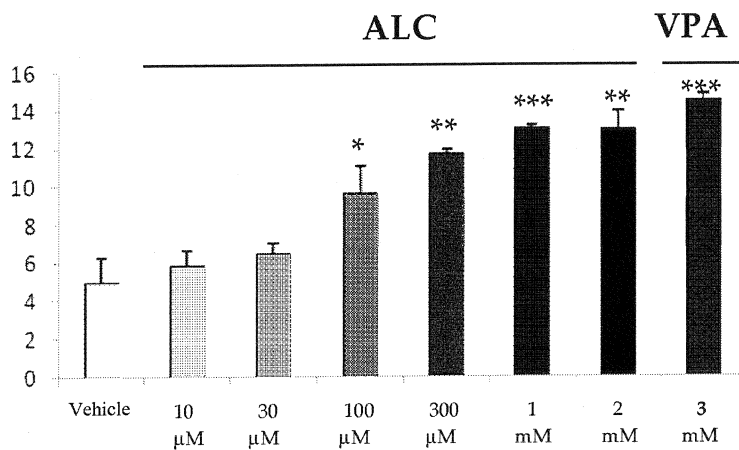


FIGURE 1

% MAP2-positive cells (new neurons)



ALC= acetyl L-carnitine; VPA= valproic acid.

Mean $\pm$ SD (n=3 exp, in triplicates).

Student's- test; * p<0.05; ** p<0.01; *** p<0.001; vs vehicle.

Figure 1 shows that ALC has a concentration-dependent strengthening effect on the differentiation of stem cells/adult progenitors to mature and immature neurons. In fact, ALC has a marked concentration-dependent hippocampal neurogenic activity as demonstrated by the increase in the percentage of MAP-2 positive cells in culture.

FIGURE 2

MAP2-positive cells (new neurons), nuclei are counterstained with Draq5

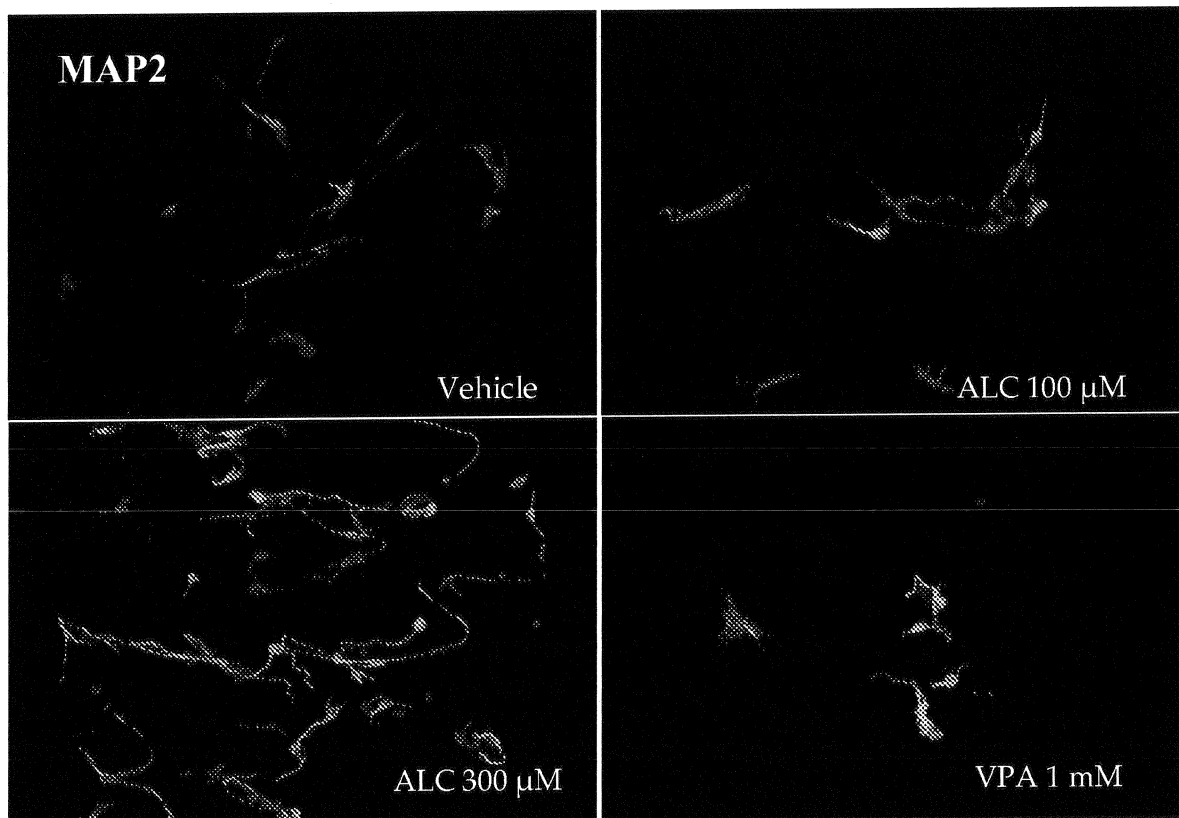
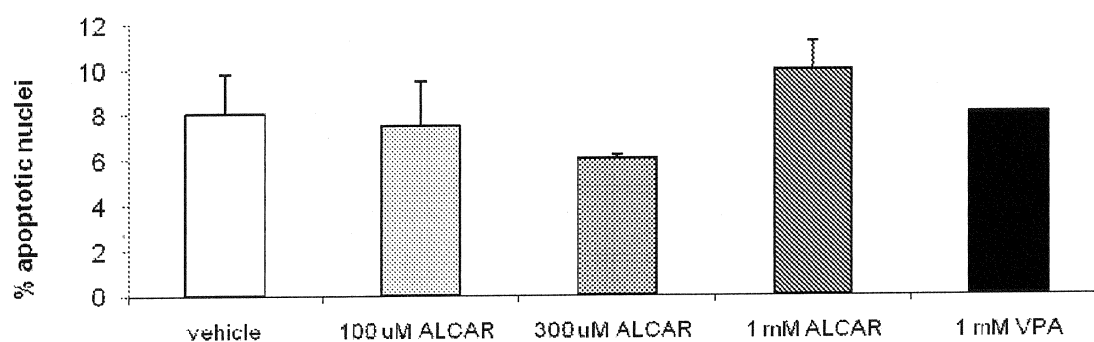


Figure 2 shows the hippocampal neurogenic activity of ALC (MAP2-positive cells i.e. new neurons) evaluated by MAP-2 staining in cultured neurons by immunofluorescence staining. Nuclei were stained with Draq5.

FIGURE 3

% of apoptosis after ALC (acetyl L-carnitine) and VPA (valproic acid) incubation



ALCAR= acetyl L-carnitine.

Figure 3 shows that the neurogenic activity of ALC is not correlated to neuroprotective activity. In fact, at the concentrations that induced neurogenesis, ALC (100 μ M; 300 μ M or 1 mM) did not reduce the percentage of apoptotic nuclei.

FIGURE 4

% of necrosis after ALC (100 μ M; 300 μ M; and 1 mM) and VPA incubation

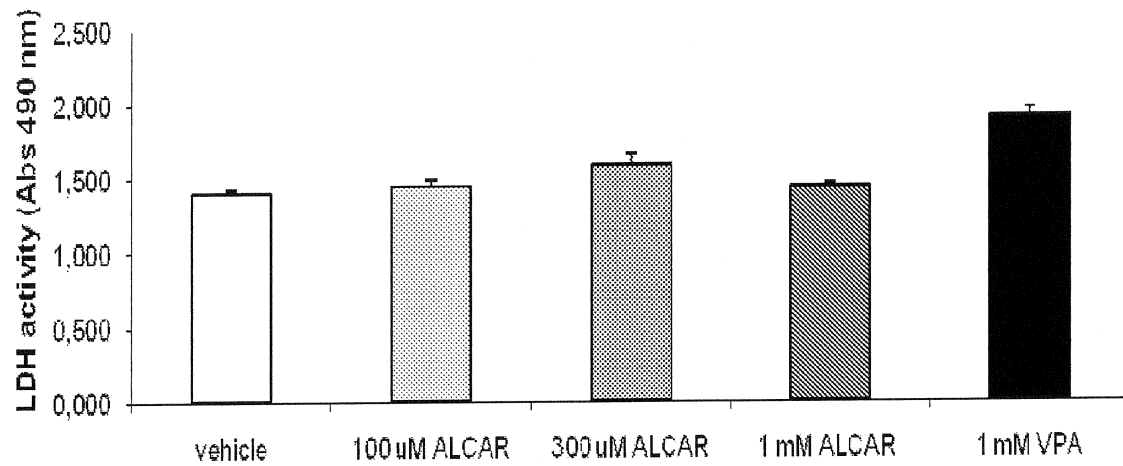
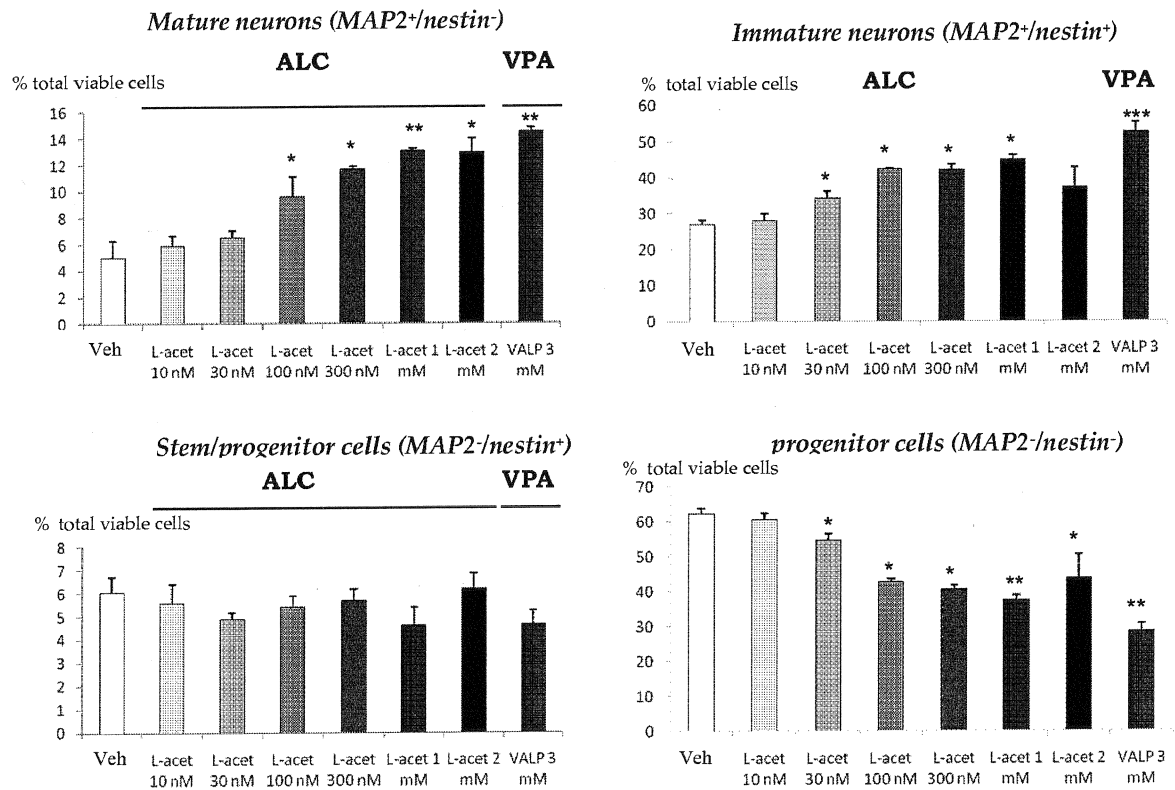


Figure 4 shows that the neurogenic activity of ALC is not correlated to neuroprotective activity. In fact, at the concentrations that induced neurogenesis, ALC (100 μ M; 300 μ M or 1 mM) did not reduce the activity of the LDH enzyme, an index of necrosis, in the culture medium.

FIGURE 5

Specific effect of ALC on cells at various stages of differentiation



Synonymous: acetyl L-carnitine=ALC=ALCAR=L-acet.

Mean $\pm$ SD (n=3 exp, in triplicates).

Student's- test; * p<0.05; ** p<0.01; *** p<0.001; vs vehicle.

Figure 5 shows the specific effect of ALC on cells at various stages of differentiation. Mature neurons are positive for MAP2 and negative for nestin, immature neurons are positive for both MAP2 and nestin, stem progenitor cells are negative for MAP2 and positive for nestin, progenitor cells are negative for both MAP2 and nestin.

FIGURE 6

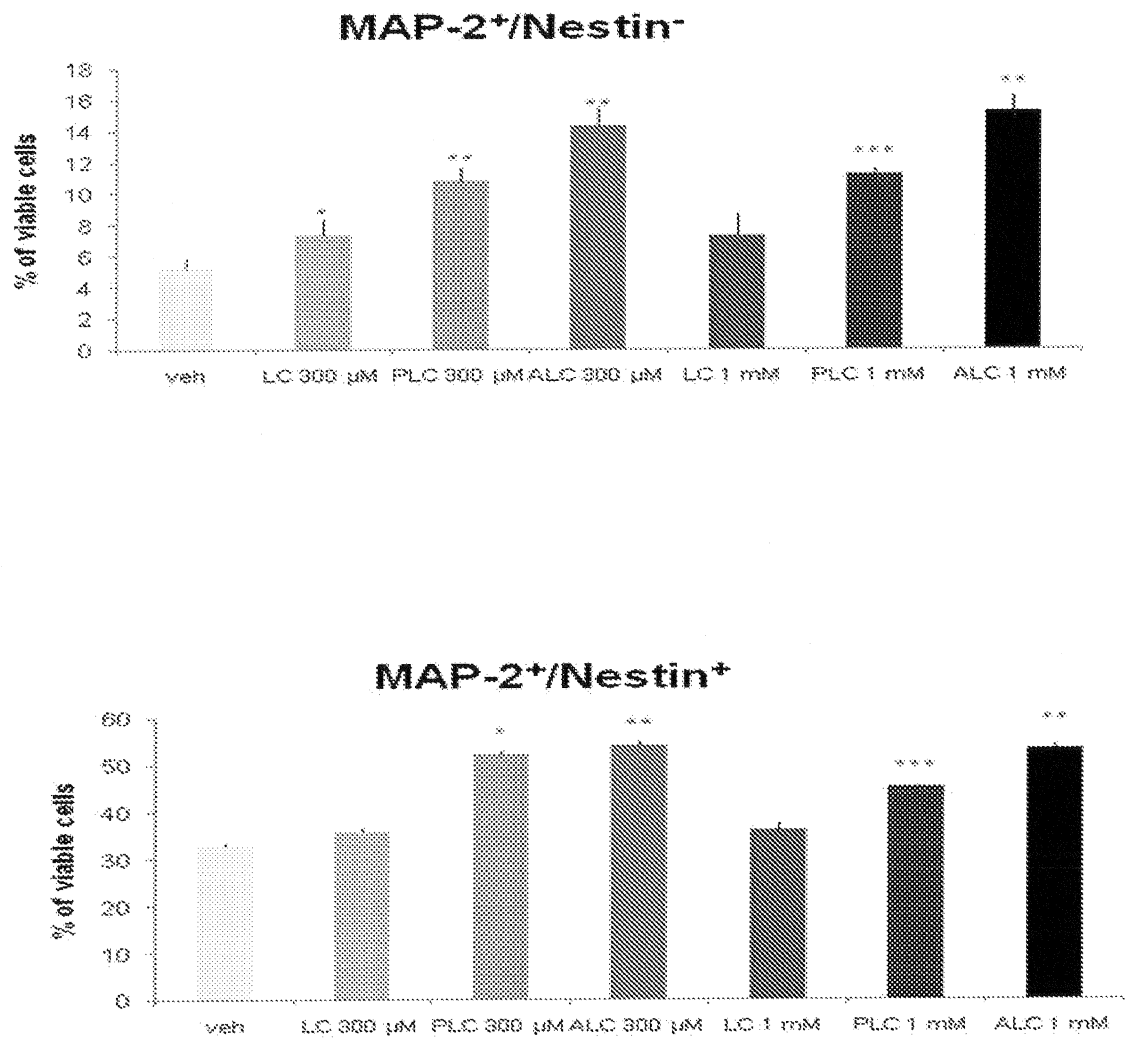
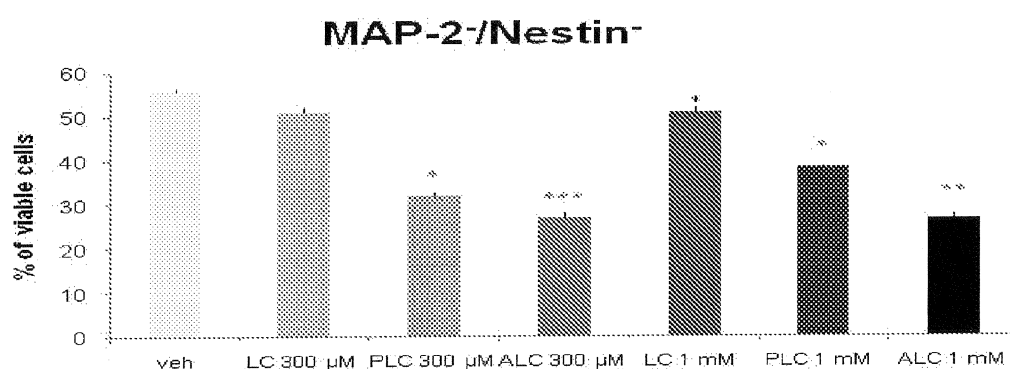


FIGURE 6 (continued)



In Figure 6 is shown that PLC and ALC are activator of NPC neuronal differentiation. In fact PLC increased the number of both mature neurons and immature neurons and reduced the number of a subpopulation of undifferentiated stem/neural progenitors, although in a lesser extent compared to ALC. On the contrary, LC had no effect.

Moreover the results demonstrated that ALC and PLC effects on NPC differentiation were not due to neuroprotective effects on newly generated immature or mature neurons.