separated according to trial type. Shaded regions indicate standard error of the mean. Bin width is 10 cm. (G)

#import data  
z\_scored <- read.csv("Figure1\_G\_0100.csv", sep=",", header=TRUE)   
colnames(z\_scored) <- c("mouse","day","trialtype","location")  
z\_scored$trialtype <- factor(z\_scored$trialtype)  
  
# z\_scored stop data for trial types  
z\_scored\_b <- filter(z\_scored, trialtype == 1)  
z\_scored\_nb <- filter(z\_scored, trialtype == 2)  
z\_scored\_p <- filter(z\_scored, trialtype == 3)  
  
# Plot all data  
g1 <- ggplot(data=z\_scored,aes(x = day,y = location)) +  
 aes(colour = factor(trialtype)) +  
 stat\_summary(fun.data = "mean\_se")  
  
# Plot each trial type  
g2 <- ggplot(data=z\_scored\_b,aes(x = day,y = location)) +  
 geom\_point() +  
 stat\_summary(fun.data = "mean\_se", colour="red")  
g3 <- ggplot(data=z\_scored\_nb,aes(x = day,y = location)) +  
 geom\_point() +  
 stat\_summary(fun.data = "mean\_se", colour="blue")  
g4 <- ggplot(data=z\_scored\_p,aes(x = day,y = location)) +  
 geom\_point() +  
 stat\_summary(fun.data = "mean\_se", colour="green")  
  
grid.arrange(g1, g2, g3, g4, ncol=2, nrow=2)



Spatial stopping behavior, quantified by the difference between the z score at the start of the track and at the entrance to the reward zone, plotted as a function of training day for each trial type.

z\_b\_model <- lmer(location ~ day + (1|mouse), data=z\_scored\_b)  
z\_b\_model\_null <- lmer(location ~ 1 + (1|mouse), data=z\_scored\_b)  
anova(z\_b\_model,z\_b\_model\_null)

## Data: z\_scored\_b  
## Models:  
## z\_b\_model\_null: location ~ 1 + (1 | mouse)  
## z\_b\_model: location ~ day + (1 | mouse)  
## Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)  
## z\_b\_model\_null 3 1180.6 1190.1 -587.32 1174.6   
## z\_b\_model 4 1040.2 1052.8 -516.09 1032.2 142.47 1 < 2.2e-16  
##   
## z\_b\_model\_null   
## z\_b\_model \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

z\_nb\_model <- lmer(location ~ day + (1|mouse), data=z\_scored\_nb)  
z\_nb\_model\_null <- lmer(location ~ 1 + (1|mouse), data=z\_scored\_nb)  
anova(z\_nb\_model,z\_nb\_model\_null)

## Data: z\_scored\_nb  
## Models:  
## z\_nb\_model\_null: location ~ 1 + (1 | mouse)  
## z\_nb\_model: location ~ day + (1 | mouse)  
## Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)  
## z\_nb\_model\_null 3 1177.7 1187.2 -585.84 1171.7   
## z\_nb\_model 4 1141.8 1154.4 -566.89 1133.8 37.907 1 7.42e-10  
##   
## z\_nb\_model\_null   
## z\_nb\_model \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

The difference varied as a function of day for beaconed (p < 2.2 x10-16, χ(1)2 = 142.5 , likelihood ratio test) and non-beaconed groups (p = 7.4 x10-10, χ(1)2 = 37.9).

z\_scored\_groups <- subset(z\_scored, day > 17 & day < 23)  
am\_fg <- aov(location ~ trialtype + Error(mouse/trialtype), data=subset(z\_scored\_groups, trialtype != 1))  
summary(am\_fg)

##   
## Error: mouse  
## Df Sum Sq Mean Sq  
## trialtype 1 270.4 270.4  
##   
## Error: mouse:trialtype  
## Df Sum Sq Mean Sq  
## trialtype 1 27.48 27.48  
##   
## Error: Within  
## Df Sum Sq Mean Sq F value Pr(>F)  
## trialtype 1 2.5 2.49 0.078 0.781  
## Residuals 50 1593.7 31.87

Probe trials on days 18-22 did not differ from non-beaconed trials (p = 0.78, F(1,50) = 0.08).

Figure 2. Path integration uses motor-related movement signals (A, E) Schematic of track designs used to test a decrease (A) or an increase (E) in the gain between motor and visual reference frames. For standard trials, for every 60 cm mice run on the treadmill, the visual track moves 60 virtual units (VU). On reduced gain trials, for every 60 cm mice run, the visual track moves 30 VU. For increased gain trials the visual track moves 120 VU for every 60 cm mice run. (B, F) Example plots of stop locations from single mice for trials in which the gain between treadmill movement and visual update of the track is reduced by 0.5 (B) or increased by x 2 (F). , (C, G) Average of z-scored stop locations across all mice for control probe trials (x 1) and trials on which the gain is reduced (C) or increased (G). Averaged data is plotted as ± SEM (N = 5 mice for x 0.5 gain, N = 4 mice for x 2 gain). (D, H) To quantify the effects of the gain change we compared, for each trial type, the ratio of stops in the location of the reward zone in the visual reference frame (orange) to the sum of the number of stops in the reward zone in the visual and motor reference frames (green). The ratio is modified by reducing (p = 0.046, paired t-test)(D) or increasing gain (p = 0.0073)(H). Thus, on trials with reduced gain (B-D), or increased gain (F-H) stops occur in anticipation of the reward zone location in the motor reference frame.

Figure 3. Path integration becomes less accurate with increasing distance (A) For tracks of increasing length the distance from the start zone to the reward zone increases as indicated. The length of other parts of the track does not change. (B) Z-scored probability of stopping during probe trials as a function of location for three tracks of increasing length. (C) Mean success rate at obtaining rewards as a function of distance from the start of the track to the reward zone separated according to trial type. The success rate depended on distance to the reward zone (p = 2.72 x 10-8, F(1,90) = 37.1) and on trial type (p <10-16, F(2,90) = 56.3). Accuracy of probe trials differed from beaconed trials (p < 10-6). Success rate depended on distance to the reward zone for probe trial data (χ2(1) = 22.8, p = 1.8 x 10-6), but not for beaconed trial data (χ2(1) = 1.87, p = 0.17).

1. Mean of the most frequent stop location plotted as a function of distance. The most frequent stop location depended on distance to the reward zone (p < 10-16, F(1,90) = 265.5, 2-way repeated measures ANOVA), but was independent of trial type (p = 0.11, F(2,90) = 2.2).

Figure 4. Targeted expression of TeLC to L2SCs abolishes their synaptic output (A) Example of a sagital section from the brain of a Sim1Cre mouse following injection of AAV-TeLC-eGFP into the MEC. Scale bar is 1 mm. (B) Schematic of experiment to test the effect of TeLC expression on synaptic output from L2SCs. AAV-FLEX-ChR2-mCherry and either AAV-FLEX-TeLC-eGFP or AAV-FLEX-eGFP were injected into the MEC of Sim1Cre mice. Synaptic output from L2SCs was evaluated by recording light evoked response of granule cells in the dentate gyrus. (C) Examples of membrane potential responses of dentate gyrus granule cells to optogenetic activation of L2SCs expressing ChR2 and either GFP (left) or TeLC-eGFP (middle). Responses are present in all neurons from control animals (n = 10 neurons, N = 5 mice) and were absent in all neurons from animals expressing TeLC-eGFP (n = 8 neurons, N = 4 mice). The peak response was reduced by expression of TeLC-eGFP (right)(p = 0, percentile bootstrap comparison of control and TeLC-eGFP groups, test statistic = 2.125, 95 % confidence interval [1.69, 4.29]). Circles are individual neurons, diamonds are the population average. Two neurons from two control mice were excluded from the plot and statistical analysis as they showed very large responses that reached action potential threshold preventing their quantification.

Figure 5. Inactivation of L2SCs impairs estimation of location (A) Day of the experiment on which each mouse from TeLC (N = 10) and control groups (N = 6) met the performance criteria to graduate from stage 1 (beaconed and non-beaconed trials) to stage 2 (beaconed, non-beaconed and probe trials) as a function of mean intensity of GFP fluorescence in layer 2 of the dorsal MEC (left), and proportion of mice that had graduated to stage 2 as a function of training day (right). The graduation day correlated with fluorescence intensity for the TeLC group (p = 0.00077, robust least squares regression), but not the control group (p = 0.38; comparison of GFP and TeLC-GFP groups: p = 0.0012, for statistical analysis see Experimental Procedures). (B) Examples of rasters of stopping locations on day 17 of training for a control mouse, and for mice with high and low expression levels of TeLC (lTeLC and hTeLC). Black dots indicating stopping location are absent on some trials because the animal did not stop. (C) Mean z-scored probability of stopping as a function of track location during beaconed trials for GFP only control (left), lTeLC (centre) and hTeLC mice (right) on days 1-5 and days 15-19. (D) Comparison of mean z-scored probability of stopping for trained mice (days 15-19) for each group on beaconed trials (left) and probe trials (right). (E) The difference, between the start of the track and the start of the reward zone, in the probability of stopping (StopsL2-L1)(locations L1 and L2 are indicated in Figure 1A) increased with training for GFP mice (p = 1.4 x 10-10, χ(1)2 = 41.2, likelihood ratio test) and lTeLC mice (p = 5.2 x 10-8, χ(1)2 = 29.6), but not for hTeLC mice (p = 0.89, χ(1)2 = 0.017). (F) Analysis of spatial strategy for beaconed trials during days 15-19. The mean location of the first stop (left) differed between control (GFP) and all TeLC mice (lTeLC and hTeLC combined)(p = 0.021, percentile bootstrap, test statistic = 16.1, confidence interval [2.9, 26.1]), and hTeLC mice differed from control mice (p = 0.01, percentile bootstrap corrected for multiple comparisons, test statistic = 22.2, 95% confidence interval [6.4, 28.6]), but there was no significant difference between lTeLC and control mice (p = 0.09, test statistic = 13.1, 95% confidence interval [-0.23, 26.8]). StopsL2-L1 (right) differed between control and all TeLC mice (lTeLC and hTeLC combined)(p = 0.00052, test statistic = 11.75, 95% confidence interval [3.26, 16.1]), and hTeLC and lTeLC mice differed from control mice (hTeLC: p = 0.0, test statistic = 12.1, 95% confidence interval [8.4, 18.1]; lTeLC: p = 0.034, test statistic = 5.42, 95 % confidence interval [1.1, 14.1]). (G) Running speed in the black box at the end of the track increased with training for all groups of mice (GFP: p = 3.5 x 10-11, χ(1)2 = 43.7; lTeLC: p = 0.0013, χ(1)2 = 10.4; hTeLC: p = 6.5 x 10-6, χ(1)2 = 20.3). During week 4 there was no difference between groups in their running speed within the black box (adjusted p = > 0.7 for all comparisons, percentile bootstrap test). (H) Analysis of spatial strategy for probe trials during days 15-19. The first stop location (left) differed between lTeLC and GFP groups (p = 0.045, test-statistic = 17.9, 95% confidence interval [0.64, 34.5]). StopsL2-L1 during probe trials (right) did not differ significantly between lTeLC and control mice (p = 0.097, test statistic = 10.7, 95% confidence interval [-1.2, 12.2]).

Figure 6. Layer 2 stellate cells are required for object-location recognition (A, D) Schematized organization of the object-location (A) and object recognition (D) experiments. In the test phase of the object-location experiment one object is moved to a novel location (A), whereas in the object recognition experiment a novel object is introduced at a familiar location (D). (B, E) The discrimination index for control mice (Control) differed significantly from mice with output from L2SCs inactivated (TeLC) in the object-location experiment (p = 0.022, unpaired t-test, df = 14, t = 2.58)(B), but not the object recognition experiment (p = 0.19, unpaired t-test, df = 14, t = 1.37)(E). (C, F) Total exploration times in the sample and test phases did not differ between animals in the object location experiment (sample phase p = 0.78, df = 14, t = 0.28; test phase p = 0.17, df = 14, t = 1.45)(C) or in the object recognition experiment (sample phase p = 0.27, df = 14, t = -1.14; test phase p = 0.66, df = 14, t = -0.45)(F), indicating that different recognition scores do not result from differences in overall exploration.