Grooming behaviour in honey bees is compromised by the interaction between sublethal exposure to neonicotinoids and *Varroa destructor*

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Stressors

Biotic

Abiotic

Pathogens

Weather

Chemicals

V. destructor

Viruses (e.g. DWV)

AFB, EFB

Insecticides

Acaricides

Fungicides

V. destructor

Ectoparasitic mite that feeds on the fat body of larvae, pupae and adult bees

V. destructor facilitates maintaining an open wound, transmission of viruses and immunosuppression

Immunosuppression: cellular (decreasing haemocytes) and humoral (down-regulation of immune related genes)

Rosenkranz et al., 2010

http://beeaware.org.au/archive-pest/varroa-mites

Deformed wing virus

Vectored by *V. destructor* (Bowen-Walker et al., 1999)

Decrease longevity, causes weight loss, and inability to fly (De Miranda et al., 2010)

Affects learning (Iqbal and Mueller, 2007)

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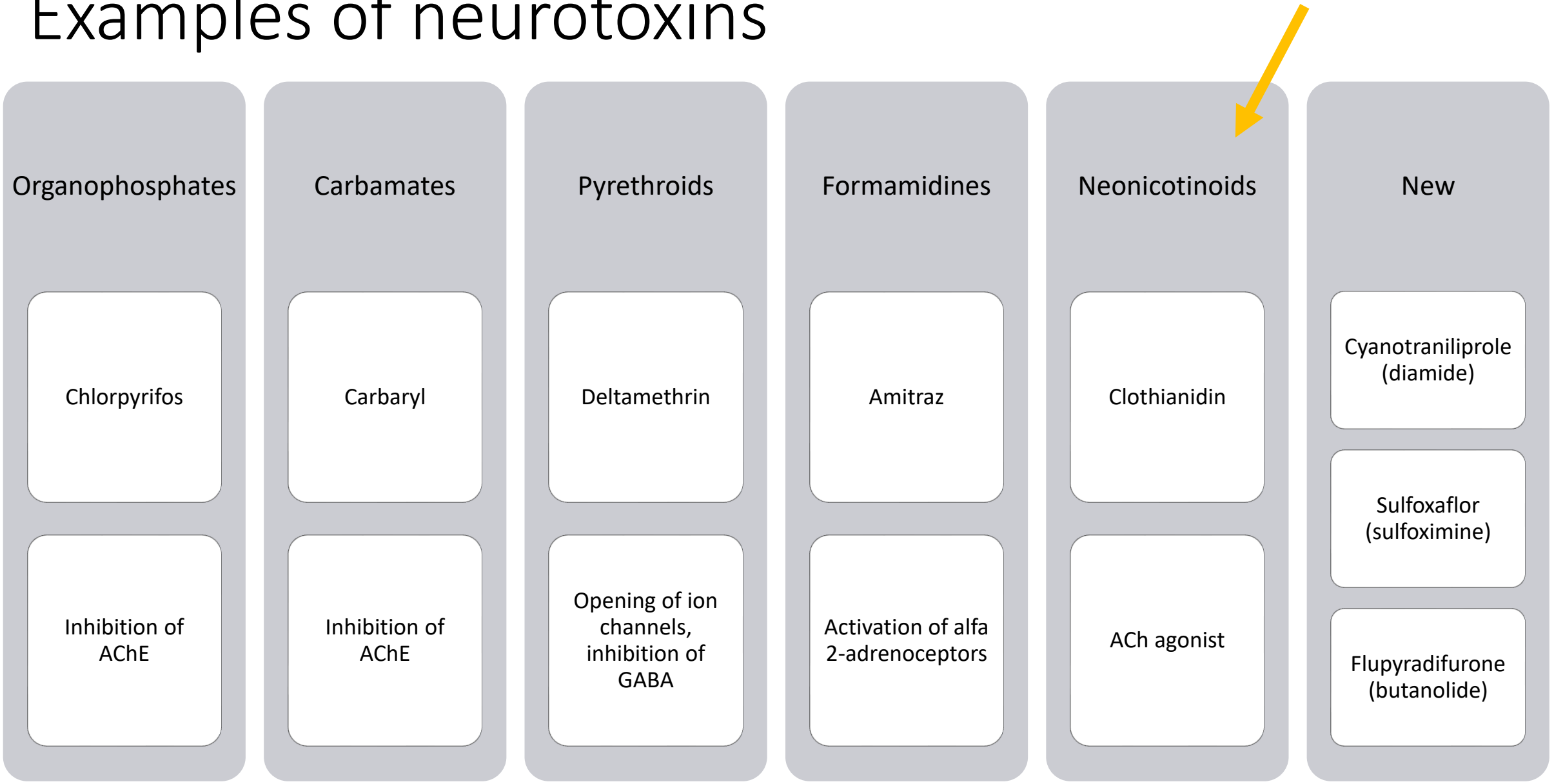
Examples of neurotoxins



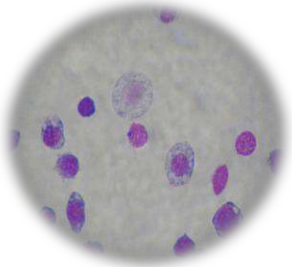
Costa et al., 2008

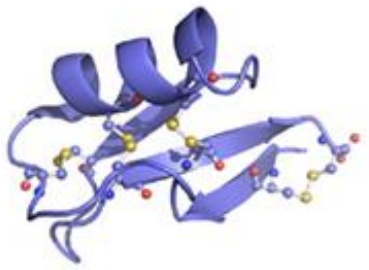
Exposure to multiple sublethal doses of clothianidin (systemic insecticide)

https://www.canolawatch.org/2015/05/21/top-10-things-to-look-for-after-emergence/
https://www.country-guide.ca/2014/11/12/neonicotinoids-for-pest-management/45112/

Defense mechanisms in honey bees

- Individual immunity
  - Cellular immunity
    - Haemocytes
  - Humoral immunity
    - AMPs





- Social immunity
  - Hygienic
  - Grooming
    - Social grooming
    - **Individual grooming**

Grooming behaviour

- Effective restrain of *V. destructor* population growth (Guzman-Novoa et al., 2012)
- Grooming intensity is important vs *V. destructor* (Guzman-Novoa et al., 2012)
- And *Acarapis woodi* (Pettis and Pankiw, 1998)
- Honey bee genotypes vary in their ability to express grooming (Guzman-Novoa et al., 2012; Bak and Wilde, 2015)
- Genes associated with grooming were identified (*neurexin*) (Arechavaleta-Velasco et al., 2012; Hamiduzzaman et al., 2017)
- Selection based on traits associated with grooming (Rinderer et al., 2010; Hunt et al., 2016; Morfin et al., unpublished)

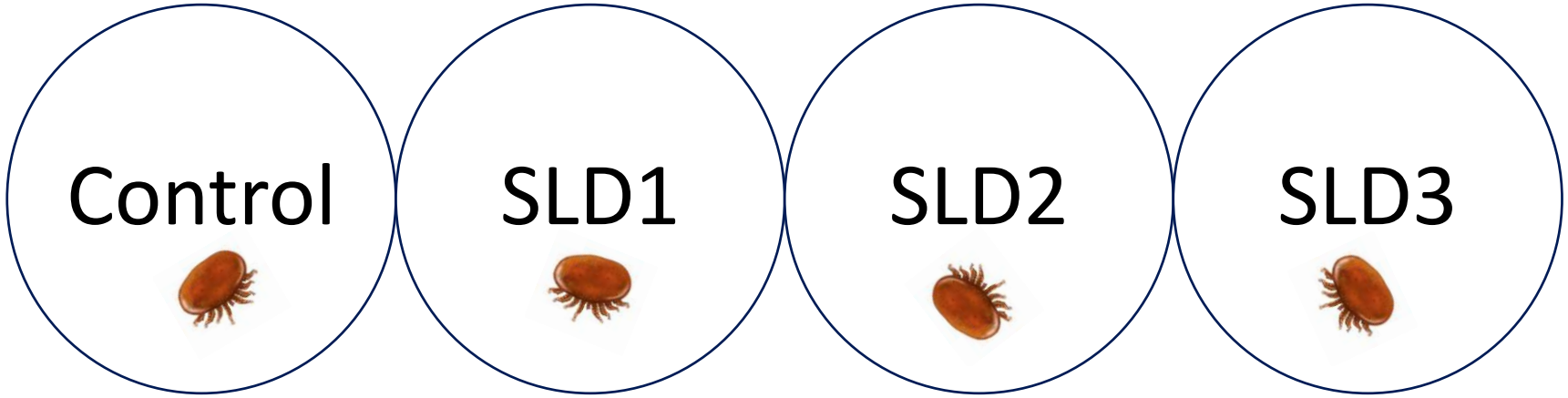
?

Is grooming behaviour affected by stressors (*V. destructor* and clothianidin)?

?

Is there an interaction between *V. destructor* and clothianidin?

Methods



Grooming

- NEB <24h
- n=40
- 7 days

Clothianidin

V. destructor

- 1 mite/bee

- No grooming
- Grooming: light/intense

Classification

RNA analyses

- RNAseq

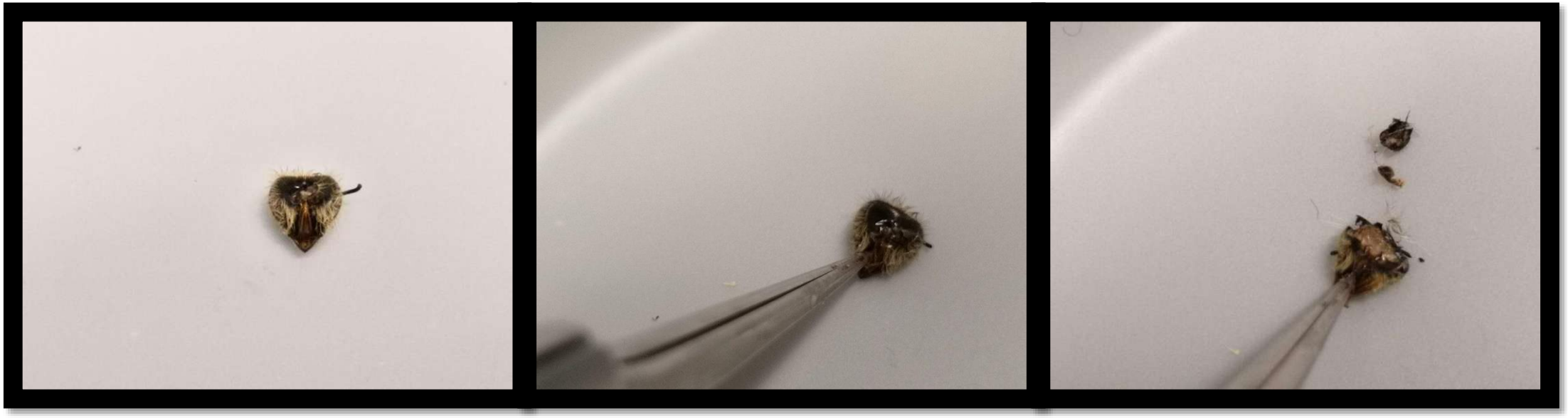
Frames in incubator

Newly emerged bees

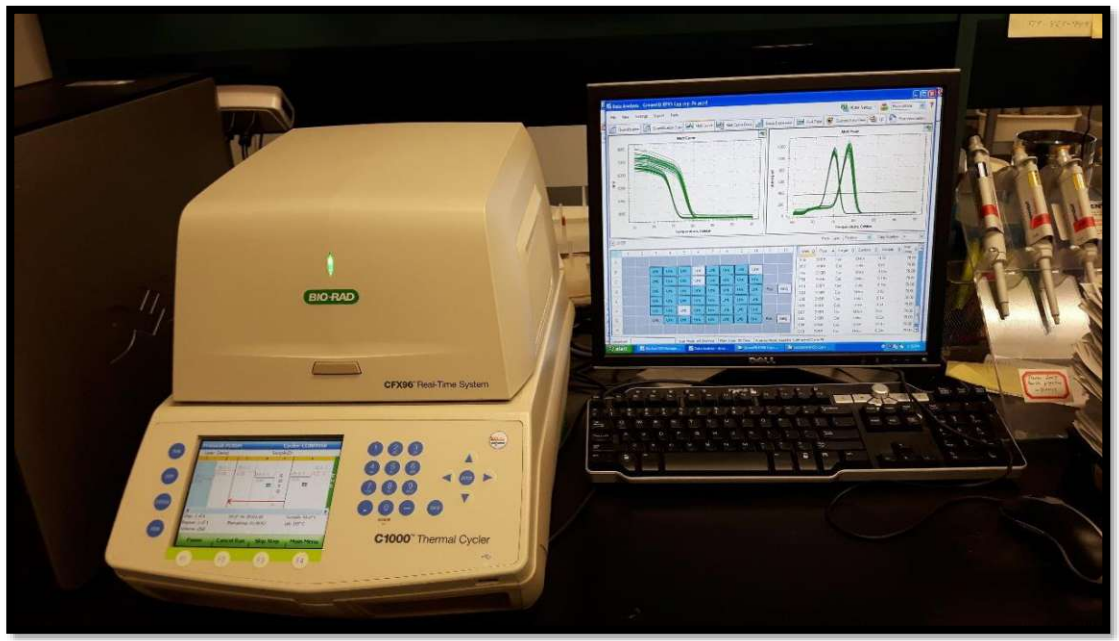
Hoarding cages in incubator

Results

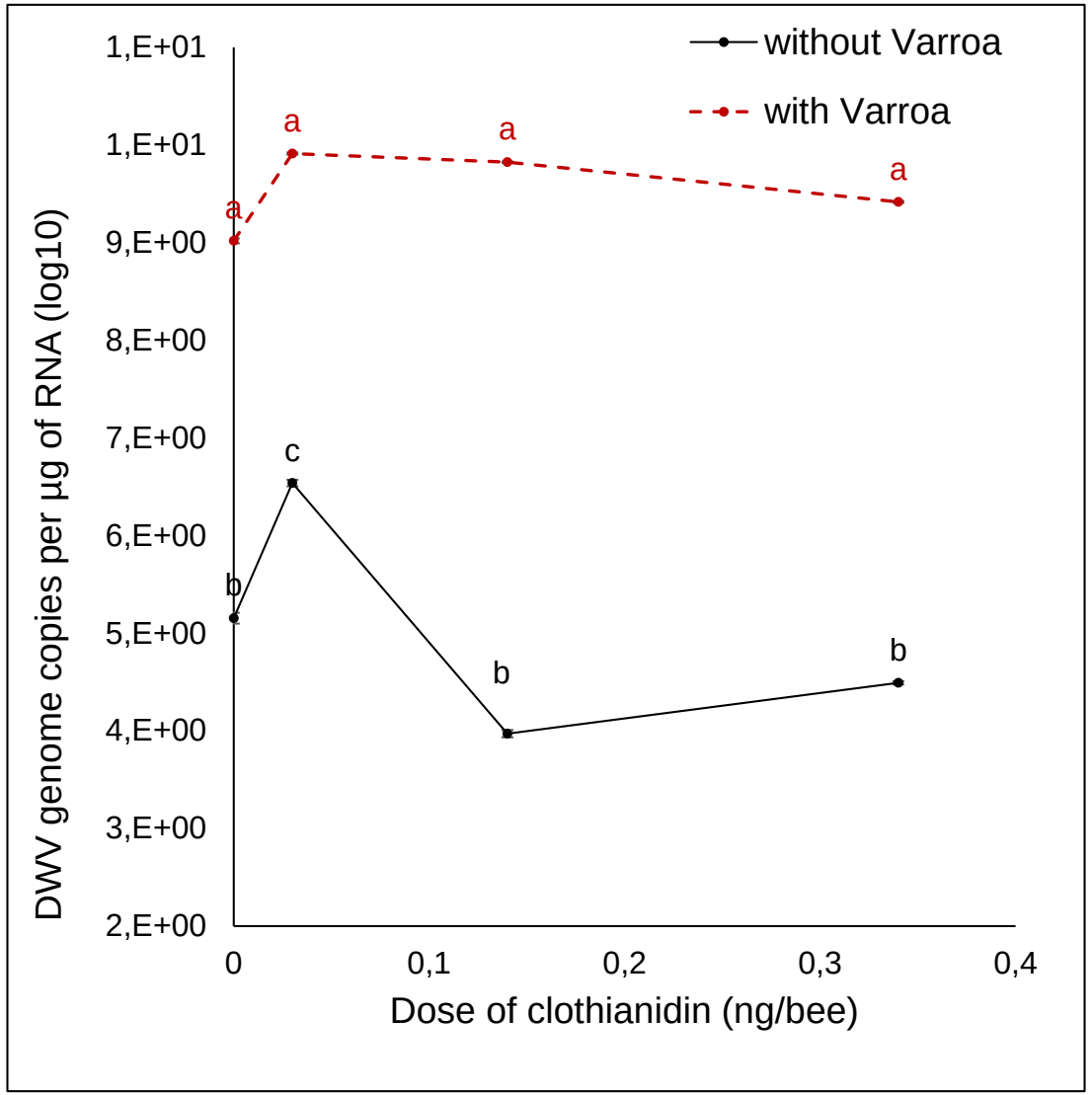
Treatment	Proportion of intense groomers
Control (0 ng)	0.72
SLD1	0.55
SLD2	0.57
SLD3	**0.39***
0 ng + V. destructor	0.56
SLD1 + V. destructor	0.53
SLD2 + V. destructor	**0.41***
SLD3 + V. destructor	**0.37***



Dissection of honey bee brains



Quantification of DWV, Guzman's lab (UofG)


without Varroa
with Varroa
DWV genome copies per µg of RNA (log10)
1,E+01
1,E+01
9,E+00
8,E+00
7,E+00
6,E+00
5,E+00
4,E+00
3,E+00
2,E+00
a
a
a
a
c
b
b
b
0
0,1
0,2
0,3
0,4
Dose of clothianidin (ng/bee)

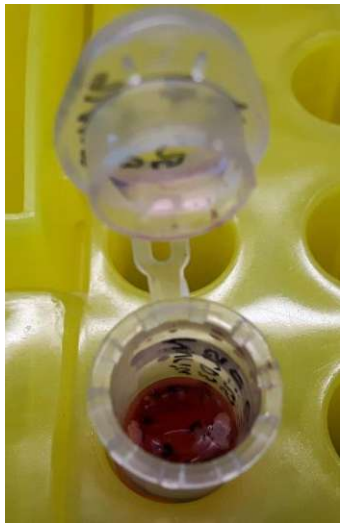
RNAseq

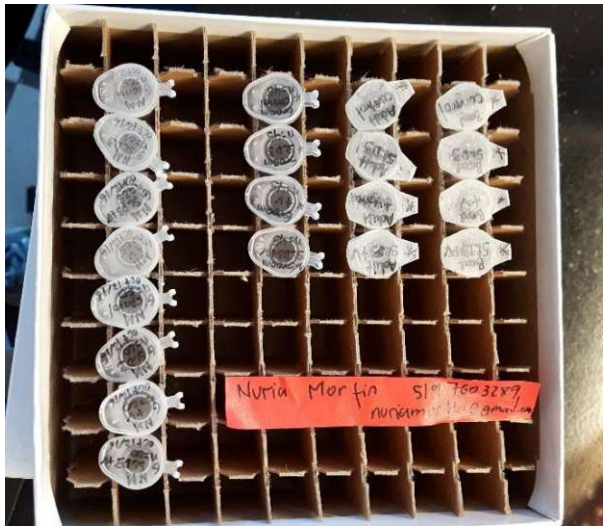
RNA extraction
- From brains

RNAseq
- Génome Québec Innovation Centre

Bioinformatics
- Canadian Centre for Computational Genomics

Results
- DEG = Differentially Expressed Genes compared to control
- Gene description
- Gene Ontology (biological pathways)





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AATTCTGCAAGAAACTTGAAAAATTCGTTGAAAGTAATGCACGAATGGAAATATATAGAT
TATGATTTCGGTAGCGAAGAAAAAAGACAAGCTGCGATTCAATCTGACGAATATGACCAT
ACGAAAAATTATCCATTCGACGTCGATCAATGGCGTGGTAAAATTTTCTTATTTTAAATT
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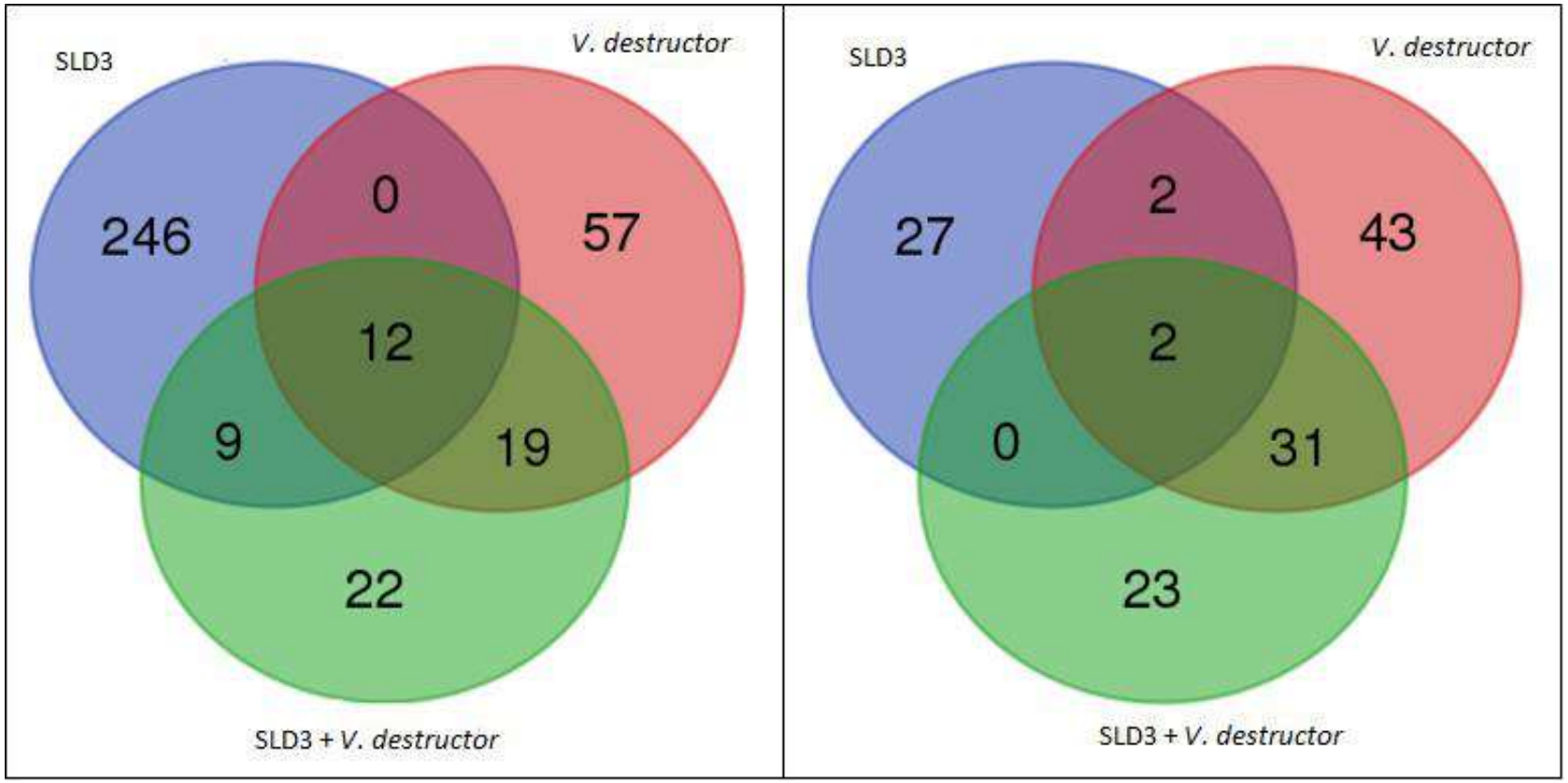
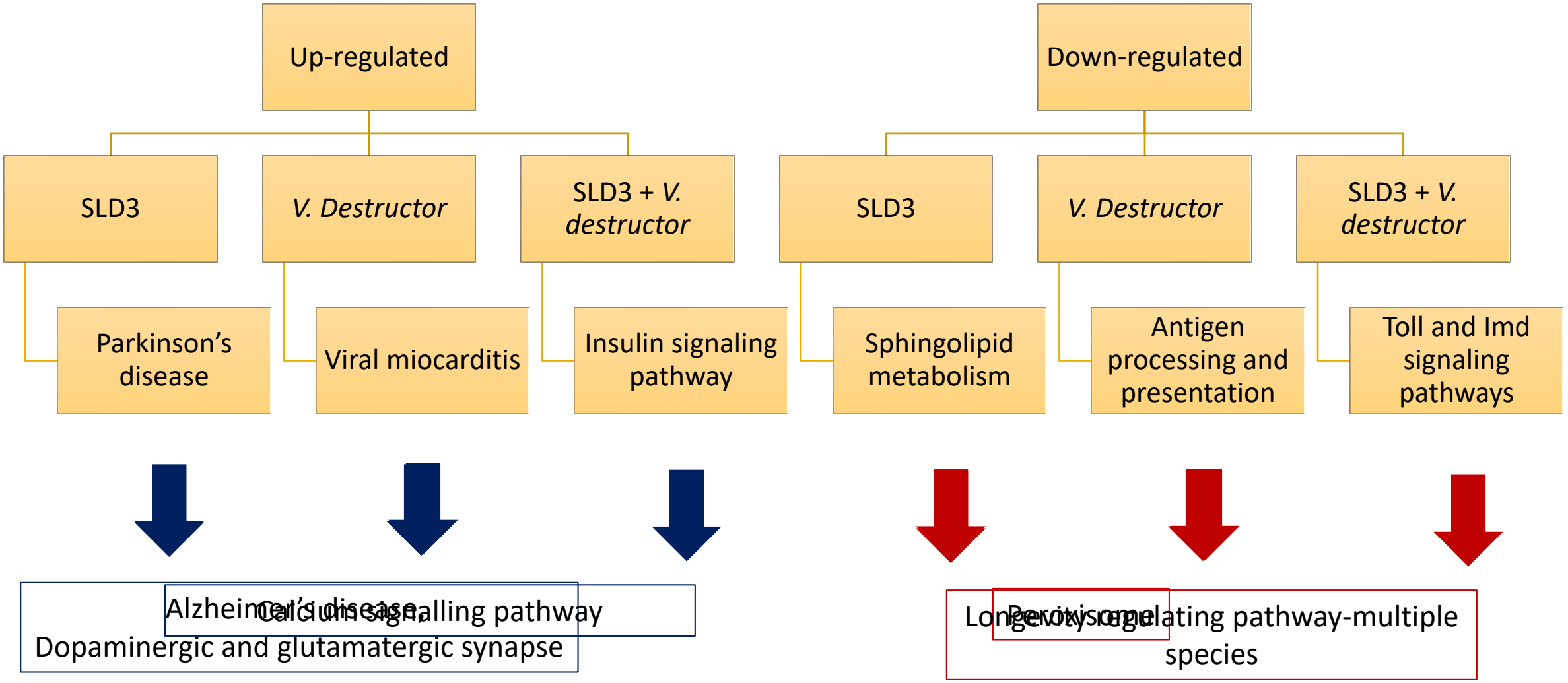
RNAseq, grooming behaviour



Up-regulated DEGs

Down-regulated DEGs

Biological pathways associated with DEGs



- Do stressors affect grooming behaviour?
- Is there an interaction between *V. destructor* and clothianidin?

Conclusion

Clothianidin decreased the proportion of groomers and intense groomers

Interaction between *V. destructor* and clothianidin

- Proportion of intense groomers
- Number of up and down regulated DEGs

Never underestimate *V. destructor*!

- Defense mechanisms
- DWV
- DEGs
- Biological pathways

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Effects of sublethal doses of clothianidin and/or *V. destructor* on honey bee (*Apis mellifera*) self-grooming behavior and associated gene expression

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Thanks for your attention ☺