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Characterization of dorsal root ganglia of golden Syrian hamsters (*Mesocricetus auratus*) in a physiological and potential pathological state

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Introduction

Dorsal root ganglia (DRG) harbor neurons transmitting somatosensory signals from the periphery to the central nervous system (CNS). Satellite glial cells (SGCs) tightly envelope these neurons and thereby regulate and maintain a stable environment [1, see illustration]. As a response to injurious processes, SGCs display signs of activation and dedifferentiation and influence neuronal excitability and sensory signaling [2, 3]. Recently, sensory abnormalities with detectable molecular changes in thoracic DRG have been reported in SARS-CoV-2 infected hamsters [4]. However, knowledge about the cellular composition, protein expression profile and potential changes under pathological conditions of DRG, especially SGCs, in hamsters is still scarce and should be investigated in more detail.

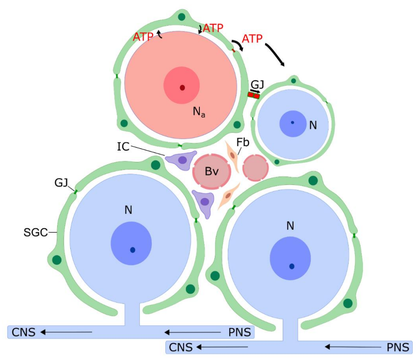


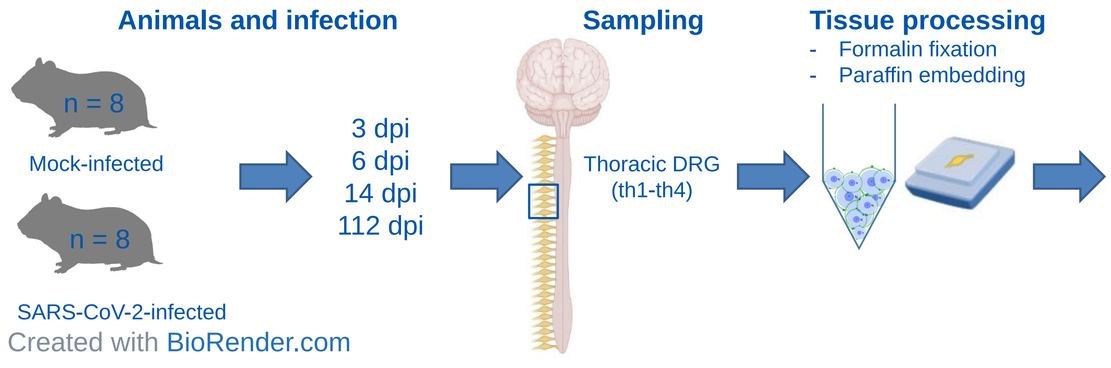
Illustration: I.Zdora, (2023): *Phenotypic characteristics of satellite glial cells of sensory ganglia with special reference to species-specific variations and commonalities*, PhD thesis. University of Veterinary Medicine Hannover and modified according to: Hanani, M. & Spray, D. C. Emerging importance of satellite glia in nervous system function and dysfunction. Nature Reviews Neuroscience 21, 485-498, doi:10.1038/s41583-020-0333-z (2020). Abbreviations: CNS=central nervous system, PNS=peripheral nervous system, SGC=satellite glial cell, N=neuron, Bv=blood vessel, IC=immune cell, Fb=fibroblast, GJ=gap junction, N_a=activated neuron, ATP=adenosinetriphosphate.

Hypothesis & Aims

Hypothesis: DRG of hamsters are similarly composed as described for other animal species. Furthermore, DRG represent a plastic functional unit and specifically SGCs adapt their expression profile in response to peripheral injurious processes such as pulmonary infection with SARS-CoV-2.

Aims: A thorough histomorphological (Fig. 1) and phenotypical (Tab. 1; Fig. 2) characterization of thoracic DRG of mock-infected as well as SARS-CoV-2-infected hamsters during the acute (3 and 6 days post infection (dpi)), subacute (14 dpi) and chronic phase (112 dpi) with respect to potential inflammatory changes (Fig. 3) and/or an altered expression profile of SGCs.

Materials & Methods



(1) Histomorphological examination

(2) Immunohistochemical examination

Features	Marker	Analysis
Viral protein	- SARS-CoV-2 nucleocapsid protein (NP)	Quantitative
SGC specific	- Glutamine synthetase (GS) - Inwardly rectifying potassium channel (Kir 4.1) - Glial fibrillary acidic protein (GFAP)	Semiquantitative
Immune cells	- Iba1 (macrophages) - CD3 (T-lymphocytes)	Quantitative (number of positive cells/mm^2 DRG*)
Others	- Vimentin (Vim) - Periaxin (PRX)	Semiquantitative

* Analyzed using QuPath-0-4-3, Bankhead, P. et al. QuPath: Open source software for digital pathology image analysis. Scientific Reports (2017).https://doi.org/10.1038/s41598-017-17204-5

Results

(1) Morphology of DRG of hamsters

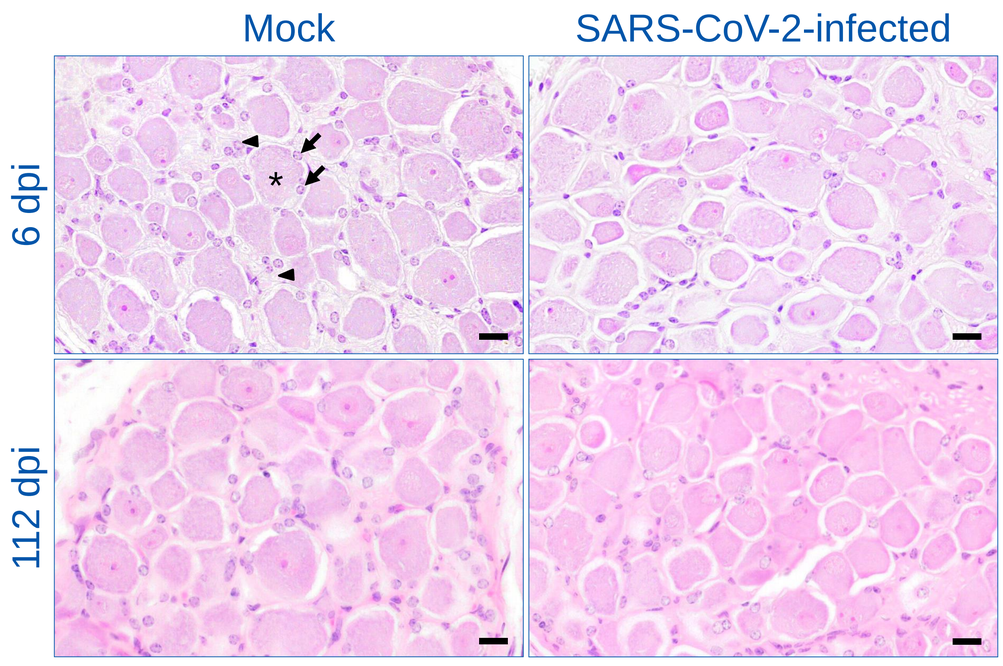


Fig. 1: Dorsal root ganglia (DRG) of mock and SARS-CoV-2-infected hamsters at 6 (acute) and 112 (chronic) dpi. The cell bodies of the pseudounipolar, sensory neurons (asterisk) are tightly surrounded by multiple satellite glial cells (SGCs; arrows). In between SGC-neuron units, afferent nerve fibers, connective tissue as well as small blood vessels and few immune cells are visible (arrowheads). No differences between DRG of mock and infected animals were detected. The clefts between neuronal bodies and surrounding SGCs are most likely shrinking artefacts due to tissue processing. H&E stain. Scale bars: 20µm.

(2) Immunohistochemical results

Marker	SGCs of mock-infected hamsters				SGCs ofSARS-CoV-2-infected hamsters			
dpi	3	6	14	112	3	6	14	112
GS	+++	+++	+++	+++	+++	+++	+++	+++
Kir 4.1	+++	++	+++	++	+++	+++	+++	++
GFAP	+++	++	+++	+++	+++	++	+++	+++
Vim	+	++	++	+	++	+	+	+
PRX	-	-	-	-	-	-	-	-

Table 1: Semiquantitative analysis of mock-infected and SARS-CoV-2-infected hamster DRG. The percentage of immuno-positive SGCs surrounding sensory neurons were estimated semiquantitatively with - = 0 %, + = 1-33 %, ++ = 34-70% and +++ >70% positive SGCs.

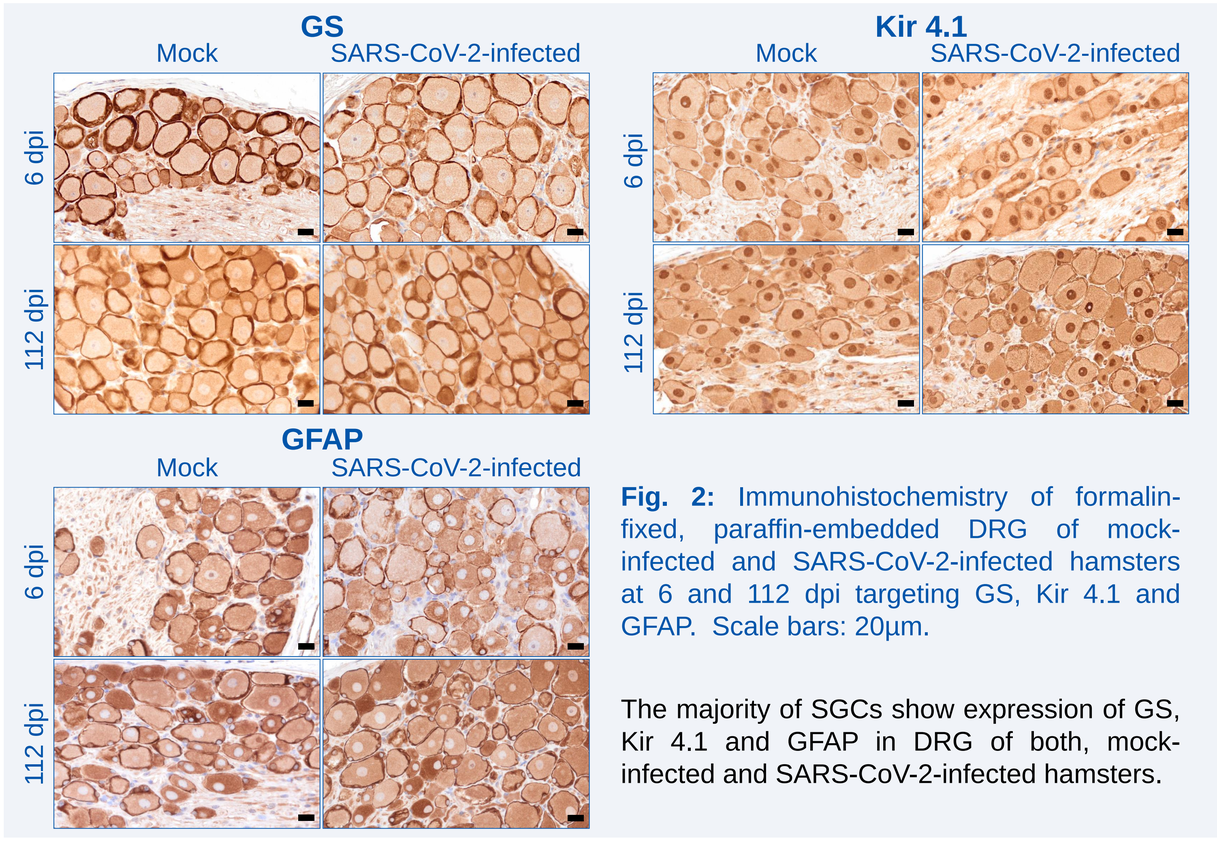


Fig. 2: Immunohistochemistry of formalin-fixed, paraffin-embedded DRG of mock-infected and SARS-CoV-2-infected hamsters at 6 and 112 dpi targeting GS, Kir 4.1 and GFAP. Scale bars: 20µm.

The majority of SGCs show expression of GS, Kir 4.1 and GFAP in DRG of both, mock-infected and SARS-CoV-2-infected hamsters.

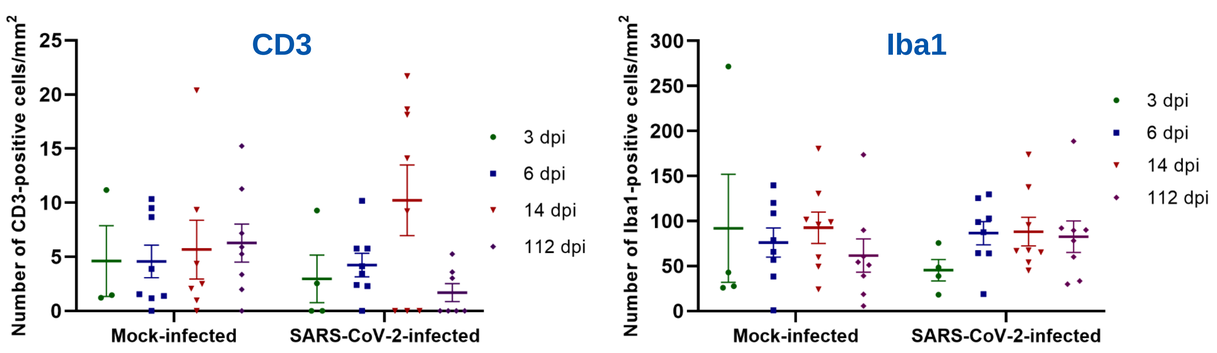


Fig. 3: Statistical analysis of the number of CD3- and Iba1-positive cells/mm^2 DRG revealed no significant differences between mock- and SARS-CoV-2-infected hamsters or different time points within a group. Single dot plots display mean values (horizontal line) and standard error of the mean (vertical line). Each point represents the individual value of each animal examined.

No positive signal for SARS-CoV-2 NP was detected within DRG at 3, 6, 14 and 112 dpi.

Conclusion & Outlook

DRG of hamsters show a similar architectural composition compared to other species [5, 6]. Several SGCs surround one neuronal soma forming discrete functional units interspersed by few immune cells, blood vessels, connective tissue cells and Schwann cells wrapping around afferent nerve fibers. In concordance with dogs, pigs and mice, the majority of SGCs of hamsters express GS and Kir 4.1 [5, 6]. Interestingly, in contrast to mice, but analogously to dogs and pigs, SGCs of hamsters express GFAP [5, 6]. Therefore, unlike in mice, GFAP cannot be used as a marker of activated SGCs in DRG of hamsters. No differences regarding protein expression levels of GS, Kir 4.1 and GFAP were detected in SGCs following SARS-CoV-2 infection using immunohistochemistry. Few immune cells (macrophages, T-lymphocytes) were detectable in DRG of infected and mock-infected hamsters without significant differences between groups or time points indicating that these cells represent resident local immune cells. No SARS-CoV-2 NP was detectable. Whether other phenotypic changes in DRG that could affect both neurons and SGCs can be observed in response to a SARS-CoV-2 infection causing somatosensory abnormalities within the PNS of SARS-CoV-2-infected hamsters, will be the subject of further investigations using additional markers (e.g. targeting transient receptor potential vanilloid channel (TRPV1), purinergic receptor P2X7, doublecortin).

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