Abnormal neuronal migration results in a spectrum of brain malformation disorders that lead to premature death or carry life-long morbidity. Generating neuronal migration cell culture systems from animal models of disease or chemically mimicking the disease state allows neuroscientists to visualise the movement dynamics of diseased cells and to investigate the cellular basis of the neurological deficit in question. Its accessibility and well characterised cells types ensure neuronal migration cultures generated from the developing cerebellum are amongst the most widely studied models of central nervous system formation. In this report, a system of imaging granule neuron precursor (GNP) migration in an organotypic slice culture from early postnatal mouse cerebellum is described. An enhanced green fluorescent protein (eGFP) tagged retroviral vector was used to label GNPs in vitro. Subsequently, conventional fluorescence microscopy visualised all the stages of their migration. This method enables the movements of large numbers of GNPs to be recorded over long periods in highly preserved slice cultures that retain their region specific signalling cues. New light is shed on the movement dynamics, both in rates and modes of migration, of GNP processes and cell bodies across the various lamina of the developing cerebellum. This report adds to our current understanding of cerebellar development and details a reproducible and cost effective method of visualising and imaging neuronal migration.

**Key words:** Granule neuron migration, slice culture, microscopy, cerebellum.

**Abbreviations:** GNP - granule neuron precursor; EGL - external granular cell layer; ML - molecular layer; PCL - purkinje cell layer; IGL - internal granular cell layer; eGFP – enhanced green fluorescent protein; P - postnatal day; DIC – differential interference contrast.
**Introduction**

During healthy brain development, new born neurons migrate along predefined paths using cell intrinsic and region specific extrinsic cues to find their destinations in the grey matter. Here, they differentiate and make functional synaptic connections. Abnormal neuronal migration in the brain results in a spectrum of cortical malformation disorders that lead to premature death or carry lifelong morbidity. Consequently, generating migration culture models in vitro and manipulating them to mimic the disease state is a common investigatory tool for researchers. For decades, the cerebellum has been used as a model of central nervous system migration. Its well-characterised cell types and defined cytoarchitecture make it an excellent system to investigate the cellular and molecular mechanisms underlying the path finding capabilities of diseased versus healthy neurons. In vivo 3H-thymidine autoradiography systems initially established the timing and general pattern of granule neuron precursor (GNP) movement (Miale and Sidman, 1961; Altman, 1969) from their origins in the external granular cell layer (EGL), migration through the molecular layer (ML), Purkinje cell layer (PCL) and differentiation in the internal granular cell layer (IGL). Directly visualising GNP migration using dissociated single cell and reaggregation culture models subsequently allowed the dynamics of synapse formation, process extension and cell body movements of GNP's to be described (Trenknew and Sidman, 1977; Hatten, 1985; Hagata and Nakatsuji, 1990). At the time, these assays revolutionised our understanding of neuronal migration, yet they do not recapitulate the directional nature of migration from the EGL to the IGL observed in vivo; for example, GNP's on glial conduits in vitro often reverse their direction of migration (Rivas and Hatten, 1995). Indeed, studies demonstrate that there are tissue specific cues that influence the migration of GNP's, some of which may derive from Purkinje cells (Kerjan et al., 2005; Cameron et al., 2007; Guan et al. 2007). In addition, there are significant variations in modes and rates of migration for cerebellar GNP's as they move in the EGL initiate migration at the base of the EGL, transit through the ML and PCL, and finally terminate in the IGL (Komuro and Rakic, 1995, 1998; Komuro et al., 2001). To further examine these issues, a migration assay that more accurately reflected the in vivo scenario needed to be established. Organotypic slice cultures from postnatal mouse cerebellum have been used to monitor the migration of GNP's labelled in vitro with lipophilic dyes (Komuro and Rakic, 1995; for reviews see Hatten, 1999 and Komuro and Yacubova, 2002; Umeshita et al., 2007). While these studies offer a significant advancement in our understanding of the spatiotemporal mechanics of cerebellar laminar formation, they are sometimes limited by the constraints imposed on them by the labelling methodologies employed and the need for confocal microscopy. Imaging assays may include a shortage of labelled cells and phototoxicity, which can result in short video recordings. In the present study, we describe a cerebellar slice culture system using a retrovirus to label dividing GNP's in large numbers in the EGL and then monitoring their migrations using conventional fluorescence microscopy and time-lapse video recordings for periods of up to 24 hr. We show that GNP's extend a long leading process prior to initiating and during migration that often extended into the ML and PCL. We also demonstrate that the migration velocities of GNP's were similar in the EGL, ML and PCL although some cells paused at the EGL – ML border. It was also evident that GNP's did not migrate continuously into and through the ML and frequently changed direction and migrated back toward to EGL. These findings add to our current knowledge of the modes and tempos of cerebellar neuronal migration while the experimental platform described could serve as a useful platform for investigators to compare migration parameters in healthy and manipulated models of neuronal migration disorders.

**Materials and Methods**

**Preparation of organotypic slices**

Cerebella were dissected from postnatal day (P) 6 mice and the choroid plexus and meninges were removed in a petri dish containing 5ml of ice cooled calcium-magnesium free Hanks balanced salt solution (CMF-HBSS). The isolated cerebellum was then transferred to another 10 cm petri dish containing 5 ml of a 1:1 solution of ice cooled slice buffer medium (90% CMF-HBSS, 0.5% NaHCO3, 5% 1M Hepes, 1% C6H12NaO6 0.5% glucose, 1% L-glutamate and 2% penicillin/streptomycin [P/S]) and slice culture medium (49% BME, 25% CMF-HBSS, 20% horse serum, 1% glucose, 1% glutamine, 2% B-27 and 2% P/S). Using a wide bore plastic transfer pipette, the cerebellum was then transferred to the stage of a tissue chopper and oriented such that one cerebellar lobe was perpendicular to the blade chopping surface (Mcllwain, CA). Excess medium was blotted away using blotting paper and 300 μm thick slices were serially sectioned in the sagittal plane beginning at the outer surface of the cerebellum through to the vermis. Using a wide bore transfer pipette, slices were then placed back into a 1:1 solution of 37 °C slice buffer and slice culture medium. The most intact and foliated slices were identified and were placed on a millicell organotypic membrane insert (MatTec, MA) using a transfer pipette and placed in an imaging dish (Figure 1): a 14 mm diameter hole was punched in the centre of petri dishes ( Falcon, MA), and a 22 mm coverslip (Thomas Science, NJ) was attached with wax (a mixture of paraffin and Vaseline in the ratio of 3 to 1) to the bottom of the dish to cover the hole (Edmondson and Hatten, 1987). 1.4 ml of slice culture medium was then added to the well and the slices were cultured in an incubator at 37 °C and 5% CO₂.

**Infection of slices with pNIT-GFP**

A replication defective retrovirus (pNIT) expressing enhanced green fluorescent protein (eGFP) (Kakita and Goldman, 1999) was used to label migrating GNP’s. After 1 hr in culture, the dish was withdrawn from the incubator and placed in a flow cabinet, the insert was removed and placed on blotting paper for up to 5 min to allow excess medium to vapourise from around the slice. One to 2 μl of concentrated virus was placed on each slice along the primary and preculminate fissures with a P2 micropipette tip. The titre of the virus was at least 5 × 10⁶ cfu / ml (Kakita and Goldman, 1999). After 24 hr of inoculation with the virus at 37 °C / 5% CO₂, GNP's were extensively infected along the entire length of the EGL. P6 represents the peak period of GNP expansion in the cerebellum in the mouse and these represent the vast majority of all proliferating cells in the cerebellum.

**Imaging of eGFP expressing GNP’s**

The dish was then placed on a motorised, X-Y-Z controlled stage of a Zeiss Axiovert 200M inverted fluorescence microscope surrounded by a Plexiglass incubation chamber. The incubation chamber was custom built for this microscope and encased the stage, objectives, filter cube turret, condenser system, and beam splitter. The temperature was set at 37°C, via a temperature regulator and warm air was blown into the chamber via a vinyl tube connected to the rear of the chamber. Temperature adjustments were made automatically by the regulator via feedback from
an electronic thermometer attached to the stage. Intense pNit retrovirus expression permitted low-power imaging to be employed, allowing greater cell sampling through a thick tissue depth, with minor phototoxicity. Fluorescent images (Mercury lamp; X-CITE 120, EXFO) of infected folia viewed with a GFP filter were obtained using a x10 objective lens (0.2 N.A) every 10 min for up to a 24 hr recording period. Multiple positions along each folia, on multiple folia and on multiple slices could be observed. Determining the number of positions was dependant on the quality of the labelling, the desired increments between each time point and hard drive storage limitations. In the present study, 20 to 25 optical Z-planes at 1 μm increments were acquired from two or three positions on one folia of one slice. This allowed for focus drift and/or movement of the cells through different planes in the tissue. At each time point, one image using DIC optics was captured. Subsequent comparison of images acquired using the GFP and DIC channels at each time point allowed the orientation and location of migrating GNPs within the cerebellar cortical layers to be determined throughout the recording period.

### Analysis of GNP movements

The position of eGFP-labelled GNPs radially migrating from the EGL into the ML was identified at each time point by the track object module in MetaMorph Image Analysis Software (Molecular Devices, Sunnyvale, CA). The mean rate of GNP migration was determined by MetaMorph software. Migration rates were measured from 127 GNPs undergoing migration in five cerebellar slices taken from individual cerebella. The time GNPs spent paused as they migrated from the EGL into the ML, PCL, and IGL was calculated by counting the number of frames where no GNP movement was detected throughout the duration of the movie, and comparing this to the total number of frames. The direction of movement was determined by analysing the direction of GNP radial migration from one frame to the next frame, and determining if the cell was undergoing forward or reverse movements, or was paused, throughout the movie and comparing these to the total number of frames. Movement parameters were measured from 118 GNPs undergoing migration in five cerebellar slices taken from individual cerebella. Data are represented as means ± standard deviation (SD).

### Results

#### eGFP expression in GNPs

After 24 hr of viral infection, GNPs were extensively labelled across all cerebellar cortical layers (Figure 2(A)). A greater number of labelled cells were present in EGL and ML. Given that the prevailing cell type undergoing mitosis at P6 are GNPs in the EGL, and that labelled cells have processes consistent with neuronal features, it was concluded that the vast majority of eGFP expressing cells in the slice culture were migrating GNPs. As the beginning of the recording period, cells were migrating tangentially (asterisks) or initiating radial migration in the EGL (red arrows) and radially migrating through the ML, PCL and IGL (green, yellow and blue arrows respectively) on both sides of the folium (Figure 2(A)).

While the extent of GNP infection varied among viral preparations, eGFP was consistently expressed throughout the cytoplasm of labelled cells. Figure 2(B) shows the full length of the leading processes (orange arrows), the trailing processes (blue arrows), and the orientation of the cell bodies of two granule neurons either side of the folia, residing in the ML and IGL (green and red asterisks respectively) (Figure 2(B)). The expression of eGFP was greatest in the cell body and in the portion of its leading process immediately distal to the cell soma. Moreover, eGFP expression was typically more intense in the leading process than in the trailing process. A distinctive feature identified with this method is that granule neurons initiating migration from the EGL appeared to extend a long process that spanned much of the ML prior to nuclear eggress from the EGL (Figure 2(A), red arrows). In addition, some granule neurons in the ML had long processes that extended to the base of, or into, the IGL during their migration (Figure 2(A), green arrows).

#### The rate and modes of GNP migration in slice cultures

This technique allowed continuous visualisation of the entire migration of GNPs from tangential migration in the EGL to subsequent radial migration through the ML, PCL and into the IGL. Using the track object tool in MetaMorph, the velocity of migrating GNPs was measured radially through the ML, PCL and IGL. Cells migrated along a range from 3.0 to 18.1 μm/hr. The average rate of migration was 8.9 ± 1.8 μm/hr (Figure 2(C)). There was no significance difference in migration velocities as cells transitioned from the EGL to the ML (8.9 ± 2.3 μm/hr), migrated through the ML (7.9 ± 3.0 μm/hr), and then through the PCL into the IGL (8.9 ± 2.5 μm/hr) (Figure 2(C)).

#### Tangential migration in the EGL

After 24 hr of inoculation with virus, GNPs were present in the upper and lower portions of two adjacent EGLs (Figure 3 (A-J)) and exhibited varying...
degrees of mobility. Some cells tangentially migrated large distances throughout the length of the recording period (Figure 3 (A-J), blue arrow). Other cells displayed slight back and forth movements (Figure 3 (A-J), green arrow) or were stationary during large portions of the recording period, often for > 12 hr (Figure 3 (A-G), yellow arrow). Mobile GNPs typically displayed elongated cell bodies and short leading and trailing processes (Figure 3 (B-J), blue arrow, Figure 3 (C-E), red arrow). These cells moved tangentially in both directions through the EGL, and occasionally reversed their direction of migration (Figure 3 (D-H), red arrow).

**Figure 4:** GNP undergoing radial migration from the EGL through the ML and into the IGL over a 24 hr recording period. (A–C) The GNP leading process (asterisks) extended into the ML and PCL prior to the initiation of radial migration from the EGL. (D–J) While the cell body is in the ML, the process extended into the IGL (K–L). The leading process retracted when the cell body migrated into the KII (B–E). In some frames, the leading process may be identified in the EGL (crosses). Scale bars are 50 µm. Time intervals are 2 hr.

**Figure 5 (A–P):** GNP undergoing slow progression from the EGL (yellow arrow). (E–P): GNP exiting the EGL rapidly and migrating directly from the EGL to the IGL (red arrow). Scale bars are 50 µm. Time intervals are 1 hr.

**Figure 6 (A–N):** Time-lapse imaging of a GNP stationary or displaying back and forth movements in the ML (asterisk). Time intervals are 1 hr. Scale bars are 50 µm. (O): Mean percentage frames GNPs spent paused, migrating toward the EGL, or toward the IGL.

**Figure 4:** GNP undergoing radial migration from the EGL through the ML and into the IGL over a 24 hr recording period. (A–C) The GNP leading process (asterisks) extended into the ML and PCL prior to the initiation of radial migration from the EGL. (D–J) While the cell body is in the ML, the process extended into the IGL (K–L). The leading process retracted when the cell body migrated into the KII (B–E). In some frames, the leading process may be identified in the EGL (crosses). Scale bars are 50 µm. Time intervals are 2 hr.

**Figure 5 (A–P):** GNP undergoing slow progression from the EGL (yellow arrow). (E–P): GNP exiting the EGL rapidly and migrating directly from the EGL to the IGL (red arrow). Scale bars are 50 µm. Time intervals are 1 hr.

**Figure 6 (A–N):** Time-lapse imaging of a GNP stationary or displaying back and forth movements in the ML (asterisk). Time intervals are 1 hr. Scale bars are 50 µm. (O): Mean percentage frames GNPs spent paused, migrating toward the EGL, or toward the IGL.

**Degrees of mobility:** Some cells tangentially migrated large distances throughout the length of the recording period (Figure 3 (A-J), blue arrow). Other cells displayed slight back and forth movements (Figure 3 (A-J), green arrow) or were stationary during large portions of the recording period, often for > 12 hr (Figure 3 (A-G), yellow arrow). Mobile GNPs typically displayed elongated cell bodies and short leading and trailing processes (Figure 3 (B-J), blue arrow, Figure 3 (C-E), red arrow). These cells moved tangentially in both directions through the EGL, and occasionally reversed their direction of migration (Figure 3 (D-H), red arrow).

**GNPs extend a long leading process prior to initiating and during migration:**

GNPs extended long leading processes during radial migration (Figure 4). Figure 4 shows a GNP cell body in the EGL while its leading process was evident in the ML (asterisk in Figure 4 (A)). As the GNP initiated migration from the EGL into the ML, the leading process extended into the PCL and the IGL (asterisk in Figures 4 (B-D)). A trailing process was present extending into the EGL (cross in Figures 4 (B-E)). As the cell soma migrated through the ML its leading process extended further into the IGL (Figures 4 (D-F)). The cell then paused at the PCL / ML border (in this instance for 2 - 4 hr). The GNP then crossed the PCL and entered the IGL (Figures 4 (I-L)). Its leading process was then retracted (asterisk in Figures 4 (K-L)).

**Migration tempos into the ML differ:**

Some GNPs migrated continuously from the EGL into the ML, PCL and IGL without pausing or changing direction (red arrow, Figures 5 (E-P)). We also observed some GNPs displaying a much slower egress from the EGL (yellow arrow, Figures 5 (A-P)). During this time, the cell body remained mostly stationary, extending and retracting short processes (Figures 5 (A-E)). Upon initiation of radial migration, a leading process extended deeper into the ML (Figures 5 (A-P)). A trailing process was also visualised extending in to the EGL (asterisk, Figure 5 (M)). After exiting the EGL, the leading process remained in front of the cell body, extending into
the ML and the PCL while the cell migrated through the ML (Figures 5 (M-P)).

Directionality of GNP movements in ML
We also monitored GNP direction of movement in the ML, PCL and IGL, and revealed that GNPs often displayed forward and reverse modes of migration, that are most apparent in the ML (asterisk in Figure 6 (A-N)). GNPs move towards the IGL 2.7 times more frequently than towards the EGL and spent 48.6 ± 3.4% of the recording period paused in the ML (Figure 6 (O)).

Discussion
Live imaging of GNPs in slice cultures using conventional fluorescence microscopy
The use of porous membrane inserts in preference to glass as growth substrates for slice cultures has proved advantageous in preserving the three-dimensional architecture of the central nervous system (Fenili and Boni, 2003; for review see Miyata et al., 2005). Experiments undertaken using these inserts usually entail placing the insert into a well and aligning slice cultures on the membrane. However, the membrane component of these inserts is raised within a plastic casing to allow for an air-medium interface, thus distancing the specimen from the glass as growth substrates for slice cultures (Komuro and Rakic, 1995; Komuro et al., 2001). However, in our study, due to the lower power of the objectives we used and high multiplicity of infection of the pNIT retrovirus, a greater field of view could be analysed and a much larger number of cells visualised within one experiment. In addition, the strong eGFP expression obtained resulted in far less artificial tissue background than occurs with Dil labelling of GNPs in the EGL of cerebellar slices (Komuro et al., 2001). While the data presented by these authors is not disputed, our experimental approach allowed for a much longer recording period (up to 24 hr), a greater sample size, and an extended representation of GNP migration in the EGL.

We also examined the transition of GNPs from tangential to radial migration. As described previously and as seen in this communication, this change in migration modality took place at the EGL/ML border (Komuro and Rakic, 1995; Komuro et al., 1995), where we observed the formation of a leading process into the ML for radial migration, with movement of the cell body along this process as it extended through the PCL and IGL. In agreement with Komuro et al. (2001), labelled GNP cells often quickly transitioned from the EGL to the ML. However, we also observed GNPs where this transition was delayed by an extended paused period (up to 12 hr); in these instances, the cell body remained at the EGL/ML border reorienting its cell cytoplasm, prior to establishing a definitive leading process and egress of the cell soma from the EGL. Our experimental paradigm allows for a much longer recording period and, as such, cells can be observed undergoing tangential migration, transitioning and then undertaking radial migration, in one recording period.

After exiting the EGL, the range of GNP migration rates are broadly similar to those described by Komuro and Rakic (1995). We report an average migration rate of 8.9 ± 2.3 μm/hr, which is in line with their findings, albeit performed at P7 (9.6 ± 2.3μm/hr), one day later than our experiments were performed. These do not compare favourably with the rates of migration observed by another report, where at P8, the rates were found to range from 17-54 μm/hr for slow and fast moving cells respectively (Umeshima et al., 2007). Komuro and Rakic (1995) suggested that as the cerebellum ages, the rate of GNP migration increases in line with the expansion of the central nervous system and the greater distance migrating cells need to travel. Nonetheless, an average of 54 μm/hr seems very fast and implies that in general GNPs have the capacity to migrate through all layers of the cerebellum within 6 to 10 hr, depending on its developmental stage. Although we did observe some fast moving cells, GNP usually did not migrate from the EGL to the IGL within 10 hrs. Moreover, we observed salutatory and back and forth movements of GNPs migrating through the EGL was clearly visualised, as previously described (Komuro et al., 2001). However, in our study, due to the lower power of the objectives we used and high multiplicity of infection of the pNIT retrovirus, a greater field of view could be analysed and a much larger number of cells visualised within one experiment. In addition, the strong eGFP expression obtained resulted in far less artificial tissue background than occurs with Dil labelling of GNPs in the EGL of cerebellar slices (Komuro et al., 2001). While the data presented by these authors is not disputed, our experimental approach allowed for a much longer recording period (up to 24 hr) rather than 6 hr), a greater sample size, and an extended representation of GNP migration in the EGL.

We also examined the transition of GNPs from tangential to radial migration. As described previously and as seen in this communication, this change in migration modality took place at the EGL/ML border (Komuro and Rakic, 1995; Komuro et al., 1995), where we observed the formation of a leading process into the ML for radial migration, with movement of the cell body along this process as it extended through the PCL and IGL. In agreement with Komuro et al. (2001), labelled GNP cells often quickly transitioned from the EGL to the ML. However, we also observed GNPs where this transition was delayed by an extended paused period (up to 12 hr); in these instances, the cell body remained at the EGL/ML border reorienting its cell cytoplasm, prior to establishing a definitive leading process and egress of the cell soma from the EGL. Our experimental paradigm allows for a much longer recording period and, as such, cells can be observed undergoing tangential migration, transitioning and then undertaking radial migration, in one recording period.

After exiting the EGL, the range of GNP migration rates are broadly similar to those described by Komuro and Rakic (1995). We report an average migration rate of 8.9 ± 2.3 μm/hr, which is in line with their findings, albeit performed at P7 (9.6 ± 2.3μm/hr), one day later than our experiments were performed. These do not compare favourably with the rates of migration observed by another report, where at P8, the rates were found to range from 17-54 μm/hr for slow and fast moving cells respectively (Umeshima et al., 2007). Komuro and Rakic (1995) suggested that as the cerebellum ages, the rate of GNP migration increases in line with the expansion of the central nervous system and the greater distance migrating cells need to travel. Nonetheless, an average of 54 μm/hr seems very fast and implies that in general GNPs have the capacity to migrate through all layers of the cerebellum within 6 to 10 hr, depending on its developmental stage. Although we did observe some fast moving cells, GNP usually did not migrate from the EGL to the IGL within 10 hrs. Moreover, we observed salutatory and back and forth movements of GNPs during radial migration, as previously described (Komuro and Rakic, 1995; Umeshima et al., 2007). Direct comparison of movement dynamics with Komuro and Rakic (1995) is difficult due to paucity of cells analysed and the shorter recording period in their study, two cells
over 50 mins, as compared to 118 cells over 12 - 24 hr in our study. Notwithstanding this, a slow moving cell they describe exhibited a similar ratio of forward to backward movement (~ 3:1), although the amount of time both their cells were paused did not compare. In addition, Umeshima et al (2007) showed a GNP rapidly migrating back towards the EGL from the ML (see SI Movie 3; Umeshima et al., 2007), but this was not commented on by the authors.

We have also visualised details of leading process movement, which concur with previous observations in dissociated cell cultures (Edmondson and Hatten, 1987) and in a slice culture (Umeshima et al., 2007). However, the long length and dynamic movements of the leading process that precedes GNP cell soma translocation is a novel observation that has not been previously characterised in slice cultures. For instance, we observed that the leading process of GNPs may extend well in front of the cell body (up to 250 - 300 μm), reaching the PCL while the cell body was still in the lower EGL, through the PCL into the IGL while the cell body was in the ML and to the bottom of the IGL while the cell body was moving through the PCL. This process eventually retracted and we have observed the subsequent formation of granule neuron dendrites (data not shown). These findings suggest that GNPs are interpreting layer specific migration cues that direct their movements via the explorations of the leading process and its growth cone.

**Conclusion**

In this communication, a transferrable and updated model of GNP migration that accurately reflects modes of GNP movements during the postnatal development of the cerebellum is described. GNPs were clearly identifiable and their movements tracked over long periods of time (> 24 hr) at short intervals without losing resolution or focus, using fluorescent microscopy. As such, this migration model represents an efficient method to monitor cell migration and represents a solution to the current constraints imposed on real time microscopy by costly imaging modalities and non-specific cell labelling techniques. The stable expression of eGFP in these cultures and long recording periods has revealed novel information on morphologic transitions between cell layers and dynamics of cell soma and process movement during GNP migration in cerebellar cortex. Moreover, this methodology, while optimised using postnatal cerebellar slices, is not confined to just the cerebellum and may also be used to resourcefully record neuronal or glial migration in spinal cord and brain slices during development. The potential use of a multi-well dish large enough to accommodate membrane inserts in each well also permits simultaneous recordings from a variety of preparations in different wells. It is anticipated that this model of GNP migration will complement the repertoire of imaging techniques used to monitor cell activities in diseased and healthy states.

**Acknowledgements**

This work was performed under the mentorship of Dr. Phyllis Faust, Columbia University and supported in part by funding from the National Institutes of Heath, Bethesda, MD.

**References**


Fenili D, De Boni U (2003) Organotypic slices in vitro: repeated, same-cell, high-resolution tracking of neuronal and glial migration in brain cortical and brain slices during development. The potential use of a multi-well dish large enough to accommodate membrane inserts in each well also permits simultaneous recordings from a variety of preparations in different wells. It is anticipated that this model of GNP migration will complement the repertoire of imaging techniques used to monitor cell activities in diseased and healthy states.

Dr. Barry was awarded a PhD in neuroscience from the Department of Anatomy and Neuroscience, University College Cork, Ireland in 2005. He subsequently conducted postdoctoral research at the Department of Pathology and Cell Biology, Columbia University, New York, where the research outlined here was initiated. Denis undertook postdoctoral research fellowship positions at the Centre for Research into Infectious Diseases and the Conway Institute of Biomolecular and Biomedical Research, University College Dublin. In 2013, Dr Barry was appointed to the position of Assistant Professor in the Department of Anatomy, Trinity Biomedical Sciences Institute, Trinity College, Dublin. Here his research is based on furthering our knowledge of developmental neuroscience and spinal cord injury.