**Supplementary Material**

**A New Tool for the Analysis of the Effect of Intracerebrally Injected Anti-Amyloid-β Compounds**

Supplementary Material 1: Additional Methods

# Animals

 For this study, we used 50-day-old male APP-transgenic mice (n = 8, APPPS1-21) [1]. All mice were housed in the animal care facility of the Department of Comparative Medicine at the University Hospital in Oslo (Norway) with mean temperature of 21-22°C, 12-h day/night cycle, and free access to food and autoclaved water. All experiments were conducted in accordance with the guidelines for animal experiments of the EU (Directive 2010/63/EU) and local animal ethics guidelines.

# Osmotic pumps and surgical techniques

 Intracerebroventricular surgery with mini-osmotic pumps was performed when mice reached an age of 50 days and the mini-osmotic pumps assured 42 days continuous delivery at a rate of 0.15 µl/h. Before surgery, ALZET® mini-osmotic pumps (model 2006) were loaded with 1x phosphate-buffered saline (PBS), which served as control. The pumps were then connected to the brain infusion kit (Brain Infusion Kit 3; ALZET®,Cupertino, CA, USA), and primed in sterile saline at 37°C for 60 h before implantation. The silicone spacers for proper fixation of the cannula were prepared based on our previously published method [2].

 Mice were anesthetized by subcutaneous injection of Zoletil (Vibrac, France) using a dosage of 2.5 µl/g body weight. Fully anesthetized mice were placed in a stereotaxic apparatus (Stoelting, USA) on a heating pad to maintain normal body temperature. Veterinary ophthalmic gel (Lubrithal, Dechra, UK) was applied on the eyes during surgery. Then, a midline incision was made to expose the skull, and a small subcutaneous pocket was opened on the back of the body using a hemostat. A hole was drilled into the skull and the mini-osmotic pumps were implanted into the subcutaneous pocket. The cannula with the attached silicone spacer was implanted into the left lateral ventricle of the brain (coordinates: anteroposterior –0.8 mm, mediolateral –2 mm, dorsoventral –1.5 mm from bregma). The base of the cannula with the silicone spacer was glued on the cleaned skull using an instant adhesive (Locitite 454, DURECT Corporation ALZET, CA, USA). After removing the tab from the infusion cannula, the skin was stitched with a suture thread (Ethilon, 5-0, AgnThos, Sweden). At the end of the surgery, mice were removed from the stereotaxic frame, put back to individual cages, and placed on a heating water apparatus until they woke up. After 50 days, the mini-osmotic pumps were removed, and mice were sacrificed.

# Tissue harvesting

 When mice reached an age of 100 days, they were sacrificed by cervical dislocation and transcardially perfused with PBS. The brains were removed immediately after perfusion, fixed in 4% paraformaldehyde and transferred into 1x PBS supplemented with 0.01% sodium azide after seven days.

# Statistical analysis

 All statistical analyses were performed using Prism (GraphPad Software, San Diego, USA). For the statistical analysis of the immunohistochemical quantification of plaques by distance groups RP and PSL one-way analysis of variance (ANOVA) followed by *post hoc* Holm-Sidak's (parametric) and Kruskal-Wallis followed by *post hoc* Dunn’s (un-parametric) tests were used. In other cases of the immunohistochemical quantification of plaques were analyzed with Student’s *t*-test. Data are presented as mean values (14 IHC stained brain sections from eight animals, i.e., 1-2 per animal) ± standard error of the mean (S.E.M.). Statistical significance was set at p < 0.05.

# Mathematical calculations

 In our Excel tool “Pump\_Animals\_Analyzing\_Tool\_Template.xltm” (provided in Supplementary Material 3), we use different mathematical calculations as described in the following sections. Our calculations follow basic geometry.

##  Slide calculations (worksheet “Slides”)

 In the following sections, we describe the equations and calculations behind the columns in the worksheet “Animals” (Supplementary Table 1).

**Supplementary Table 1.** Equations and calculations behind the columns in the worksheet “Animals”.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Column** | **Value** | **Column** | **Value** | **Column** | **Value** |
| X[H1] | $$x\_{1}$$ | X[I2] | $$x\_{RP2}$$ | b[SL(I1)] | $$b\_{PSL1}$$ |
| Y[H1] | $$y\_{1}$$ | Y[I2] | $$y\_{RP2}$$ | Side[I1] |  |
| X[H2] | $$x\_{2}$$ | m[HSL] | $$m\_{HSL}$$ | X[SL(I2)] | $$x\_{HSL2}$$ |
| Y[H2] | $$y\_{2}$$ | b[HSL] | $$b\_{HSL}$$ | DeltaX[SL(I2)] | $$Δx$$ |
| X[I1] | $$x\_{RP1}$$ | X[SL(I1)] | $$x\_{HSL1}$$ | b[SL(I2)] | $$b\_{PSL2}$$ |
| Y[I1] | $$y\_{RP1}$$ | DeltaX[SL(I1)] | $$Δx$$ | Side[I2] |  |

### Hemisphere straight line (HSL)

 Using the general formula for a straight line

|  |  |  |
| --- | --- | --- |
|  | $$y=mx+b$$ | (1) |

where *m* is the slope and *b* the y-intercept of the line, and two given points on that line

$$P\_{1}\left(x\_{1}, y\_{1}\right)$$

$$P\_{2}(x\_{2}, y\_{2})$$

defined previously in AxioVision (hemisphere border reference points), we can calculate *m* and *b* and thus, the equation of the hemisphere dividing straight line (HSL):

$$m\_{HSL}=(y\_{2}-y\_{1})/(x\_{2}-x\_{1})$$

$$b\_{HSL}=y\_{1}-(m\_{HSL}×x\_{1})$$

$$y=m\_{HSL}x+b\_{HSL}$$

### Hemisphere determination

 $P\_{HSL}\left(x\_{HSL}, y\_{HSL}\right)$ is the point on HSL that has the same y-value as the previously in AxioVision defined reference point $P\_{RP}\left(x\_{RP}, y\_{RP}\right)$. $P\_{RP}$ is either the ipsilateral injection point or the contralateral corresponding point. Then we can calculate $x\_{HSL}$ as follows:

$$x\_{HSL}=(y\_{RP}-b\_{HSL})/m\_{HSL}$$

To determine, in which hemisphere $P\_{RP}$ is located, we calculate the difference Δx:

$$Δx=x\_{RP}-x\_{HSL}$$

If Δx is negative, the reference point $P\_{RP}$ is in the left hemisphere, if Δx is positive, $P\_{RP}$ is in the right hemisphere. Together with the slide-specific information which side contains the injection channel (as given in the worksheet “Animals”), Δx is used to categorize each hemisphere as ipsi- or contralateral.

 Note: This calculation is performed twice, once for each reference point.

### Parallel straight line (PSL)

 The injection or reference channel is defined as the line parallel to the hemisphere border that goes through $P\_{RP}$ (injection or reference point, respectively). Thus, we can calculate $b\_{PSL}$ using $m\_{HSL}$ and $P\_{RP}$

$$b\_{PSL}=y\_{RP}-(m\_{HSL}×x\_{RP})$$

and use (1) to obtain the equation for the parallel straight line (PSL):

$$y=m\_{PSL}x+b\_{HSL}$$

 Note: This calculation is performed twice, once for each reference point.

##  Plaque calculations (worksheet “Measured plaques”)

 In the worksheet “Measured plaques”, we calculate for each plaque the distance from the reference point $P\_{RP}$, the distance from the PSL, and to which size category (small, medium, or large, as defined in the worksheet “Main”) they **belong (Supplementary Table 2).**

**Supplementary Table 2.** Calculations behind the columns in the worksheet “Measured plaques”.

|  |  |
| --- | --- |
| **Column** | **Value** |
| X[P] | $$x\_{Q}$$ |
| Y[P] | $$y\_{Q}$$ |
| X[HSL] | $$x\_{HSLQ}$$ |
| DeltaX | $$Δx$$ |
| Side[P] |  |
| d[RP] | $$d\_{RP}$$ |
| d[PSL] | $$d\_{PSL}$$ |

### Hemisphere determination

 Let $Q(x\_{Q}, y\_{Q})$ be a plaque and $P\_{HSLQ}(x\_{HSLQ}, y\_{Q})$ the point on HSL that has the same y-value as the plaque (similar to section 5.1.2). Then we can calculate $x\_{HSLQ}$ as follows:

$$x\_{HSLQ}=(y\_{Q}-b\_{HSL})/m\_{HSL}$$

To determine, in which hemisphere $P\_{RP}$ is located, we calculate the difference Δx:

$$Δx=x\_{Q}-x\_{HSLQ}$$

If Δx is negative, the plaque is in the left hemisphere, if Δx is positive, the plaque is in the right hemisphere. Together with the slide-specific information which side contains the injection channel (as given in the worksheet “Slides”), Δx is used to categorize each hemisphere as ipsi- or contralateral.

### Distance from reference point

 The distance $d\_{RP}$ of the plaque Q from the reference point $P\_{RP}$ is calculated using the Pythagorean theorem:

$$d\_{RP}=\sqrt{(x\_{RP}-x\_{Q})^{2}+(y\_{RP}-y\_{Q})^{2}}$$

### Distance from PSL

 The distance $d\_{PSL}$ is the distance of the plaque Q from the PSL (see section 5.1.3), a line that is parallel to the HSL and that goes through $P\_{RP}$. $d\_{PSL}$ is calculated using the Hesse normal form to calculate the distance of a point from a line:

$d\_{PSL}=\frac{\left|m\_{PSL}×x\_{Q}-y\_{Q}+b\_{HSL}\right|}{\sqrt{m\_{HSL}^{2}+1}}$

### Distance categories

 Depending on the values of $d\_{RP}$ and $d\_{PSL}$, each plaque is categorized according to the five defined distance categories for RP and PSL. The user can change these categories.

# References

[1] Radde R, Bolmont T, Kaeser SA, Coomaraswamy J, Lindau D, Stoltze L, Calhoun ME, Jaggi F, Wolburg H, Gengler S, Haass C, Ghetti B, Czech C, Holscher C, Mathews PM, Jucker M (2006) Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO Rep* **7**, 940-946.

[2] Sike A, Wengenroth J, Upite J, Bruning T, Eiriz I, Santha P, Biverstal H, Jansone B, Haugen HJ, Krohn M, Pahnke J (2017) Improved method for cannula fixation for long-term intracerebral brain infusion. *J Neurosci Methods* **290**, 145-150.