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Tech-Trends Application Note Volume 5, Series 1

Rapid and efficient genetic manipulation of gyrencephalic carnivores using *in utero* electroporation

Abstract

Background
Higher mammals such as primates and carnivores have highly developed unique brain structures such as the ocular dominance columns in the visual cortex, and the gyrus and outer subventricular zone of the cerebral cortex. However, our molecular understanding of the formation, function and diseases of these structures is still limited, mainly because genetic manipulations that can be applied to higher mammals are still poorly available.

Results
Kawasaki, et. al. developed and validated a rapid and efficient technique that enables genetic manipulations in the brain of gyrencephalic carnivores using *in utero* electroporation. Transgene-expressing ferret babies were obtained within a few weeks after electroporation. GFP expression was detectable in the embryo and was observed at least 2 months after birth. This technique was useful for expressing transgenes in both superficial and deep cortical neurons, and for examining the dendritic morphologies and axonal trajectories of GFP expressing neurons in ferrets. Furthermore, multiple genes were efficiently co-expressed in the same neurons.

Conclusion
This method promises to be a powerful tool for investigating the fundamental mechanisms underlying the development, function and pathophysiology of neuronal structures which are unique to higher mammals.

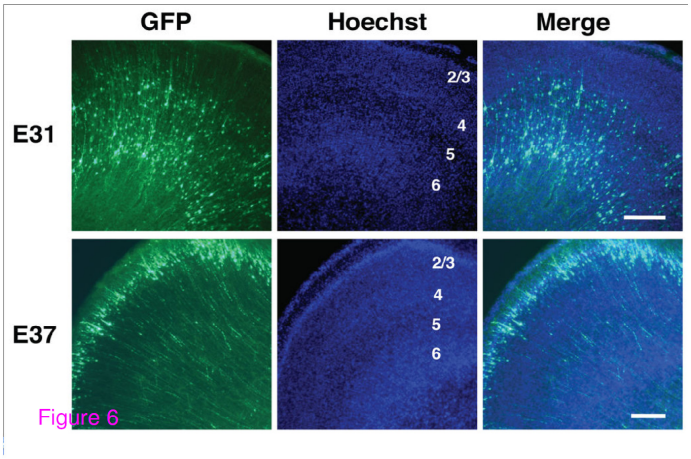

Figure 6

Figure 6: Distribution of GFP-positive neurons in the ferret cerebral cortex. *In utero* electroporation was performed at either E31 or E37 to express GFP in the cerebral cortex, and the ferret brain was dissected out at P10-15. Coronal sections were stained with Hoechst 33342 to reveal cytoarchitectonic structures of the cortex. Cortical layers are indicated with numbers in Hoechst images. When *in utero* electroporation was carried out at E31, GFP positive neurons were preferentially found in layers 5 and 6 (upper panels). In contrast, *in utero* electroporation at E37 resulted in layer 2/3 being transfected (lower panels). Scale bars, 200 μm

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In utero electroporation procedure for ferrets

Kawasaki et. al. established a procedure of *in utero* electroporation for ferrets by modifying that for rodents. Pregnant ferrets were anesthetized with sodium pentobarbital, and their body temperature was monitored and maintained using a heating pad. The uterine horns were exposed and kept wet by adding drops of PBS intermittently. The location of embryos was visualized with transmitted light delivered through an optical fiber cable. It is important to use transmitted light for visualizing embryos in the uterus. The pigmented iris was visible, and this enabled us to assume the location of the lateral ventricle. Approximately 2-5 µl of DNA solution (0.5-1 mg/ml) was injected into the lateral ventricle at the indicated ages using a pulled glass micropipette. Because the position and shape of the placenta in ferrets are more obscure compared with those in mice, care should be taken not to damage the placenta with glass micropipettes. Each embryo within the uterus was placed between tweezer-type electrodes with a diameter of 5 mm. Square electric pulses (50-150 V, 50 ms) were passed 5 times at 1 s intervals using an electroporator (ECM830, BTX). Higher voltages resulted in higher transfection efficiency. Care was taken to quickly place embryos back into the abdominal cavity to avoid excessive temperature loss. The wall and skin of the abdominal cavity were sutured, and the embryos were allowed to develop normally. Experiments were repeated at least three times and gave consistent results.

Source: Kawasaki, H. et. al., Rapid and efficient genetic manipulation of gyrencephalic carnivores using in utero electroporation. *Molecular Brain* 2012, **5**:24

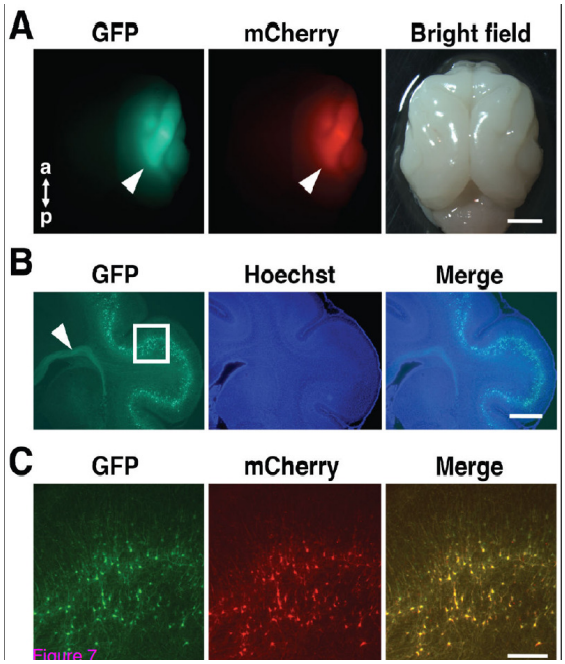

Figure 7 Double labeling using *in utero* electroporation. Cortical neurons were transfected with pCAG-GFP and pCAG mCherry using *in utero* electroporation at E33. The brain was dissected out at P20, and coronal sections were made. **(A)** GFP signals and mCherry signals were clearly observed on the brain. **(B)** Numerous GFP-positive neurons were found in deep cortical layers. GFP-positive axons were also clearly visible even without GFP immunostaining (arrowhead). The area within the white box is magnified and shown in **(C)**. Note that most GFP-positive neurons were also positive for mCherry **(C)**. The morphology of GFP-positive neurons was clearly visible even without GFP immunostaining. Scale bars, 5 mm **(A)**, 1 mm **(B)** and 200 µm **(C)**

Platinum Tweezertrode, 5 mm Diameter
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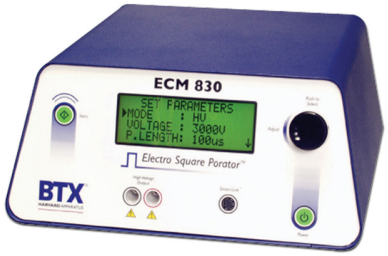

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