Passive transfer of IgG from patients with long-COVID neurological symptoms induces tactile allodynia in mice

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Introduction

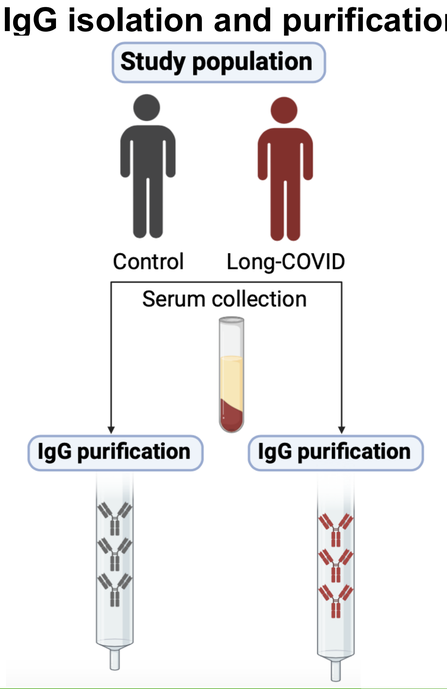
Between 15% and 30% of SARS-CoV-2-infected people still experience neurological symptoms (memory impairment, attention deficits, pain) more than 4 months after the onset of COVID-19. This condition, known as long-neuro-COVID, is poorly understood and might be explained by a persisting autoimmune response against nervous-derived self-antigens. The aim of this study is to determine whether IgG autoantibodies from long-COVID patients can bind to central and peripheral nervous system epitopes and reproduce neuropsychiatric symptoms upon transfer into mice.

Methods

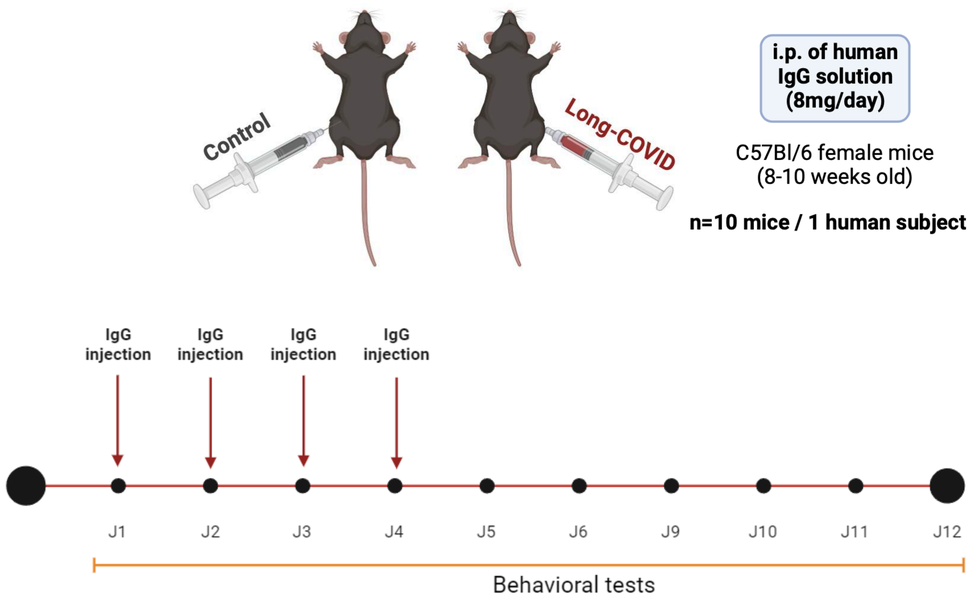
1. Recruitment of patients

/	/	Control (n=6)	Long-COVID (n=9)	/	/	Control (n=6)	Long-COVID (n=9)
Age (years)	Mean	47,4 (27-59)	47,5 (29-64)	**Visual selective attention:**			
				Time (ms; percentiles)	>P2,3 **<P2,3**	6 0	6 **3**
				Omissions (percentiles)	>P2,3 **<P2,3**	6 0	7 **2**
Sex	Female Male	6 0	9 0	**Auditory selective attention:**			
				Time (ms; percentiles)	>P2,3 **<P2,3**	6 0	4 **5**
				Omissions (percentiles)	>P2,3 **<P2,3**	6 0	5 **4**
Moca	>26/30 **<26/30**	6 0	6 **3**	**Go/No go:**			
				Time (ms; percentiles)	>P2,3 **<P2,3**	6 0	5 **4**
				Omissions (percentiles)	>P2,3 **<P2,3**	6 0	6 **3**
SDMT	>-2 **<-2**	6 0	8 **1**	**Beck depression inventory**	**>4** <4	6 0	7 **2**
STROOP (percentiles)	>P2,3 **<P2,3**	6 0	6 **3**	**Pain intensity** (/10)	Mean	0,5 (0-1)	5 (2-7)
D2 test (percentiles)	>P2,3 **<P2,3**	6 0	6 **3**				

2. IgG isolation and purification

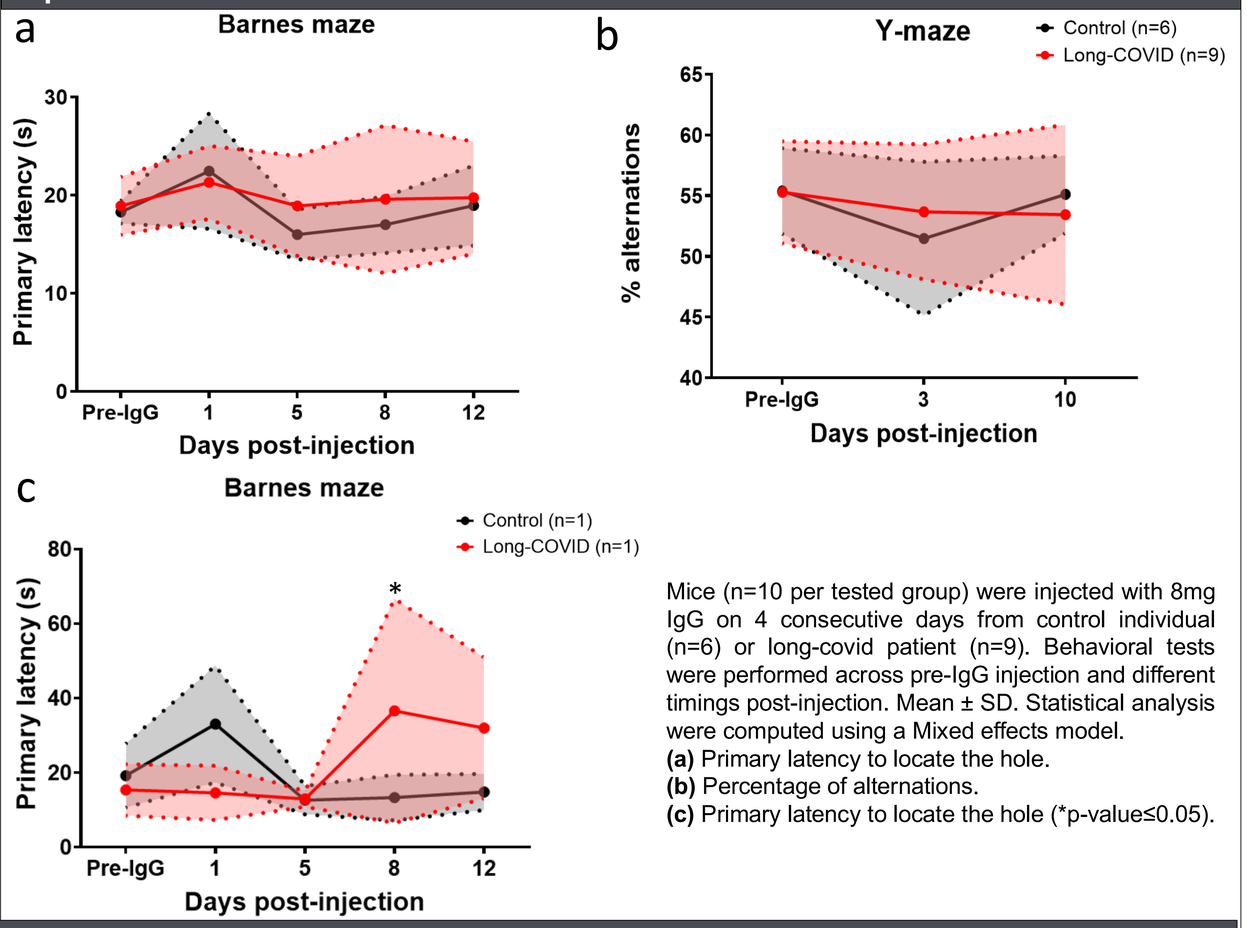


3. IgG injection into C57Bl/6 mice



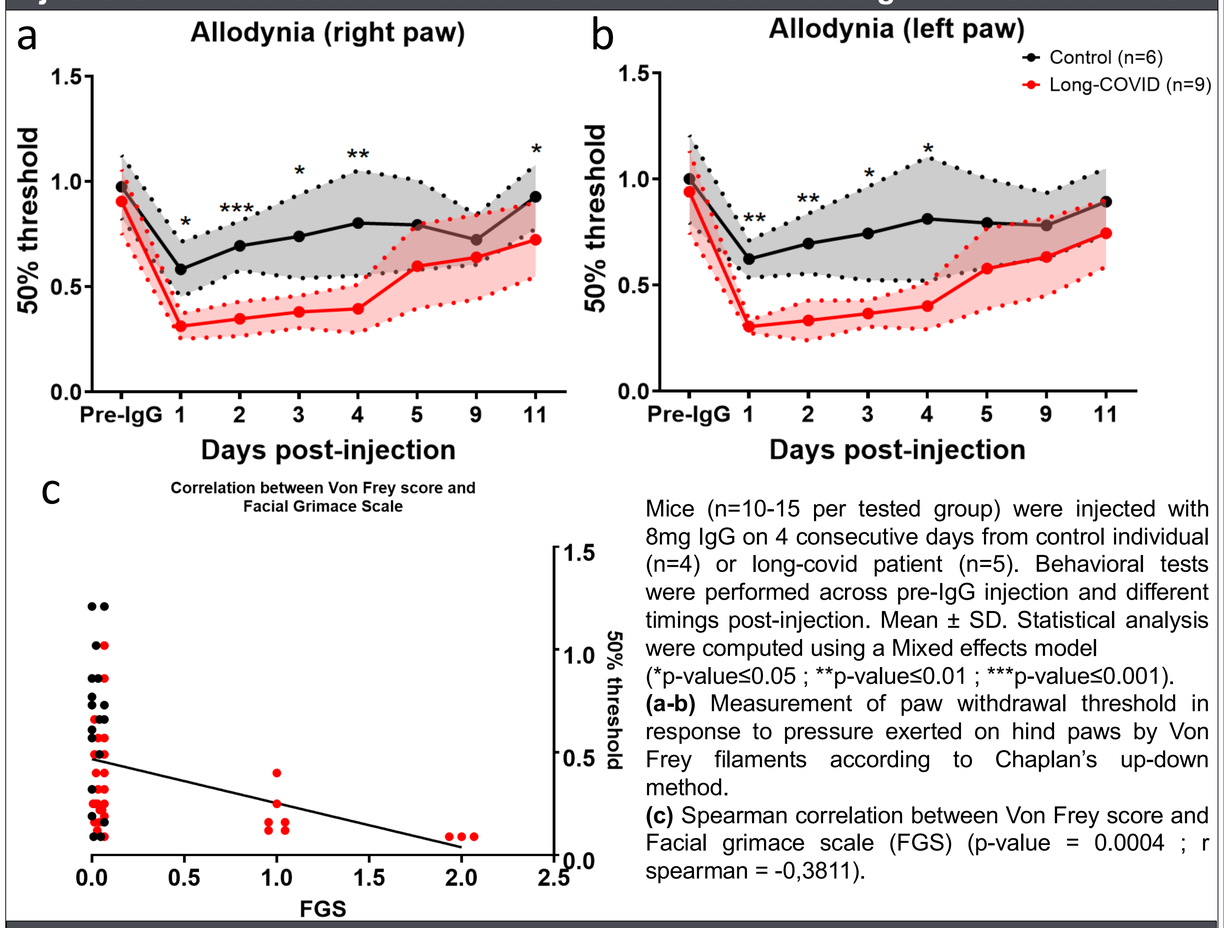
Results

FIGURE 1: Passive transfer of IgG from long-covid patients to mice did not induce a significant memory impairment, except for one patient suffering from cognitive impairment.



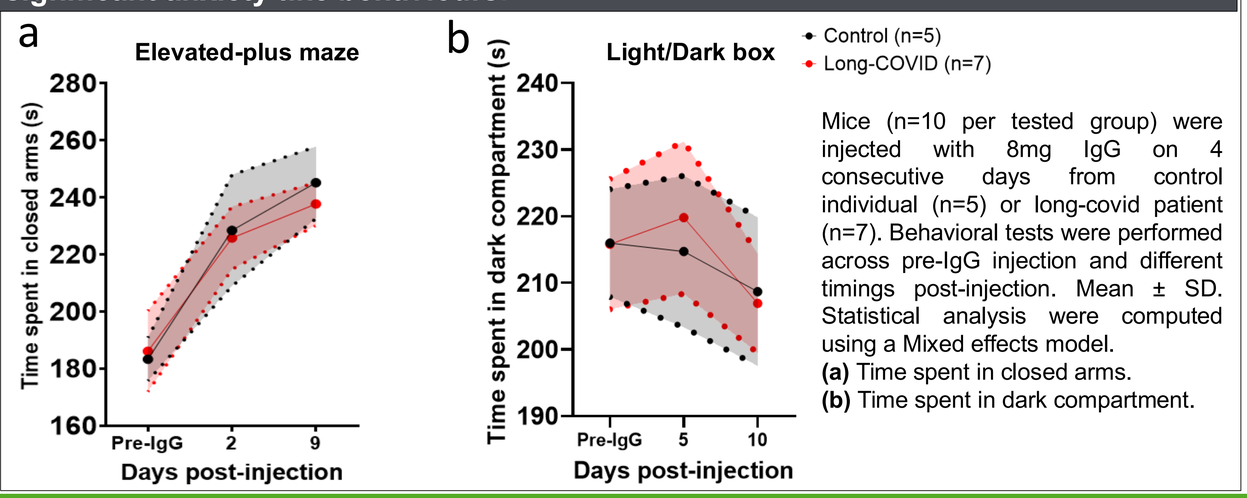
Mice (n=10 per tested group) were injected with 8mg IgG on 4 consecutive days from control individual (n=6) or long-covid patient (n=9). Behavioral tests were performed across pre-IgG injection and different timings post-injection. Mean ± SD. Statistical analysis were computed using a Mixed effects model.
(a) Primary latency to locate the hole.
(b) Percentage of alternations.
(c) Primary latency to locate the hole (*p-value≤0.05).

FIGURE 2: Passive transfer of IgG from long-covid patients to mice induced a significant decrease of paw withdrawal threshold during the first week post-injection. This decrease correlated with an increased Facial grimace scale score.



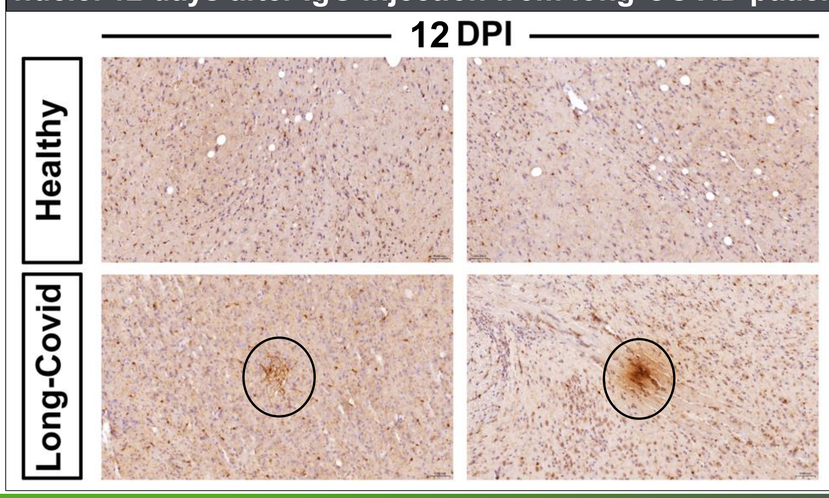
Mice (n=10-15 per tested group) were injected with 8mg IgG on 4 consecutive days from control individual (n=4) or long-covid patient (n=5). Behavioral tests were performed across pre-IgG injection and different timings post-injection. Mean ± SD. Statistical analysis were computed using a Mixed effects model (*p-value≤0.05 ; **p-value≤0.01 ; ***p-value≤0.001).
(a-b) Measurement of paw withdrawal threshold in response to pressure exerted on hind paws by Von Frey filaments according to Chaplan's up-down method.
(c) Spearman correlation between Von Frey score and Facial grimace scale (FGS) (p-value = 0.0004 ; r spearman = -0,3811).

FIGURE 2: Passive transfer of IgG from long-covid patients to mice did not induce a significant anxiety-like behaviours.



Mice (n=10 per tested group) were injected with 8mg IgG on 4 consecutive days from control individual (n=5) or long-covid patient (n=7). Behavioral tests were performed across pre-IgG injection and different timings post-injection. Mean ± SD. Statistical analysis were computed using a Mixed effects model.
(a) Time spent in closed arms.
(b) Time spent in dark compartment.

FIGURE 4: Presence of activated microglia (Iba1⁺ cells) clusters in mice thalamus nuclei 12 days after IgG injection from long-COVID patients.



Characterization of mice brain microglia using anti-Iba1 immunohistochemistry. Scale bar represents 0,05 mm.

Conclusion

Mice injected with IgG from long-COVID patients showed no difference with the control group in terms of anxiety behaviors, as well as no impairment of spatial memory. Mice receiving IgG from long-COVID patients displayed a significant decrease of paw withdrawal threshold in both hind paws which correlated with increased FGS score. Clusters of activated microglia were detected in thalamus of these mice 12 days after IgG injection. These data show that IgGs from long-COVID patients can hyperactivate the nociceptive pathways and produce, in mice, pain-related symptomatology. Further analysis will aim at identifying the PNS or CNS targets.