

# Turning preferences in the corner turning test and the status of proinflammatory cytokines in the right-left brain hemispheres in the lipopolysaccharide-induced neuroinflammation model\*

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## ABSTRACT

**Aims:** Neuroinflammation is described as an inflammatory response within the nervous system. The study aimed to investigate the behavior of rats in the corner turning test and the distribution of proinflammatory cytokines between the right/left hemispheres in the neuroinflammation model induced by lipopolysaccharide. Furthermore, locomotor activity, anxiety-like behaviors, and stress-related Harderian gland secretion were also analyzed.

**Methods:** 12 adults male Wistar albino rats were divided into control (n=6) and neuroinflammation animals (n=6). The neuroinflammation group received a single dose of 2 mg/kg intraperitoneally LPS, and 1 ml/kg physiological saline was administered to the control group. Six hours after LPS administration, open field test was performed for 5 minutes. The corner rotation test protocol was performed 6 and 24 hours after LPS administration. Subjects were sacrificed 24 hours after LPS administration. TNF- $\alpha$  and IL-1 $\beta$  were analyzed in the prefrontal cortex, striatum, and serum. All procedures were conducted with the approval of the Balıkesir University Experimental Animals Ethics Committee (Date: 22.11.2023, Decision No: 2023/10-5). Results were analyzed using the students' T test.

**Results:** In the open field test, locomotor activity decreased ( $p < 0.0001$ ), while anxiety-like behaviors increased ( $p < 0.05$ - $p < 0.0001$ ). Harderian gland secretion significantly increased by 4-5 points in the neuroinflammation group ( $p < 0.0001$ ). No difference was observed between the control and LPS groups in the corner-turning test ( $p > 0.05$ ). TNF- $\alpha$  and IL-1 $\beta$  significantly increased compared to the control group ( $p < 0.01$ - $p < 0.0001$ ). There was no difference in proinflammatory cytokines between the right hemispheres ( $p > 0.01$ ).

**Conclusion:** In the neuroinflammation group, anxiety-like behaviors and proinflammatory cytokines increased. Stress-related spotting around the eyes and nose increased. According to the corner-turning test, no significant difference was observed. More studies are needed in neuroinflammation models with the corner-turning test, which examines damage to the hemispheres in neurological experiments.

**Keywords:** Anxiety-like behaviors, corner-turning test, neuroinflammation, open-field test, right-left hemisphere differences

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## INTRODUCTION

Inflammation is the complex reaction to damage or injury caused by endogenous or exogenous stimuli in tissue. Inflammation involves processes that eliminate the cause of the injury and repair the damage in the tissue.<sup>1</sup> This process provides a protective effect on the body.<sup>2</sup> Excessive and prolonged inflammation, on the other hand, contributes to the pathophysiology of many disorders.<sup>3</sup> Neuroinflammation is the inflammatory response in the brain and spinal cord.<sup>4</sup> Astrocytes, microglia, endothelial cells in the blood-brain barrier, and neurons are important cells involved in the inflammatory process.<sup>5</sup> Lipopolysaccharide (LPS) is a large molecule found in the outer membrane of gram-negative bacteria.<sup>6</sup> LPS triggers the immune system's activation.<sup>7</sup>

LPS can cross the blood-brain barrier and thus is often administered subcutaneously and intraperitoneally to create a rodent neuroinflammation model.<sup>7</sup> Systemic administration of LPS has been shown to increase anxiety-depression-like behaviors and cognitive impairments in rats.<sup>8,9</sup> In addition to behavioral studies, alterations in the cytokine profile have been investigated in different hemispheres and regions of the brain.<sup>10,11</sup>

The corner turn test is a behavioral test used to confirm damage occurrence and assess the healing process's prognosis in models where unilateral lesions or damage have been induced in the central nervous system. This test is an essential assessment of its ability to turn backward. Rats with damage

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to the brain's right hemisphere turn to the right, while those with damage to the left hemisphere turn to the left (ipsilateral turning). In healthy control groups, lesions' presence or absence and turning backward from both the right and left sides are equal and random. In studies investigating different brain pathologies, the corner turn test is repeatedly can be conducted to confirm lesions' presence or absence and examine the recovery process without sacrificing the animals. Its low cost and reusability are significant advantages.<sup>12,13</sup>

No studies investigate rat behavior in the corner turning test in a neuroinflammation model created with systemic LPS administration. The study aims to identify the difference in the degree of behavioral impairments, including turn-corner preference, locomotor activity, exploring, and anxiety-like behaviors after systemic LPS administration. In addition to analyzing the preference for corner turning, the study also aimed to analyze proinflammatory responses in different neuroanatomical regions of the left and right hemispheres.

## METHODS

### Animals

12 Wistar Albino rats (adult, male) were purchased from Balıkesir University. The groups were divided into control (n=6) and the lipopolysaccharide-induced neuroinflammation group (n=6). The animals were fed ad libitum. This study was approved by the Balıkesir University Experimental Animals Ethics Committee (Date: 22.11.2023, Decision No: 2023/10-5). All procedures were carried out in accordance with the ethical rules and the principles.

### Neuroinflammation Model

A single dose of Lipopolysaccharide (Sigma Aldrich, *Escherichia coli* O111:B4) was administered intraperitoneally at 2 mg/kg.<sup>7</sup> The control group received physiological saline at 1 ml/kg.

### Open Field Test

The open field test (OFT) investigates general locomotor activity and anxiety-like behaviors.<sup>14</sup> For 5 minutes, a camera recorded behaviors. After the experiment, the OFT was cleaned with 70% alcohol. The total distance traveled (horizontal movement) and total rearing number (total vertical movement) were analyzed for locomotor activity.

A decrease in time spent in the central zone, number of entries into the central zone, and number of unsupported rearing numbers is a sign of increased anxiety-like behavior. Furthermore, prolonged rearing latency time indicates an increase in anxiety behaviors.

### Corner Turning Test

Performed to confirm unilateral damage to the striatum and cortex or to examine the recovery process.<sup>15</sup> While control animals turn equally to the right or left, test animals with damage turn backward toward the side with greater damage (ipsilateral turning). Animals will be approached to a 30-degree corner formed by two plexiglass walls. The animals' turn to return from the corner and exit, either to the left or right, will be recorded. Twenty trials will be conducted. (A

30-second rest period will be applied between each trial) Furthermore, the number of turns to the left and right will be recorded. The number of turns to the left or right will be expressed as a percentage of the total number of turns. Example of formula:  $[(\text{left turns})/(\text{total turns})\times 100]$ .<sup>15</sup>

### Harderian gland secretion score (Chromodacryorrhoea Scale)

The Harderian gland is located near the eyes of various animals, including rats and mice. Under stress and disorders, the secretion of the Harderian gland increases in rats, causing discoloration around the eyes and nose.<sup>16</sup> This condition is called chromodacryorrhoea. The chromodacryorrhoea's grading scale ranges from zero to five, with five representing severe black-red staining extending to the eye-nose area. According to the Chromodacryorrhoea scale, score 0: No drops present; score 1: A single red-black drop of 1 mm or smaller; score 2: A drop larger than 1 mm or several small red-black drops; score 3: Assigned when several large drops or numerous small red-black drops form, score 4: Assigned when approximately 25-50% of the eye or nose is covered by red-black drops, score 5: Assigned when red-black drops cover more than 50% of the eye area or nose.<sup>16</sup>

### Anesthesia and Molecular Analysis

The animals were euthanized under anesthesia with 50 mg/kg ketamine+10 mg/kg xylazine, and the heart was punctured to drain the blood. The prefrontal cortex and striatum were dissected according to the rat brain atlas.<sup>17</sup> The removal of brain tissues was performed on an ice block and completed within 2 minutes. Tissue samples were stored at -80 °C until analysis. The tissues were then homogenized for molecular analysis. Proinflammatory cytokines were analyzed in the subjects' prefrontal cortex and striatum, obtained from both the right and left hemispheres. In addition, inflammation markers were analyzed in the serum. ELISA kits were used according to the manufacturer's instructions (TNF- $\alpha$  (BT Lab, catalog no: E0764Ra), IL-1 $\beta$  (BT Lab, catalog no: 0119Ra).

### Statistical Analysis

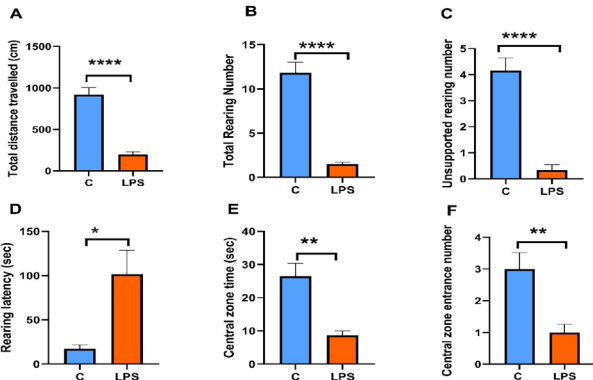
Behavioral and molecular results were assessed for normal distribution using the Shapiro-Wilk test because the number of subjects in each group was less than 15. Since the data showed a normal distribution, it was analyzed using student's T test. The results of the study are presented as mean $\pm$ SEM. Differences were considered statistically significant at the p<0.05 level. According to the Source Equation method for preclinical studies, a two-group design with six subjects in each group is sufficient.<sup>18</sup>

## RESULTS

### Behavioral Results

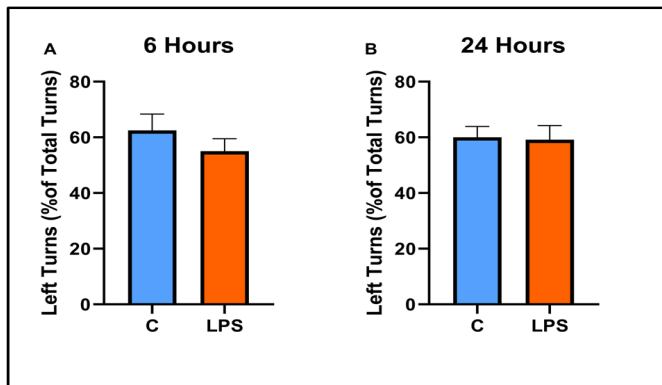
The open-field test was used to examine locomotor activity and anxiety-like behaviors, as shown in **Figure 1**. Following LPS administration, locomotor activity in both the vertical (total rearing number) and horizontal directions (total distance traveled) was reduced in the experimental group compared to the control group (p<0.0001). Similarly, the number of unsupported rearing numbers (p<0.0001), the time

spent in the central zone ( $p < 0.01$ ), and the number of entries into the central zone (0.01) were significantly reduced in the LPS group. Rearing latency time was prolonged in the LPS group compared to the control group ( $p < 0.05$ ). The Corner turning percentages in the LPS group were analyzed 6 hours and 24 hours later and are presented in **Figure 2**. There was no difference between the groups regarding corner turning percentages in both time intervals ( $p > 0.05$ ). The Harderian gland score was dramatically increased in the LPS group compared to the control group ( $p < 0.0001$ ), (**Figure 3**).



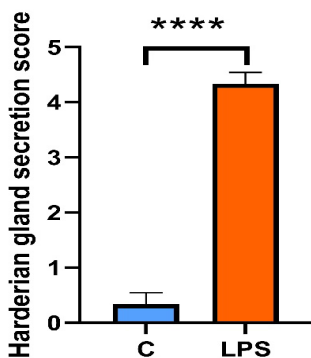
**Figure 1.** Behavioral findings in the open field test in the experimental groups (A) Total distance travel, (B) Total rearing number, (C) Unsupported rearing number, (D) Rearing latency, (E) Central zone time, (F) Central zone entrance number values in the graphs are presented as the means±SEMs (student's T test, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$ )

C: Control group, LPS: Lipopolysaccharide group SEMs: Scanning electron microscopy



**Figure 2.** Behavioral findings in the corner turning test in the experimental groups (A) Left turns -6 hours, (B) Left turns-24 hours values in the graphs are presented as the means±SEMs (student's T test)

C: Control group, LPS: Lipopolysaccharide group, SEMs: Scanning electron microscopy

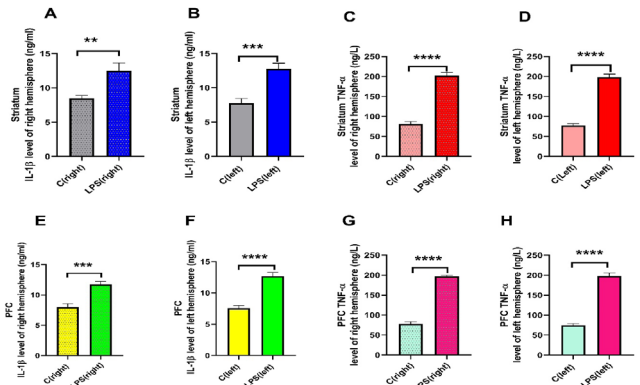


**Figure 3.** The behavioral finding of Harderian gland secretion in the experimental groups, Harderian glands secretion score values in the graph is presented as the means±SEMs (student's T test, \*\*\*\* $p < 0.0001$ )

C: Control group, LPS: Lipopolysaccharide group, SEMs: Scanning electron microscopy

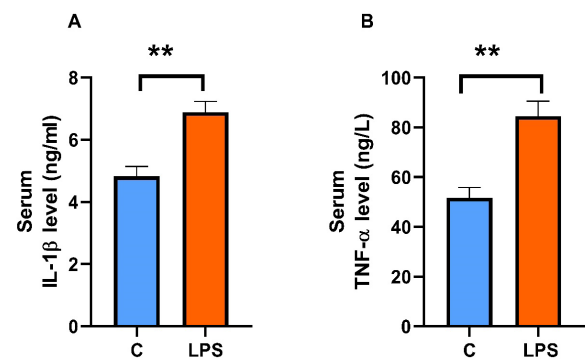
**Molecular Results**

The levels of proinflammatory cytokines were examined in the neuroinflammation model created with the LPS application and are shown in **Figure 4**. TNF- $\alpha$  increased significantly in both the right and left striatum ( $p < 0.0001$ ). Similarly, TNF- $\alpha$  rose considerably in the right and left prefrontal cortex ( $p < 0.0001$ ). According to IL-1beta results, IL-1beta increased significantly in both the right ( $p < 0.01$ ) and left ( $p < 0.001$ ) striatum of the LPS group. Similarly, IL-1beta increased remarkably in the right ( $p < 0.001$ ) and left ( $p < 0.0001$ ) prefrontal cortex (**Figure 4**). TNF alpha and IL-1 beta levels in serum were significantly higher in the LPS group compared to the control group ( $p < 0.001$ ), (**Figure 5**).



**Figure 4.** Molecular findings in the experimental groups (A) Striatum IL-1 $\beta$  of the right hemisphere, (B) Striatum IL-1 $\beta$  of the left hemisphere, (C) Striatum TNF- $\alpha$  of the right hemisphere, (D) Striatum TNF- $\alpha$  of the left hemisphere, (E) PFC IL-1 $\beta$  of the right hemisphere, (F) PFC IL-1 $\beta$  of the left hemisphere, (G) PFC TNF- $\alpha$  of the right hemisphere, (H) PFC TNF- $\alpha$  of the left hemisphere, values in the graphs are presented as the means±SEMs (student's T test, \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ )

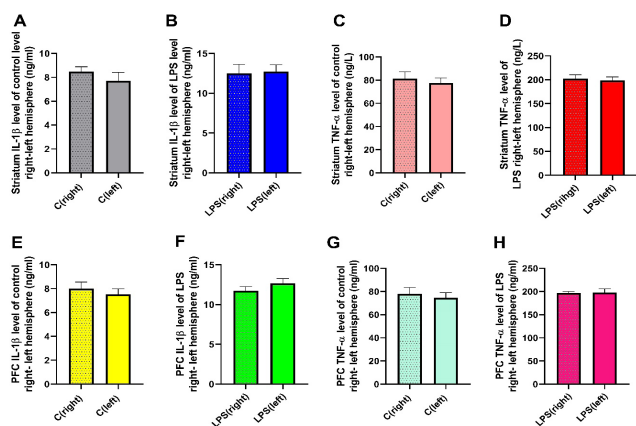
C: Control group, LPS: Lipopolysaccharide group, PFC: Prefrontal cortex, TNF- $\alpha$ : Tumor necrosis factor-alpha, IL-1 $\beta$ : Interleukin-1beta, SEMs: Scanning electron microscopy



**Figure 5.** Molecular findings in the experimental groups (A) Serum IL-1 $\beta$ , (B) Serum TNF- $\alpha$  values in the graphs are presented as the means±SEMs (student's T test)

C: Control group, LPS: Lipopolysaccharide group, TNF- $\alpha$ : Tumor necrosis factor-alpha, IL-1 $\beta$ : Interleukin-1beta, SEMs: Scanning electron microscopy

The study investigated whether there was a difference between the left and right hemispheres in the prefrontal cortex and striatum. In the control group, TNF-alpha and IL-1beta results were similar in both brain regions, with no significant difference observed ( $p > 0.05$ ). Similarly, in the LPS group, the proinflammatory cytokine profile was similar in the right and left hemispheres ( $p > 0.05$ ), (**Figure 6**).



**Figure 6.** Molecular findings in the experimental groups (A) striatum IL-1β of the control right-left hemisphere, (B) Striatum IL-1β of the LPS right-left hemisphere, (C) Striatum TNF-α of the control right-left hemisphere, (D) Striatum TNF-α of LPS the right- left hemisphere, (E) PFC IL-1β of the control right-left hemisphere, (F) PFC IL-1β of LPS right-left hemisphere, (G) PFC TNF-α of the control right-left hemisphere, (H) PFC TNF-α of LPS right-left hemisphere, values in the graphs are presented as the means±SEMs (student's T test)

C: Control group, LPS: Lipopolysaccharide group, PFC: Prefrontal cortex, TNF-α: Tumor necrosis factor-alpha, IL-1β: Interleukin-1beta, SEMs: Scanning electron microscopys

## DISCUSSION

The present study investigated different behavioral patterns and a detailed proinflammatory cytokine profile in a neuroinflammation model induced by LPS.

Six hours after LPS administration, a significant decrease in locomotor activity was observed in rats during the open field test. The animals exhibited minimal movement in both vertical and horizontal directions. Similar reductions in locomotor activity have been reported in other neuroinflammation studies. The increased proinflammatory response in the brain is likely to contribute to suppressing locomotor activity.<sup>19,20</sup>

According to open-field test results, anxiety-like behaviors increased in the subjects. The subjects avoided the center of the open field and did not want to enter the central area. In addition, unsupported rearing, a research behavior, decreased drastically in the subjects. The rearing behavior latency time performed by the subjects to explore the new environment occurred much later. The findings obtained are consistent with those of other neuroinflammation studies.<sup>21,22</sup> Also, the subjects' stress-related Harderian gland secretion increased, and black-red spotting showed in their eyes and noses. Similar findings were observed in the neuroinflammation model conducted by Çalışkan et al.<sup>7</sup> in 2024.

The corner-turning test is frequently preferred in brain injury models. After lesion formation, motor functions in the contralateral extremity are impaired. For instance, motor dysfunction occurs in the right leg and arms after left hemisphere lesions. Therefore, rats with left hemisphere damage move their left extremities. In the corner-turning test, subjects turn to the left if the brain lesion is on the left side and to the right if it is on the right (ipsilateral turning).<sup>12,15</sup>

The behavior of subjects in the corner turning test was analyzed for the first time in the literature in a systemic high-dose neuroinflammation model. No ipsilateral dominant turning was observed in the subjects; they turned both to the

right and left sides. This indicates that the LPS administered systemically affects both hemispheres behaviorally similarly. In the 6-hour and 24-hour results, rats administered with LPS exhibited turning to both the right and left sides, similar to the control group.

To investigate whether neuroinflammation occurs in subjects, cytokines such as TNF-α and IL-1β are frequently measured.<sup>23,24</sup> In the present study, high levels of TNF-α and IL-1β were found in the PFC, striatum, and serum after high-dose LPS administration. Neuroinflammation occurred in both the right and left hemispheres.

According to the results of the neuroinflammation group, proinflammatory cytokine levels between the right and left hemispheres were similar. Systemic LPS administration led to a proinflammatory cytokine response in both the right and left hemispheres. This finding is consistent with the corner-turning test. The absence of differences between the right and left hemispheres in the corner turning test and the neuroinflammation group indicates that systemic LPS induces a homogeneous inflammatory response in the brain hemispheres. Therefore, researchers can dissect PFC or striatum in the right or left hemisphere following systemic LPS administration.

This study analyzed the prefrontal cortex and striatum regions, which play an important role in anxiety pathology.<sup>7,18</sup> Both brain regions exhibited parallel data without differences between the right and left hemispheres. Certain factors, such as BDNF, were demonstrated to be transported between the PFC and striatum via anterograde transport pathways.<sup>25</sup> A similar mechanism may also be present for the proinflammatory cytokines discussed in this study.

Systemic administration of LPS not only affects the central nervous system, but also the peripheral organ systems. LPS can also cause inflammation and damage to vital organs such as the liver, lungs, kidneys, and heart.<sup>26,27</sup>

Koc et al.<sup>27</sup> reported that systemic LPS administration resulted in elevated liver function enzymes, impaired antioxidant systems, and histopathological changes. Similar histopathological abnormalities and oxidative stress were also observed in the lungs and kidneys in the same study. Researchers investigating changes in behavioral patterns following systemic LPS administration should consider the possibility that other organs may also be affected.

The hypothalamic-pituitary-adrenal axis may be activated due to systemic inflammation. Indeed, it has been demonstrated that different glucocorticoids increase following LPS administration.<sup>28,29</sup> The timing of LPS administration and the time of animal sacrifice are critically important.

When LPS is administered in the early morning and the rat is sacrificed exactly 24 hours later, the morning glucocorticoid peak may suppress inflammation. Researchers cannot determine whether the suppression of inflammation is due to the agent administered for therapeutic purposes or due to the morning rise in glucocorticoids. Therefore, sacrificing the animal at a time closer to midday, when glucocorticoid production is near its plateau, may be beneficial.<sup>7</sup>

In recent years, research has focused on inflammasomes, which are protein complexes associated with intracellular inflammation. In particular, the NLRP3 inflammasome is activated by LPS.<sup>30</sup> TNF- $\alpha$  activates independently of the NLRP3 inflammasome. IL-1 $\beta$ , on the other hand, increases its secretion upon activation of the intracellular NLRP3 inflammasome.<sup>30</sup> Therefore, the co-action of these two pro-inflammatory cytokines may provide insights into two distinct inflammatory pathways. Potential anti-inflammatory agents developed for therapeutic or prophylactic purposes could selectively suppress one of these pathways. Consequently, investigating the co-action of both pro-inflammatory cytokines would be beneficial for validating the model.

### Limitations

The present study has some limitations. The first limitation is that it was conducted only on male subjects. Experiments should also be performed on female rats for translational medicine and public health. This limitation, mainly due to budget and time constraints, should be addressed in future studies. Another limitation is that the subjects' dominant paw (right or left) was not examined. In future studies, a paw preference test should be conducted to identify dominant paw use, an indicator of brain lateralization. Additionally, other proinflammatory cytokines and different neuroanatomical regions should also be investigated.

### CONCLUSION

In the present study, a high dose of 2 mg/kg LPS increased anxiety-like behaviors and harderian gland secretion. In the corner turn test, the subjects turned to both the right and left sides. TNF- $\alpha$  and IL-1 $\beta$  levels were significantly elevated in the right-left PFC and striatum in the neuroinflammation group. In the neuroinflammation group, proinflammatory cytokine elevations were similar in both the right and left hemispheres. This similarity, along with the turning behavior from both sides in the corner turn test, indicates that the neuroinflammation model induced by systemic LPS administration exhibits a homogeneous effect on the central nervous system.

### ETHICAL DECLARATIONS

#### Ethics Committee Approval

The study was carried out with the permission of the Balıkesir University Experimental Animals Ethics Committee (Date: 22.11.2023, Decision No: 2023/10-5).

#### Informed Consent

The informed consent form was not obtained because of the study's experimental design.

#### Referee Evaluation Process

Externally peer-reviewed.

#### Conflict of Interest Statement

The authors have no conflicts of interest to declare.

### Financial Disclosure

The authors declared that this study has received no financial support.

### Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

### Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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