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TITLE: Establishing mixed neuronal and glial cell cultures from embryonic mouse brains to study infection and innate immunity.

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SUMMARY:
This protocol presents a novel way of generating central nervous system cell cultures from embryonic day 17 mouse brains for neuro(immuno)logy research. This model can be analyzed using various experimental techniques, including RT-qPCR, microscopy, ELISA, and flow cytometry.

ABSTRACT:
Models of the central nervous system (CNS) must recapitulate the complex network of interconnected cells found *in vivo.* The CNS consists primarily of neurons, astrocytes, oligodendrocytes, and microglia. Due to increasing efforts to replace and reduce animal use, a variety of *in vitro* cell culture systems have been developed by others to explore innate cell properties which will allow the development of therapeutics for CNS infections and pathologies. Whilst certain research questions can be addressed by human based cell culture systems such as (induced) pluripotent stem cells, working with human cells has its own limitations with regards to availability, costs and ethics.
Here we describe a novel protocol for isolating and culturing cells from embryonic mouse brains. The resulting mixed neural cell cultures mimic several of the cell populations and interactions that are found in the brain *in vivo*. Compared to current equivalent methods this protocol more closely mimics the characteristics of the brain and also garners more cells, therefore allowing for more experimental conditions to be investigated from one pregnant mouse. Further, the protocol is relatively easy and highly reproducible. These cultures have been optimized for use at various scales, including 96-well based high throughput screens, 24-well microscopy analysis and 6-well cultures for flow cytometry and qRT-PCR analysis. This novel culture method is a powerful tool to investigate infection and immunity within the context of some of the complexity of the CNS with the convenience of *in vitro* methods.

INTRODUCTION:

Improving our understanding of the central nervous system (CNS) is critical to improve therapeutic options for many neuroinflammatory and neurodegenerative diseases. The CNS, a complex network of interconnected cells within the brain, spinal cord, and optic nerve, is primarily made up of neurons, oligodendrocytes, astrocytes, and their own innate immune cells, the microglia[1]. An *in vitro* approach can often drastically reduce the numbers of mice required to do meaningful research; however, the complex nature of the CNS makes it impossible to recapitulate the *in vivo* situation using cell lines. Mixed neural cell cultures provide an extremely valuable research tool to investigate neuro(immuno)logy questions in a relevant model, in line with the Replacement, Reduction and Refinement (3Rs) principles[2,3].

Thomson et al (2008) described a cell culture method using prenatal spinal cord cells that differentiate into all the aforementioned main CNS cell types[4]. This system has also got synapse formation, myelinated axons, and nodes of Ranvier. The main limitation of this culturing method is that, being spinal cord, it does not usefully model the brain and the cell yields from embryonic day 13 (E13) spinal cords are constricting, thus limiting the number of experimental conditions that can be investigated. Therefore, we aimed to develop a new cell culture system that recapitulates the characteristics of the brain with increased cell yield to reduce the requirement for animals.

Using Thomson et al (2008) as a starting point we have developed a cell culture model derived purely from the prenatal mouse brains. These cultures have all the same cell populations, interconnectivity, and treatment options as the spinal cord cultures except there is less myelination by comparison. However, having a CNS *in vitro* model with approximately three times higher cell yield is more efficient, requiring fewer mice and less time processing embryos. We optimized this novel culture system for multiple downstream applications and scales, including glass coverslips for microscopy analysis and various sizes of plastic well plates, including 96 well for high-throughput research.

PROTOCOL:

Schematic Diagram

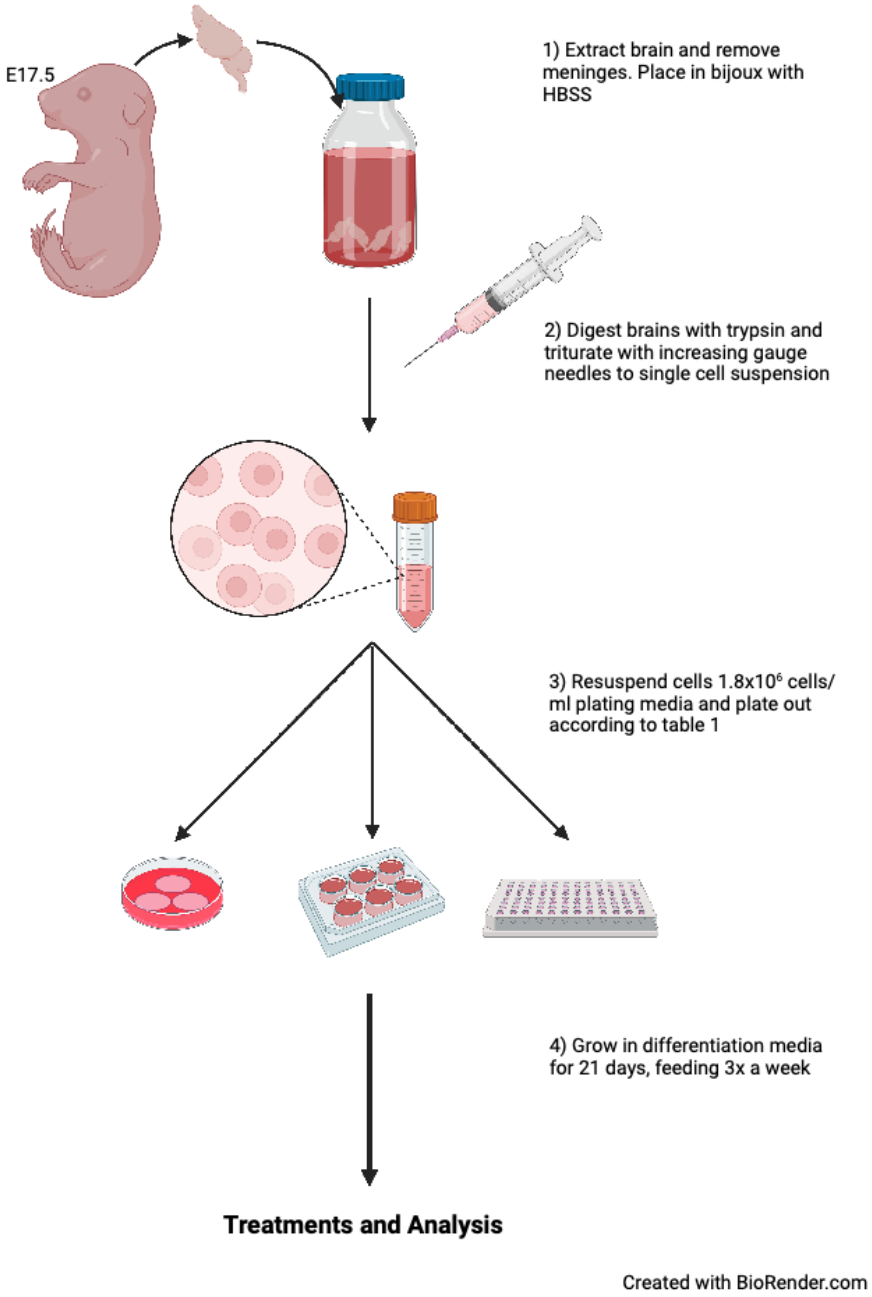

Figure 1: Schematic overview of described method to generate mixed neuronal and glial cultures.

Protocol:

All animal experiments should be performed compliant with local law and guidelines for animal use. Animals were housed in specific-pathogen free conditions in accordance with the UK Animals Scientific Procedures Act 1986, under the auspices of a UK Home Office Project License and were approved by the local Ethical Review Committee at the University of Glasgow. For this study, in-house bred adult C57BL/6J mice were used. The use of young females (8-12 weeks) is recommended due to the higher success rate of pregnancy; males can be reused for multiple rounds of breeding. Figure 1 represents a schematic overview of the described method to generate mixed neuronal and glial cultures.

1) Prepare the required tissue culture consumables

Table 1: Required volumes for preparation of coated tissue culture plastics.

Required format	BA-PLL/well	Water for wash	Volume cells in Plating Media (PM) to plate out	Topping up Media	Remove/add when feeding cultures (3x/week)
6 well format (9.6cm^2)	1000 µL	1000 µL	1000 µL	-400 µL +600 µL DM+	-500 µL Media +600 µL DM+/-
96 well format (0.32 cm^2)	100µL	100 µL	50 µL	+60 µL DM+	-50 µL Media +60 µL DM+/-
Coverslip in dish format	20 mL per 200 coverslips	20 mL	100 µL per coverslip	+600 µL DM+ +300 µL PM	-500 µL Media +600 µL DM+/-

1.1) Prepare the plates and/or dishes containing microscopy coverslips inside a Class 2 Safety cabinet. Sterilize all reagents or autoclave to ensure sterility during the culture period.

1.2) Add the appropriate volume BA-PLL (13.2 µg/mL poly-L-lysinehydrobromide (PLL) in boric acid buffer (BA) (50mM boric acid, 12.5mM sodium tetraborate, pH 8.5)) to each well (1000 uL/well in 6-well plate, 100ul in 96-well format, volumes summarized in Table 1). For microscopy coverslips, add 20 mL BA-PLL to a 9 cm diameter tissue culture dish containing 200 sterile coverslips, swirl to distribute evenly.

1.3) Incubate at 37°C for 1-2 hours.

1.4) Remove the BA-PLL solution from each well or dish containing microscopy coverslips, and wash by adding 20 mL sterile water, swirling the coverslips, then removing the water. Repeat this wash step three times. For a dish containing coverslips, leave sterile water in tissue culture dish on the final wash for ease of removing the coverslips.

1.5) Remove as much liquid as possible with a sterile pipette and allow to dry for at least 2 hours or up to overnight.

1.6) Coated plates can be stored at 4°C for up to 2 months.

NOTE: The boric acid with poly-l-lysine (BA-PLL) solution can be reused up to 3x, adding new PLL each time. Store BA-PLL at 4°C. Dishes are treated with BA-PLL as the PLL allows the cells to stick down and grow. Without this treatment the cells will lift after approximately one week of culture and no longer be able to differentiate.

2) Dissection of E17 embryonal brains

2.1) Cohouse one or multiple female mice with a male mouse. Check females daily for a mucus plug indicating mating has taken place. Any "plugged" female mice should be separated from the male to ensure correct start date of gestation. Mice can be weighed to confirm pregnancy or monitored visually.

2.2) Cull the pregnant mouse at E17 using appropriate methods in compliance with local animal welfare guidance and laws, for example rising carbon dioxide concentration, lethal overdose of anesthetic or dislocation of the neck.
NOTE: The chosen method must not disrupt the embryos. For this study, exposure to a rising concentration of carbon dioxide gas followed by confirmation of death by severing the femoral artery was used to cull the pregnant dams.

2.3) Place the culled, pregnant mouse on its back on a dissection board; while pinning it down is not required it might make it easier for unexperienced researchers. Pinch the midline of the abdomen using forceps. Using sharp scissors, cut open the abdomen through the skin and the peritoneum over the midline from genitalia to ribcage, being careful not to puncture the uterus.

I. The mouse uterus consists of two horns, each typically containing 1-5 embryos. Remove the uterus containing the embryos from the mother and immediately place on ice.

2.4) Cut through the yolk sack on the side of the placentas, being careful to not damage the embryos and remove the embryos from their yolk sack.

2.5) Immediately decapitate the embryos, adding the heads into a dish with Hanks' Balanced Salt Solutions (HBSS) without calcium (Ca^{2+}) and magnesium (Mg^{2+}) (HBSS-/-) on ice.
NOTE: If genotyping is required, one can remove the tail at this stage for genetic analysis. When multiple genotypes are expected, the heads of each embryo should be kept separately for culturing.

2.6) Using angled forceps, position the head on its side facing left.

2.7) Pierce the eye with one edge of the forceps, holding the chin firmly with the other edge.

2.8) Starting at the nape, gently tear the skin of the scalp along the midline towards the tip of the snout.

2.9) Entering through the spinal cord, noticeable as a white oval, use the angled forceps to crack open the skull along the midline exposing the brain.

2.10) Gently peel the skull away on the side facing upwards, exposing the brain.

2.11) Lift the brain out of the skull, disposing of the skull once the brain is completely removed.

2.12) Remove the meninges using the forceps, which are noticeable as a thin membrane with dense blood vessels.

2.13) Place the brains into a bijou containing 2 mL HBSS-/- on ice.

2.14) Repeat steps 2.6) to 2.13) with the remaining brains, adding up to 4 brains per bijou.

2.15) Add 250 µL 10x trypsin to the bijou and triturate the brains by shaking the bijou. Incubate for 15 minutes at 37 °C.
NOTE: All steps from this point forward should be performed in a sterile tissue culture hood.

2.16) Thaw 2 mL of Soybean Trypsin (SD) Inhibitor (Leibovitz L-15, 0.52 mg/mL trypsin inhibitor from soybean, 40 µg/mL DNase I, 3mg/mL BSA fraction V) per bijou containing up to 4 brains from -20° C by placing it at 37 °C.

2.17) Add 2 ml SD inhibitor to each bijou containing brains (per bijou containing up to four brains), shaking the bijou again to disperse it evenly.
NOTE: SD inhibitor decreases the activity of the trypsin to prevent unnecessary digestion of the samples to preserve cell viability.

2.18) Without centrifugation, remove 2 mL of the supernatant from each bijou transferring it into a 15 mL falcon tube, being careful not to transfer cell clumps.

2.19) Triturate the remaining cells in the bijou with a 19 G needle attached to a 5 mL syringe by aspirating the suspension twice. This will create a thick mucus-like mixture.

I. Repeat twice more using a 21 G needle. If there are clumps remaining, triturate once more with the 21 G needle.

2.20) Transfer the cells from the bijou to the same 15 mL centrifuge tube (from 2.18) using a 23 G needle.

2.21) Centrifuge at 200 x *g* at room temperature (RT) for 5 minutes.

2.22) Remove all the supernatant using a 5 ml stripette and transfer it to another 15 mL falcon being careful to not disturb the loose pellet at the bottom which contains the required cells.

2.23) Centrifuge the supernatant again at 200 x *g* at RT for 5 minutes.
NOTE: This step is not essential, but if you require many cells or had few embryos one could perform this step to recover as many cells as possible from the supernatant.

2.24) Using 10 mL of plating media (PM) (49% Dulbecco's Modified Eagle Medium (DMEM), 1% Penicillin/Streptomycin (Pen/Strep), 25% horse serum, 25% HBSS with Ca^{2+} and Mg^{2+} (HBSS+/+)), combine and resuspend the two pellets together to create a whole cell suspension.

2.25) Count cells using trypan blue and either a hemocytometer or digital cell counter and dilute the cell suspension with PM to a concentration of $1.8x10^6$ cells/mL.

3) Plating the cells:

3.1) Add the required volume of cell suspension to the required format as detailed in Table 1: 1000 µL per well in 6-well format, 50 µL per well in 96-well format or 100 µL per coverslip.

3.2) Incubate for 2-4 hours at 37 °C with 5-7% CO_2. Check cells have adhered using an inverted microscope.

3.3) Top cells up by removing the media and topping up with new differentiation media (DM+: DM- including 10µg/ml insulin. DM-: DMEM, 1% Pen/Strep, 50 nM hydrocortisone, 10 ng/mL biotin, 2.5 mL 100x N1 media supplement). Volumes as detailed in Table 1. Press down any floating coverslips using a sterile pipette tip.

4) Maintaining the cultures:

NOTE: These cultures require feeding thrice weekly to support optimal growth and differentiation. Cultures will reach the optimum health and maturity for experiments on days in vitro (DIV) 21. Cells can be kept in culture for up to 28 days, after which the cultures quickly degenerate.

4.1) Three times per week until DIV12, replace part of the supernatant with fresh DM+ by removing 500 µL per well in 6-well format, 50 µL per well in 96-well format or 500 µL per dish containing 3 coverslips, and adding 600 µL per well in 6-well format, 60 µL per well in 96-well format or 500 µL per coverslip dish (Table 1).

4.2) Three times per week from DIV13 onwards, replace part of the supernatant with fresh DM- by removing 500 µL per well in 6-well format, 50 µL per well in 96-well format or 500 µL per dish containing 3 coverslips, and adding 600 µL per well in 6-well format, 60 µL per

well in 96-well format or 500 µL per coverslip dish (Table 1).

REPRESENTATIVE RESULTS:

Microscopy

Cultures grown on glass coverslips are ideal to analyze by microscopy. To visualize the development of the cultures, coverslips were fixed in 4% PFA at multiple timepoints from DIV0 (once cells were attached) until DIV28. The cultures were stained for immunofluorescence imaging as previously described[5] using three different staining combinations: NG2 (immature oligodendrocytes) and Nestin (neuronal stem/progenitor cells) as developmental markers, SMI31 (axons), MBP (myelin) and NeuN (neuron cell body) as neuronal markers or CNP (oligodendrocytes), GFAP (astrocytes), and Iba1 (microglia) as glial markers (Figure 2).

The various cell types were quantified using CellProfiler pipelines (based on https://github.com/muecs/cp/tree/v1.1). Each individual data point was generated from an average of 10 images taken from 3 coverslips per timepoint. For quantifying quantity of astrocytes percentage field of view was used instead of number of cells. This was due to difficulties differentiating between the individual cells as they frequently overlap. (Figure 2M-N). The cultures reach peak maturity and cell density at DIV21, after which the cultures start to degrade (Figure 2N).

Importantly, these cultures can easily be treated with drugs such as potential therapeutics or used to trace *in vitro* infections. In this example, cultures on coverslips were transferred to a 24 well plate and infected with the highly neurotropic virus Semliki Forest virus (SFV) (strain SFV6)[6] which expresses zsGreen in infected cells. To ensure low level infection, we used a Multiplicity of Infection (MOI, or number of added virus particles per cell) of 0.05 as titrated on BHK cells. After 0-72 hours post infection (hpi), cultures were fixed in 4% PFA and stained for analysis by immunofluorescent imaging. Figure 3 illustrates that in line with *in vivo* infection, SFV mainly infects oligodendrocytes and neurons[6].

Reverse Transcription (RT)-quantitative (q)PCR

In addition to microscopy, our CNS cultures can be used for analysis by molecular methods such as RT-qPCR of mRNA responses to treatment. To further investigate the innate antiviral response, 6 well plate cultures were treated with a range of doses of the potent antiviral cytokine interferon beta (IFN-β) for 24 hours. Cultures were lysed with guanidium-thiocyanate/phenol and the RNA isolated, converted to cDNA and analyzed by qPCR as previously described[7]. Using this method differential expression of many genes can be measured. Here, upregulation of *Ccl5* was quantified against the housekeeping gene *18s*. CCL5 is a chemotactic cytokine (chemokine) involved in the inflammatory response. Indeed, here, IFN-β treatment results in an upregulation of *Ccl5* mRNA in the cultures (Figure 4A).

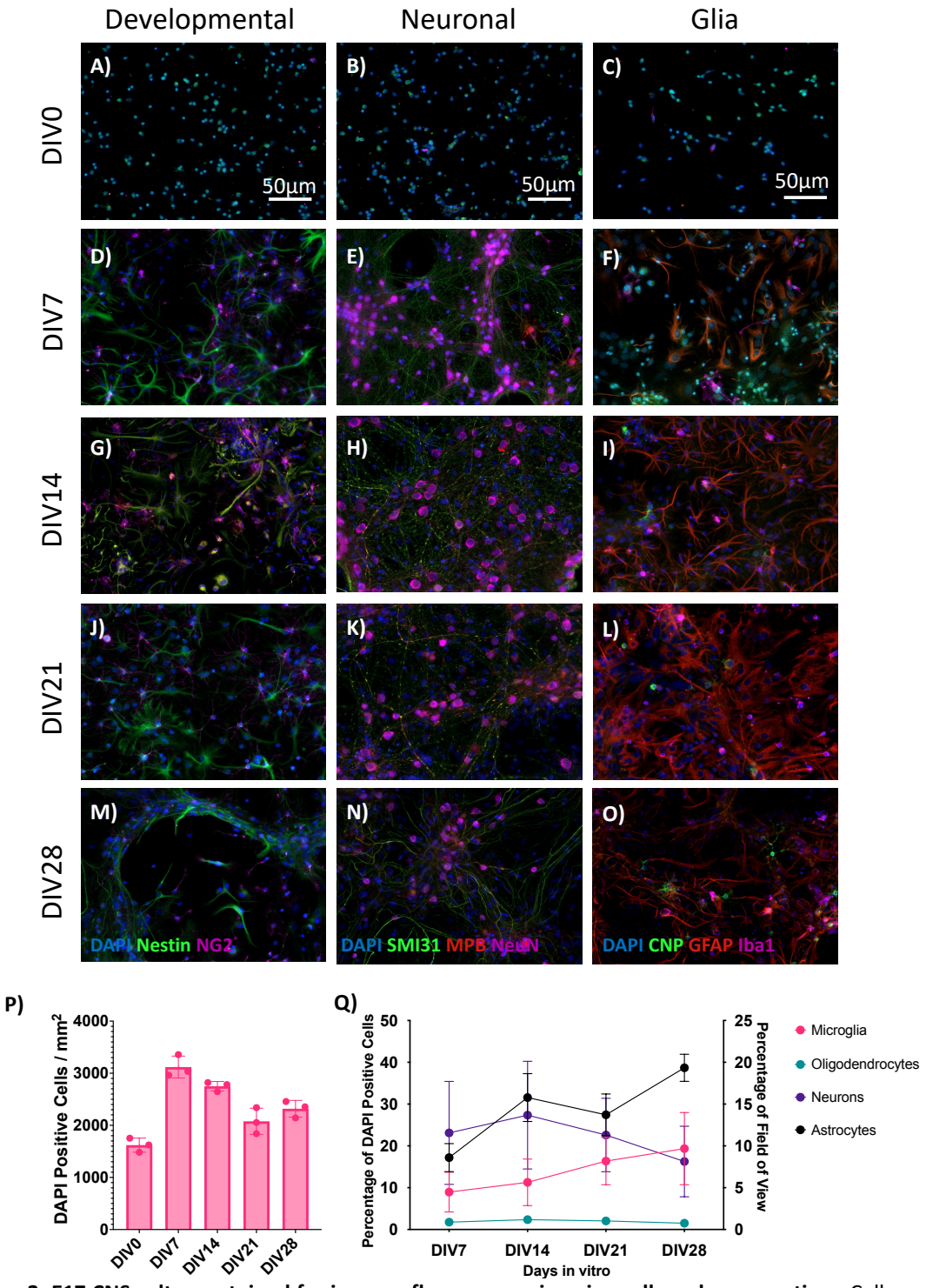

Figure 2: E17 CNS cultures stained for immunofluorescence imaging cell markers over time. Cells were fixed with 4% PFA before being permeabilized and stained to visualize different populations. Representative images of A,D,G,J,M) developmental markers NG2 and Nestin; B, E,H,K,N) neuronal markers SMI31 (axons), MBP (myelin) and NeuN (neuron cell body); and C,F,I,L,O) glial markers CNP (oligodendrocytes), GFAP (astrocytes), and Iba1 (microglia). P) Counts of DAPI per mm^2, n=3, each n from technical triplicates of 10 images per triplicate. Q) Cell counts as percentage of DAPI positive cells (microglia, oligodendrocytes and neurons) and percentage of field of view (astrocytes), n=3, each n from technical triplicates of 10 images per triplicate. Error bars are ±SEM.

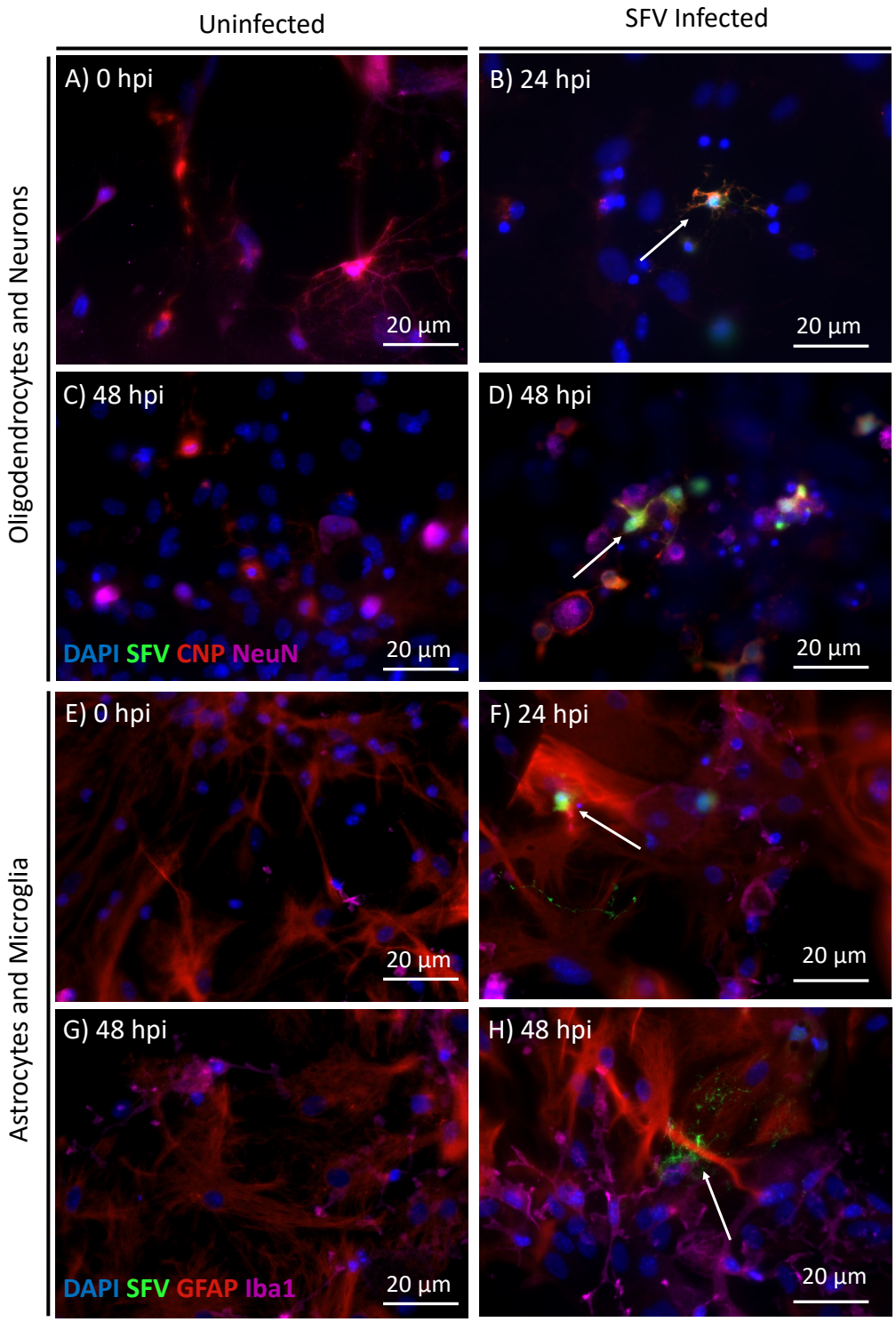

Figure 3: Semliki Forest virus (SFV) infection of E17 CNS cultures over time. Representative images of uninfected control cultures (A, D); and cultures infected with 0.03 MOI SFV (B-C, E-F). Cells were infected for 0-48 hours and stained with CNP (oligodendrocyte marker) and NeuN (neuron marker) or GFAP (astrocytes) and Iba1 (microglia). White arrows indicate an infected cell. This strain of SFV expresses the green fluorescent protein zsGreen to enable tracing of viral infection.

ELISA

The 96-well format of cultures is an excellent tool for high throughput screenings of treatments. To investigate that the upregulation of *Ccl5* mRNA results in expression of CCL5 protein we measured CCL5 in the supernatant of the cultures by ELISA. In this example, 96 well cultures were treated in duplicate with 27 incremental doses of IFN-β to generate a dose response curve (Figure 4B). In line with the increasing mRNA expression (Figure 4A), there is an increase in CCL5 released in the supernatant. As expected, Figure 4C demonstrates a clear correlation between mRNA and protein expression.

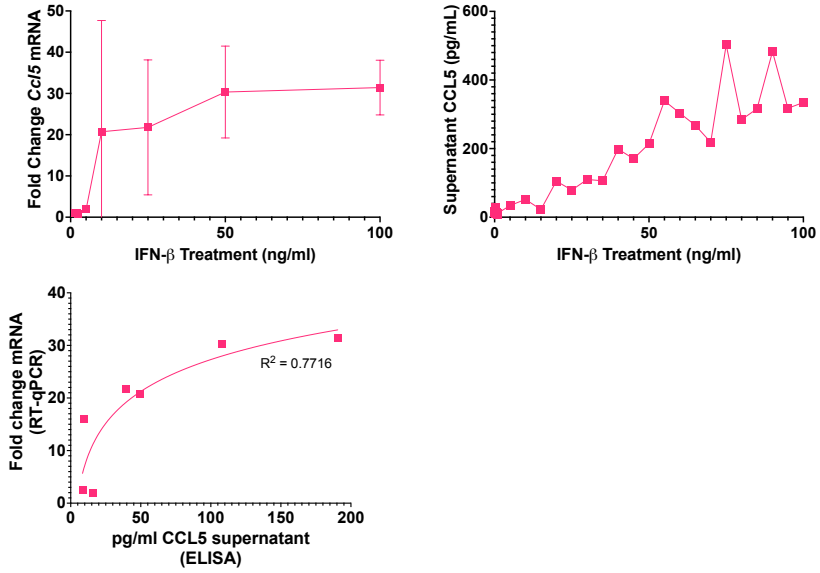

Figure 4: CCL5 mRNA and protein expression of E17 CNS cultures following IFN-β treatment. Cells were treated in the 6 well plate format (mRNA) or the 96 well plate format (protein) for 24 hours with a range of doses of IFN-β. A) Expression of Ccl5 mRNA after treatment with 0-100 ng/mL IFN-β, biological n=2, each taken from technical triplicates. B) CCL5 concentration in supernatant following 0-100 ng/mL IFNβ treatment, biological n=1, taken from technical triplicates. C) cellular mRNA Ccl5 upregulation vs CCL5 protein concentration in supernatant with log trendline. Error bars are ±SEM.

Flow Cytometry

Flow cytometry is a powerful tool to investigate expression of many intra- and extracellular markers simultaneously. However, analyzing complex highly interactive cultures by flow cytometry can be challenging due to cell damage and death when taking cells from culture to single cell suspension for processing and analysis. Comparing a variety of protocols using 0.05-0.5% Trypsin-EDTA, 1-10x trypsin without EDTA and gentle dissociation reagent, we found that treating the 6 well plate cultures with 0.05% Trypsin/EDTA for 10 minutes at 37 °C with gentle agitation we were able to lift the cells with gentle trituration to create a single cell suspension. Especially for flow cytometric analysis of CNS cells, it is critical to be gentle during cell preparation, minimize wash steps and take great care to keep cells on 4 °C or ice at all times.

To assess viability and cell types in this single cell suspension, cells were stained with a viability dye, and fluorescently labelled antibodies against CD45, CD11b, O4, ACSA-2, and CD24 to allow

visualization of each individual cell population in the cultures (Figure 5). Around 70% cell viability was obtained from this method, averaging approximately 2x10^6 viable, single cells per 6 well plate (Figure 5C). In line with the microscopy results (Figure 2N), there were large numbers of microglia, neurons and astrocytes whilst oligodendrocytes were the least abundant.

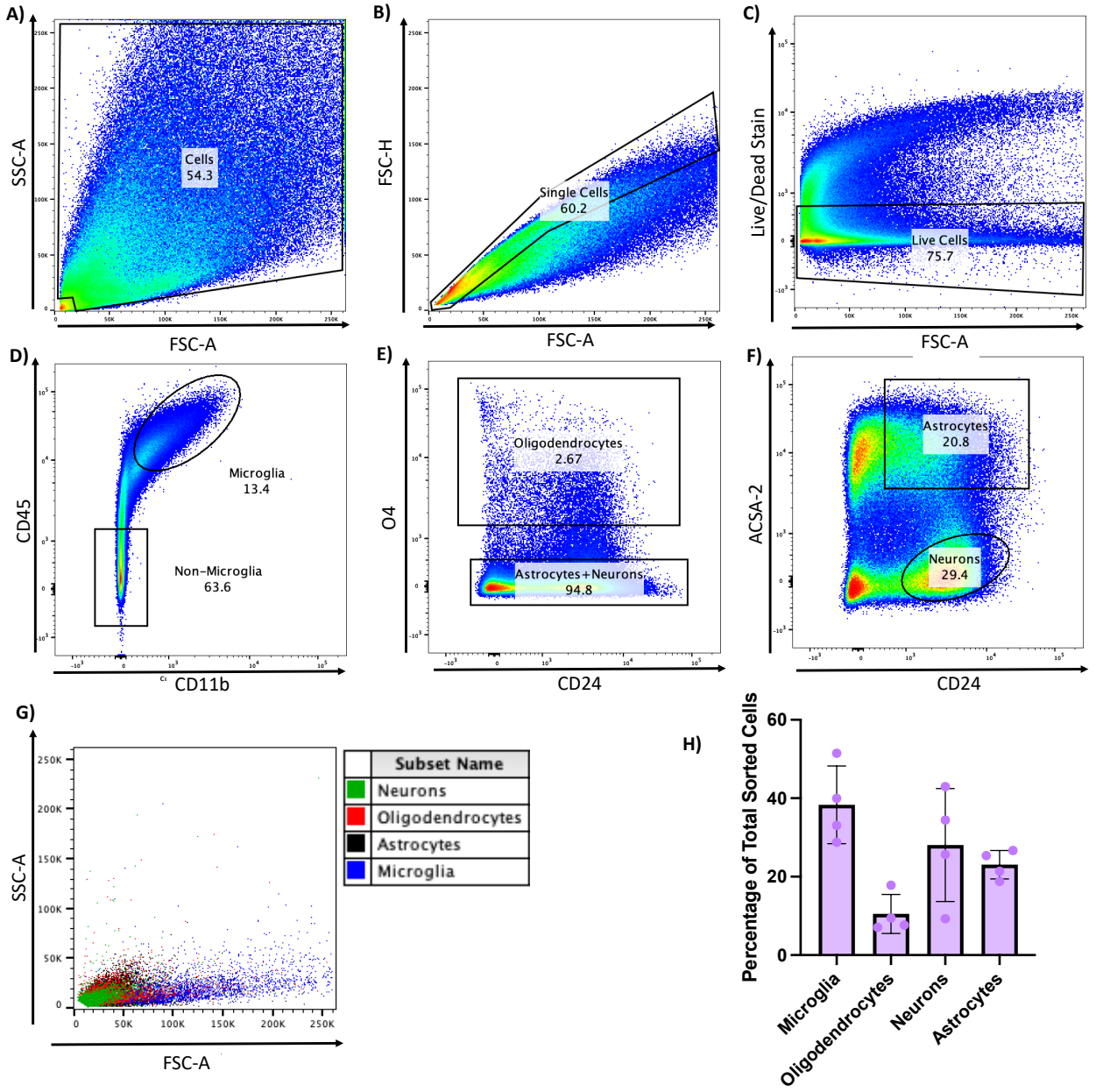

Figure 5: Flow cytometry gating strategy to generate single cell populations from E17 CNS cultures. Cells were dissociated using 0.05% trypsin-EDTA and stained with appropriate antibodies then sorted into individual cell populations. A-C) Gating strategy to sort for live, single cells. D) Strategy to select for microglia ($CD45^+CD11b^+$). E) Strategy to select for oligodendrocytes ($CD45^-$ $CD11b^-$ $O4^+$) cells. F) Strategy to sort for astrocytes ($CD45^-$ $CD11b^-$ $O4^-$ $ACSA\text{-}2^+$ $CD24^+$) and neurons ($CD45^-$ $CD11b^-$ $O4^-$ $ACSA\text{-}2^-$ $CD24^+$). G) Individual cell populations overlaid on each other showing discrete populations. H) Individual cell types as a percentage of the total cells sorted n=4. Error bars are ±SEM.

DISCUSSION:

The CNS is a complex network which spans from the brain down to the spinal cord and consists of many cell types, predominantly neurons, oligodendrocytes, astrocytes and microglia[1]. As each cell has an important role in the maintaining homeostasis and generating appropriate responses to challenges in the CNS[8–10] a culture system that contains all these cell types is a useful and versatile tool to investigate how the brain might react to a stimulus. The ability to study these cells, and their interactions, in an *in vitro* context means a great variety of techniques can be employed to investigate various experimental questions easily. We have outlined a quick and efficient method to obtain and culture the main CNS cell types from the prenatal brain which can mimic individual cell populations in the adult mouse brain[11].

Previously established protocols for generating CNS cultures use E13.5 spinal cords[4,5]. In addition to having a suitable model for the spinal cord, it is highly relevant to have a model that recapitulates the brain. Therefore, brains from these E13.5 mice were originally used to generate the CNS cultures described here using the same protocol. However, after DIV14 the cells started to form large aggregates with densely packed neuronal cell bodies. While astrocytes were present it is not clear if they were evenly distributed. It is unclear if the cells in the center of these cell bodies would receive sufficient nutrients and oxygen from the culture medium as the cells outside would. Neurons, when suffering from oxygen and nutrient deprivation, are liable to go through apoptosis and release stress signals to glial cells[12] which might lead to a proinflammatory phenotype[13]. Neurogenesis was thought to be a likely cause of these densely packed aggregates, and this phase subsides by E17[14]. When brains from E17 mouse embryos were used the cells still formed networks but did not cluster into the large aggregates seen in E13.5 and were therefore regarded as a more suitable age for the brain cultures to be generated from.

Our technique also results in a greater number of cells obtained from one pregnancy (averaging $1x10^7$ cells/brain compared to 3-$4x10^6$ cells/spinal cord)[7]. For an average pregnant mouse with 8-10 embryos, this correlate to up to 54 different experimental conditions in the 6 well plate format, or 150 experimental conditions for microscopy (compared to 19 and 64 respectively from spinal cord). As such, these CNS cultures are a great tool to reduce the required number of mice[15]. Especially the 96-well format can be easily used for high throughput assays, for instance enabling screening of drug candidates prior to testing in animals, vastly reducing the number of required animals for such studies. Hopefully, this will contribute to improving the low success rate of CNS drugs tested in animals prior to clinical trials in humans[16,17].

The cultures reach peak maturity by DIV21. Cell counts increase up until this timepoint, after which the cells start to degenerate with decreasing numbers of each type of cell. This was measured both by looking at the quantified cell numbers (Figure 2N) as well as the quantity of pyknotic nuclei (dense DAPI stains) (Figure 2M). While the developmental markers Nestin and NG2 are still expressed on DIV14 (Figure 2G) and DIV21 (Figure 2J), this is due to some of the astrocytes going through astrogliosis which upregulates nestin[18] while some of the oligodendrocytes are not fully mature and therefore still express some NG2[19]. Based on our microscopy validation, only a small portion of the cells still express developmental markers compared to the fully mature cells by DIV21 (Figure 2I, L) and therefore by this time the cultures

are largely mature. Of note, *in vivo* the density of microglia is highly variable across the murine CNS. The level of microglia as defined by Iba1 expression using microscopy is at the high end of this density in our cultures[20]. The high percentage of microglia that survive the flow cytometry procedure is likely due to microglia being more robust than other CNS cells, which generally have delicate extensions and are therefore more likely to be damaged during flow cytometry preparation.

It only takes 3 weeks following dissection until the cells are ready for experimentation which is shorter than most human-based *in vitro* methods such as organoids or iPSC derived models[21]. As long as *in vivo* research is required to ensure the safety of new therapeutics, using *in vitro* cell culturing methods such as ours will aid testing toxicity and efficacy efficiently before testing in animals, reducing the requirement for animals and enhance the translation of research from *in vitro* to *in vivo.* Hopefully this will eventually lead to more efficient translation to human based clinical trials as well. Ultimately these cultures were created to allow the study of the CNS. While *in vitro* systems cannot fully express the complexity of the CNS, primary cell derived cultures more accurately represent *in vivo* CNS properties[22,23]. *In vitro* approaches can allow for a more reductive approach to investigate the CNS in the absence of infiltrating immune cells[24] and the blood brain barrier integrity[25]. Removing this layer of complexity can make it easier to unravel mechanisms. As such, our culturing system is a useful tool to answer research questions that complements *in vivo* animal research.

The ability to harvest, isolate, and culture CNS cells has already resulted in great advances in our understanding of the innate CNS[15]. This protocol and video demonstrate the dissection of E17 mouse brains and the resulting trituration and culturing of the cells to generate a cell culture system containing all the main cell types of the CNS. Multiple molecular techniques have been employed on these cultures showcasing the effectiveness these cultures have for investigating the CNS.

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DISCLOSURES:
Authors have nothing to disclose.

MATERIALS:

Name of Material/ Equipment	Company	Catalogue Number	Comments/Description
Boric Acid	Sigma	B6768-500G	For boric acid buffer
Sodium Tetraborate	Sigma	221732-100G	For boric acid buffer
Poly-L-Lysinehydrobromide	Sigma	P1274	For Boric acid / poly-L-lysine solution to coat coverslips
HBSS w/o Ca Mg	Sigma	H9394-500ML	For brains to be added to
10x Trypsin	Sigma	T4549-100ML	To digest tissue
Leibovitz L-15	GIbco	11415-049	For SD Inhibitor
Trypsin inhibitor from soybean	Sigma	T9003-100MG	For SD Inhibitor
DNase I	Thermofisher	18047019	For SD Inhibitor, can use this or the other Dnase from sigma
DNase I	Sigma	D4263	For SD Inhibitor, can use this or the other Dnase from thermofisher
BSA Fraction V	Sigma	A3059-10G	For SD Inhibitor
DMEM High glucose, sodium pyruvate, L-Glutamine	Gibco	21969-035	For DM+/-, and for plating media
Pen/Strep	Sigma	P0781-100ML	For DM+/-, and for plating media
Horse Serum	Gibco	26050-070	For plating media
HBSS w Ca Mg	Sigma	H9269-500ML	For plating media
Hydrocortisone	Sigma	H0396	For DM+/-
Biotin	Sigma	B4501	For DM+/-
N1 media supplement	Sigma	N6530-5ML	For DM+/-
Insulin	Sigma	I1882	For DM+
Coverslip	VWR	631-0149	To plate out cells for microscopy
35mm TC Dish	Corning	430165	Plate out 3 PLL coated coverslips per 1 35mm dish
140mm TC Dish	Fisher	11339283	Put 8 35mm dishes per 1 140mm dish
Angled forceps	Dumont	0108-5/45-PO	For dissection
Dissection Scissors	Sigma	S3146-1EA	For dissection
Fine forceps	Dumont	0102-SS135-PO	For dissection
7mL Bijoux	Fisher	DIS080010R	To put brains intp
5mL syringe	Fisher	15869152	For trituration of sample
18G needle	Henke Sass Wolf	4710012040	For trituration of sample
21G needle	BD	304432	For trituration of sample
23G needle	Henke Sass Wolf	4710006030	For trituration of sample

15mL Falcon	Sarstedt	62554502	To collect cells into pellet for resuspension in plating media
6 well plate	Corning	3516	To plate out cells for RT-qPCR, and flow cytometry
96 well plate	Corning	3596	To plate out cells for high-throughput testing
NG2	Sigma	AB5320	Immature oligodendrocytes
Nestin	Merck	MAB353	Neuronal stem/progenitor cells
SMI31	BioLegend	801601	Axons
MBP	Bio-Rad	MCA409S	Myelin
NeuN	Thermofisher	PA578499	Neuronal cell body
CNP	Abcam	AB6319	Mature oligodendrocytes
GFAP	Invitrogen	13-0300	Astrocytes
Iba1	Alpha-Laboratories	019-1971	Microglia
eBioscience Fixable Viability Dye eFluor 780	Thermofisher	65-0865-14	Live / Dead stain
Trizol	Thermofisher	15596026	For lysing cells for RT-qPCR

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