Metafectene Pro® transfection of primary cultures of rat cerebellar granule neurons.

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The cells were isolated from 8 days old male rat pups according to the procedure described by *Gallo et al.* (1982) and cultured in 35 mm dishes. These cultures are shown to at least 80 % cerebellar granule neurons with the rest being different glial cells, mostly astrocytes, and other types of neurons. As we previously have had problems transfecting neuronal cultures it is important to investigate which cell types are actually transfected using a given protocol.

Brief protocol:
The cells were transfected according to the manufacturers protocol except as specified below. using 2 µl Metafectene Pro and 1 µg DNA, of which 0.5 µg were pmaxFP-Green-N (Amaxa AG, Cologne, Germany) and the rest empty CMV plasmid or 1 µg luciferase gene.

The cells were transfected in either regular medium or using Locke's buffer with 25 mM K^+ (134 mM NaCl, 25 mM KCl, 3.6 $NaHCO_3$, 2.3 mM $CaCl_2$, 5.6 mM glucose, pH 7.4).

For the dishes transfected in regular medium, the Metafecten Pro and DNA were mixed with BME (without antibiotics or serum) and incubated for 20 minutes before the solutions were added to the dishes containing regular medium (BME with glutamine, 25 mM KCl, gentamycine and foetal calf serum). The solution was left on the cells for 5 hours before being removed and replaced by conditioned medium from other dishes.

For the dishes transfected in Locke's buffer with 25 mM K^+ the Metafecten Pro and DNA were mixed with this buffer and incubated for 20 minutes before the solutions were added to the dishes containing the same buffer. The original medium of these dishes was saved. After 5 hours the solution was removed and the original medium was put back on the dishes.

All experiments were done 48 hours later.

Results:
Using luciferase as a measurement of transfection efficiency between using medium and Locke's solution, we found that the overall transfection efficiency were about twice as high for Locke's when compared to medium.

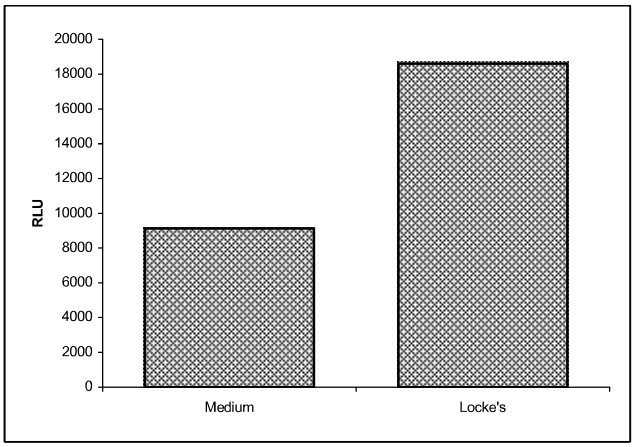

Figure 1 Comparison of dishes transfected using medium or Locke's buffer as described above. Each bar is the average of two dishes transfected with luciferase.

In order to establish whether the transfected cells were cerebellar granule neurons or other cell types, the cells transfected with pmaxFP Green were examined in a fluorescence microscope, and each fluorescent cell were classified as either cerebellar granule neuron (CGN)or non-cerebellar granule neuron (non-CGN). About a third of the fluorescent cells were classified as CGN (small, spherical cell body, preferably with fluorescent neuritis), whereas the rest were classified as non-CGN. About twice as many cells were transfected using Locke's compared to medium.

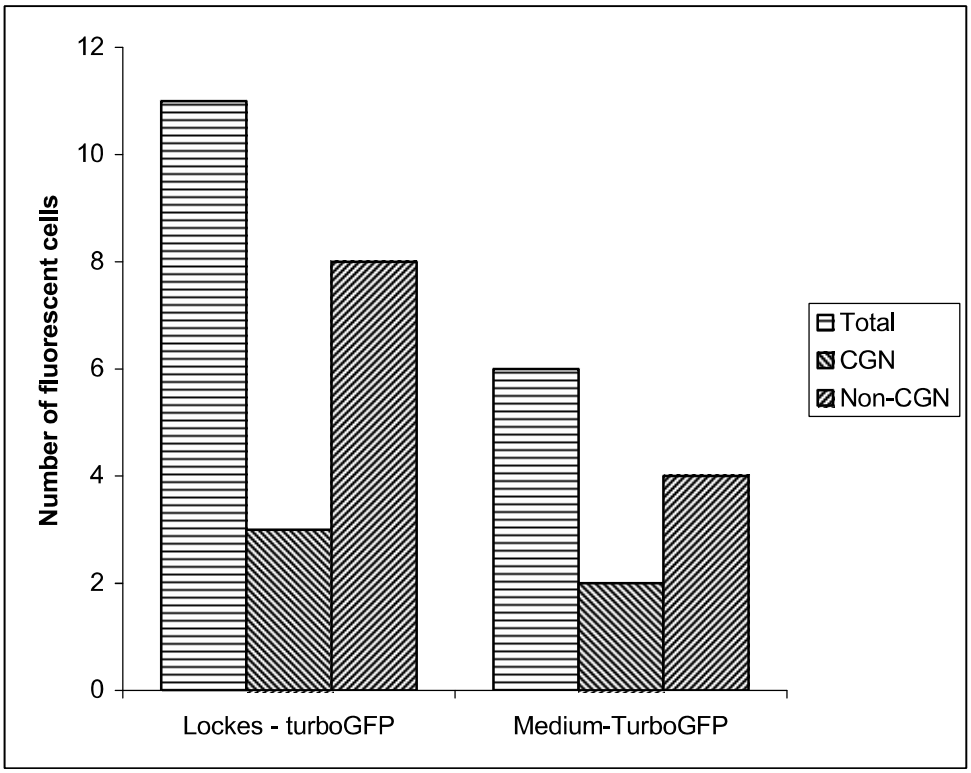

Figure 2 Count of fluorescent CGN and non-CGN cells using the two different transfection methods described above. CGN=cerebellar granule neuron.

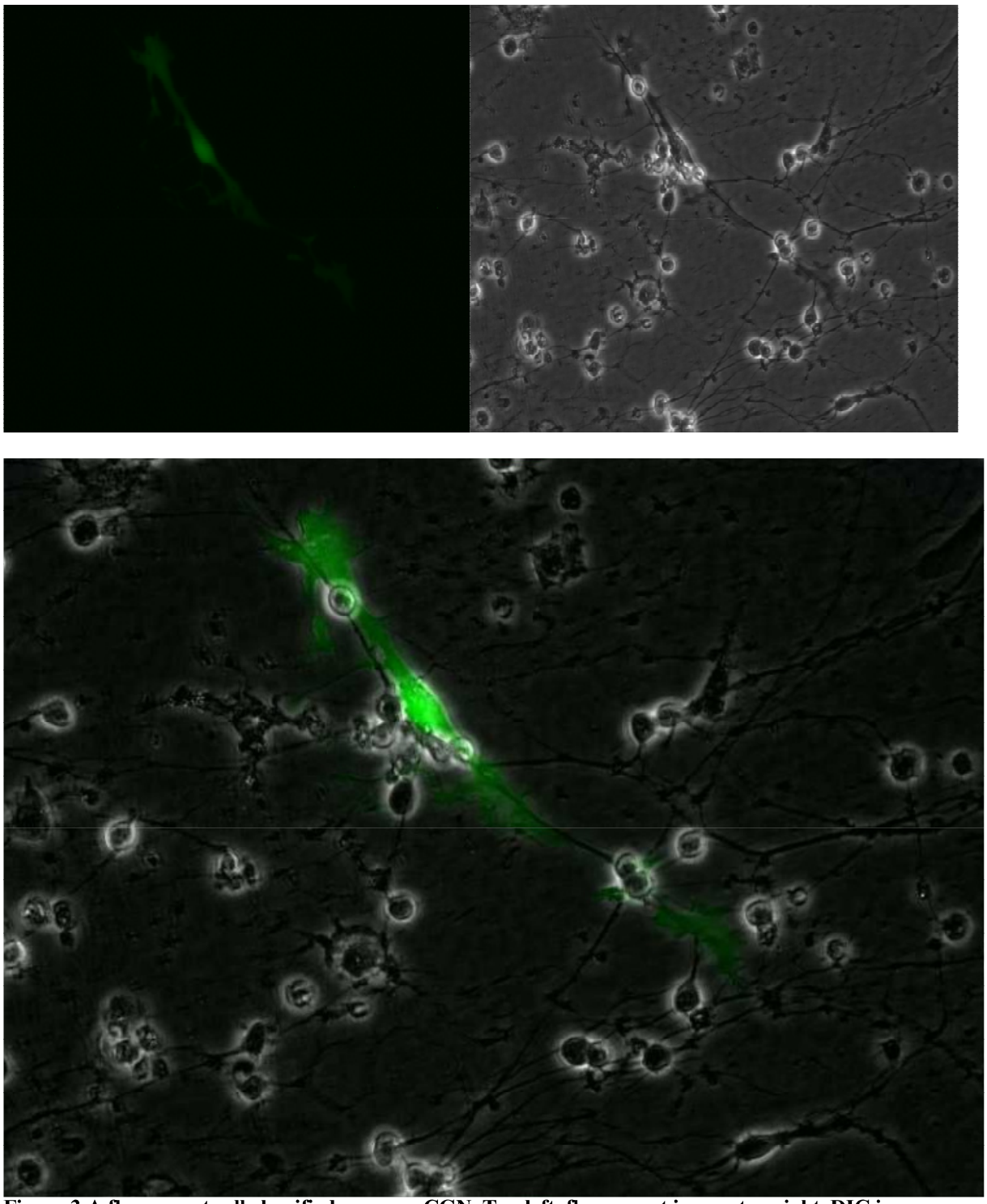

Figure 3 A fluorescent cell classified as a non-CGN. Top left, fluorescent image, top right, DIC image. Bottom merge.

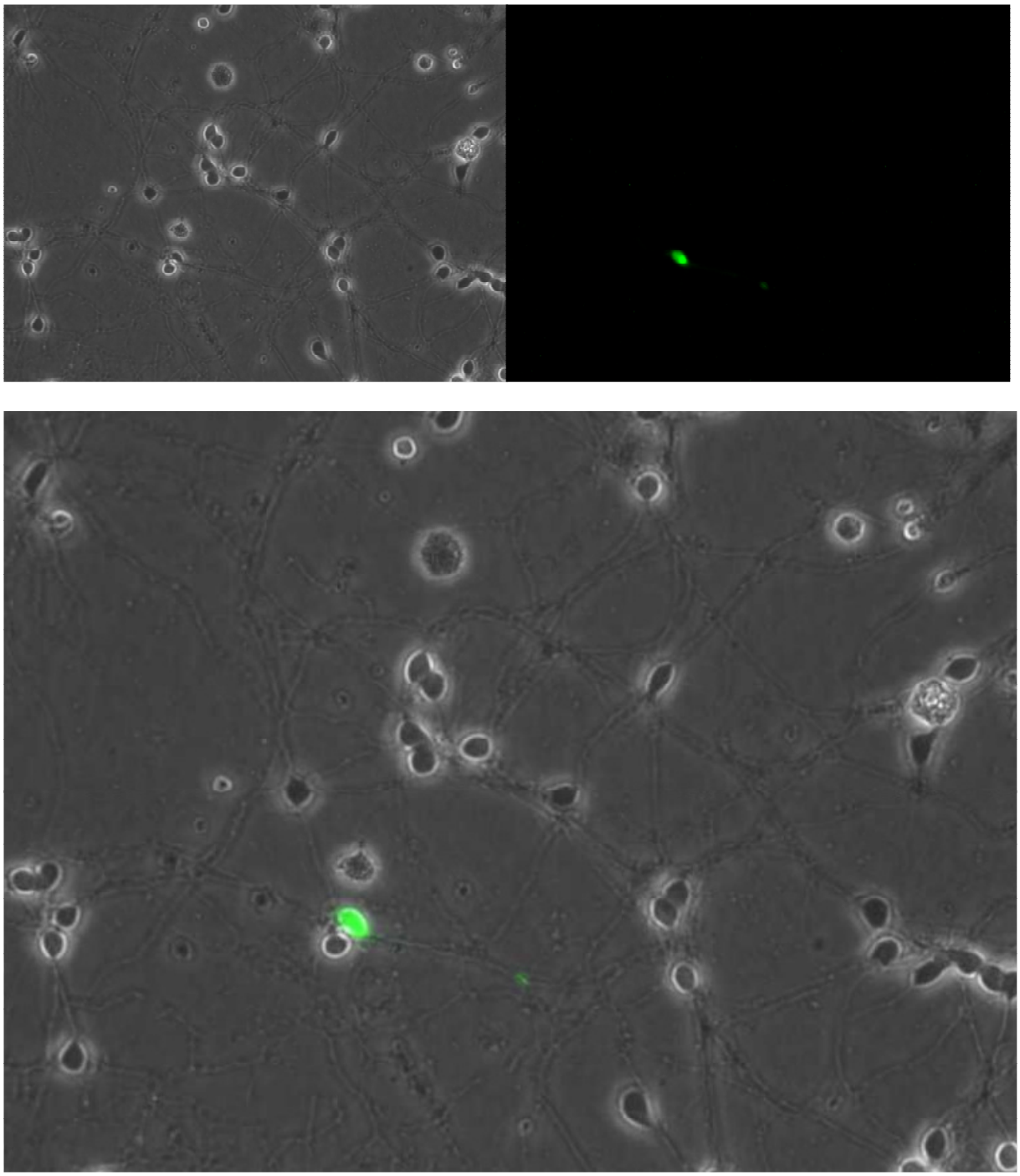

Figure 4 A fluorescent cell classified as a CGN. Top right, fluorescent image, top left, DIC image. Bottom merge.