Role of the Neuropeptide OFQ/N in Neuroinflammation

Paisley Payne, Marialaina Nissenbaum, Alexander Kusnecov

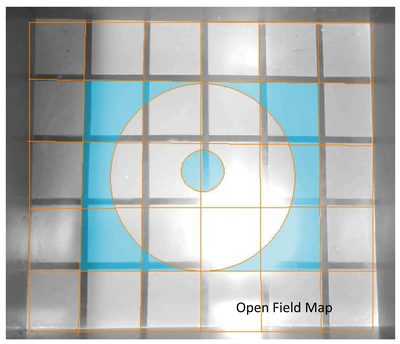
RUTGERS
Aresty Research Center for Undergraduates

Abstract

Bacterial lipopolysaccharide (LPS) is an endotoxin that activates the immune system and enables macrophages to release proinflammatory cytokines which induce stress-like effects in the brain. In the current study, the goal was to determine whether the inflammatory and behavioral response to LPS differed in mice deficient for the neuropeptide OFQ/N, or its receptor, NOP/ORL. OrphaninFQ/nociceptin (OFQ/N) has several functions, and may be important in containing the magnitude of the stress response. Male and female mice with a C57/BL6 background were grouped by genotype (wild-type, OFQ knockout, or ORL-1 knockout) and treated with an acute injection of LPS (5ug) or saline control. Two hours after injection, mice were tested for behavior in the open field (OF) and responsiveness to a novel object (OF/NO test). One hour after completion of the 10 minute OF/NO test, animals were perfused and brains and spleen collected. Spleens were measured for the cytokine interleukin-1β (IL-1β), while brains were assayed for immediate early gene expression (data collection in progress). Wildtype mice responded to LPS by showing delayed entrance into the inner zone surrounding a novel object stimulus. The response of ORL-1 KO mice treated with LPS also showed a significant decrease in distance travelled in the NO/OF, compared to the saline control. In contrast, for OFQ knockouts there was no significant difference in travel distance between LPS and Saline-treated groups. However, latency to enter the inner zone (i.e. where the novel object was located) was highest for LPS treated OFQ knockouts, and was also significantly different between LPS and saline-treated ORL knockouts. Finally, the IL-1β cytokine levels in knockout animals were higher in LPS treated animals compared to saline, confirming the proinflammatory response to LPS. In conclusion, deletion of ORL-1 receptor seems to cause the most significant change in behavior, suggesting that it has a protective function against immune challenge with LPS.

Background

When depressed, every organism displays a natural set of symptoms similar to those seen in infectious illness (eg,. lethargy, fatigue, appetite loss and reduced motivation). We suspect that depression stems from overactive microglial and cytokine responses which are normally regulated by the OFQ/N system. Depression and sickness behavior have similar symptoms. To induce stress, the mice were injected with LPS which enables macrophages to release pro-inflammatory cytokines such as interleukin-1β (IL-1β), which has been identified as a cytokine that may cause sickness behavior during inflammation, septic shock and wound healing.



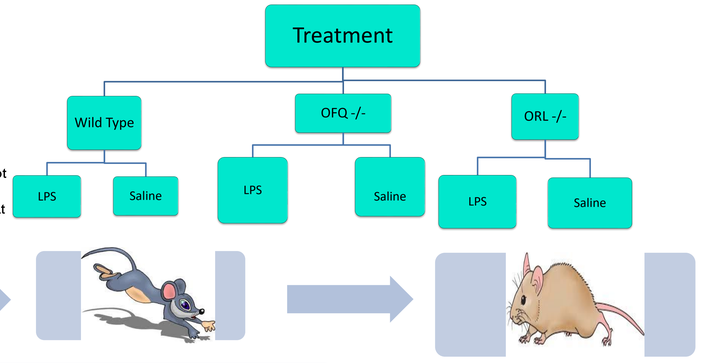
Methods and Materials

SUBJECTS

- 30 young adult mice C57BL/6 (aged 4-6 months)
- OFQ KO (n=6; 2 M, 4 F)
- ORL KO (n=7; 3 M, 4 F)
- WT (n=17; 10 M, 7 F)

STATISTICAL ANALYSIS

Repeated measures ANOVA and means tables compared treatment and genotype to: latency to enter inner zone in each stage (Fig. 1&2), time spent in the border zones (Fig. 3&4), total distance travelled (Fig. 5&6). Total distance by treatment and genotype was significant ($p<0.01$). Mean speed, latency to enter inner zone, and border time was not significantly different among groups, however a limiting factor of this study is the small group sizes, which represent low statistical power, as well as the reason subjects were collapsed across sex for the purpose of this analysis.



Steps 1 & 2
- Home cage
- Injection of LPS/Saline

Steps 3 & 4
- 90 mins. in cage
- 10 mins in OF/NO

Steps 5 & 6
- Home Cage for 90 mins
- Perfusion

Results

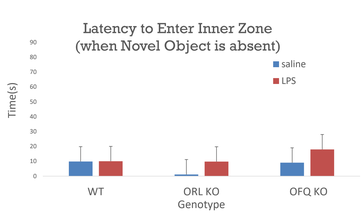


Figure 1: The OFQ KO treated with LPS took longer to enter the inner zone than the control group. The saline treated ORL KO hesitated the least to enter the inner zone.

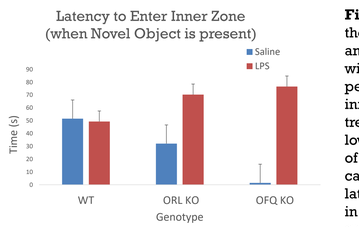


Figure 2: In the presence of the novel object, The ORL KO and OFQ KO groups treated with LPS had a longer latency period before entering the inner zone. The OFQ KO treated with saline had the lowest latency period of any of the groups. LPS did not cause a significant change in latency to enter the inner zone in the wildtype control groups.

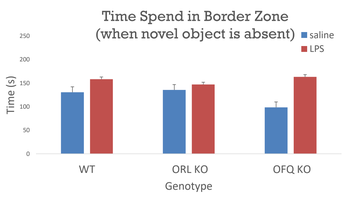


Figure 3: The OFQ KO spent less time in the border zone when injected with saline than when injected with LPS. The WT groups and ORL KO groups were not significantly different.

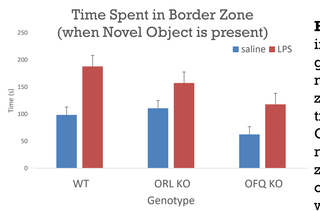


Figure 4: Animals injected with LPS in each genotype group spent more time in the border zone than their saline-treated controls. WT and ORL KO animals spent more time in the border zone when the novel object was present than when it was absent (Fig 3).

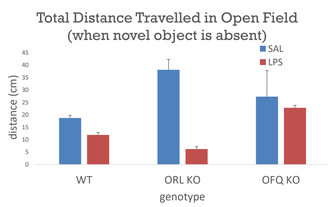


Figure 5: The ORL KO group treated with saline travelled more than double the distance of the WT saline treated group. The ORL KO treated with LPS travelled half the distance of the WT LPS treated animals.

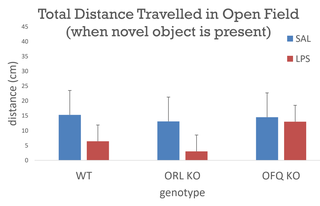


Figure 6: Animals in each group had lower total distance travelled in the presence of the novel object, when compared to their respective group in the absence of the object. This may suggest exploration or fear of the novel object. The ORL KO treated with LPS travelled the least distance, while LPS did not have this effect on the OFQ KO.

Discussion

This study has shown that the ORL KO and OFQ KO mice were at the center of the maze more often when injected with LPS. The ORL KO and the OFQ knockouts injected with LPS stayed in the inner latency zone far more than those injected with saline during the Open field test. Later, when the novel object was introduced, the knockouts injected with LPS still stayed in the middle of the maze. These animals seemed to be far less afraid of the novel object than the mice injected with saline. The knockout mice injected with saline stayed near the borders for a majority of time, suggesting that the LPS and knockout combination made the mice more brazen. OFQ knockouts injected with saline verses OFQ knockouts injected with LPS had the most substantial differences. Furthermore, during the open field tests, all the mice spent an equal amount of time at the border of the maze. However, the mice injected with LPS spent far more time at the border of the maze when the novel object was introduced. These results suggest that the mice injected with LPS moved between the inner zone and the border much more often than those injected with saline. This suggests their behavior was less inhibited than mice injected with saline. Ultimately, this suggests the mice injected with LPS had less anxiety than those not injected. This seems to suggest that mice without ORL and OFQ do not show sickness behaviors induced by LPS. Therefore, these two systems may be causing the sickness responses such as lethargy, fatigue, and lack of motivation that are seen in depression.

Future Direction

In the future, we aim to expand on this preliminary data to determine if there is significance among groups as suggested by the trends that have been observed in current experiment. Further, we want to explore changes in microglial cell morphology and proliferation. We also plan to assay several proinflammatory cytokines (specifically, IL-6 and TNFα) to determine if there is an overabundance of these cytokines in relation to behavioral or immunological stress.

References

Awada, R., Rondeau, P., Grès, S., Saulnier-Blache, J. S., Dhellencourt, C. L., & Bourdon, E.Autotaxin protects microglial cells against oxidative stress. Free Radical Biology and Medicine. *52*(2), 516-526. (2012). doi:10.1016/j.freeradbiomed.2011.11.014

Cerretti, D., Kozlosky, C., Mosley, B., Nelson, N., Ness, K. V., Greenstreet, T., Et, A. Molecular cloning of the interleukin-1 beta converting enzyme. Science, *256*(5053), 97-100. (1992). doi:10.1126/science.1373520

Grandi, D., Massi, M., Guerrini, R., Caló, G., & Morini, G. Role of nociceptin/orphanin FQ receptors in the decrease of mucosal mast cells caused by acute stress in the rat colon. Life Sciences, 89 (19-20), 735-740. (2011). doi:10.1016/j.lfs.2011.08.015

M. Mallimo, Elyse, & Kusnecov, Alexander W. The Role of Orphanin FQ/Nociceptin in Neuroplasticity: Relationship to Stress, Anxiety and Neuroinflammation. Frontiers in Cellular Neuroscience, 7. (2013). doi:10.3389/fncel.2013.00173

Urbach-Ross, D., & Kusnecov, A. W. Effects of acute and repeated exposure to lipopolysaccharide on cytokine and corticosterone production during remyelination. Brain, Behavior, and Immunity, *21*(7), 962-97. (2007).doi:10.1016/j.bbi.2007.03.010

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