Iron overload impairs the migratory ability of a model of immature and migratory GnRH neurons

Steffani L, Di Nitto F, °Dongiovanni P, °Rametta R, °Valenti L, °Fargion S, Magni P., Ruscica M.

Dep. of Pharmacological and Biomelecular Sciences and °Dep. of Pathophysiology and Transplantation, Università degli Studi di Milano
liliana.steffani@unimi.it, paolo.magni@unimi.it, massimiliano.ruscica@unimi.it

BACKGROUND

Iron is essential for proper brain development in the fetal and early neonatal period. Iron represents a micronutrient for cellular metabolism and aerobic respiration, but cellular iron overload produces toxic build-up in many organs (including the brain) via free radical formation. In thalassaemic patients with pubertal failure, iron overload is the most important factor afflicting the hypothalamic-pituitary axis, leading to hypogonadotrophic hypogonadism and growth failure.

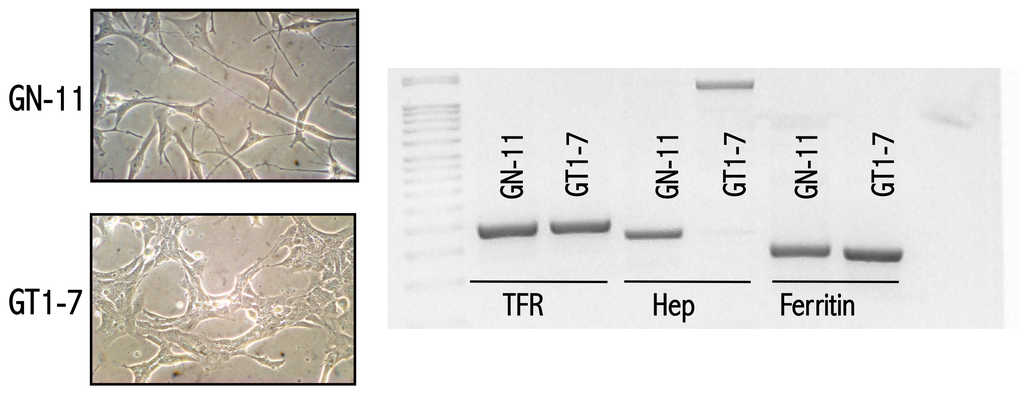
AIM - To investigate the mechanisms of iron toxicity in *in vitro* GN-11 cells, a model of immature and migratory GnRH neurons

METHODS

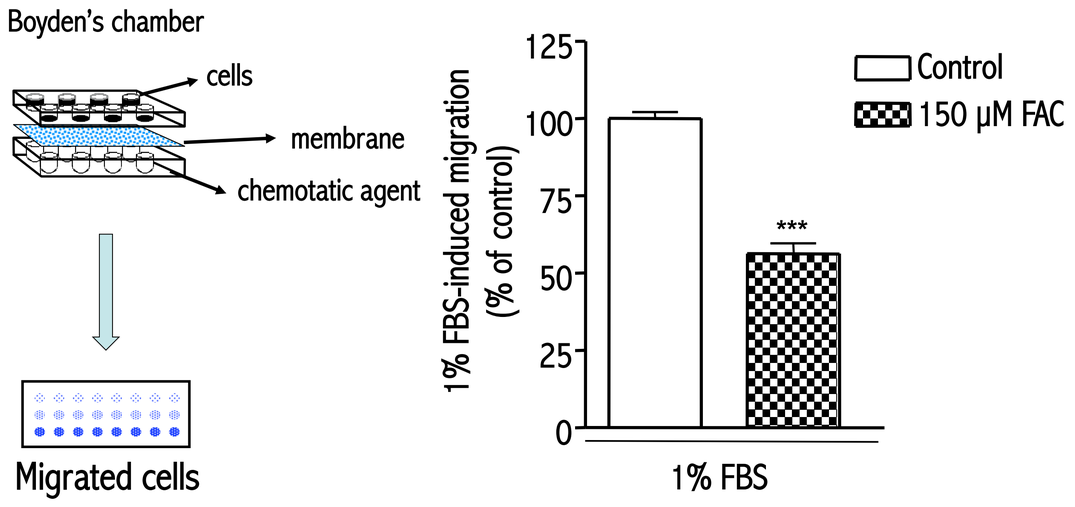
Mouse GN-11 cells (immature GnRH neurons with migratory ability) were used. Hepcidin, ferritin and transferrin receptor gene expression was evaluated by PCR. GN-11 chemotaxis was assessed by Boyden chamber assay. Activation of chemomigration-related cell signaling (extracellular signal-regulated kinase (ERK), 5' adenosine monophosphate-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC)) was evaluated by Western blot analysis (WB).

RESULTS

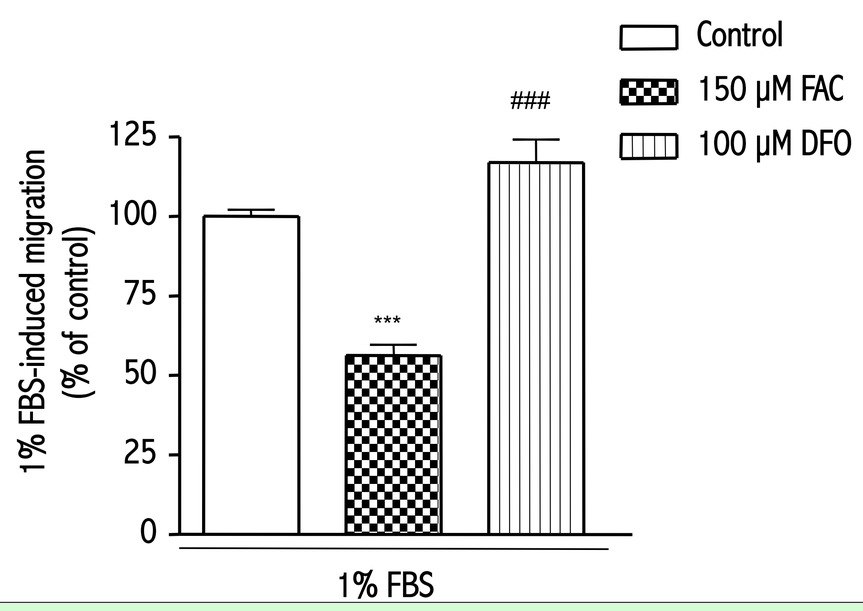
1. GN-11 cells express hepcidin (Hep), ferritin and transferrin receptor (TFR) genes



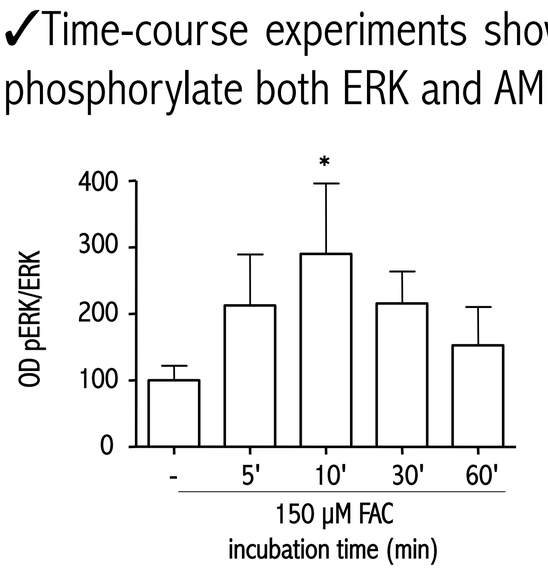
1. 150 µM ferric ammonium citrate (FAC) treatment inhibited (-35%, $p<0.05$) FBS-induced chemo-migration of GN-11 cells

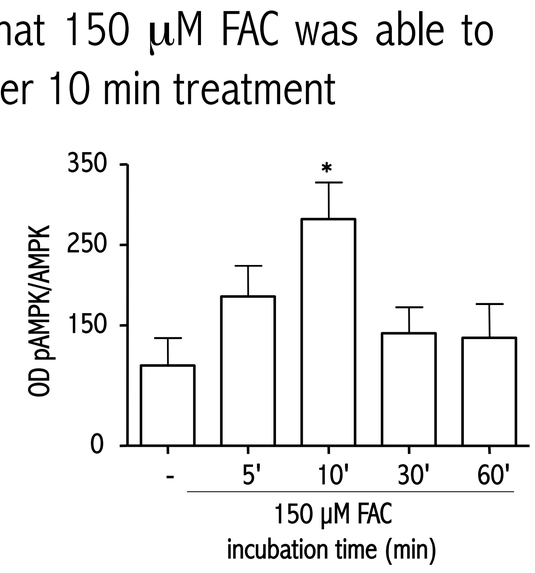


✓ Pre-treatment with 100 µM deferoxamine (DFO), a specific iron chelator, rescued the FAC effect on cell-migration



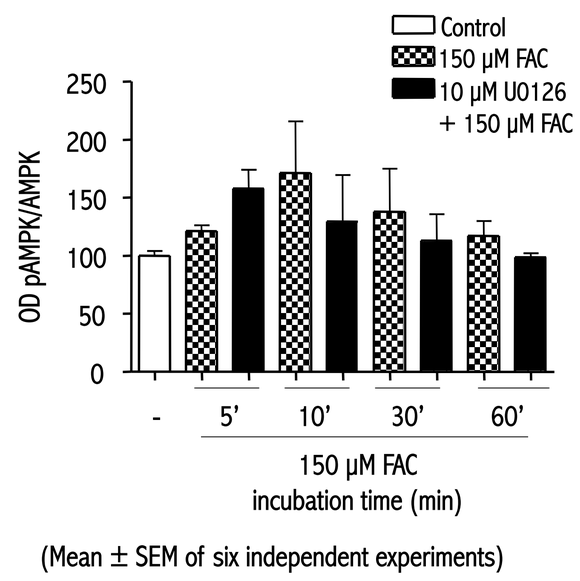
✓Time-course experiments showed that 150 µM FAC was able to phosphorylate both ERK and AMPK after 10 min treatment

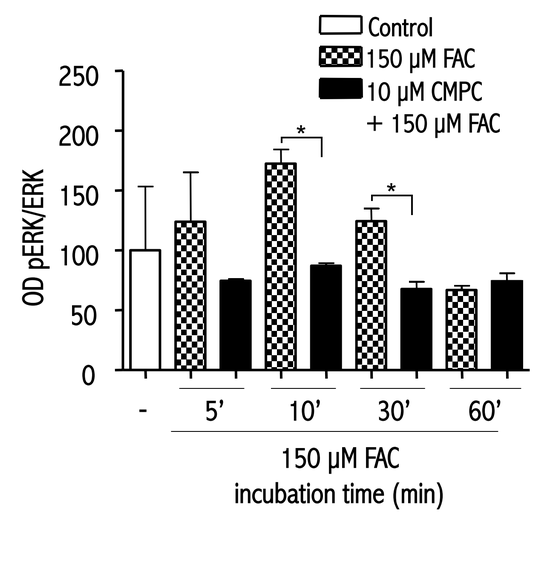




(Mean ± SEM of six independent experiments)

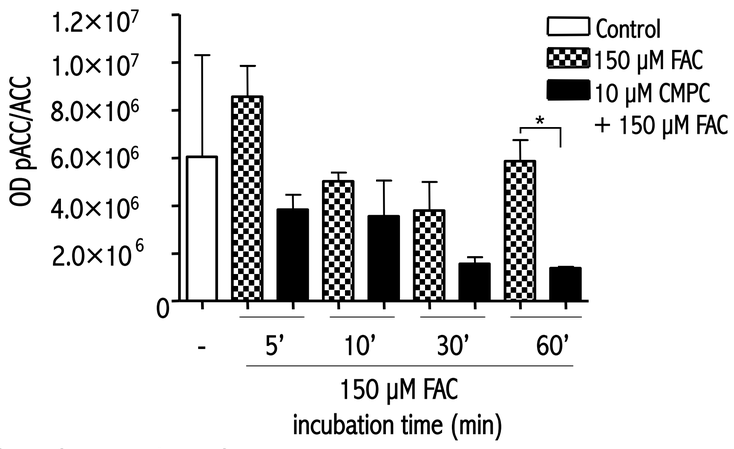
✓ Specific ERK and AMPK inhibitors, U0126 and Compound C (CMPC), respectively, abolished FAC-mediated signaling





(Mean ± SEM of six independent experiments)

✓ CMPC (10 µM) counteracted FAC-driven phosphorylation of acetyl-CoA carboxylase (ACC), an AMPK downstream protein



(Mean ± SEM of six independent experiments)

CONCLUSIONS - Iron negatively affects neuron migration via ERK and AMPK. Among the consequences of this event, iron overload may impair migration of GnRH neurons from the olfactory placode into forebrain and hypothalamus, where they promote reproductive competence.