Identifying the Metabolic Fuel Requirements of Adult Neural Progenitor Cells

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Introduction:

Energy metabolism is necessary for cellular activity. This is especially true in the brain. The brain comprises about 2% of body weight, but accounts for approximately 25% of glucose uptake and 20% of oxygen consumption.

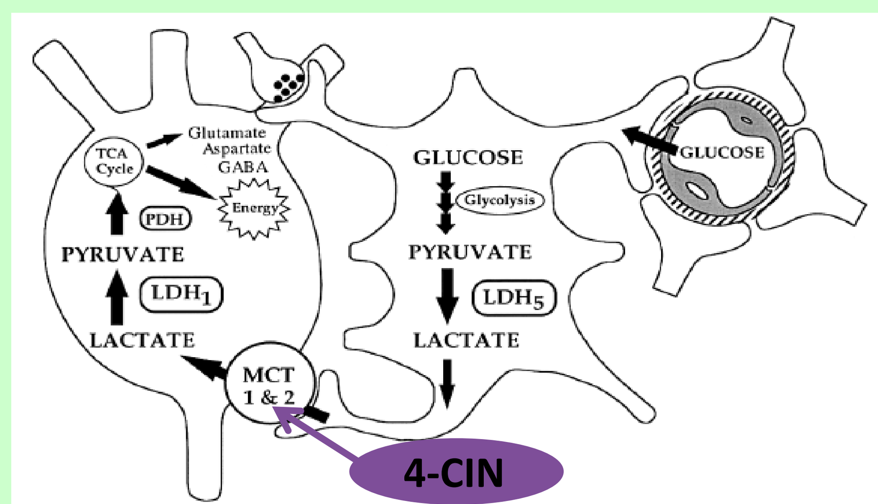
Neural progenitor cells (NPCs) are specialised stem cells found within specific regions of the adult brain, mainly the sub ventricular zone (SVZ). Like all other cells, NPCs require a metabolic substrate to generate energy for cell division.

The lab found that these cells maintain their aerobic respiration rates needed for proliferation when grown in zero levels of glucose, suggesting that another metabolic fuel is their primary energy source.

A further study found that the enzymes needed for fatty acid oxidation were found within the specific areas of the brain where neurogenesis takes place, suggesting that fatty acids could be the primary energy source of these stem cells.

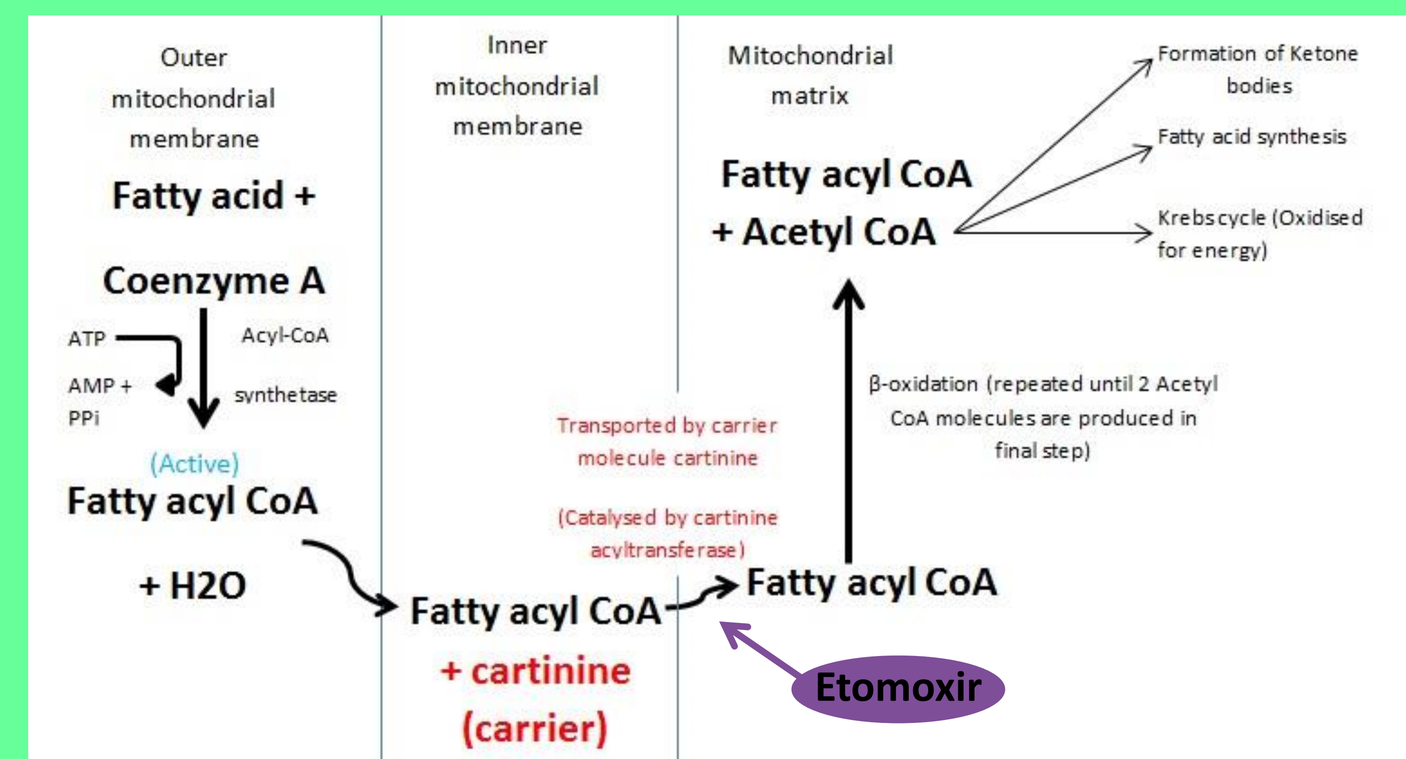
Aims:

To investigate the effects of limiting glucose and fatty acid oxidation on the level of cellular proliferation both *in vivo*, within the SVZ of adult mouse brains. *In vitro* experiments were also carried out on cultured NPCs collected from 3 month old mice to assess the effects of inhibiting the oxidation of fatty acids and increasing their availability, on NPC proliferation and survival.



Adapted from Pellerin, 1998.

Figure 1. Showing the basis of glucose metabolism . This diagram shows the mechanism by which neuronal activity links to glucose metabolism. Glutamate release stimulates astrocytes to uptake glucose from nearby capillaries. Astrocytes metabolise glucose to lactate, making it available to neurons. Proposed by Pellerin & Magistretti, 1994.



Scheme 1. Mechanism of fatty acid oxidation. Free fatty acids are transported across both mitochondrial membranes and oxidised. Products are used for further fatty acid synthesis, energy production via the Krebs Cycle or to form ketone bodies eventually used as a metabolic fuel. Fatty acyl CoA is used as a starting substrate for the next round of β-oxidation.

References: http://2012books.lardbucket.org/books/introduction-to-chemistry-general-organic-and-biological/s23-06-stage-ii-of-lipid-catabolism.html

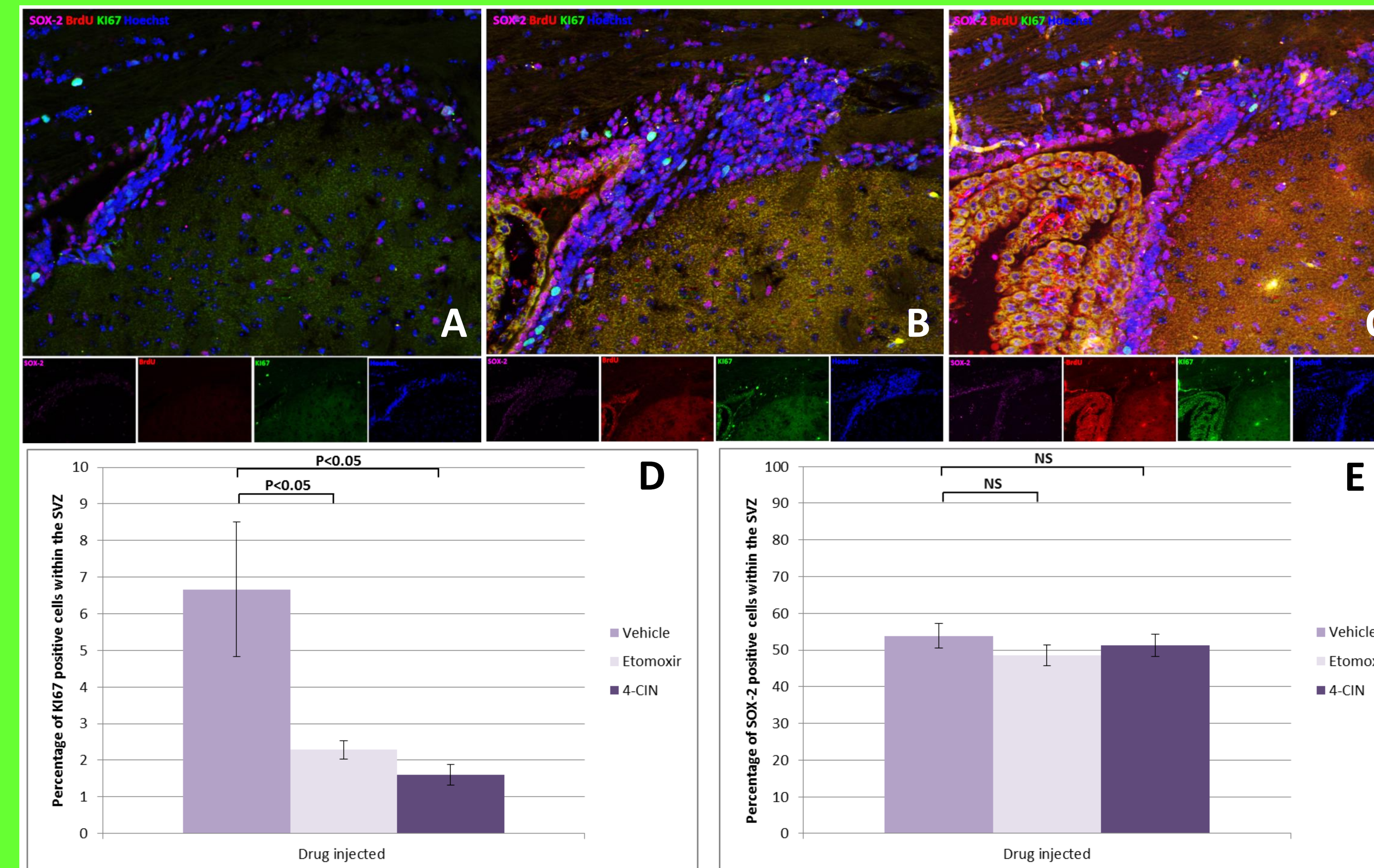


Figure 2. Treatment of NPCs with a Fatty acid oxidation inhibitor (Etomoxir) and a Lactate transporter inhibitor (4-CIN) leads to a decrease in proliferation (KI67+) whilst having no effect on neural progenitor cell presence (SOX-2+) within the SVZ of mice. Mice were injected with 50mg/kg BrdU and PBS (A), 40mg/kg etomoxir (B) or 40mg/kg 4-CIN (C) intraperitoneally for 3 days. Tissue was then collected and stained for KI67, SOX-2 and BrdU marking. A hoeschst stain was also used for a total cell count. A significant decrease was observed in the percentage of proliferating cells when treated with both etomoxir ($P<0.05$) and 4-CIN ($P<0.05$) (D). No difference was observed in the percentage of cells when treated with both etomoxir ($P>0.05$) and 4-CIN ($P>0.05$) (E).

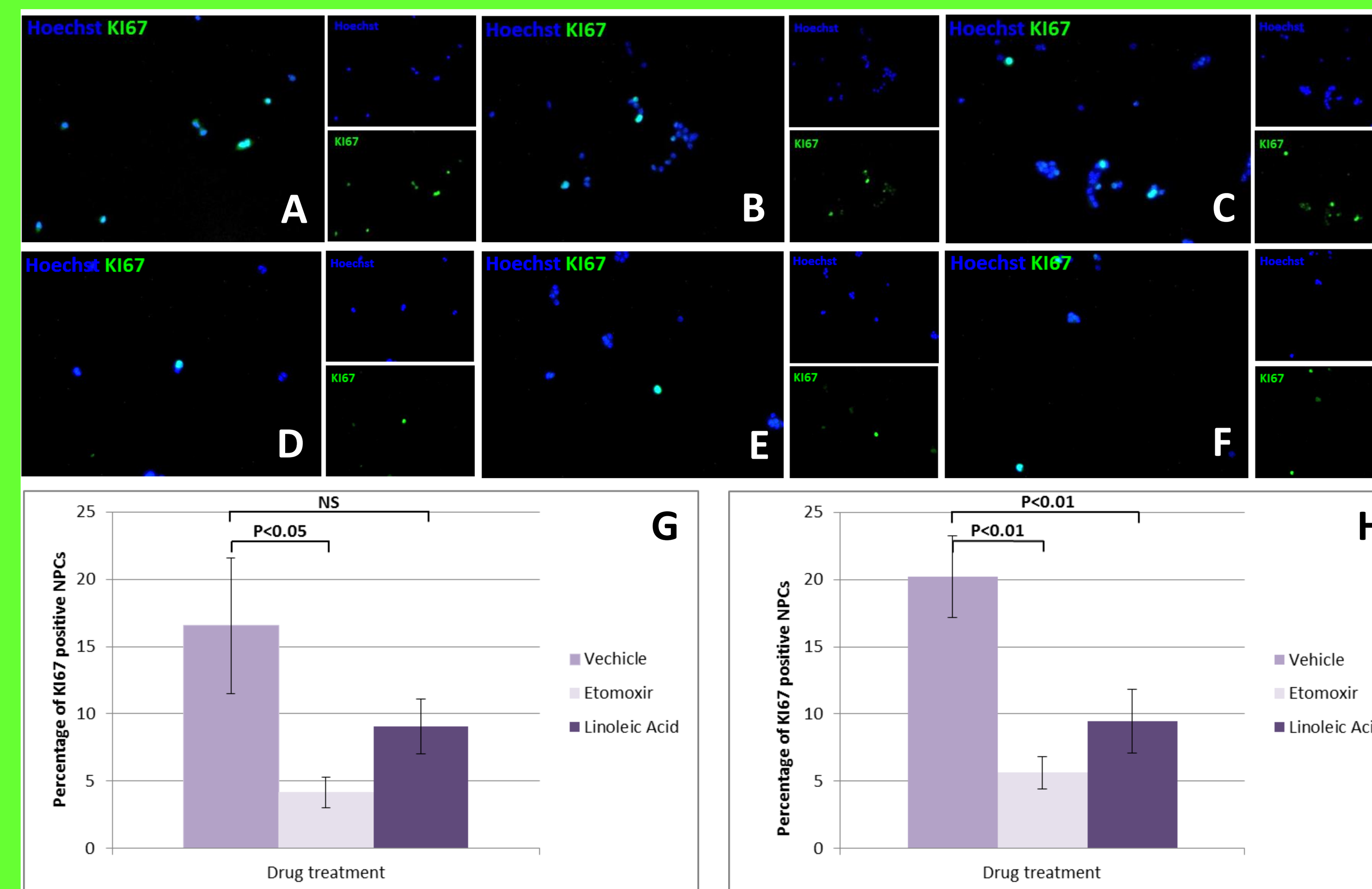


Figure 3. Treatment of extracted NPCs with a Fatty acid oxidation inhibitor (Etomoxir) and an Unsaturated fatty acid (Linoleic Acid) leads to a decrease in proliferation (KI67+) of NPCs. Sample photomicrographs show 3 month old mice NPCs from 2 passages, 14 (A/B/C) and 15 (D/E/F) treated with PBS (A,D) 100µM etomoxir (B,E) or 100µM linoleic acid (C,F) and stained for both total cell number using pan nuclear marker Hoechst and KI67 marking. The percentage of KI67+ proliferating cells from passage 14 decreased significantly upon treatment with etomoxir ($P<0.05$) however no significant change was seen upon treatment with linoleic acid ($P>0.05$)(G). Percentages of proliferating cells from passage 15 significantly decreased upon treatment when treated with both etomoxir ($P<0.01$) and linoleic acid ($P<0.01$)(H).

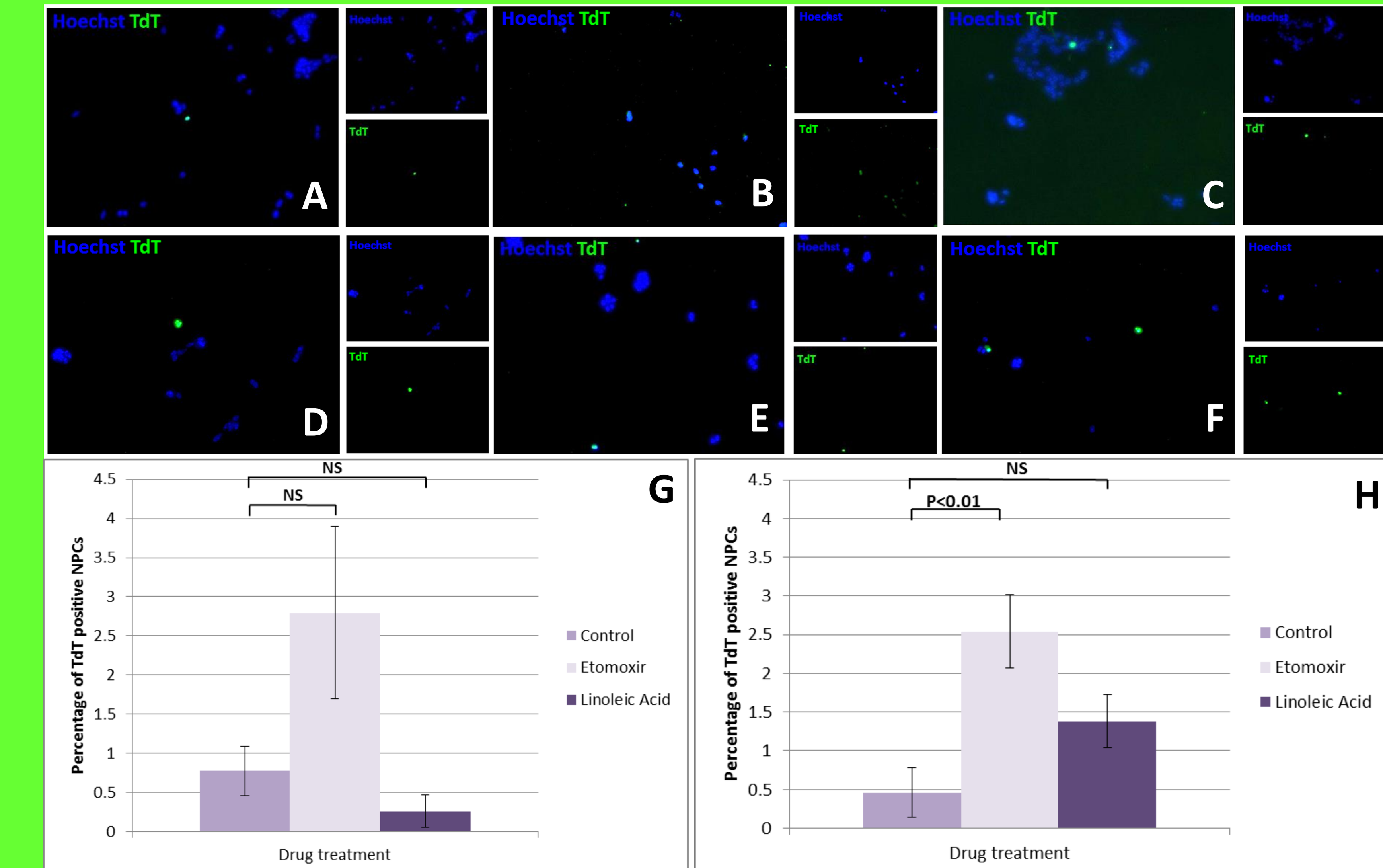


Figure 4. Treatment of extracted NPCs with a Fatty acid oxidation inhibitor (Etomoxir) causes a slight increase in apoptosis (TdT+) of NPCs whilst treatment with an Unsaturated fatty acid (Linoleic Acid) has no effect. Sample photomicrographs show 3 month old mice NPCs from 2 passages, 14 (A/B/C) and 15 (D/E/F) treated with PBS (A,D), 100µM etomoxir (B,E) or 100µM linoleic acid (C,F) and stained for both total cell number using pan nuclear marker Hoechst and TdT apoptotic cell marking. Treatment of cells from passage 14 with both etomoxir and linoleic acid did not cause a significant change in the percentage of TdT+ apoptotic cells ($P>0.05$) (G). However, in cells from passage 15, etomoxir treatment caused a significant increase in the percentage of TdT+ apoptotic cells ($P<0.01$) with no significant different seen in treatment with linoleic acid ($P>0.05$)(H).

Conclusions:

Overall, the results show that in *vitro*, inhibiting fatty acid metabolism leads to a significant decrease in proliferation when compared with a control sample.

When fatty acid availability was increased, proliferation decreased but to a much lesser extent and this decrease was not significant. However, in a slightly later passage of these cells, the decrease seen was significant. I also found that treatment with Etomoxir has a slight effect on cell survival.
In vivo the results show that inhibiting both fatty acid and glucose metabolism leads to a significant decrease in cell proliferation whilst having no effect on a marker of progenitor cell identity. Showing that neither treatment caused neural progenitor cell s to undergo differentiation.

These data suggest that fatty acids are likely to be an energy source required by these cells for proliferation. However, data also suggests these may also be able to use glucose as metabolic fuels.
Further study involving a larger sample size will be needed to provide a more reliable result.

Implications: Outcomes of this research may provide an insight into ways in which the effect of degenerative brain diseases could be reversed. Conversely, knowledge of the fuel requirements of these cells could also be used to inhibit the growth rate of adult gliomas.