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Effects of empagliflozin and L-ornithine L-aspartate on behaviour, cognitive function, and physical performance in mice with non-alcoholic steatohepatitis

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INTRODUCTION

- **non-alcoholic fatty liver disease (NAFLD)** has a number of extrahepatic complications, which include cerebrovascular disease, cognitive and behavioural alterations, and accelerated brain aging
- patients with NAFLD are at a 4 times higher risk to develop **cognitive impairment**, and tend to have decreased **physical performance**[1]

AIM

- to evaluate the effects of **empagliflozin (EMPA)** and **L-ornithine L-aspartate (LOLA)** on cognitive function and physical performance in a mouse model of non-alcoholic steatohepatitis (NASH)

METHODS

Experimental groups: C57BL/6 male mice

- **Intact** (I) 0.9% NaCl q.d. p.o. — n = 10
- **Control** (C) NASH + 0.9% NaCl q.d. p.o. — n = 14
- **EMPA** NASH + 2 mg·kg^{-1} EMPA q.d. p.o. — n = 14
- **LOLA** NASH + 1.5 g·kg^{-1} LOLA q.d. p.o. — n = 14

NASH induction:

- a western diet + weekly intraperitoneal carbon tetrachloride (0.32 mg·kg^{-1}) for 6 months[2]

Cognitive function assessment:

- open field (OF) test
- elevated plus maze (EPM)
- Barnes maze (BM)
- light/dark box (LDB) test[3]

Physical performance assessment:

- weight-loaded (7.5% of b.w.) forced swim (FS) test
- triple weight-loaded exhaustive swim (TES) test

RESULTS

Animal survival

The mean lethality rate was 45.2% among all NASH groups and was not affected significantly by either of the drugs (Fig. 1).

NASH-related cognitive impairment and memory deficit

Control mice exhibited decreased movement speed (p<0.01), and increased total freezing time (p<0.01) and the numbers of head dips (p<0.05) and rearing episodes (p<0.05) in the OF, EPM, and LDB tests, indicating anxiety-like behaviour (Figs. 2-4). Control mice also had higher error percentages and latencies to find the target hole (p<0.05) on Day 12 vs. Day 5 compared to Intact animals, indicating a spatial memory retention deficit (Fig. 5).

EMPA increased the number of grooming bouts and time spent in the light chamber of the LDB (p<0.05), and LOLA increased the time spent in the open arms of the EPM (p<0.05) (Figs. 2-4). The spatial memory deficit was partially rescued by both EMPA and LOLA (Fig. 5).

NASH-related decrease in physical performance

Control mice had poorer performance than Intact specimens (p<0.05, p<0.01) in both swimming tests. LOLA restored normal performance levels in the FS test, and improved post-exercise recovery (p<0.01) at 45 min after the start in the TES test (Fig. 6).

Fig. 2. Open field test results
* — p < 0.05; ** — p < 0.01

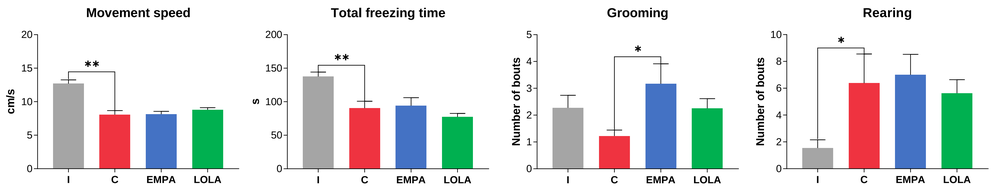


Fig. 3. Elevated plus maze results
* — p < 0.05; ** — p < 0.01

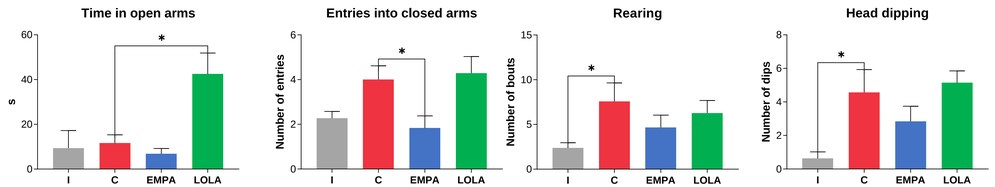
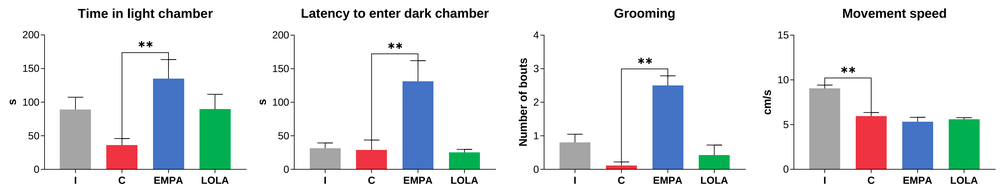


Fig. 4. Light/dark box test results
* — p < 0.05; ** — p < 0.01



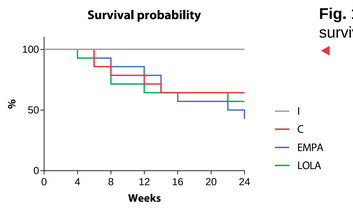


Fig. 1. Kaplan-Meier survival analysis results.

Fig. 5. Barnes maze results.
* — p < 0.05; ** — p < 0.01

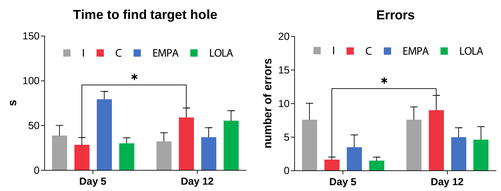
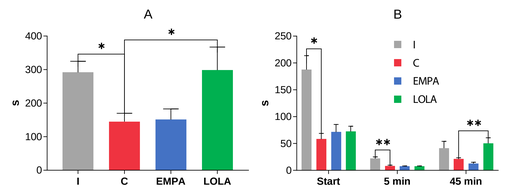


Fig. 6. Forced swim (A) and triple weight-loaded exhaustive swim (B) tests results.
* — p < 0.05; ** — p < 0.01



CONCLUSIONS

- experimentally induced **NASH** causes anxiety-like behaviour, impairs long-time memory, and decreases physical performance in C57BL/6 mice
- daily administration of **EMPA** (2 mg·kg^{-1}) or **LOLA** (1.5 g·kg^{-1}) ameliorates NAFLD-related cognitive deficit
- daily administration of **LOLA** also improves physical performance and post-exercise recovery

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