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METHODS PAPER

Primary neuron and astrocyte cultures from postnatal *Callithrix jacchus*: a non-human primate in vitro model for research in neuroscience, nervous system aging, and neurological diseases of aging

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Abstract The ability to generate in vitro cultures of neuronal cells has been instrumental in advancing our understanding of the nervous system. Rodent models have been the principal source of brain cells used in primary cultures for over a century, providing insights that are widely applicable to human diseases. However, therapeutic agents that showed benefit in rodent models, particularly those pertaining to aging and age-associated dementias, have frequently failed in clinical trials. This discrepancy established a potential "translational gap" between human and rodent studies that may at least partially be explained by the phylogenetic distance between rodent and primate species. Several non-human primate (NHP) species, including the common marmoset (*Callithrix jacchus*), have been used extensively in neuroscience research, but in contrast to rodent models, practical approaches to the generation of primary cell culture systems amenable to molecular studies that can inform in vivo studies are lacking. Marmosets are a powerful model in biomedical research and particularly in studies of aging and age-associated diseases because they exhibit an aging phenotype similar to humans. Here, we report a practical method to culture primary marmoset neurons and astrocytes from brains of medically euthanized postnatal day 0 (P0) marmoset newborns that yield highly pure primary neuron and astrocyte cultures. Primary marmoset neuron and astrocyte cultures can be generated reliably to provide a powerful NHP in vitro model in neuroscience research that may enable mechanistic studies of nervous system aging and of age-related neurodegenerative disorders. Because neuron and astrocyte cultures can be used in combination with in vivo approaches in marmosets, primary marmoset neuron and astrocyte cultures may help bridge the current translational gap between basic and

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clinical studies in nervous system aging and age-associated neurological diseases.

Keywords *Callithrix jacchus* · Neuroscience · Nervous system aging · Neurological diseases

Introduction

Physiologically relevant animal models that recapitulate mechanisms and manifestations of human disease must be used to define the core etiology of these diseases. It has become increasingly apparent in the last decade that the translatability of studies focused on aging and age-associated neurological diseases performed in traditional animal models such as rodents and invertebrate species is challenging [1, 2]. This is likely due at least in part to large phylogenetic differences between invertebrates or rodents and humans. As potential alternative models, non-human primates (NHP) are physiologically and functionally more similar to humans than rodents or invertebrates, which may have advantages for preclinical translation [3, 4]. The common marmoset (*Callithrix jacchus*) is a small (350–450 g) New World monkey that lives in tight-knit social groups and has a relatively short lifespan (maximum lifespan of approximately 22 years) as compared to many other NHP. These characteristics increase practicality of biomedical research using marmosets [4, 5], particularly for the study of aging and age-associated diseases. Marmosets undergo age-related cognitive decline [6–10], and have been used extensively in major research areas of neuroscience research, including developmental neurobiology [11, 12], aging [5, 13], and age-associated neurological disease [7, 10, 14, 15], providing a strong foundation for studies of aging of the nervous system. However, and in contrast to similar studies in rat and mouse models [16–19], in vivo functional studies in marmoset monkeys cannot be informed by conspecific in vitro studies because practical approaches to the isolation and culture of primary neurons and astrocytes from marmosets have not yet been reported [20, 21].

Devising a practical approach to generate a physiologically and translationally relevant in vitro primate neuronal model is of significant value and high potential impact for the neuroscience field. While induced pluripotent stem cells (iPSCs) generated from human somatic cells can be differentiated into neurons and glial cells to study the pathobiology of diseases and screen for therapeutic targets, the use of these cells introduces their own unique challenges and limitations [22]. iPSC cultures are cost-intensive, and the generation of functional neurons can take months [20, 23]. Furthermore, during reprogramming, the donor cell type can lead to preferential lineage-based differentiation to certain cell types that may acquire unknown epigenetic alterations [24–28]. In some instances, this heterogeneity can limit the ability of iPSC-derived neuronal cultures to recapitulate the physiology of primary cells [29, 30]. The vast majority of in vitro electrophysiological studies of neuron function have been performed in primary cultures of embryonic rat or mouse neurons [31, 32]. The availability of primary neurons from an NHP species is expected to enable physiological and molecular studies that may reveal important differences in neuronal physiology and function between rodents and primates, yielding data with high translational potential [4].

Development of primary neuronal cell cultures has generally required embryonic brain collection, which in NHP models is complicated by practical and ethical concerns. Marmoset mothers generally have twin births, with triplets and quadruplets also occurring at times [33]. However, it is rare for a marmoset mother to successfully rear more than two offspring at a time and additional offspring not accepted by the mother must either be hand-reared or fostered, or if these options are not available, humanely euthanized. Infants with serious health problems at birth that make their survival unlikely are also slated for humane euthanasia. These euthanized animals represent a potential source of opportunistic tissue sampling from animals during early development. We report here the isolation and culture of highly pure primary neurons and astrocytes from brain tissues of postnatal day 0 (P0) infants. To our knowledge, this is the first description of an approach to the generation of primary neurons from marmosets that does not require cesarean section of pregnant females and sacrifice of viable marmoset embryos [20]. The method described here should be widely available to researchers at institutions where breeding marmoset colonies are maintained. The substantially higher yield of viable primary cells from a P0 marmoset brain allows for numerous experiments to be performed. Thus, primary marmoset neurons and astrocytes from marmosets may provide a powerful in vitro model system with high translational potential for neuroscience research and for research focused on central nervous system aging and age-associated neurodegenerations.

Materials and methods

Marmoset husbandry

Marmoset parents used in this study were housed within a colony at the Barshop Institute for Longevity and Aging Studies at the University of Texas Health Science Center at San Antonio (UTHSCSA). The UTHSCSA Institutional Animal Care and Use Committee (IACUC) regularly monitored marmoset housing and animal conditions to ensure all guidelines for the health and safety of the animals were met. This research was reviewed and approved by the UTHSCSA IACUC and experiments were conducted in compliance with the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals and adhered to the American Society of Primatologists (ASP) principles for the ethical treatment of non-human primates. Animals were housed in male/female pairs (or as part of existing family units including older offspring) and all pregnancies were opportunistic and not part of breeding plan. At birth, offspring were assessed for health by laboratory and veterinary staff and animals deemed for medical euthanasia as likely low chance of postnatal survival. Offspring deemed unlikely to survive were immediately euthanized by overdose of isoflurane as administered by UTHSCSA veterinary staff.

Isolation and culture of primary marmoset neurons

Brains were immediately collected from euthanized P0 marmosets. The brain was dissected and the meninges and large surface vessels were removed. The cerebral cortex (including the hippocampus) was isolated. Tissue was rinsed in cold Hank's buffered saline solution (HBSS, Sigma-Aldrich, St. Louis, MO, USA) and then cut into small pieces. The tissue was pelleted and resuspended in 0.25% trypsin-EDTA (Gibco, Pittsburgh, PA, USA) with 1 U/mL DNase I (Thermo Fisher, DN0521, Waltham, MA, USA) and incubated for 10 min at 37 °C. An equal volume of Neurobasal media with 20% Cosmic Calf serum (CCS, HyClone, Logan, UT, USA) was added and the tissue was mechanically disrupted by passing through a serological pipette. The tissue was pelleted and resuspended in Neurobasal media (Gibco, Pittsburgh, PA, USA) containing 2% CCS (HyClone, Logan, UT, USA), 1 mM GlutaMAX (Gibco, Pittsburgh, PA, USA), 2% B-27 supplement (Gibco, Pittsburgh, PA, USA), and 1% penicillin and streptomycin (Gibco, Pittsburgh, PA, USA). The cell suspension was further disrupted by passing through a serological pipette and then filtered through a 70-μm cell strainer. The filtered cell suspension was then plated on 50 μg/mL poly-D-lysine-coated plates (P7886, Sigma-Aldrich, St. Louis, MO, USA). Primary marmoset neurons were plated at 450,000 cells per well in 24-well plates, 2.5 million cells per well in 6-well plates, or 25,000 cells per well in 96-well plates. Cultures were incubated in a 5% CO_2 incubator at 37 °C for 45 min. The medium was then replaced with Neurobasal media (Gibco, Pittsburgh, PA, USA) containing 2% B-27 supplement (Gibco, Pittsburgh, PA, USA), 1 mM GlutaMAX (Gibco, Pittsburgh, PA, USA), and 1% penicillin and streptomycin (Gibco, Pittsburgh, PA, USA). Fifty percent of the culture media was then replaced with fresh media every 2 days for approximately 3 weeks until neurons reached maturity.

Characterization of primary marmoset neuronal cultures

Immunocytochemistry was performed to characterize cultured primary marmoset neurons after they reached maturity at 21 days in vitro (DIV). Cells were fixed in 4% v/v paraformaldehyde, rinsed with HBSS, and permeabilized with 0.1% Triton X-100. Cells were blocked with 5% bovine serum albumin in TBS with 0.1% Tween20 at room temperature for 1 h and then incubated in primary antibody overnight at 4 °C. Primary antibodies specific for neuronal markers as well as for markers for other major brain cell types were used (e.g., astrocytes, vascular endothelial cells, oligodendrocytes; Table 1). Cultures were then probed with appropriate secondary antibodies conjugated to Alexa Fluor 488 or Alexa Fluor 594 and counterstained with DAPI to identify the nucleus. Images were obtained with a Zeiss LSM 710 confocal microscope. Brightfield images were obtained with a ZOE Cell Imager (Bio-Rad, Hercules, CA, USA).

Isolation and culture of primary marmoset astrocytes

The protocol for isolating primary marmoset astrocytes was based on methods previously described by Lin et al. (2007) and Chen et al. (2016) [34, 35]. Brains were immediately collected from euthanized P0

Table 1 Primary antibodies specific for neuronal markers as well as for markers for other major brain cell types

Cell marker	Antibody	Company	Cat #
Neurons	NeuN	Millipore	MAB377
Neurons	Synaptophysin	Abcam	ab32127
Neurons	Neurofilament	Abcam	ab24575
Neurons	MAP 2	Abcam	ab32454
Microglia	Iba1	Wako	019-19741
Endothelial cells	CD31	Abcam	ab28364
Astrocytes	Aquaporin-4	Millipore	AB2218
Astrocytes	GFAP	Millipore	MAB360
Oligodendrocytes	Myelin basic protein (MBP)	Novus	NB110-79873

marmosets. The brain was removed and cut into small pieces in 0.25% trypsin-EDTA (Gibco, Fisher Scientific, Pittsburgh, PA, USA) and incubated for 25 min at 37 °C. An equal volume of media DMEM-F12 (ATCC, Manassas, VA, USA) with 10% Cosmic Calf serum (HyClone, Logan, UT, USA) was added to the homogenate. The tissue was passed through a 10-mL serological pipette 10–15 times and then additional media were added. The tissue was pelleted and then resuspended in a small amount of media. The tissue suspension was again passed through a 10-mL serological pipette 10–15 times. The tissue was further dissociated by passing it through a glass Pasteur pipette. The cell suspension was then filtered through a 40-μm cell strainer. The filtered cell suspension was plated in a T75 flask with 12 mL of media and incubated in a 37 °C incubator with 5% CO_2. The media were changed on the second day and the cells were grown to confluency (1–2 weeks) changing the media every 1–2 days. Cells were then passaged twice (using 0.25% trypsin-EDTA) to remove any contamination of other cell types to obtain a purified culture. Astrocytes were frozen down in complete astrocyte media plus 20% Cosmic Calf serum and 5% DMSO in a controlled rate cell freezing container in − 80 °C and then transferred to liquid nitrogen storage to cryopreserve the astrocytes. Astrocytes can be successfully cultured after cryopreservation using standard methods.

Characterization of primary marmoset astrocyte cultures

Marmoset astrocytes were plated in a 96-well plate and grown to confluency. Immunocytochemistry was performed to characterize the isolated primary marmoset astrocyte cultures. Briefly, cells were washed with HBSS and fixed in 4% v/v paraformaldehyde for 20 min, rinsed with HBSS, and permeabilized with 0.1% Triton X-100 for 10 min. Cells were incubated in 10% goat serum for 1 h at room temperature and then incubated in primary antibody overnight at 4 °C. Primary antibodies specific for astrocytes as well as for other cell types in the brain were used (e.g., neurons, endothelial cells, oligodendrocytes). The antibodies used to characterize marmoset astrocyte cultures are shown in Table 2. Cultures were then probed with appropriate secondary antibodies conjugated to Alexa Fluor 488 and counterstained with DAPI to identify the nucleus. Images were obtained with a Nikon Eclipse Ti inverted microscope.

Quantitative analyses of protein expression by immunoassay

To validate immunoreactivity of antibodies used to characterize our cultures for reactivity with marmoset brain tissue, we used brain tissue from a young (3-year-old) marmoset lysed in RIPA buffer (sc-24948, Santa Cruz Biotechnologies, Dallas, TX, USA) and sonicated briefly and centrifuged at 12,000×*g* to remove cellular debris. These protein lysates were then used in capillary electrophoresis immunoassays (Protein Simple Wes system [36, 37]). To determine whether other non-target cell types were isolated with the astrocytes, primary antibodies specific for Iba-1, PSD-95, myelin basic protein (MBP), and CD31 were used (see Tables 1 and 2). Data was analyzed using Compass in SW software (ProteinSimple, San Jose, CA, USA).

Table 2 Antibodies used to characterize marmoset astrocyte cultures

Cell marker	Antibody	Company	Cat #
Astrocytes	Aldolase C	EnCore	MCA-4A9
Astrocytes	Aquaporin-4	Millipore	AB2218
Astrocytes	EAAT-2	Santa Cruz	sc-365634
Astrocytes	GFAP	Millipore	MAB360
Endothelial cells	CD31	Abcam	ab28364
Neurons	PSD-95	Millipore	MABN1190
Oligodendrocytes	Myelin basic protein (MBP)	CST	78896

Results

Isolation and characterization of primary marmoset neuron cultures

Viable primary neurons from P0 marmoset infants were successfully isolated and cultured. Each P0 infant brain yielded an average of 3×10^7 cells, sufficient to plate among twelve 6-well plates. We used various other culture vessel formats in our studies with comparable success. For all of these culture formats, well-to-well reproducibility was high, with uniformly comparable densities for neurons in all wells and across plates. At day 1 in vitro (DIV1), cultures contained some remaining cellular debris but by DIV4 most debris had been eliminated by medium changes (Fig. 1a). By DIV7, primary marmoset neurons in culture showed increased neurite growth with increased length and branching. At DIV19, neurons had increased soma area and significant outgrowth of neurites forming highly interconnected networks (Fig. 1a). In contrast to cultures obtained from P0 infants, maturation of neuron cultures obtained from P1 and P2 infants stalled after 1 week and cells lost viability after 10 days (data not shown). Interestingly, primary cultures from a P0 infant born 2 weeks after its due date could not be established. DIV21 neuron cultures from P0 marmoset brains were immunoreactive for neuron-specific markers including nuclear antigen Fox-3/Rbfox3/hexaribonucleotide binding protein-3 (NeuN), synaptophysin (SYP), neurofilament (Neurofil), and microtubule-associated protein 2 (MAP 2) (Fig. 1b). Remarkably, cultured primary marmoset neurons were supported by a monolayer of aquaporin-4 (AQP4) and glial fibrillary acidic protein (GFAP)–positive astrocytes that developed concomitantly with neuronal maturation and became quiescent when they reached confluency (Fig. 1c). Astrocytes that become quiescent and remain in neuron cultures have also been reported in human neuron isolation procedures [38].

Neurons and astrocytes were evenly distributed in the cultures and the ratio of neurons to astrocytes remained constant throughout neuron maturation. Microglia, endothelial cells, and oligodendrocytes were largely absent from neuron cultures as we were unable to detect immunoreactivity for ionized calcium-binding adaptor molecule 1 (IBA1, microglia), cluster of differentiation 31 (CD31, endothelial cells), and myelin basic protein (MBP, oligodendrocytes) in these cultures (Fig. 1d). Lack of immunoreactivity against these markers in neuron cultures did not arise from a lack of reactivity of these antibodies against marmoset proteins, as antibodies used to detect expression of IBA1, CD31, and MBP in cultures showed reactivity in marmoset brain tissue (Supplemental Figure 1A). Incubation with secondary antibodies did not result in immunoreactive signals (Fig. 1e). Of note, neuron-astrocyte mixed cultures remained viable for up to 35 days. Taken together, these data indicate that cultures of neurons and quiescent astrocytes with remarkable long-term viability can be established from P0 postnatal marmoset brain using the methods described.

Isolation and characterization of primary marmoset astrocyte cultures

Viable primary astrocytes were isolated and cultured from P0 marmoset infant brains (Fig. 2a). Each P0 infant brain yielded a sufficient number of cells to plate two 75-mL flasks. We used all these culture vessel formats in our studies with comparable success. All culture formats yielded well-to-well reproducibility,

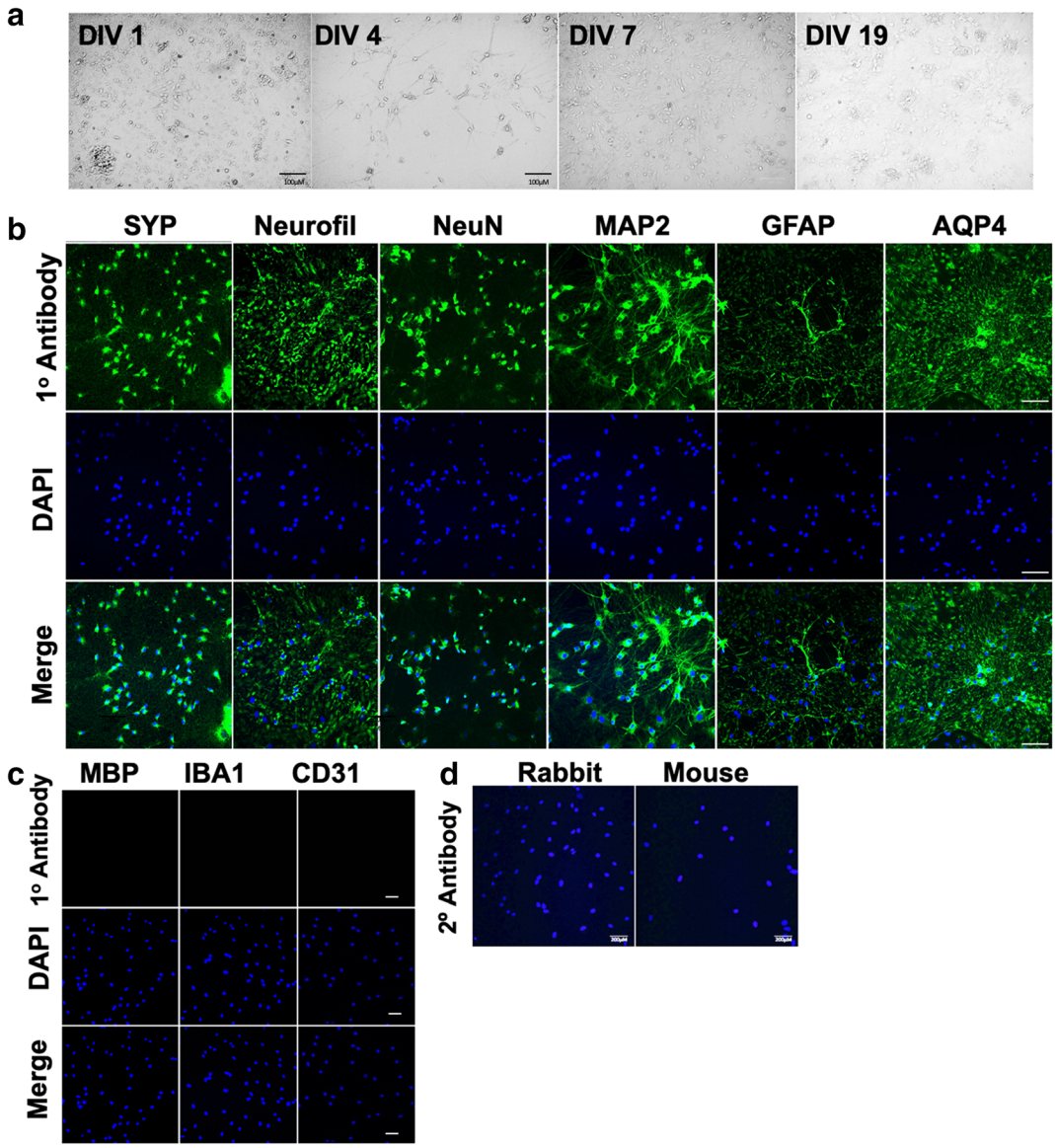

Fig. 1 Characterization of neuron cultures obtained from postnatal day 0 marmoset brain. **a** Representative wide-field images of primary neurons isolated from P0 marmoset brains cultured to maturity from day 1 in vitro (DIV1) to DIV19. **b** Representative images of immunocytochemistry studies in neuron cultures. Neuron cultures show immunoreactivity for the neuron-specific markers synaptophysin (SYP), neurofilament (Neurofil), neuronal nuclear protein (NeuN), and microtubule-associated protein (MAP 2). **c** Cells with astrocyte-like morphology in neuron cultures show immunoreactivity for astrocyte markers glial fibrillary acidic protein (GFAP) and aquaporin-4 (AQP4). Astrocytes in neuron cultures stop proliferating after reaching confluency. **d** Neuronal cultures do not contain microglia, endothelial cells, and oligodendrocytes as indicated by absence of immunoreactivity for ionized calcium-binding adaptor molecule 1 (IBA1), cluster of differentiation 31 (CD31), and myelin basic protein (MBP), respectively. **e** Secondary antibodies alone do not contribute to fluorescent signals in our studies. Scale bars represent 200 μm

with uniformly comparable densities for astrocytes. Astrocytes attached to the culture substrate at DIV4 underwent morphological changes typical of human astrocytes during early passaging [39], acquiring a spindle-like fusiform morphology. By DIV7, marmoset astrocytes in culture acquired a flat, polygonal formation with complex projections associated with a mature state in human or mouse astrocytes [40, 41]. Cultures were strongly immunoreactive for astrocyte markers including GFAP, aquaporin-4, aldolase

C, and excitatory amino acid transporter 2 (EAAT2, also known as GLAST) (Fig. 2b) but did not show detectable immunoreactivity for markers for other brain cell types such as CD31 (endothelial cells), post-synaptic density protein 95 (PSD-95, neurons), and MBP (oligodendrocytes) (Fig. 2c). The antibodies used for CD31, PSD-95, and MBP were reactive to marmoset brain tissue (Supplemental Figure 1B). Thus, a lack of immunoreactivity against CD31, PSD-95, and MBP in astrocyte cultures did not arise from a lack of reactivity of these antibodies against marmoset proteins. No immunoreactive signal was observed when primary marmoset astrocyte cultures were stained with secondary antibodies alone (Fig. 2d). Together, these data show that viable cultures of pure astrocytes can be established from P0 postnatal marmoset brains using the methods described. Furthermore, cultured marmoset astrocytes can be frozen in media and 5% DMSO for long-term storage. We have found efficient growth of thawed cell aliquots without significant loss in viability until passage eight (data not shown). Thus, primary marmoset astrocytes provide an advantageous and more translationally relevant alternative to rodent astrocyte cultures for in vitro studies.

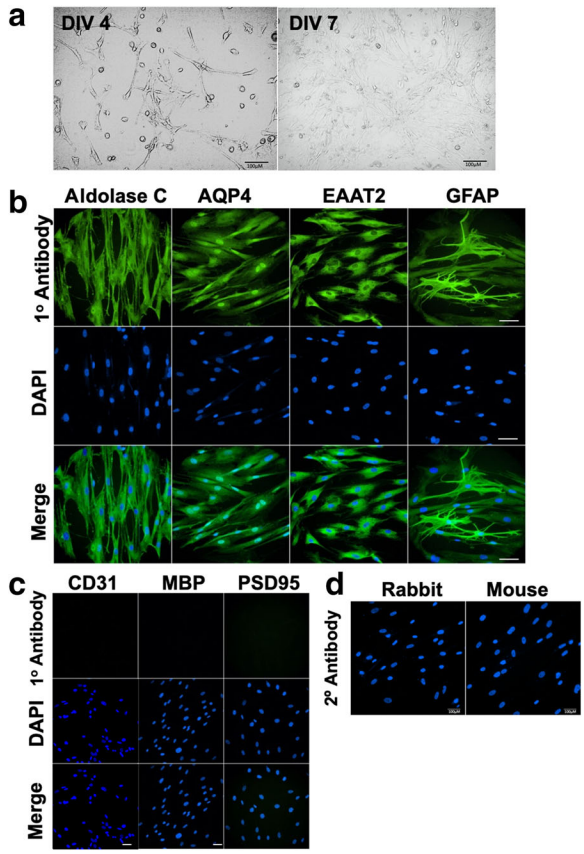

Fig. 2 Characterization of astrocyte cultures obtained from postnatal day 0 marmoset brain. **a** Representative wide-field images of primary marmoset astrocytes grown to confluency from day 4 in vitro (DIV4) to DIV7. **b** Representative images of immunocytochemistry studies in astrocyte cultures. Astrocyte cultures show strong immunoreactivity for astrocyte-specific markers Aldolase C, aquaporin-4 (AQP4), excitatory amino acid transporter 2 (EAAT2), and glial fibrillary acidic protein (GFAP). **c** Astrocyte cultures do not contain endothelial cells, oligodendrocytes, and neurons as indicated by absence of immunoreactive signals from cluster of differentiation 31 (CD31), myelin basic protein (MBP), and post-synaptic density protein 95 (PSD95) respectively. **d** Secondary antibodies alone do not contribute to fluorescent signals in our studies. Scale bars represent 100 μm

Discussion

We report a practical method for the generation of primary cultures of neurons and astrocytes from an NHP species, the common marmoset. Generation of neurospheres capable of differentiation into neurons, oligodendrocytes, and astrocytes [42] and cultures of pure astrocytes from dissociated V1 [43] marmoset tissue at postnatal day 14 have been reported. To our knowledge, however, this is the first time that pure, two-dimensional cultures of neurons and astrocytes have been successfully established from a postnatal NHP. The method for primary culture of marmoset neurons and astrocytes we describe broadens the scope of neuroscience research to include an in vitro model for neurons and astrocytes from an NHP species that can be used in preclinical studies with high translational value. A prior report by Tokuno et al. used cesarean section to harvest marmoset embryos [20].

In addition to their phylogenetic proximity with human cells, an important advantage of primary cultures of neurons from marmosets as compared to those generated from rodents is in their stability and the high reproducibility of the cultures. Fetal neuron-astrocyte cultures from mice cannot be practically established because the cultures are overwhelmed by continuing astrocyte proliferation, requiring treatment with cytosine arabinoside (cytarabine, ARA-C) to block this process [44]. ARA-C, however, reduces viability of primary neurons in culture and may have other impact on neuron function, particularly in long-term cultures. Marmoset astrocytes in postnatal marmoset primary neuron cultures, in contrast, arrest proliferation as they reach confluency. This eliminates the requirement for

treatment with ARA-C or other cytostatic compounds. Furthermore, postnatal marmoset primary neuron-astrocyte cocultures remain viable and stable for at least 35 days.

Pathological dysfunction of astrocytes plays a critical role in the development of various neurological diseases, such as age-related tau astrogliopathy (ARTAG) [45], multiple sclerosis [46], and Alzheimer's disease (AD) [47, 48]. Astrocytes in human and non-human primate brains are more abundant and include interlaminar astrocytes; they also have more complex structures, with a greater abundance of astrocyte processes, and display faster propagation of calcium signaling than rodent astrocytes [49, 50]. These and other important differences make primary astrocyte cultures obtained from P0 marmosets a powerful tool for translational research. Importantly, primary P0–derived marmoset astrocytes can be cryopreserved for future use.

Limitations of our method are the relative scarcity of breeding colonies of marmosets, and the requirement for substantial flexibility in the logistics for collection and processing of P0 marmoset brains. It is hard to predict exactly when a birth will occur, and whether non-viable offspring will be present. Most deliveries are in the early morning hours. In all studies, we collected tissue within 12 h of birth. Of note, primary neuronal cultures established from tissue collected after 24-h post parturition (P1) were non-viable. We also observed high variability in the quality and viability of cultures obtained from pregnancies which were overdue. Postnatal tissue collection, however, reduces potential health complications for the mother associated with the approach reported by Tokuno et al. [20] where cesarean section was used to harvest embryos.

Marmosets are increasingly used for aging and biomedical research [51–53]. This small, non-human primate is a practical model for neurodegenerative diseases such as Parkinson's [54] and potentially AD [55–57]. As the use of marmosets in neuroscience (including studies focused on brain aging and on age-associated neurological diseases) expands, tools such as primary postnatal marmoset neuron and astrocyte cultures will be critical to complement in vivo studies. Neuronal cultures obtained from post-natal marmosets can be made widely available for electrophysiology and transwell-based co-cultures, as well as 3D and organoid culture systems that can be used in powerful translational research focused on aging and neurodegenerative diseases. Because P0 marmoset primary neuron and astrocyte preparations have very high yields, they may be suitable for the performance of high-throughput screens. This could potentially enable highly translatable drug discovery efforts targeting brain aging and age-related neurological diseases. This expectation is also based on important intrinsic advantages of the postnatal marmoset cultures that include, but are not limited to, their well-to-well reproducibility and intrinsic stability, the latter owing to the capacity of postnatal marmoset astrocytes to become quiescent in culture conditions, and to the very high cell yields that can be reproducibly obtained from each P0 brain as mentioned above. Thus, we expect that primary postnatal marmoset neurons and astrocytes will provide a powerful novel tool that will help advance studies with exceptional translational potential, widely applicable to neuroscience research, including prominent studies in nervous system aging and age-associated neurological diseases.

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Compliance with ethical standards The UTHSCSA Institutional Animal Care and Use Committee (IACUC) regularly monitored marmoset housing and animal conditions to ensure all guidelines for the health and safety of the animals were met. This research was reviewed and approved by the UTHSCSA IACUC and experiments were conducted in compliance with the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals and adhered to the American Society of Primatologists (ASP) principles for the ethical treatment of non-human primates.

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