Supplementary Material

Alpha Synuclein Pre Formed Fibrils Injected into Prefrontal Cortex Primarily Spread to Cortical and Subcortical Structures

SUPPLEMENTARY MATERIALS

Lipopolysaccharide injection challenge

Systemic infection increases risk of developing delirium and dementia in humans [1–3] and peripheral injections of lipopolysaccharide (LPS) are widely used as a model of systemic infection and resultant behavioral changes in rodents [4–6]. At 16 mpi, 0.5 mg/kg LPS (Sigma #L2880, St. Louis, MO) diluted in 0.9% sterile saline was injected intraperitoneally (IP) in five mice with PFC monomers and five mice with PFC PFFs. This dose of LPS was chosen as previous studies demonstrated accelerated decline in rodent models of tauopathy and prion disease [7,8]. The remaining three mice with PFC monomers and three mice with PFC PFFs were injected with equivalent volumes of saline. Behavior in the open field, food burying assay, and nest building was then assessed (Supplementary Figure 1A-D). At 21 mpi, a lower dose (0.25 mg/kg LPS) was injected IP to all mice to examine acute effects of the challenge in the open field and rapid reversal Barnes maze 48 h prior to perfusion (Supplementary Figure 1E-G). In addition to imaging synuclein and tyrosine hydroxylase, separate brain sections were blocked for 1 h in 2% normal goat serum (NGS) in PBST (0.3% Triton X-100 in 1x PBS) and then incubated for approximately 20 h in mouse anti-GFAP (EMD Millipore #MAB3402, Burlington, MA) diluted to 1:500 in blocking solution and rabbit anti-IBA1 (FUJIFILM Wako #019-19741) diluted to 1:1000 in blocking solution. Sections were washed three times and then incubated with Alexa Fluor 488 goat anti-mouse (Invitrogen #A11001, Waltham, MA) and Alexa Fluor 568 goat anti-rabbit (Invitrogen #A11011) for 1 h at room temperature. Sections were washed and mounted with ProLong Diamond Antifade Mountant (Invitrogen #P36961) on Superfrost microscope slides (Fisher Scientific). All sections were imaged as described in the main manuscript.

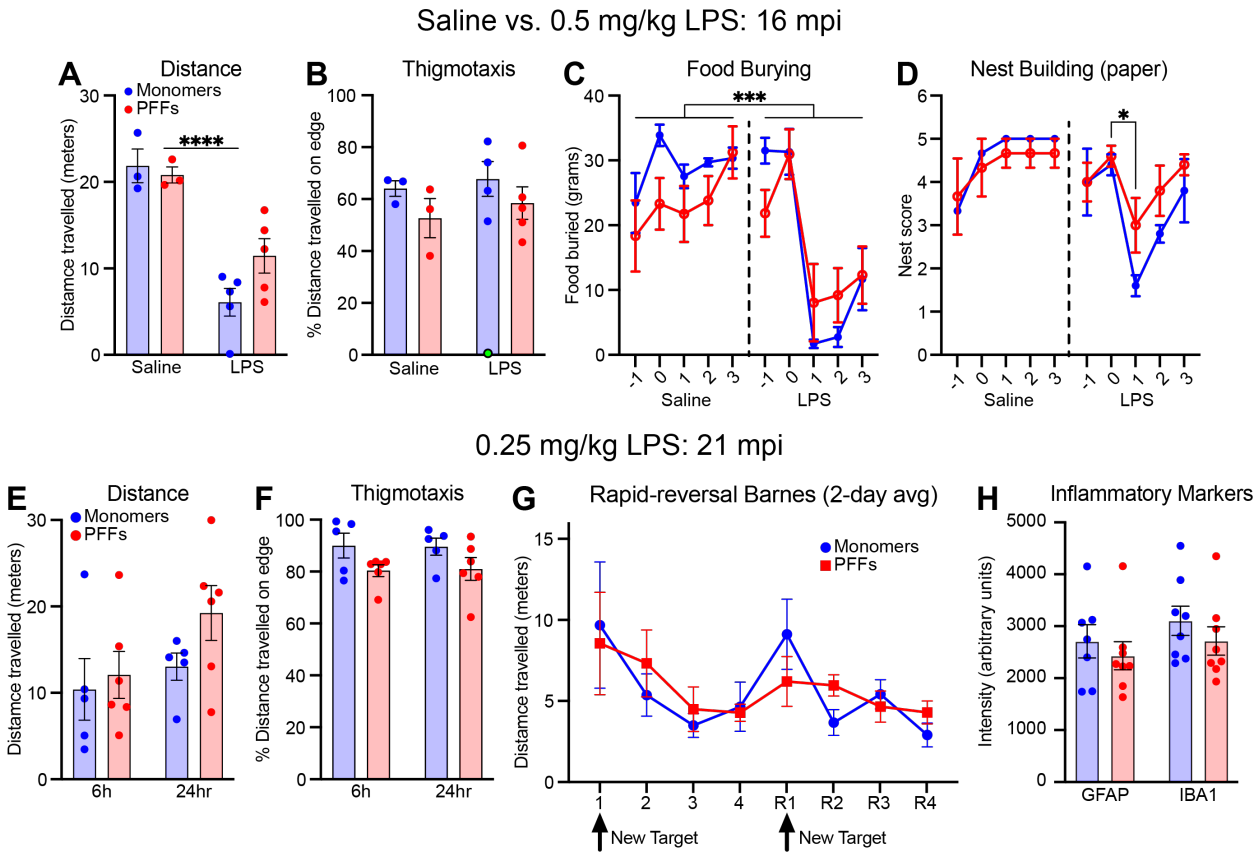
Statistics

Behavioral tasks after LPS challenge were analyzed using two- or three-way ANOVA or Friedman Rank Sum test (nest building only) followed by separate Mann-Whitney *U* tests corrected for multiple comparisons.

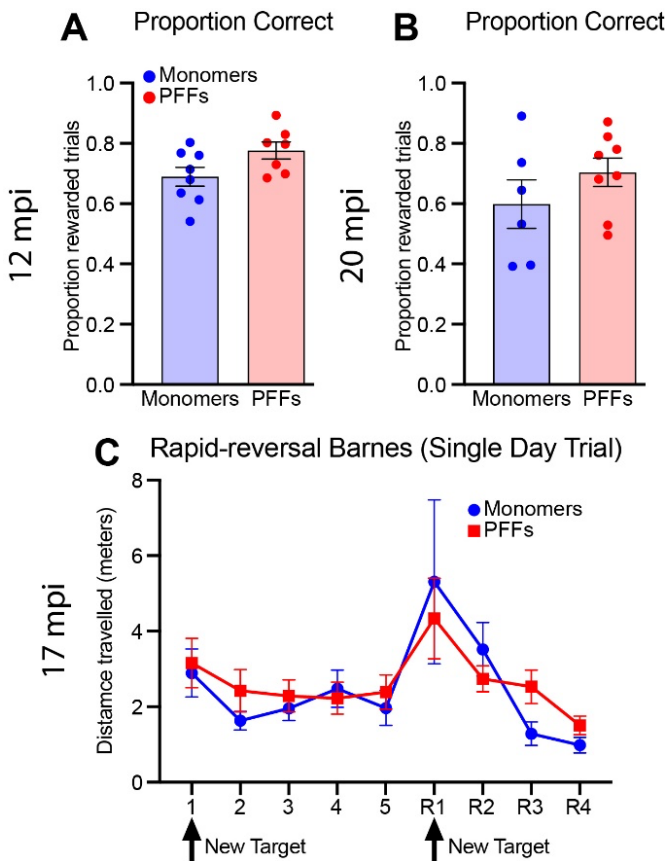
SUPPLEMENTARY RESULTS

We tested if mice previously injected with PFFs were more susceptible to peripheral immune activation by lipopolysaccharide (LPS) injections, which may induce a delirium-like challenge as in previous literature [4–6]. At 16 mpi, five mice with PFFs in the PFC and five mice with monomers in the PFC were injected intraperitoneally (IP) with 0.5 mg/kg LPS. The remaining three mice in each group received equivalent volumes of saline. We then tested behavior in the open field, food burying, and nest building assays. Although LPS caused a significant decrease in distance travelled (Fig. 5A; two-way ANOVA; $F_{(1, 12)} = 44.82$; $p = < 0.0001$) and food burying (Fig. 5C; three-way ANOVA; $F_{(1, 12)} = 25.64$; $p = 0.0003$), we found no evidence of an enhanced response in PFF injected mice (Fig. 5A; distance travelled $F_{(1, 12)} = 1.33$; $p = 0.27$; Fig. 5B; two-way ANOVA; thigmotaxis $F_{(1, 11)} = 0.52$; $p = 0.49$; Fig. 5C; food burying $F_{(1, 12)} = 1.32$; $p = 0.27$). Unlike 12 mpi, there was no significant effect of PFFs on thigmotaxis ($F_{(1, 11)} = 2.46$; $p = 0.15$), possibly due to a lack of statistical power, as the pattern remained similar even in the presence of LPS (saline power: monomers vs. PFFs = 0.53; LPS power: monomers vs. PFFs = 0.13). Similarly, although LPS impaired nest building ($\chi^2(4) = 12.18$, $p = 0.02$), this response was not enhanced in PFC PFF mice (Fig. 5D; 1-day post LPS injection; Mann-Whitney *U*; $p = 0.16$). These exploratory results suggest that PFC PFF mice are not more susceptible to peripheral immune challenge. This is further supported by a final lower dose challenge of 0.25 mg/kg LPS given at 21 mpi. PFC PFF mice were not more susceptible to LPS challenge in the open field or rapid reversal Barnes maze.

While the LPS challenge induced significant changes in behavior over a range of tasks (Supplementary Figure 1A-D), the experiment was underpowered to detect smaller differences between monomer treated control and PFF treated mice (Supplementary Figure 1A-G). Thus, conclusions based on these experiments are limited due to lack of statistical power.



Supplementary Figure 1. Exploratory investigation of inflammatory challenge with lipopolysaccharide (LPS). At 16 months post injection (mpi), five mice with PFFs in the PFC and five mice with monomers in the PFC were injected intraperitoneally (IP) with 0.5 mg/kg LPS. The remaining three mice in each group received equivalent volumes of saline. A) Distance travelled and B) thigmotaxis during the open field after either saline or LPS injection. There was a main effect of LPS on distance traveled, but this effect was not different for monomer and PFF mice. One monomer mouse treated with LPS was excluded from thigmotaxis analysis as no movement was recorded during the assay. C) Amount of food buried (in grams) during the food burying assay and D) nest building score (shredded paper only) for 2 days before and 3 days after either saline or LPS injection. At 21 mpi, all remaining mice were injected with 0.25 mg/kg LPS. E) Distance travelled and F) thigmotaxis during the open field test. PFC PFF injected mice did not differ from controls in distance travelled (meters) either 6- or 24-h post LPS injection (two-way repeated-measures ANOVA; timepoint: $F(1, 9) = 5.115$; $p = 0.05$; treatment: $F(1, 9) = 1.272$; $p = 0.29$). However, PFC PFF mice did display a non-significant trend for reduced thigmotaxis (two-way repeated-measures ANOVA; timepoint: $F(1, 10) = 0.003$; $p = 0.96$; treatment: $F(1, 9) = 4.096$; $p = 0.07$), regardless of timepoint post injection ($F(1, 10) = 0.003$; $p = 0.96$). G) Average total distance travelled averaged across two-days post LPS of the rapid reversal Barnes maze (two-way repeated-measures ANOVA; trial: $F(2.626, 21.01) = 2.667$; $p = 0.08$; treatment: $F(1, 8) = 0.034$; $p = 0.86$). H) Fluorescent intensity (arbitrary units) of GFAP and IBA1 in whole striatum. All data are expressed as mean ± SEM, and each dot represents a single mouse. *p < 0.05; ***p < 0.001; ****p < 0.0001



Supplementary Figure 2. Additional measures of interval timing performance and behavioral flexibility. Proportion of rewarded trials during the interval timing switch task at A) 12 mpi and B) 20 mpi. Unpaired t-tests revealed no differences in the proportion correct at either timepoint (12 mpi: $t(13) = 2.03$; $p = 0.06$; 20 mpi: $t(12) = 1.2$; $p = 0.25$) C) Average total distance travelled during a single day rapid reversal Barnes maze protocol. Compared to the multiple day Barnes maze protocol at 21 mpi, PFC PFF mice show a similar, but non-significant, altered pattern of behavioral flexibility with a greater distance travelled to reach the target during the reversal phase (two-way repeated-measures ANOVA; trial: $F(2.182, 30.55) = 4.232$; $p = 0.02$; treatment: $F(1, 14) = 0.2317$; $p = 0.64$). All data are expressed as mean ± SEM, and each dot represents a single mouse.

Supplementary Figure 3. Top: representative images across the entire rostral-caudal aspect of the brain in two mice treated with PFFs (one saline and one LPS treated at 16 mpi, sacrificed at 21 mpi). Bottom: representative images of two monomer control mice (one saline and one LPS treated at 16 mpi, sacrificed at 21 mpi).

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