**Supplementary Material**

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

**Supplementary Material 5:** **Presentation of calculated and measured values in diagrams performing statistical analysis**

To demonstrate the possibilities of our analysis method, we analyzed data from eight PBS-injected APPtg animals and quantified the number of plaques and other parameters in the ipsilateral compared to the contralateral hemisphere. The Excel template calculates the group means based on the number of blocks (i.e., animals, n=8) per group, while the value of each animal may be itself the mean of one or several slides (n=14 slides in total, 2 slides showed staining problems).

1. **Number of plaques**

We determined the number of Aβ plaques in the cortex comparing ipsi- and contralateral hemispheres. Similar data was obtained according to the two different distance category approaches: RP or PSL. No significant difference was observed in the total number of Aβ plaques in the cortex comparing contralateral versus ipsilateral hemispheres (p = 0.6; Supplementary Figure 1A). Multiple comparisons showed that there was no significant difference between hemispheres in the Aβ plaque number by distance category (RP approach - 1st group: p = 0.8; 2nd group: p = 0.9 ; 3rd group: p = 0.9 ; 4th group: p = 0.9; 5th group: p = 0.9; Supplementary Figure 1B; PSL approach - 1st group: p = 0.9; 2nd group: p = 0.5; 3rd group: p = 0.9; 4th group: p = 0.9; 5th group: p = 0.9; Supplementary Figure 1C). If the pump is used to administer a potentially bioactive compound, this statistical comparison will provide information about the distribution of Aβ plaques around the injection site or channel. This information could be used to understand the distribution and efficiency of the experimental compound.



**Supplementary Figure 1.** Bar graphs show quantification according to the location of Aβ plaques (n = 8, control animals treated with PBS). Quantification of total plaque number (A), plaque number by distance category (RP approach) (B), and plaque number by distance category (PSL approach) (C) in the cortex area. Student’s t-test (A), one-way analysis of variance (ANOVA) followed by Holm-Sidak's (B) and Kruskal-Wallis followed by Dunn’s (C) multiple comparisons were used. The data are presented as the mean values ± standard errors of the means (S.E.M.). Statistical significance was set at p < 0.05.

1. **Cortex area covered by plaques**

We determined the absolute area covered by Aβ plaques in the ipsilateral and contralateral hemisphere. We did not observe significant changes between the two hemispheres regarding the total plaque area (p = 0.7; Supplementary Figure 2A). Similarly, there was no significant difference between hemispheres regarding the area covered by plaques when divided into groups according to the two different distance category approaches (RP approach - 1st group: p = 0.8; 2nd group: p = 0.9; 3rd group: p = 0.9; 4th group: p = 0.9; 5th group: p = 0.9; Supplementary Figure 2B; PSL approach - 1st group: p = 0.9; 2nd group: p = 0.7; 3rd group: p = 0.9; 4th group: p = 0.9; 5th group: p = 0.9; Supplementary Figure 2C).



**Supplementary** **Figure 2.** Bar graphs show the absolute cortical area covered by Aβ plaques (n = 8, control animals treated with PBS). Quantification of cortical area covered by plaques (A), and cortical area covered by plaques according to distance category approaches RP (B) and PSL (C). Student’s *t*-test (A) and one-way analysis of variance (ANOVA) Kruskal-Wallis followed by Dunn’s (B, C) multiple comparisons were used. The data are presented as the mean values ± standard errors of the means (S.E.M.). Statistical significance was set at p < 0.05.

1. **Average plaque size**

We further performed in-depth analysis of Aβ plaque size and size distribution in the cortex comparing ipsi- and contralateral hemispheres. Comparing the overall mean plaque size in each hemisphere, we did not find a significant difference (p = 0.6; Supplementary Figure 3A). Additionally we compared the average Aβ plaque size between the two hemispheres by distance category and found no significant difference in any approach (RP approach - 1st group: p = 0.9; 2nd group: p = 0.9 ; 3rd group: p = 0.9 ; 4th group: p = 0.9; 5th group: p = 0.9; Supplementary Figure 3B; PSL approach - 1st group: p = 0.9; 2nd group: p = 0.9; 3rd group: p = 0.9; 4th group: p = 0.9; 5th group: p = 0.9; Supplementary Figure 3C). This result suggests a homogenous distribution of differently sized Aβ plaques across each hemisphere.



**Supplementary Figure 3.** Bar graphs show the mean number of Aβ plaques (n = 8, control animals treated with PBS). Quantification of plaque size (A), and plaque size according to distance category approaches RP (B) and PSL (C). Student’s t-test (A), one-way analysis of variance (ANOVA) followed by Holm-Sidak's (B) and Kruskal-Wallis followed by Dunn’s (C) multiple comparisons were used. The data are presented as the mean values ± standard errors of the means (S.E.M.).

1. **Number of plaques by size group**

We determined the number of Aβ plaques by three size groups in the cortex comparing ipsi- and contralateral hemispheres. Plaques can be categorized into small (≤400 µm2), medium (401–700 µm2) and large (>700 µm2) plaques. There was no statistically significant difference comparing the mean number of plaques by different sizes between contralateral and ipsilateral hemisphere (small: p = 0.9, medium: p = 0.9, large: p = 0.9; Supplementary Figure 4A). Additionally, we compared the number of plaques by sizes small, medium, and large divided into groups according to the two different distance category approaches. We did not observe significant changes between the two hemispheres regarding the number of plaques by small size group (RP approach -1st group: p = 0.9; 2nd group: p = 0.9; 3rd group: p = 0.9; 4th group: p =0.9; 5th group: p = 0.9; Supplementary Figure 4B; PSL approach - 1st group: p = 0.9; 2nd group: p = 0.7; 3rd group: p = 0.9; 4th group: p = 0.9; 5th group: p = 0.9; Supplementary Figure 4C). Further we did not observed a statistical significance between the two hemispheres regarding the number of plaques by medium size group (RP approach - 1st group: p = 0.7; 2nd group: p = 0.9; 3rd group: p = 0.9; 4th group: p =0.9; 5th group: p = 0.6; Supplementary Figure 4D; PSL approach - 1st group: p = 0.9; 2nd group: p = 0.5; 3rd group: p = 0.9; 4th group: p = 0.9; 5th group: p = 0.9; Supplementary Figure 4E). Similarly, there was a no statistical significance observed between both hemispheres regarding the number of plaques by large size group (RP approach - 1st group: p = 0.8; 2nd group: p = 0.9; 3rd group: p = 0.9; 4th group: p = 0.9; 5th group: p = 0.9; Supplementary Figure 4F; PSL approach - 1st group: p = 0.9; 2nd group: p = 0.3; 3rd group: p = 0.9; 4th group: p = 0.9; 5th group: p = 0.9; Supplementary Figure 4G).



**Supplementary Figure 4.** Bar graphs show the mean number of Aβ plaques (n = 8, control animals treated with PBS). Quantification of number of plaques by size groups (A), number of small plaques according to distance category approaches RP (B) and PSL (C). Number of medium plaques according to distance category approaches RP (D) and PSL (E). Number of large plaques according two distance category approaches RP (F) and PSL (G). One-way analysis of variance (ANOVA) followed by Holm-Sidak's (A, D, F) and Kruskal-Wallis followed by Dunn’s (B, C, E, G) multiple comparisons were used. The data are presented as the mean values ± standard errors of the means (S.E.M.).