

# Early-life Influenza A (H1N1) infection independently programs brain connectivity, HPA axis and tissue-specific gene expression profiles

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Supplementary Data

**Supplementary Data 1: PCR quantification of IAV (H1N1)** RNA extracted from homogenised mouse lungs was reverse transcribed using SuperScript 3 and H1N1 was amplified using the protocol of Spackman et al (Reference 89). H1N1 was not detected in any of the animals an PND 56. Ct values are given, and NA represents samples for which no amplification was observed.

mouse_ID	Ct	PCR_plate	note	DOB	generation	PND14_date	PND14_treatment	PND56_date	PND56_treatment	sex
5147	20.36	1	posControl	20/09/2019	F1	04/10/2019	Sal	21/11/2019	H1N1	f
3009	23.94	2	posControl	11/09/2018	F1	25/09/2018	Sal	06/11/2018	H1N1	m
3011	34.28	2	posControl	11/09/2018	F1	25/09/2018	Sal	06/11/2018	H1N1	f
3007	35.52	3	posControl	11/09/2018	F1	25/09/2018	Sal	06/11/2018	H1N1	m
5152	NA	2	posControl	20/09/2019	F1	04/10/2019	Sal	21/11/2019	H1N1	m
3001	NA	1	NA	05/09/2018	F1	19/09/2018	Sal	31/10/2018	Sal	m
4042	NA	1	NA	25/02/2019	F1	11/03/2019	Sal	22/04/2019	Sal	f
4048	NA	3	NA	26/02/2019	F1	12/03/2019	Sal	23/04/2019	Sal	m
5184	NA	2	NA	26/10/2019	F1	09/11/2019	Sal	14/12/2019	Sal	m
6036	NA	1	NA	11/01/2020	F1	25/01/2020	Sal	07/03/2020	Sal	f
6039	NA	3	NA	11/01/2020	F1	25/01/2020	Sal	07/03/2020	Sal	f
6062	NA	2	NA	16/02/2020	F1	02/03/2020	H1N1	12/04/2020	Sal	f
3013	NA	1	NA	14/09/2018	F1	28/09/2018	H1N1	09/11/2018	Sal	f
3015	NA	1	NA	14/09/2018	F1	28/09/2018	H1N1	09/11/2018	Sal	m
4002	NA	1	NA	09/02/2019	F1	23/02/2019	H1N1	05/04/2019	Sal	m
4007	NA	1	NA	12/02/2019	F1	26/02/2019	H1N1	11/04/2019	Sal	f



### Data 3: Microarray Immune infiltration

**Deconvolution:** In order to check for the presence of specific cellular populations and processes, we applied consensus independent component analysis (consICA) (Nazarov et al., 2019). It was already shown that the method successfully identifies variable cell subtypes and cellular processes in bulk-sample transcriptomics data, especially linked to immune and proliferative cells (Nazarov et al., 2019, Golebiewska et al., 2020). The method was applied as described in (Nazarov et al., 2019) directly on the dataset, thus avoiding using of a reference data. Number of independent components was fixed to 20.

**Results:** Our deconvolution of the data set result in tissue-specific processes (e.g. xenobiotic metabolic process for liver, FDR=1e-22, regulation of synaptic plasticity, FDR=9e-8, for cerebellum) but did not detect any immune signal. This suggests no significant effect of the treatment on immune response in the considered organs.

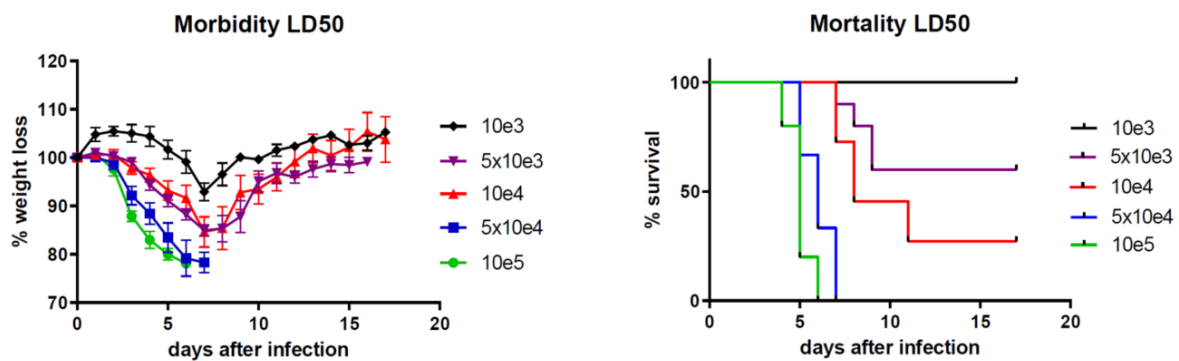
TISSUE	PROCESS	FDR
sub-cortical	chemical synaptic transmission	2e-27
cerebellum	regulation of synaptic plasticity	3e-08
sub-cortical, cerebellum, brain stem	chemical synaptic transmission	2e-27
liver	xenobiotic metabolic process	7e-25
liver	exogenous drug catabolic process	2e-24
adrenal gland	fatty acid metabolic process	6e-14
adrenal gland	steroid hormone biosynthetic process	5e-03

**Data 4:** Scoring system for IAV sickness symptoms. Template from (Koch et al., 2016) with modifications.

Feature	Symptoms	points	Day1	Day2	Day3	Day ...
Appearance	Normal (smooth coat, clear eyes/nose)	0				
	Hunched and/or fluffed	1				
	Ocular discharge and/or edema	3				
	Pale, white mucus membranes/skin	6				
	Blue mucus membranes/skin (cyanosis)	12				
Respiratory rate	Normal breathing	0				
	Increased breathing ( <i>subjective</i> recognition by experimenter)	3				
	Increased breathing ( <i>obvious</i> ; double normal rate, rapid, shallow)	6				
	Abdominal breathing (+/-gaspings or open mouth breathing)	12				
General behavior	Normal (based in baseline observations)	0				
	Decreased mobility	2				
	Ataxia, wobbly, weak	6				
	Inability to stand	12				
Provoked behavior	Normal (moves when cage is disturbed, runs from hand)	0				
	Subdued; responds to stimulation (moves away briskly)	1				
	Subdued even to stimulation (moves away slowly)	3				
	Unresponsive to gentle prodding	6				
	Does not right when placed on side within 5 seconds	12				
		<b>Weight (in grams):</b>				
		<b>% loss (compared to day 1):</b>				
Weight loss	< 10%	0				
	10-15%	2				
	15-20%	6				
	20-25%	10				
	≥25%	12				
		<b>Total Score:</b>				
		<b>Notes:</b>				
		<b>Initials of experimenter/caretaker:</b>				

Score	Interpretation
< 6	Normal
6–11	Morbid: Monitor at least 3 times per day; notify appropriate personnel immediately
≥ 12	Moribund: Notify responsible personnel immediately If any single criterion is 12 or higher = euthanize immediately

**Data 5:** LD50 morbidity and mortality



**Data 6.** MRI scan protocol. SR: Spatial Resolution, SI: Number of Slices, TE: Echo Time, TR: Repetition Time, ET: Echo Train, AVG: Number of Averages, SD: Scan Duration, NOB: number of b-value, NOD: number of diffusion direction.

<b>SEQUENCE</b>	<b>PURPOSE</b>	<b>PARAMETERS</b>
<b>SE-DTI</b>	Mapping of Fractional Anisotropy (FA)/ preferential direction of diffusion	<b>SR:</b> 0.16 × 0.16 × 1 mm; <b>SI:</b> 20; <b>TE\TR:</b> 22\800 ms; <b>AVG:</b> 5; <b>NOB:</b> 1; <b>b-value:</b> 1000; <b>NOD:</b> 7; <b>SD:</b> 49min44sec
<b>μMRI - FSE</b>	High resolution anatomy	<b>SR:</b> 0.08 × 0.08 × 0.2 mm ; <b>SI:</b> 64 ; <b>TE\TR:</b> 17\3000 ms; <b>ET:</b> 8; <b>AVG:</b> 2; <b>SD:</b> 3h31min42sec