The non-nuclear splice isoform of NFκB gene Dif modulates sensitivity to ethanol sedation in Drosophila melanogaster

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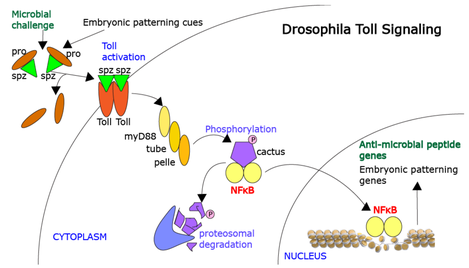
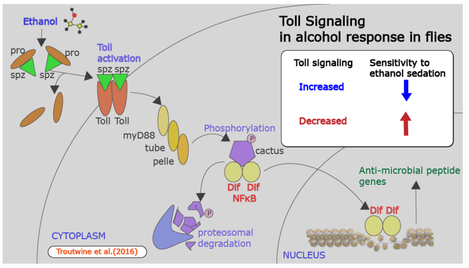
SIGNIFICANCE

- ❖ **Neuroimmune signaling and NFKBs have been implicated in ethanol response in mammalian systems**
- ❖ **The study demonstrates a novel non-nuclear NfkB protein that functions at the synapse of neurons in the brain that modulates alcohol response.**
- ❖ **Raises possibilities of functioning with neurotransmission and neuron communication and plasticity in alcohol response.**

Drosophila Toll signaling in alcohol response





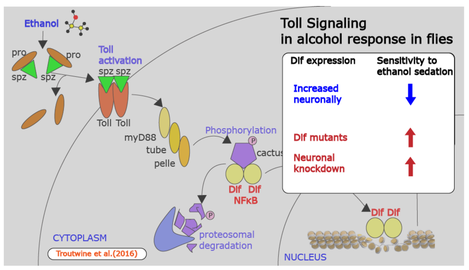


Figure 1:

- ❖ **The Toll signaling pathway functions in activating immunity upon infection by gram positive bacteria and fungi, through NfKB protein Dif (Dorsal-like immunity factor)**
- ❖ **Activating the Toll signaling pathway reduces sensitivity to ethanol sedation**
- ❖ **Dif functions neuronally to modulate sensitivity to ethanol sedation**

NFkB Dif is expressed in two protein isoforms Dif A and Dif B

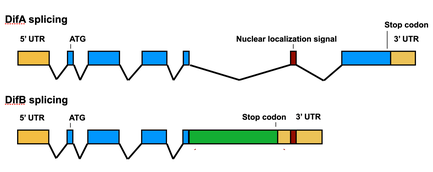


Figure 2:

- ❖ **Dif A is expected to be nuclear, having a nuclear localization signal**
- ❖ **Dif B is expected to be non-nuclear**

Mutually exclusive expression patterns of the two isoforms of the Dif gene

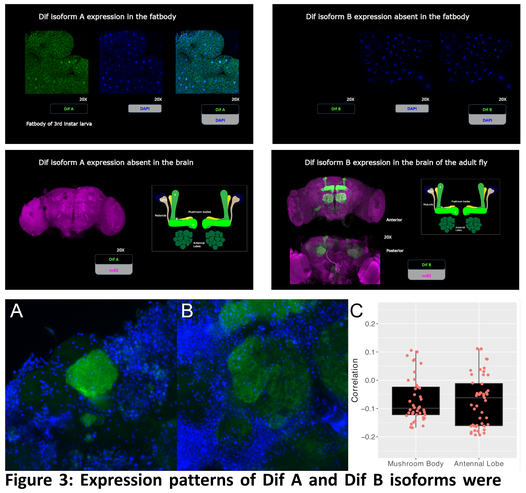
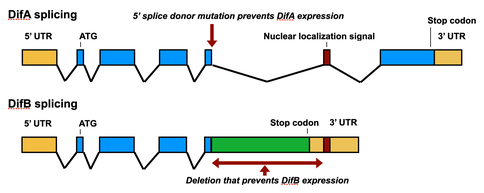


Figure 3: Expression patterns of Dif A and Dif B isoforms were investigated using antibodies against them and Immunohistochemistry

- ❖ **Dif A expression in the fatbody of the fly larva. Expression is nuclear**
  Dif B expression is absent in the fatbody
- ❖ **Dif B expression in the mushroom bodies and antennal lobes of the brain**
  Dif A expression is absent in the brain
- ❖ **Colocalization analysis confirms non-nuclear localization of the DifB protein in the brain**

The Dif A and Dif B mutant flies



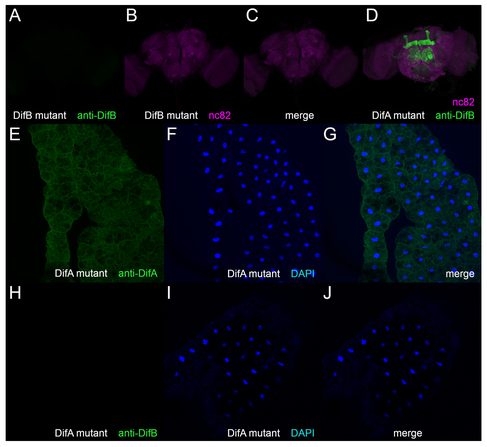
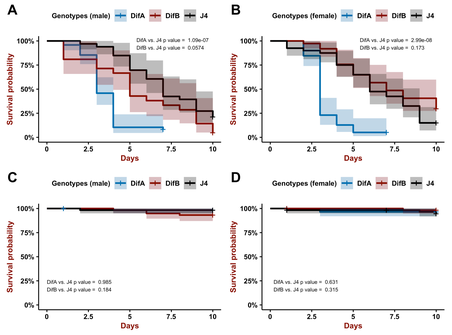


Figure 4: Characterization of the Dif A and Dif mutant flies using Immunohistochemistry

- ❖ **Dif B protein is absent in the brain of Dif B mutant flies. Dif A protein in present.**
- ❖ **Dif A protein is non-nuclear in the Dif A mutant larvae**
- ❖ **Dif B protein is not present in the Dif A mutant larvae**

Dif A isoform is involved in immune response



(A)- Male infected (C)-Male uninfected
(B)- Female infected (D)- Female uninfected

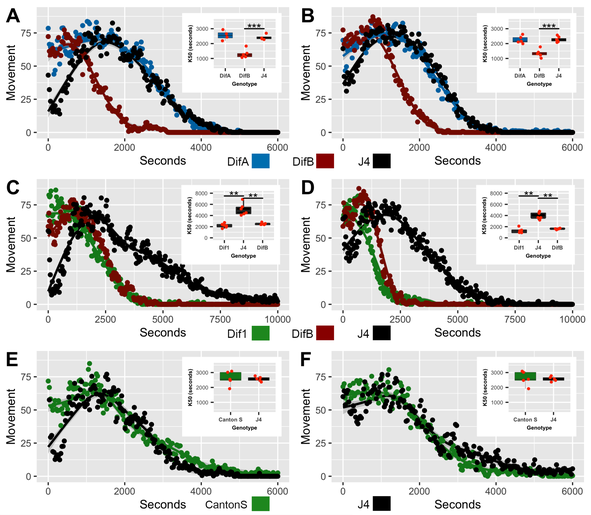
Figure 5: The loss of Dif A has a larger effect on mortality upon *Beauvaria bassiania* fungal infection

- ❖ **Dif A mutant flies have a higher death rate compared to the J4 (Parental) flies.**
- ❖ **Dif B mutant flies and the parental flies were comparable in response to infection**

Increased sensitivity to ethanol induced sedation in the Dif mutant is caused by the absence of the B splice isoform

Figure 6: Dif isoform B plays a role in resistance to ethanol induced sedation.
Sedation profiles of mutant flies and controls were compared after exposure to 35% ethanol

- ❖ **Dif B mutant flies show a higher sensitivity to ethanol induced sedation, compared to Dif A mutant flies and Parental flies.**
- ❖ **Dif null flies (where both isoforms are inactive) are comparable in ethanol sensitivity to the Dif B mutant flies.**
- ❖ **The parental genetic background J4 flies are comparable to the common wildtype strain Canton S.**



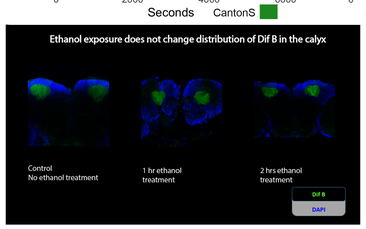
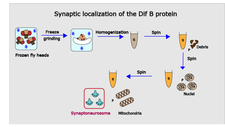


Figure 7: Dif B acts outside of the nucleus in modulating ethanol sensitivity.
Cellular localization of Dif B in the neurons of the mushroom body remains cytoplasmic in response to ethanol, during sedation.

Dif B protein is synaptic in the brain



- **A) Total homogenate**
- **B) Synaptosome fraction**
- **C) Crude nuclear fraction**

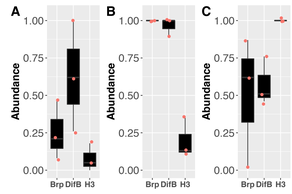


Figure 8: Synaptoneurosome preparations show the synaptic localization of the Dif B protein.
Whole fly homogenate of wildtype flies were fractionated into the synaptosome fraction and the nuclear fraction. Each was probed for presence of Dif B protein using antibody staining. Molecular markers were
Synaptosome fraction = synaptic protein Brp (Brushpilot)
Nuclear fraction= Histone H3

CRISPR mediated tagging of Dif A and Dif B isoforms in the fly

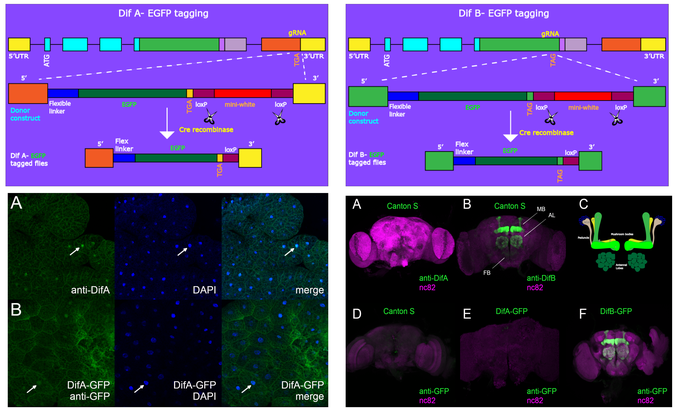
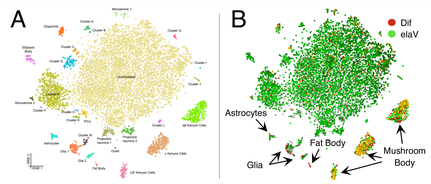


Figure 9: The two isoforms 'A' and 'B' of the Dif gene were tagged with EGFP using CRISPR gene editing.
Dif A-EGFP and Dif B-EGFP show parallel expression patterns in the fat body (Dif A) and brain (Dif B) to their native untagged gene counterparts. Characterized by immunohistochemistry using antibodies against Dif A, Dif B and EGFP.

Single Cell Transcriptomics confirm Dif expression in the mushroom bodies of the adult brain



Cell type groupings | Dif B expression patterns

Figure 10: Cell type specific expression patterns of the Dif gene (Dif A and Dif B isoforms)

- **Neuronal marker= elaV (Green)**
- **Dif gene= red**
- **Intersection= Yellow**

CONCLUSIONS

- ❖ **Mutually exclusive expression patterns of two splice isoforms of the same NfkB gene**
- ❖ **Non-nuclear isoform of the NfkB gene Dif localizes to the synapse**
- ❖ **Dif A and Dif B isoforms function in two independent characteristics of the fly**
- ❖ **Dif B functions outside of the nucleus in modulating alcohol sensitivity**