

# Behavioral analyses in the immunodeficient NOG mouse after intrahippocampal kainic acid induction of chronic epileptic seizures



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Abstract #4044

## INTRODUCTION

Behavior testing is a critical platform to characterize animal models of neurological disorders such as epilepsy, as well as to evaluate the efficacy and safety of novel therapeutics.

Severely immunodeficient mouse strains such as NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Sug</sup>/JicTac (NOG) are often selected for preclinical model development in the fields of regenerative medicine and cell therapy, as they can support human cell engraftment at a high rate of success. However, their performance on many behavioral tasks is not well characterized.

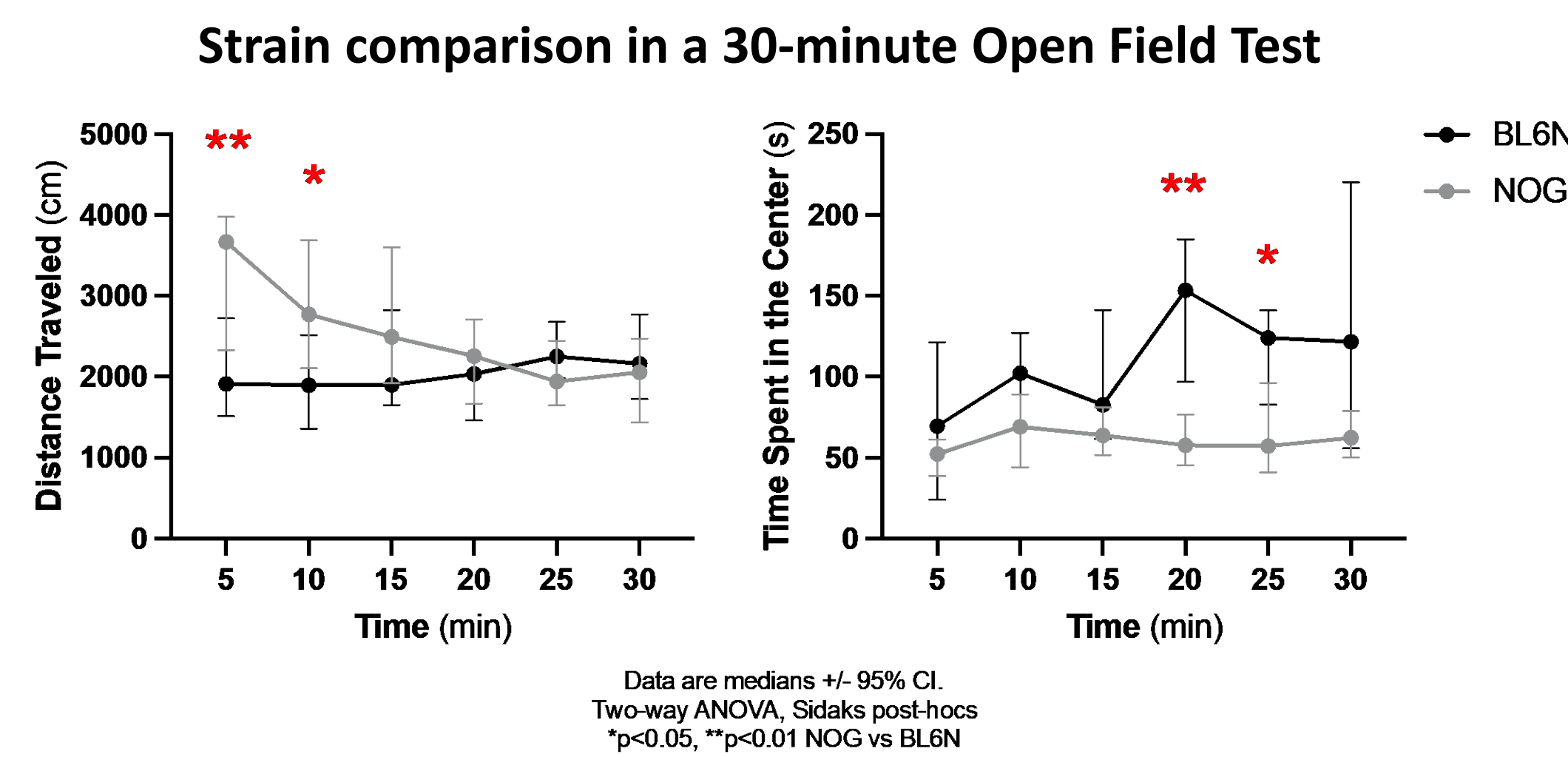
To facilitate the use of NOG mice in our preclinical studies using the intrahippocampal kainic acid (IHKA) model of epilepsy, behavior assays were optimized for exploratory activity, anxiety, and learning and memory in naïve and epileptic IHKA NOG mice. First, behavioral activity of naïve NOG mice was compared to BL6N or BL6J mice to validate assay protocols.

## Examining the locomotor and exploratory activity of NOG mice

### Method

Size: 40 x 60 cm

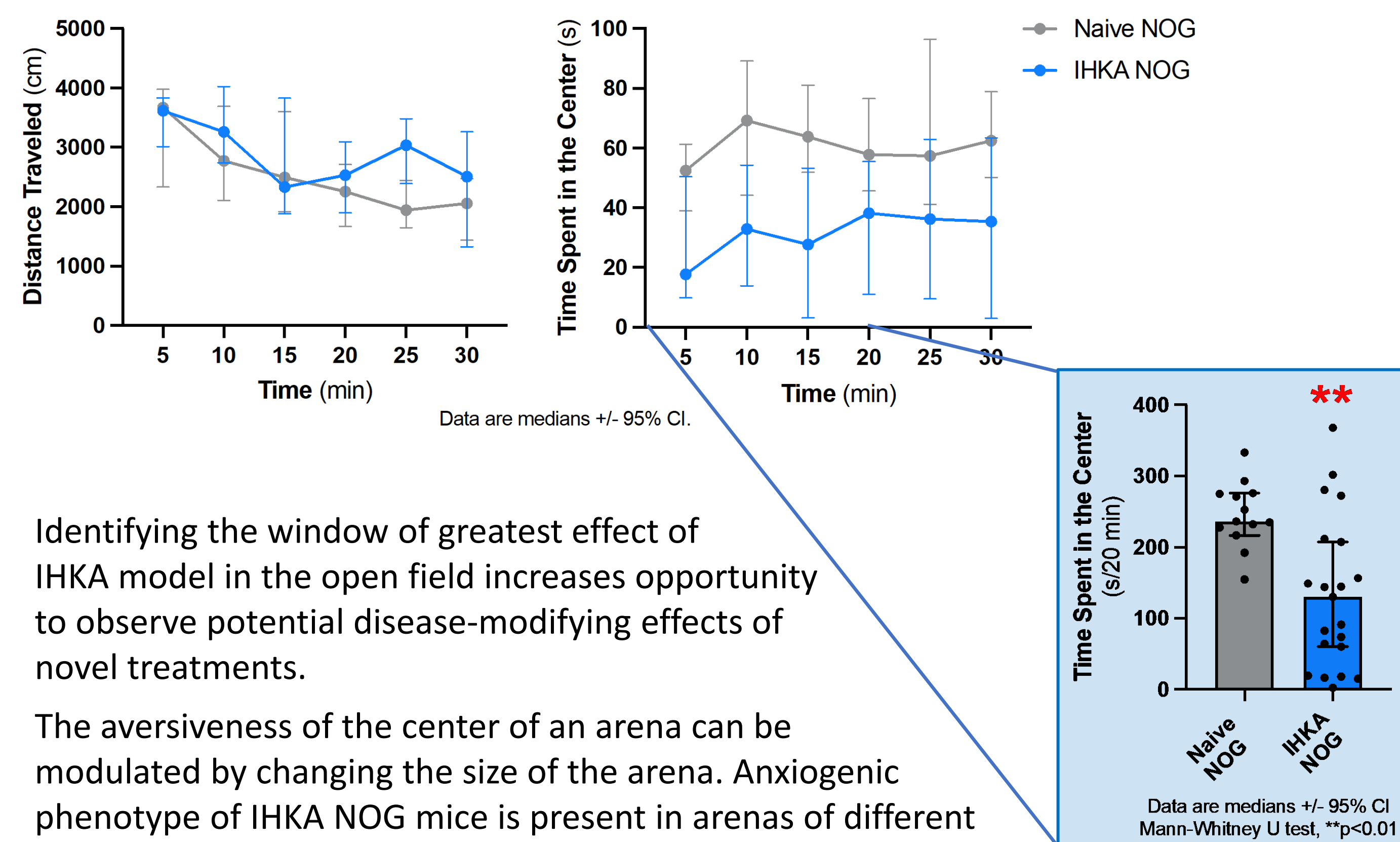
- 150 – 200 LUX
- Other arena sizes tested:
  - 23 x 30 cm
  - 40 x 40 cm



- Naïve NOG mice exhibit higher general activity and spend lower amounts of time in the center of an arena when compared to BL6N mice.

## The greatest effects of IHKA epilepsy model in NOG mice are observed in the first 20-minutes of an Open Field Test

### Epileptic vs naïve NOG comparison in a 30-minute Open Field Test



- Identifying the window of greatest effect of IHKA model in the open field increases opportunity to observe potential disease-modifying effects of novel treatments.
- The aversiveness of the center of an arena can be modulated by changing the size of the arena. Anxiogenic phenotype of IHKA NOG mice is present in arenas of different sizes (data not shown) but is most prominent in the largest arena tested.
- Mice identified in the open field as having a rotation bias of ≥ 90% are excluded from analysis and further behavioral testing.

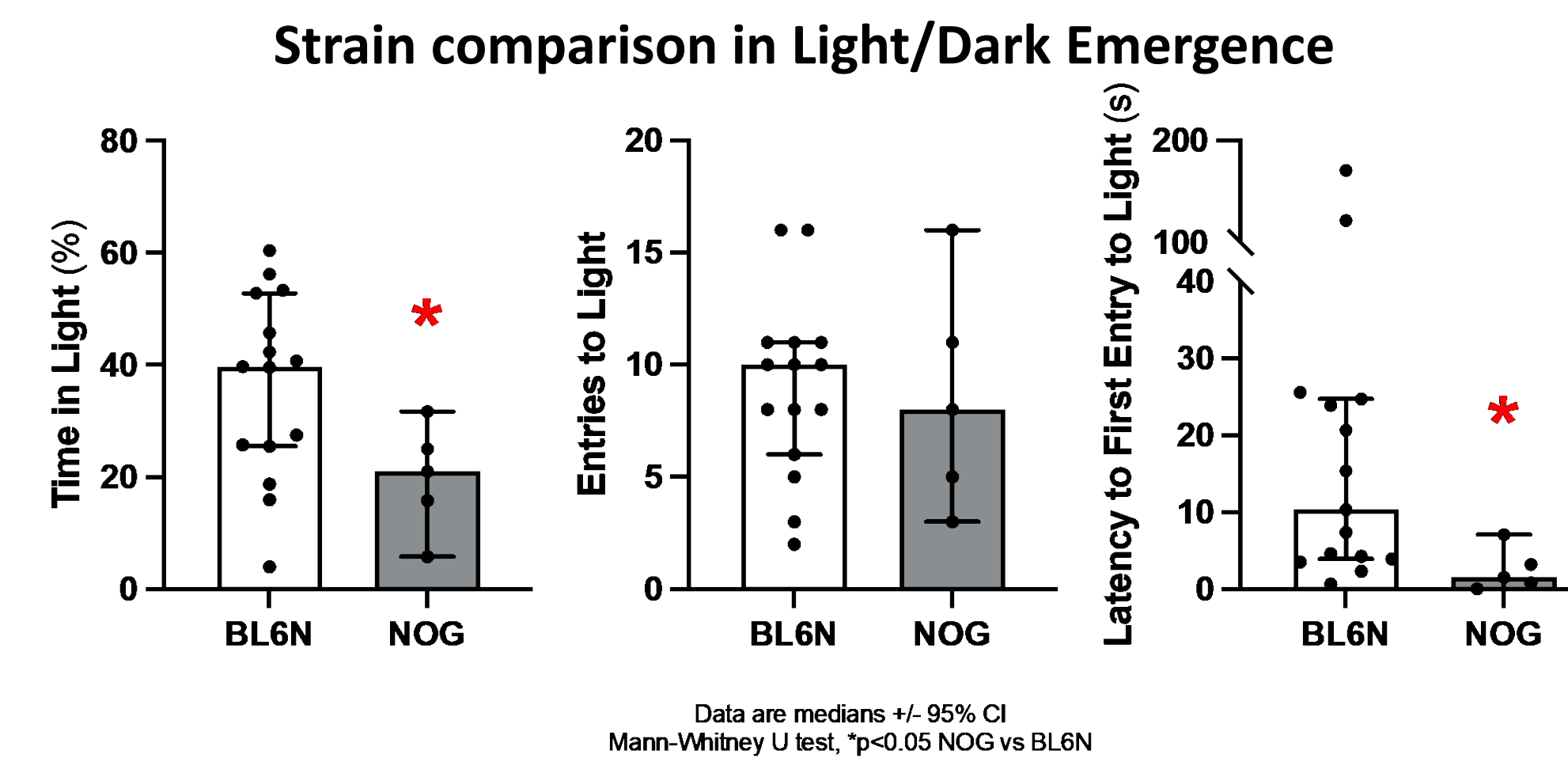
IHKA = Intrahippocampal kainic acid

## Examining anxiety-like behaviors in NOG mice

### Method

20 x 40 0 LUX	40 x 40 cm 800-850 LUX
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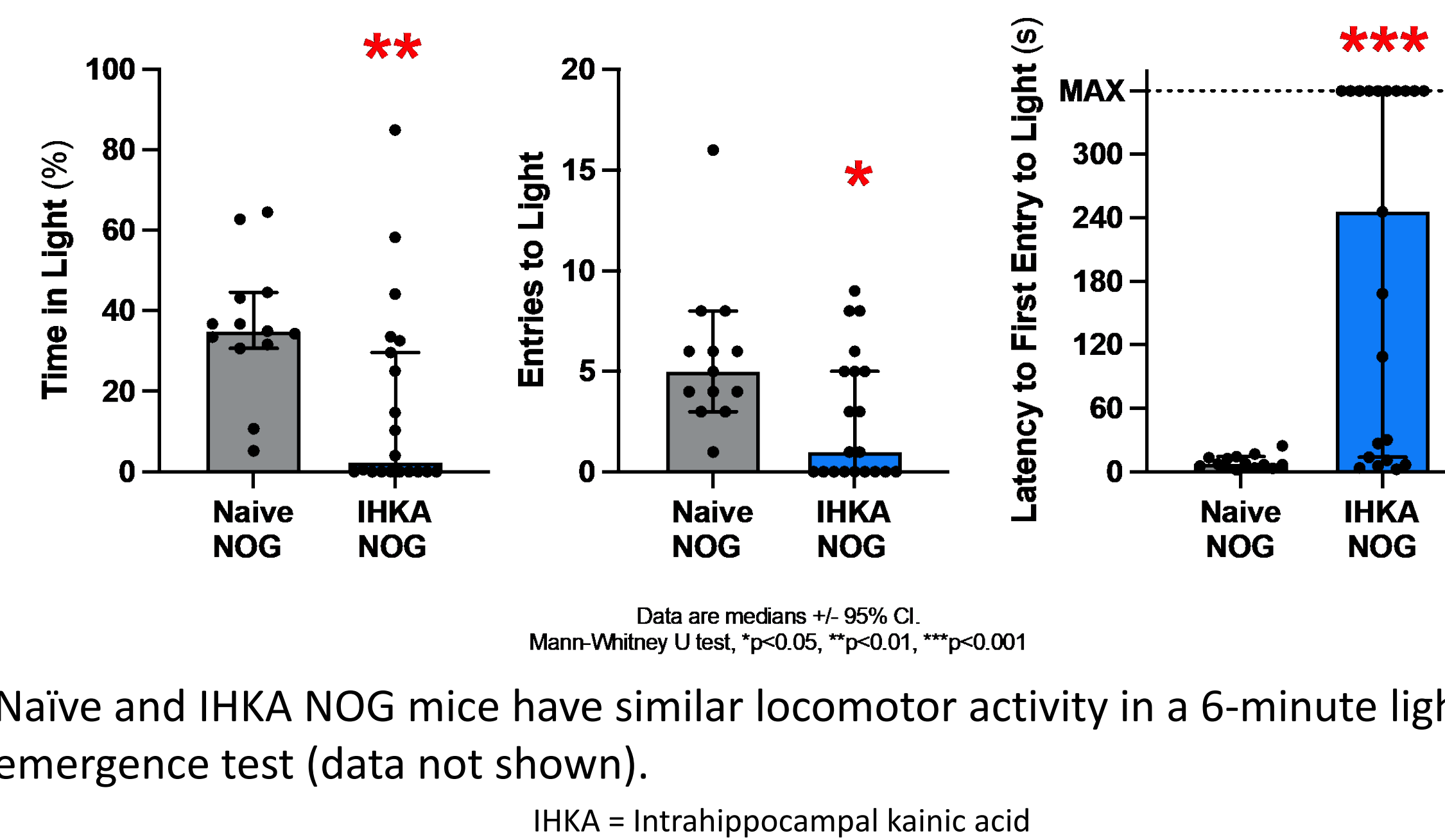
- Light/Dark Emergence
  - Animal starts in dark and is given 6 min to explore after a door is opened to light
- Other assays tested:
  - Elevated Zero Maze



- Naïve NOG mice spend less time in the light chamber and have lower latencies to emerge during the Light/Dark Emergence (LDE) test compared to BL6N mice.
- Naïve NOG mice do not consistently emerge onto open arms in Elevated Zero Maze as tested (data not shown), which does not allow for observation of anxiety-associated behaviors in preclinical models.

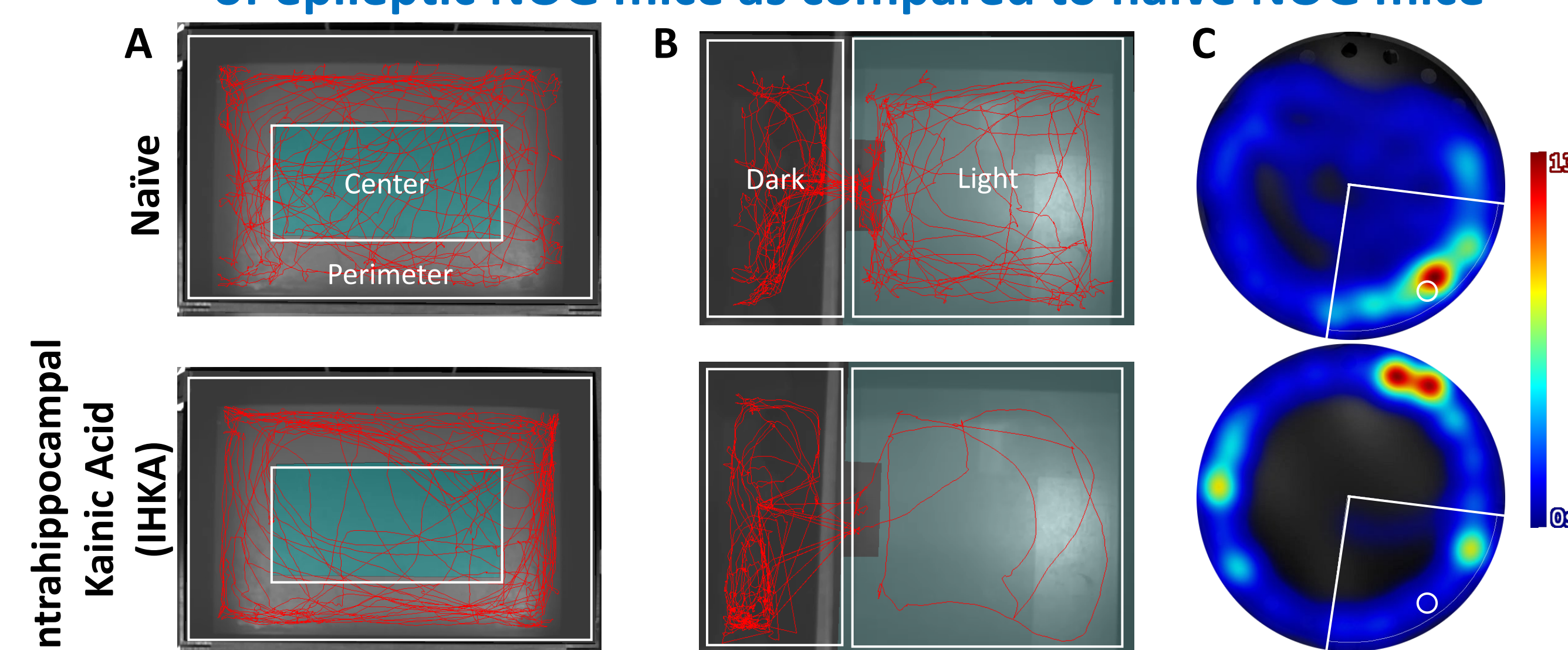
## Epileptic NOG mice exhibit anxiety-associated behaviors in LDE

### Epileptic vs naïve NOG comparison in Light/Dark Emergence



- Naïve and IHKA NOG mice have similar locomotor activity in a 6-minute light/dark emergence test (data not shown).

## Observed behavioral phenotype in anxiety and spatial memory assays of epileptic NOG mice as compared to naïve NOG mice



Representative traces of naïve NOG (top) and IHKA NOG (bottom) center point tracking in the Open Field Test (A), Light/Dark Emergence (LDE) (B), and the Barnes Maze (C). (A) Naïve NOG mice exhibit greater exploration of the center of an open field than IHKA NOG mice in a 5-minute trace. (B) Naïve NOGs make more entries into the light chamber and spend more time exploring the light chamber compared to IHKA NOGs in LDE. (C) Heatmaps of a 3-minute probe of spatial memory in the Barnes Maze reveal preferential exploration of the target hole location and quadrant by naïve NOGs compared to IHKA NOGs.

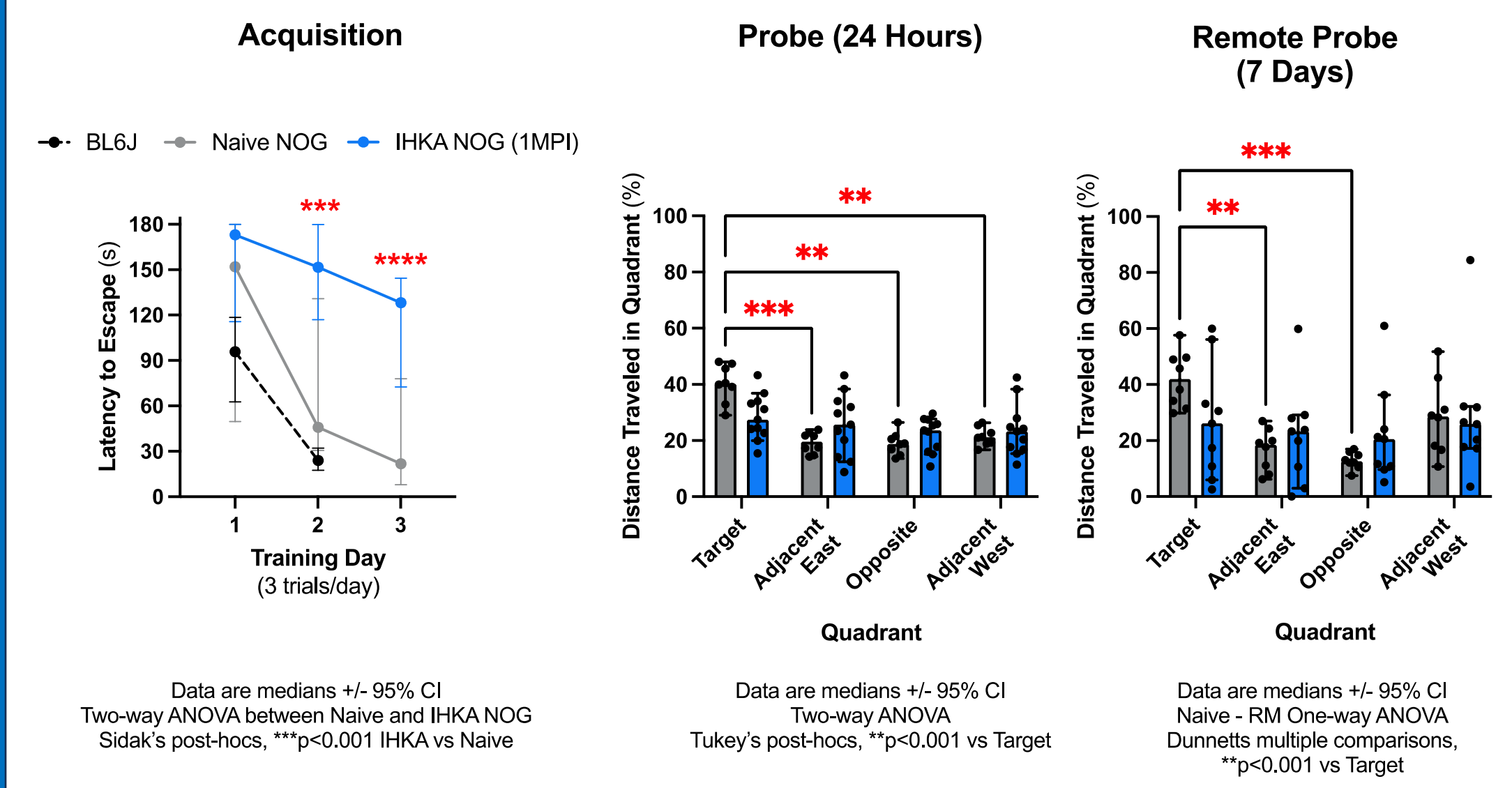
## Examining learning and memory in NOG mice

### Method

850-900 LUX  
20 holes

- Barnes Maze
  - 3 trials/day, 180s MAX
  - 15-20 min ITI
  - 180s probe 24-hours post training
- Other assays tested:
  - Novel Object Recognition/Location
  - Y-Maze Spontaneous Alternation
  - Y-Maze Two-Trial Recognition Memory

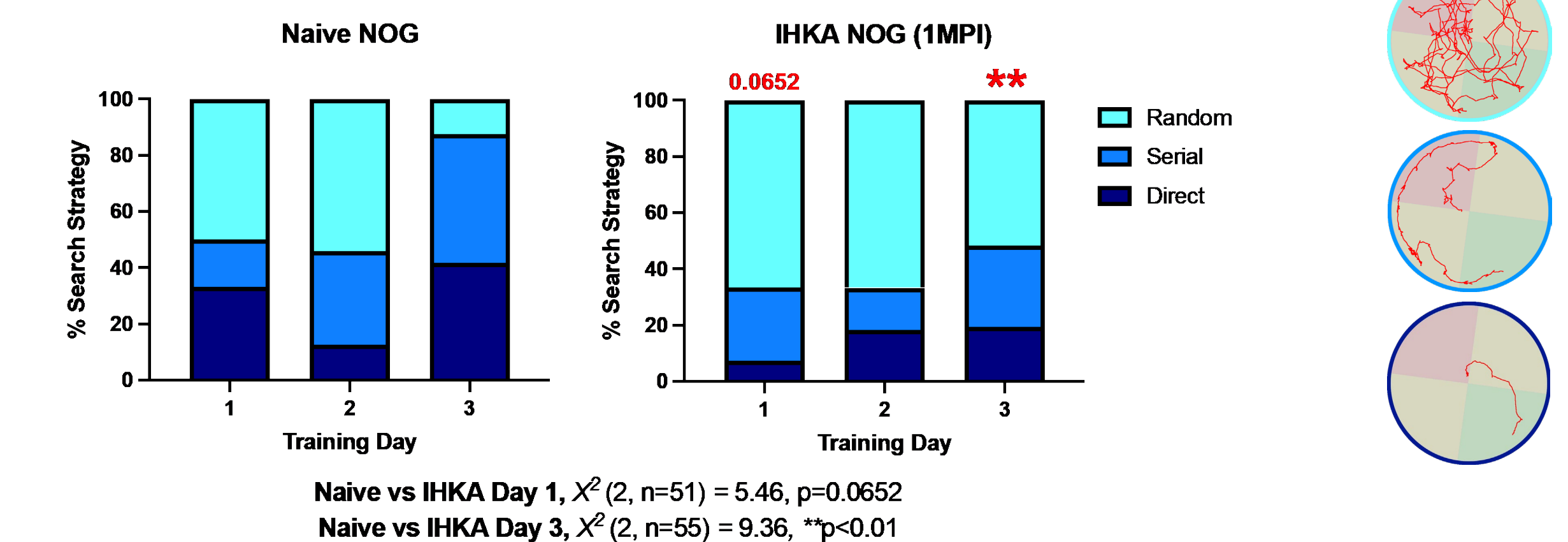
### Strain and epileptic vs naïve NOG comparison in the Barnes Maze



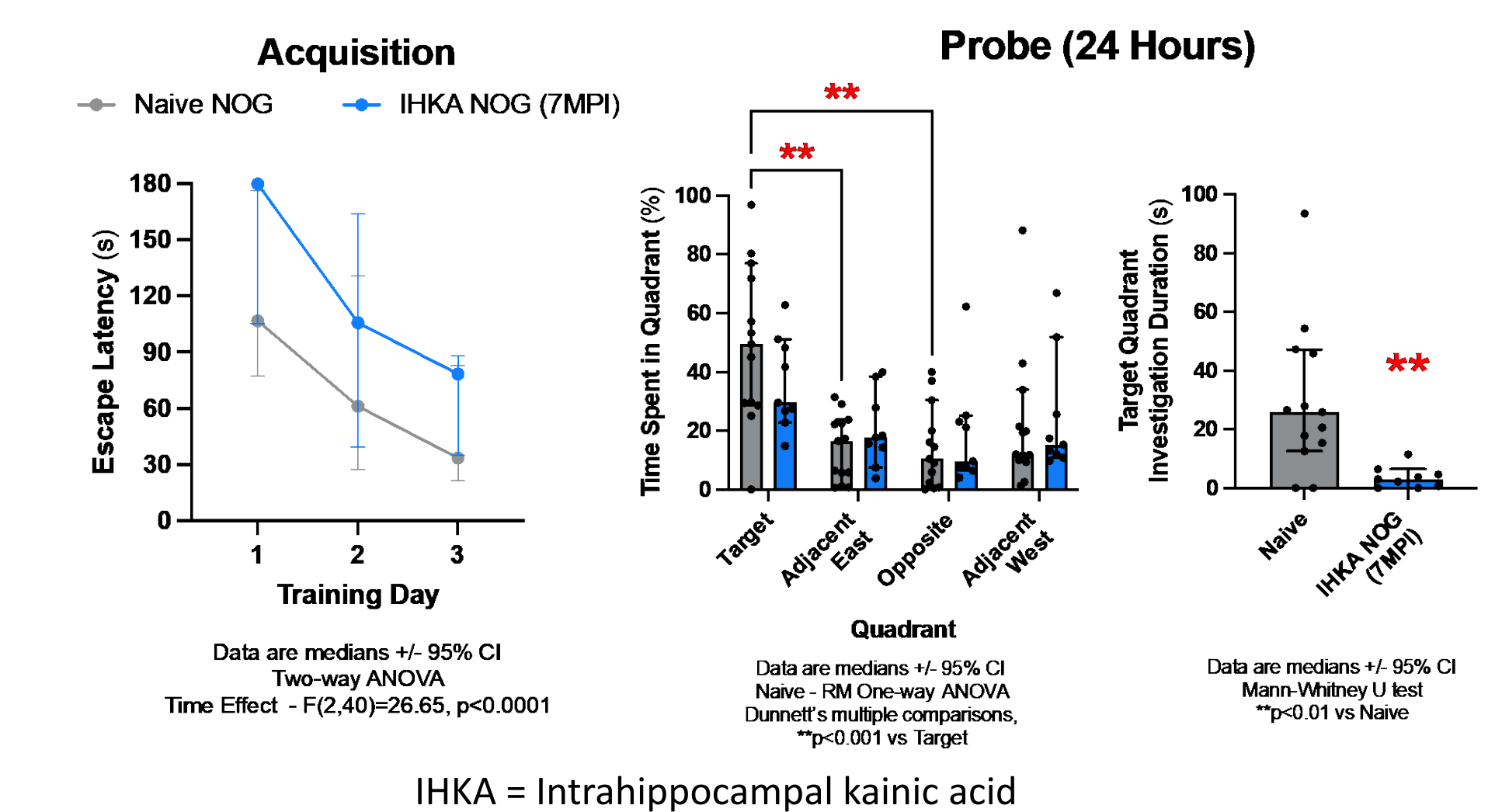
- A short protocol is sufficient for naïve NOG learning in the Barnes Maze and captures a deficit in epileptic NOG spatial learning and memory at 1-month post-induction (MPI).
- Naïve NOG mice exhibit recall of a target location in a remote probe test.
- Naïve NOG mice show low interaction times in object-based memory tasks (e.g., Novel Object Recognition/Location), do not alternate at higher than chance levels in the Y-Maze, and do not recognize a novel arm in a Y-Maze two-trial recognition memory test (data not shown).

## Naïve NOG mice navigate to the escape location with more efficient strategies than IHKA NOGs at 1-month post-induction

### Epileptic vs naïve NOG search strategy comparison in the Barnes Maze



## Spatial learning deficits are present at 7-months post-induction



## CONCLUSIONS

The Open Field Test, Light/Dark Emergence, and the Barnes Maze make the basis for a good battery of behavioral tests to assess locomotor activity, anxiety-like behavior, and spatial memory in immunodeficient NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Sug</sup>/JicTac (NOG) mice.

These results in intrahippocampal kainic acid model NOG mice recapitulate select behavioral comorbidities of epilepsy as described in immunocompetent mouse strains, improving the utility of this strain in the development of novel regenerative therapies.