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Ustaoglu, Pinar; McQuarrie, David W. J.; Rochet, Anthony; Dix, Thomas C.; Haussmann, Irmgard U.; Arnold, Roland; Devaud, Jean-Marc; Soller, Matthias

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National Brain Research Centre (NBRC), India

*CORRESPONDENCE

Matthias Soller
✉ m.soller@bham.ac.uk

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Memory consolidation in honey bees is enhanced by down-regulation of *Down syndrome cell adhesion molecule* and changes its alternative splicing

Pinar Ustaoglu^{1,2}, David W. J. McQuarrie^{1,2}, Anthony Rochet³,
Thomas C. Dix^{1,2}, Irmgard U. Haussmann^{1,4}, Roland Arnold^{2,5},
Jean-Marc Devaud^{3,6} and Matthias Soller^{1,2*}

¹School of Biosciences, College of Life and Environmental Sciences, University of Birmingham, Birmingham, United Kingdom, ²Birmingham Centre for Genome Biology, University of Birmingham, Birmingham, United Kingdom, ³Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI), CNRS, UPS, Toulouse University, Toulouse, France, ⁴Department of Life Science, Faculty of Health, Education and Life Sciences, Birmingham City University, Birmingham, United Kingdom, ⁵College of Medical and Dental Sciences, Institute of Cancer and Genomics Sciences, University of Birmingham, Birmingham, United Kingdom, ⁶Institut Universitaire de France (IUF), Paris, France

Down syndrome cell adhesion molecule (*Dscam*) gene encodes a cell adhesion molecule required for neuronal wiring. A remarkable feature of arthropod *Dscam* is massive alternative splicing generating thousands of different isoforms from three variable clusters of alternative exons. *Dscam* expression and diversity arising from alternative splicing have been studied during development, but whether they exert functions in adult brains has not been determined. Here, using honey bees, we find that *Dscam* expression is critically linked to memory retention as reducing expression by RNAi enhances memory after reward learning in adult worker honey bees. Moreover, alternative splicing of *Dscam* is altered in all three variable clusters after learning. Since identical *Dscam* isoforms engage in homophilic interactions, these results suggest a mechanism to alter inclusion of variable exons during memory consolidation to modify neuronal connections for memory retention.

KEYWORDS

Dscam, alternative splicing, learning and memory, honey bee, RNA interference (gene silencing)

Introduction

Memory consolidation is the process through which memories are stabilized and stored into long-term memory (Kandel et al., 2014). Gene expression through transcription of genes plays pivotal roles in this process (Alberini et al., 1994; Clayton, 2000; Alberini, 2009; Kandel et al., 2014). During transcription, splicing out of introns allows to alter coding

content through alternative splicing, which is particularly abundant in the brain in genes coding for ion channels and cell adhesion molecules (Soller, 2006; Vuong et al., 2016; Ule and Blencowe, 2019). From some of these genes, hundreds of different proteins can be made leading to a substantial increase of the coding capacity of the genome. However, the functional consequences of diversity generated by alternative splicing remain poorly understood.

In honey bees, an invertebrate model species for the study of learning and memory processes, requirement of transcription and the neuronal RNA binding protein ELAV during memory consolidation further suggest important roles for alternative splicing (Lefer et al., 2012; Villar et al., 2020; Ustaoglu et al., 2021). Accordingly, alternative splicing events in a number of cell adhesion molecules and ion channels among other genes have been linked to neuronal excitability, learning and memory (Beffert et al., 2005; Demares et al., 2013; Demares et al., 2014; Poplawski et al., 2016; Sengar et al., 2019; Torres-Mendez et al., 2019; Ustaoglu et al., 2021; Torres-Méndez et al., 2022). Likewise, ELAV itself is substantially alternatively spliced in honey bee mushroom bodies (brain centers for learning and memory) and its expression pattern differs between individuals, likely reflecting differences in individual experiences (Ustaoglu et al., 2021).

The *Down syndrome cell adhesion molecule* (*Dscam*) gene encodes one of the largest immunoglobulin (Ig) domain superfamily cell adhesion molecule and contains 10 Ig domains and six fibronectin type III (FN) repeats. This overall protein structure of *Dscam* is evolutionary conserved between vertebrates and invertebrates with an overall amino acid identity and similarity of 29 and 46% between humans and *Drosophila*, respectively, (Crayton et al., 2006; Schmucker and Chen, 2009; Hu et al., 2011). In arthropods, *Dscam* is massively alternatively spliced to generate tens of thousands of different protein isoforms from four clusters of mutually exclusive variable exons that vary in numbers between different species (Graveley et al., 2004; Hemani and Soller, 2012; Zhang et al., 2019). In *Drosophila*, the *Dscam* gene contains 95 alternative exons in variable cluster 4 (12 exons), 6 (48 exons), 9 (33 exons), and 17 (2 exons) to generate 36'016 different mRNAs (Schmucker et al., 2000).

Drosophila Dscam is expressed in the nervous system where its variability is used during development for neuronal wiring, and in the immune system as a pattern recognition receptor for pathogen clearance by phagocytosis (Watson et al., 2005; Hemani and Soller, 2012). The main roles in the nervous system comprise establishment of overlapping dendritic fields in the peripheral nervous system and to branch neurites in neuronal tracts (Hughes et al., 2007; Matthews et al., 2007; Soba et al., 2007). Key to these functions of *Dscam* are homophilic repulsive properties of individual identical *Dscam* isoforms, but not to other splice variants (Wojtowicz et al., 2004; Wojtowicz et al., 2007). Accordingly, dendrites of neurons can overlap if they express different *Dscam* isoforms and neuronal tracts such as the *Drosophila* mushroom body containing hundreds of neurons can split into two lobes (Wang et al., 2002; Matthews et al., 2007). Between *Drosophila* and honey bees, *Dscam* protein is highly conserved indicated by 66% identity and 76% similarity, respectively, (Hu et al., 2011).

Mechanistically, it has been proposed in *Drosophila* that individual neurons adopt a unique set of variables stochastically to comply with their fate in branching in the peripheral nervous system and in the mushroom bodies (Neves et al., 2004;

Miura et al., 2013). This model further implies that *Dscam* alternative splicing is maintained in individual neurons. In the immune system of mosquitoes, however, exposure to different pathogens altered inclusion of variables to generate variants with higher affinity (Dong et al., 2006). Accordingly, both stochastic as well as deterministic rules have been found to govern dendritic branching in *Drosophila* (Palavalli et al., 2021). Moreover, the homophilic interaction of identical *Dscam* isoforms in *Drosophila* could also be converted to attraction at low levels and in this way build novel connections (Wojtowicz et al., 2004).

Here, we examined its role in learning and memory in honey bees, where memory consolidation involves changes in gene expression (Lefer et al., 2012; Villar et al., 2020; Ustaoglu et al., 2021) and neuronal connectivity (Hourcade et al., 2010). Here, we discovered that down-regulated *Dscam* during learning enhances memory consolidation in honey bees. To characterize alternative splicing in honey bees we sequenced *Dscam* cDNAs encompassing the individual variable clusters to comprehensively update previous annotation from comparing genomic sequences (Graveley et al., 2004). This analysis reveals that honey bees have 9 exon 4, 52 exon 6, and 19 exon 10 variables. Unlike *Drosophila*, we find intra-cluster splicing in honey bee *Dscam*. When we analysed alternative splicing during the course of memory consolidation, we discovered that alternative splicing significantly changed within variable clusters. Our results indicate that changes in *Dscam* splicing can be induced upon experience likely to establish new neuronal connections associated with memory consolidation.

Results

Dscam is required for memory consolidation in honey bees after olfactory reward conditioning

To detect honey bee *Dscam*, we used a polyclonal anti-serum raised against *Drosophila Dscam* (Watson et al., 2005). In honey bees this polyclonal antibody recognizes proteins at the size of *Drosophila Dscam* of 222 and 270 kDa and three shorter bands of 100, 110, and 130 kDa (Supplementary Figure 1A). To validate that the larger band recognized by the antibody is indeed honey bee *Dscam*, we injected double stranded RNA (dsRNA) against honey bee *Dscam* into the central brain for knock-down by RNAi. A substantial reduction of $72 \pm 5\%$ and $79 \pm 10\%$ ($n = 3$ each) is achieved after 48 and 64 h of the expected bands at 222 and 270 kDa, respectively, but not of the shorter bands of 100, 110, and 130 kDa suggesting that these bands in honey bees are unspecific (Figure 1A and Supplementary Figure 1B).

To assess whether *Dscam* has a role in learning and memory, honey bees were individually trained in an associative olfactory conditioning task 2 days after injection of *Dscam* dsRNA or GFP (control) dsRNA to then measure short-term and long-term memory 1 h and 1 day after training, respectively, (Figure 1B). Both groups showed significant learning over the successive trials (RM-ANOVA, Trial effect: $F = 46.55$, $p < 0.001$), without significant effect of the treatment ($F = 2.28$, $p = 0.135$, Figure 1C left). We then tested short-term (1 h following conditioning) and long-term (24 h) memory to ask whether *Dscam* downregulation could

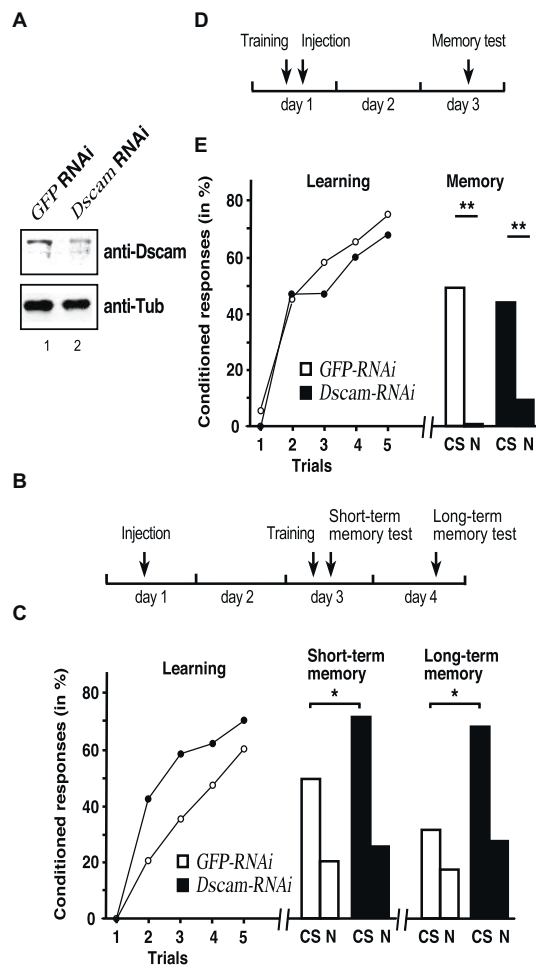


FIGURE 1

Down syndrome cell adhesion molecule (Dscam) is required for learning and memory consolidation. (A) Western blot detecting Dscam (top) or tubulin (bottom) in honey bee central brains of control GFP and Dscam RNAi 48 h after injection of dsRNA into worker honey bees. (B) Schematic of the treatment to test for Dscam's role in learning and memory consolidation. (C) Learning (left), short term (middle) and long-term memory (right) performances of control GFP RNAi (white) and Dscam RNAi (black) after injection of worker honey bees with dsRNA. CS: conditioned odor, N: novel odorant. * $p < 0.05$. (D) Schematic of the treatment control for Dscam's role in learning and memory consolidation. (E) Learning (left) and memory (right) performances of control GFP RNAi (white, $n = 39/n = 22$ at 24 h) and Dscam RNAi (black, $n = 46/n = 15$ at 24 h) after injection of worker honey bees with dsRNA. CS, conditioned stimulus, N, novel odorant ** $p < 0.01$. The source data underlying this figure are available in [Supplementary Data 1](#).

impact on memory formation despite preserved learning capacities (Figure 1C). Here, at 1 h (Figure 1C middle) honey bees from the Dscam group responded significantly more to the conditioned stimulus (CS) than those from the GFP group (Fischer's exact test: $\chi^2 = 4.72$, $p = 0.044$), while the proportions of specific responses did not differ either ($\chi^2 = 3.37$, $p = 0.118$, Figure 1C middle and left). Yet, while both groups clearly responded more to the CS than to the novel odorant 1 h after training, the difference was not significant in either case (McNemar test, GFP: $\chi^2 = 2.88$, $p = 0.090$; Dscam: $\chi^2 = 0.0588$, $p = 0.808$). The proportions of individuals displaying specific responses (i.e., responses to the CS but not the

novel odour, N) did not differ either between the groups ($\chi^2 = 2.56$, $p = 0.125$, Figure 1C middle). Thus, down-regulated Dscam levels enhanced memory performance without affecting its specificity.

At 24 h after conditioning, only a portion of honey bees survived and could be tested (GFP: $n = 22$, Dscam: $n = 15$). Both groups responded more to the CS than to the novel odorant, although the difference was not significant in the Dscam group due to a smaller sample size (McNemar test, GFP: $\chi^2 = 4.84$, $p = 0.028$; Dscam: $\chi^2 = 0.048$, $p = 0.827$, Figure 1C right). Again, the proportions of specific responses did not differ between the groups ($\chi^2 = 0.337$, $p = 0.118$), but honey bees from the Dscam group showed a marginally significantly higher response rate to the CS than those from the GFP group (Fischer's exact test: $\chi^2 = 4.36$, $p = 0.050$). Thus, down-regulated Dscam levels enhanced CS-N specific long-term memory performance.

To reject the possibility that loss of Dscam impacts on long-term memory retrieval *per se* due to a prolonged downregulation of Dscam, we performed an additional experiment in which injection was done shortly after training, so that RNAi would be effective at the time of retrieval (24 h) rather than at consolidation (Figure 1D). As expected, this treatment did not affect learning (Trial effect: $F = 48.48$, $p < 0.001$; Trial \times Treatment interaction: $F = 0.697$, $p > 0.05$; Figure 1E left). More importantly, long-term memory retrieval was intact and 2 days after training both groups responded similarly to the CS (Fischer's test: $\chi^2 = 0.729$, $p > 0.05$) and responded significantly less to the novel odorant (GFP: $\chi^2 = 5.23$, $p < 0.05$; elavl2: $\chi^2 = 7.47$, $p < 0.01$), thus indicating a preserved memory of the CS-US association and no enhancement of memory (Figure 1E right).

These results thus argue that down-regulation of Dscam during memory consolidation enhances storage of memories.

Alternative splicing analysis of honey bee Dscam reveals novel isoforms and intra-cluster splicing

Since Dscam in arthropods is abundantly alternatively spliced, we wanted to analyze Dscam alternative splicing after training, but this required evaluation of the gene annotation. The exon-intron structure of honey bee Dscam had previously been annotated based on phylogenomic conservation and comparison to cDNAs obtained from Drosophila Dscam (Graveley et al., 2004). To directly address alternative splicing in Dscam variable clusters 4, 6, and 10 in honey bees, we RT-PCR amplified the variable clusters and Illumina sequenced the amplicons. This analysis revealed strong overlap with the previous annotation, but also novel isoforms and additional intra-cluster splicing (Decio et al., 2019).

In particular, in each of the clusters we find an additional exon (exon 4.0, 6.0, and 10.0) before the previously annotated isoforms (Figures 2A–C). Exon 4.0 and 10.0 alter the frame resulting in non-productive isoforms.

In the exon 4 cluster, we find prominent use of an alternative 5' splice sites in exon 3 that splices to exons 4.1 and 4.3 (3a4.1 and 3a4.3, Figure 2D) that changes the open reading frame to generate non-function products. In addition, an additional 3' splice site is present in exon 4.5 generating an in-frame shorter isoform (Figure 2A).

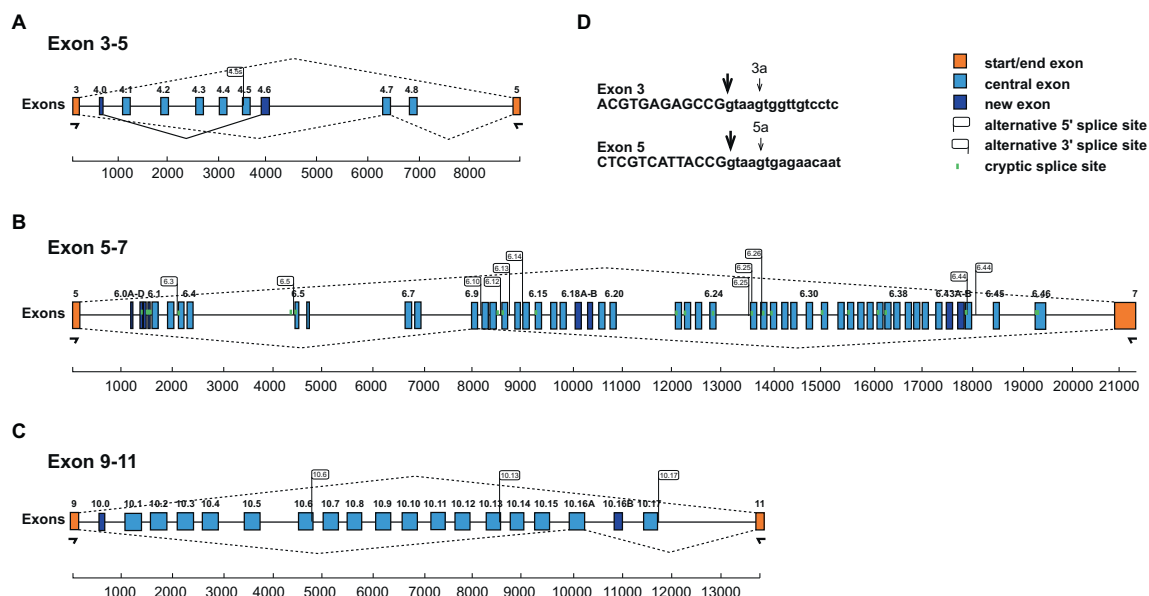


FIGURE 2

Schematic of the honey bee *Dscam* variable exon clusters 4, 6, and 10. (A–C) Gene models of *Apis mellifera Dscam* variable exon clusters 4 (A, top), 6 (B, middle), and 10 (C, bottom) depicting constant flanking exons in orange and variable exons in light blue boxes. New variable exons are indicated by dark blue boxes. Alternative 5' and 3' splice sites are indicated by right and left directed flags, respectively. Intra-cluster splicing is indicated by solid lines and dashed lines indicate representative alternative splicing. Cryptic splice sites (<1%) are indicated by vertical green lines. Exon clusters are drawn to scale with scale bars are shown at the bottom. (D) Alternative 5' splice sites in exons 3 and 5 are shown as small arrows.

In the beginning of the exon 6 cluster, we find a number of 3' splice sites, which we describe as exons 6.0A–D, but we could not detect a downstream 5' splice site by amplicon sequencing (Figure 2B). Potentially, they could include large exons using known 5' splice sites.

In the exon 6 and 10 clusters (Figures 2B, C), we identified additional variable exons (6.18B, 6.43B, and 10.16B), that maintain the reading frame and additional 5' and 3' splice sites, that are above splicing noise (>1%). In the exon 6 cluster, we find one additional 5' splice site (6.44s2) and 13 new 3' splice sites (exons 6.1s, 6.3s, 6.5s, 6.10s, 6.11s, 6.12s, 6.13s, 6.14s, 6.15s, 6.25s1, 6.25s2, 6.26s, 6.31s, 6.33s, and 6.44s). In the exon 10 cluster, we find three additional 5' splice sites (exon 10.6s, 10.13s, and 10.17s). All of these new variable exons maintain the reading frame.

In addition, we find low levels of skipping of exons in the variable clusters. Skipping of exon 4 and 10 variables maintains the reading frame, while skipping of exon 6 variables leads to truncated *Dscam* isoforms.

In summary, our expression analysis of honey bee *Dscam* mutually exclusive splicing reveals 9 exon 4 variables, 52 exon 6 variables, and 19 exon 10 variables.

Alternative splicing in *Dscam* exon 4 cluster changes in slow learners during memory consolidation

To analyse alternative splicing of *Dscam* in the variable cluster 4, we used our previously established gel-based assay, that can distinguish all eight variables and exon skipping in addition to newly discovered isoforms and reliably detect changes during

development and in adult morphs (Decio et al., 2019; Haussmann et al., 2019). When we analysed inclusion of exon 4 variables we noticed obvious differences in some honey bees, particularly ones that have learned faster than others (Ustaoglu et al., 2021) and we therefore split trained honey bees into two groups of fast and slow learners. As negative controls, we used a group of unpaired honey bees, which were presented the stimuli (CS: odorant, US: sucrose) as trained honey bees, except that the delay between the CS and US was increased to impede the learning of any association.

Upon learning differences appeared immediately particularly for exons 4.1 and 4.3s as well as exon skipping, but only in some honey bees (Figure 3A). When we quantified the inclusion levels (Figures 3B–D), significant changes were observed in exons 4.0 and 4.6s 2 h after training in the slow learners. A total of 4 h after training, significant changes were detected for exons 4.2, 4.3, 4.6s and skipped exons in slow learners (Figure 3D).

Alternative splicing in *Dscam* exon 10 variables changes most prominently in slow learners 2 h after training

To analyse alternative splicing of *Dscam* in the variable cluster 10, we also used our previously established gel-based assay, that can distinguish 13 from 18 variables (72%) and exon skipping with significant changes during development and in adult morphs (Figure 4A and Supplementary Figure 2).

Upon olfactory conditioning of honey bees, significant changes were detected immediately after training in slow learners for exon 10.12 (Figure 4B). Intriguingly, 2 h after training significant changes were detected in 62% (8 from 13) of variable exons in

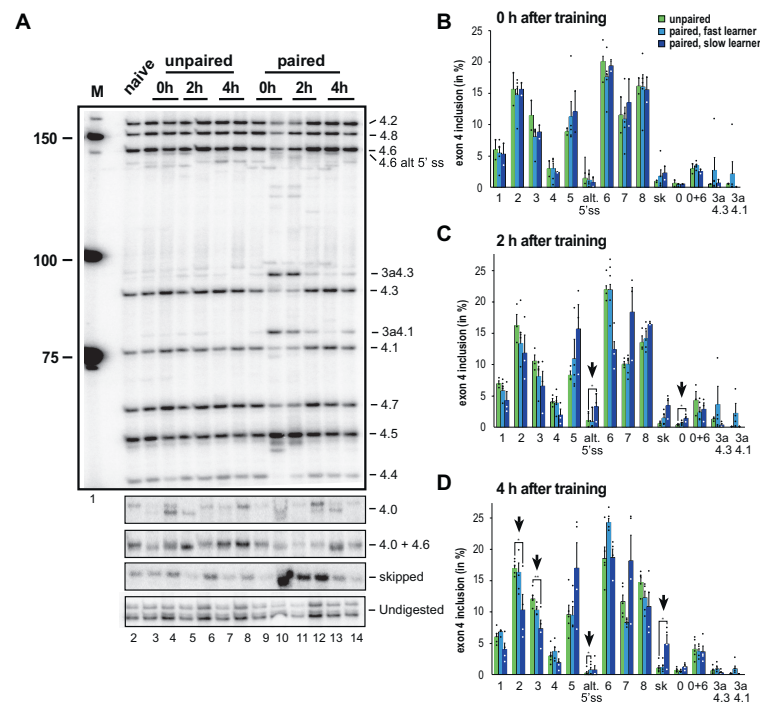


FIGURE 3

Down syndrome cell adhesion molecule (*Dscam*) variable exon 4 alternative splicing analysis during memory consolidation. (A) Representative 5% denaturing polyacrylamide gel separating ³²P-labeled alternative splice products from worker brains conditioned with unpaired (left) and paired odors and reward. Identity of splice variants are indicated at the right. M, Marker. (B–D) Analysis of alternative splicing unpaired control (green), fast (light blue) and slow learners (dark blue) indicating exon inclusion frequency (in%) for the different variables immediately after training (0 h), 2 or 4 h after training. Significant changes are indicated by vertical arrows (* $p < 0.05$; ** $p < 0.01$). The source data underlying this figure are available in [Supplementary Data 1](#).

slow learners (Figure 4C). A total of 4 h after training, significant changes were found in exons 10.7, 10.12, and 10.17 of slow learners, while fast learners show a significant increase in exon skipping (Figure 4D).

Alternative splicing in *Dscam* of more than half of exon 6 variables changes in fast learners 2 h after training

For the analysis of *Dscam* alternative splicing in the exon 6 cluster, we switched to amplicon sequencing as the gel-based assay could not sufficiently resolve the variables. For this analysis we did paired-end sequencing 2 h after training and summed up the reads for control honey bees where no paired stimulus was given, and for honey bees that either learned fast or slow.

Strikingly, from the 48 different exon 6 variables, 63% (30 out of 48), showed significant changes in fast learning honey bees compared to controls, while no significant differences were detected between controls and slow learners (Figures 5A, B).

Discussion

Alternative splicing massively expands proteomic diversity and is particularly abundant in the brain. It is most prominently elaborated in the arthropod *Dscam* gene making about three

times as many proteins than genes present in the genome (Soller, 2006; Hemani and Soller, 2012). Here, we characterize in depth the alternative splicing landscape of the honey bee *Dscam* gene and identify novel isoforms including an increased use of alternative 5' and 3' splice sites and intra-cluster splicing further expanding the diversity and shedding light on its unusual splicing regulation. *Dscam* mis-regulation has been associated with intellectual disabilities in humans associated with an additional copy of chromosome 21 (Vacca et al., 2019). In particular, an extra copy of *Dscam* in *Drosophila* causes strengthening of synapses and in a mouse model leads to excessive GABAergic synapses in the neocortex (Lowe et al., 2018; Liu et al., 2023). When we analysed learning and memory in honey bees upon reducing *Dscam* levels by RNAi we find enhanced memory storage. In addition, we find that alternative splicing of *Dscam* changes in the variable clusters during memory consolidation. Since *Dscam* homophilic interaction could stabilize existing connections (Chen et al., 2006), down-regulation of specific isoforms could loosen existing neuronal connections to stimulate formation of new ones, essentially recapitulating down-regulation of *Dscam* as obtained by RNA interference. Hence, an evolutionary conserved function of *Dscam* in learning and memory likely lies in changing expression, that in *Drosophila* can also be achieved by alterations in alternative splicing.

To store individual memory traces in the mushroom bodies, likely only few neurons are involved. It is thus surprising that *Dscam* alternative splicing changed substantially, such that

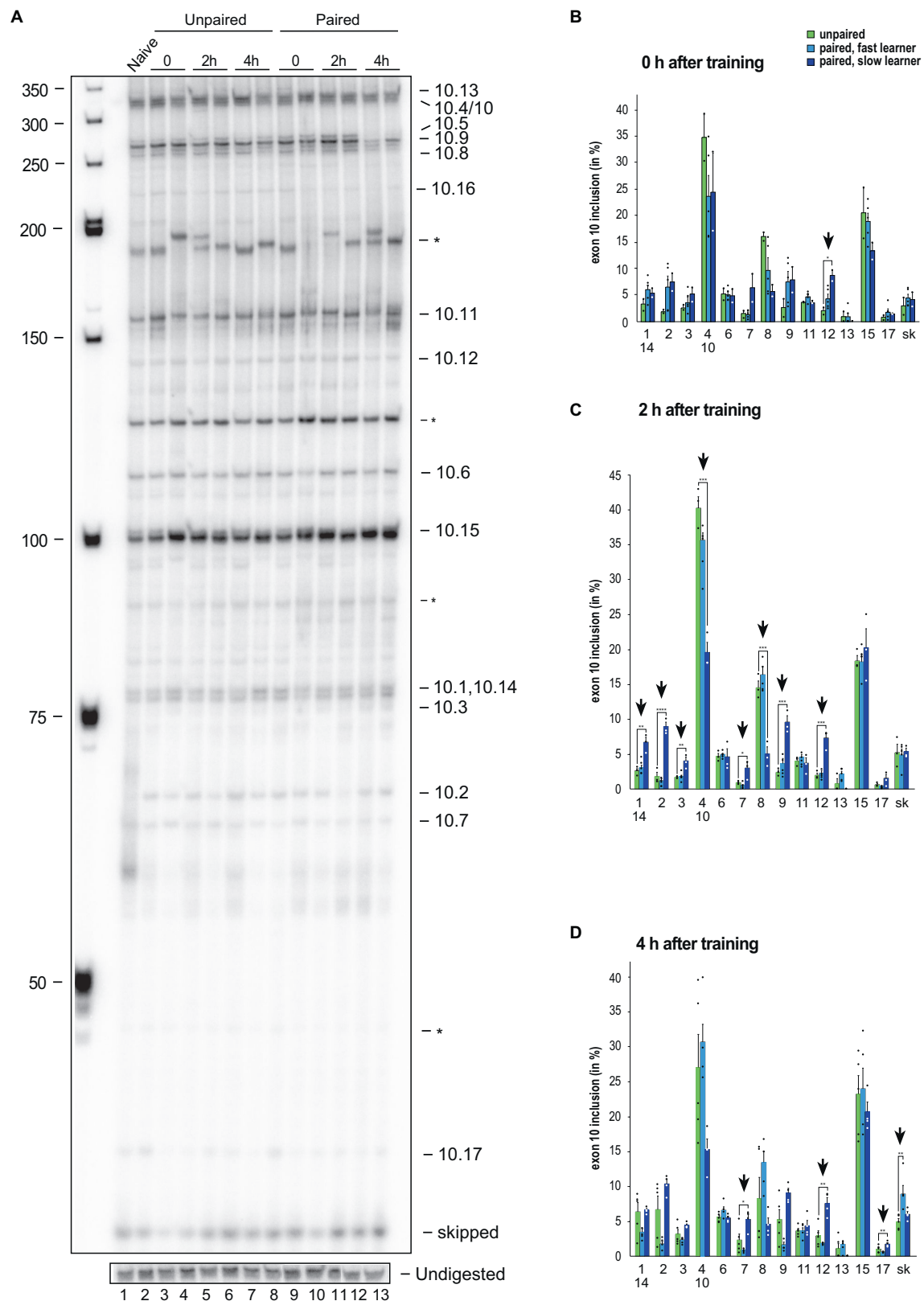


FIGURE 4

Down syndrome cell adhesion molecule (*Dscam*) variable exon 10 alternative splicing analysis during memory consolidation. **(A)** Representative 5% denaturing polyacrylamide gel separating 32 P-labeled alternative splice products from worker brains conditioned with unpaired (left) and paired odors and reward. Identity of splice variants are indicated at the right. M, Marker. **(B–D)** Analysis of alternative splicing unpaired control (green), fast (light blue) and slow learners (dark blue) indicating exon inclusion frequency (in%) for the different variables immediately after training (0 h), 2 or 4 h after training. Significant changes are indicated by vertical arrows (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). The source data underlying this figure are available in [Supplementary Data 1](#).

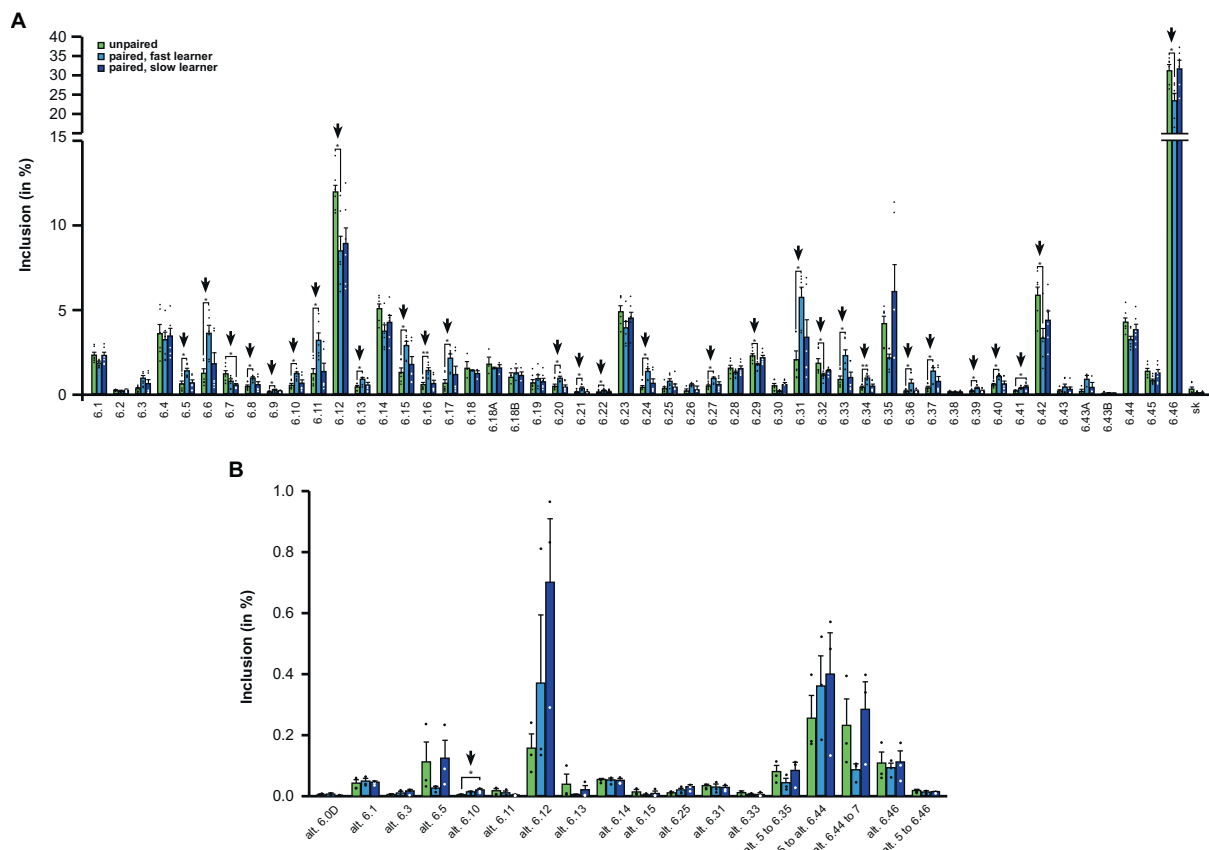


FIGURE 5

Down syndrome cell adhesion molecule (*Dscam*) variable exon 6 alternative splicing analysis during memory consolidation. (A,B) Analysis of alternative splicing unpaired control (green), fast (light blue) and slow learners (dark blue) indicating exon inclusion frequency (in%) from amplicon sequencing for the different variables 2 h after training for main variable (A) and alternative splice sites (B). Significant changes are indicated by vertical arrows (* $p < 0.05$; ** $p < 0.01$). The source data underlying this figure are available in [Supplementary Data 1](#).

significant changes could be detected in central brains. It will be of interest to follow up which neurons exactly change splicing upon learning, which might be possible with the development of long-read sequencing technology for single cell analysis to overcome current limitations in alternative splicing analysis (Zaharieva et al., 2012; Decio et al., 2019; Decio et al., 2021).

Memory consolidation requires transcription and implicates that alternative splicing can impact on the process of memory storage (Kandel et al., 2014; Ustaoglu et al., 2021). In honey bees, a first transcriptional wave occurs at the beginning of memory consolidation (Lefer et al., 2012). Of note, it is during this phase that also *Dscam* alternative splicing changes. And importantly, honey bees have displayed strikingly different splicing patterns depending on whether they actually learn or not during training. The most prominent changes occur in the exon 6 cluster, which compared to *Drosophila* has expanded by additional isoforms but also more frequent use alternative 5' and 3' splice sites, which is constituent with the more sophisticated neuroanatomy and behavioral performance of honey bees compared to *Drosophila*. In contrast, exon 4 and 10 clusters in honey bees have less isoforms. Lower diversity in these two clusters has been suggested to be associated with *Dscam*'s role as a pathogen receptor in the immune system as flies are extensively exposed to pathogens due to their life in a decomposing environment (Graveley et al., 2004).

Dscam diversity has been shown to change in mosquitos toward isoforms that recognize pathogens with higher affinity (Dong et al., 2006). Our finding that *Dscam* splicing changes upon learning further supports that the *Dscam* splicing pattern is not fixed and can change on demand. In fact, levels of *Dscam* have been found critical to neuronal function in *Drosophila* affecting nerve growth, synaptic targeting and neuronal physiology (Cvetkovska et al., 2013; Lowe et al., 2018; Hernández et al., 2023). Moreover, *Dscam* has also been identified as a target of Fragile X messenger ribonucleoprotein 1 (Fmr1) that together with mRNA modification pathways impacts on local translation important for neuronal functions such as learning and memory (Cvetkovska et al., 2013; Haussmann et al., 2022; Anreiter et al., 2023).

Consistent with subtle changes in *Dscam* expression being effective in altering nerve growth, synaptic targeting and neuronal physiology, we find changes in alternative splicing during memory consolidation. In particular, skipping of the entire variable cluster occurs for exon 4 and 10 shortly after training resulting in removal of part of the homophilic interaction domain concomitantly reducing cell adhesion (Wojtowicz et al., 2004).

Dynamic regulation of *Dscam* expression in the nervous system is somewhat unexpected based on *Dscam*'s role in mediating axonal bifurcation for exit from axonal tracts or spreading in dendritic fields based on *Dscam*'s homophilic repulsive properties.

Potentially, *Dscam*'s repulsive homophilic properties could only apply during development of the nervous system and convert to attraction to expand connectivity in mushroom bodies to consolidate memories. In fact, attractive properties of *Dscam* have been found with lower *Dscam* concentration (Wojtowicz et al., 2004). Moreover, *Dscam* has been shown roles in directed wiring of peripheral nervous system (PNS) neurons to innervate the ventral nerve cord (Chen et al., 2006). Likewise, whether homophilic binding of *Dscam* turns into repulsive or attractive cues is also dependent on interacting partners such as Netrins, Robo and Slit (Andrews et al., 2008; Liu et al., 2009; Alavi et al., 2016).

How *Dscam* mutually exclusive alternative splicing is regulated is still poorly understood. Initially, it has been proposed that base-pairing of a constant region at the beginning or end of the cluster with sequences in front of variable exons would direct inclusion of variable exons, but compelling sequence conservation is only present in the exon 6 cluster (Graveley, 2005; Haussmann et al., 2019; Ustaoglu et al., 2019). Our finding that inclusion of alternative exons from the variable clusters changes upon memory consolidation further suggests that there is a mechanism to alter inclusion of specific variables possibly to change neuronal connections for enhancing memory. Given an increasingly aging human population, deciphering the molecular mechanism underlying memory enhancement is of great interest in light of cognitive dysfunctions associated with Alzheimer's, Parkinson and other age-related diseases (Stern and Alberini, 2013; Noyes et al., 2021).

Materials and methods

Honey bees, treatment, and behavioral assays

For initial molecular analysis of honey bee *Dscam*, honey bees (*Apis mellifera*) were collected from local honey bee hives in the UK (worker honey bees were used unless otherwise specified). For developmental gene expression analysis and behavioral experiments, honey bees were taken from the experimental apiary on the university campus in Toulouse (France), on the morning of each experiment. For behavioral experiments, workers were harnessed in metal tubes following cold-anesthesia leaving access to the head, fed with 5 μ l of sucrose solution (50% weight/weight in water) and until needed kept in the dark at room temperature. They were fed in the same way on every morning and evening during the time of each experiment. Learning and memory experiments were done as described (Ustaoglu et al., 2021).

RNAi, Western analysis recombinant protein expression antibody stainings and imaging

For RNAi knockdown in honey bees, a *Dscam* DNA template of 820 bp was amplified spanning a cDNA of the most conserved part of *Dscam* (exons 11–14) with primers AM *Dscam* T7 RNAi 11F and AM *Dscam* T7 RNAi 14R containing a T7 promoter

on each side and cloned into pB SK + tango with Xho and *EcoRV* according to standard procedures (Soller and White, 2005). A 700 bp fragment for *GFP* was amplified as described (Ustaoglu et al., 2021). dsRNA was generated by *in vitro* transcription with T7 polymerase with the MegaScript kit (Ambion) for 3 h according to the manufacturer's instructions. After digestion of the template with TurboDNase (Ambion), dsRNA was phenol/chloroform extracted, ethanol precipitated and taken up in RNase free water at a concentration of 5 μ g/ μ l. The dsRNA (250 nl) was then injected into the brain through the median ocellus with a Nanoject II microinjector (Drummond).

RNAi efficiency testing for *Dscam* was done from dissected central brains by Western blotting according to standard protocols as described (Soller and White, 2005) using a polyclonal rat anti-*Dscam* antibody (1:1000, 358 against a constant part from amino acid 1650 to 2016) (Watson et al., 2005) and infrared dye coupled secondary antibodies (IRDye800CW, LI-COR) were used and detected with an Odyssey infrared imaging system (LI-COR). Tubulin was detected with a mouse anti- α tubulin antibody (1:10,000, clone DM1A, SIGMA). Quantification of Western blots was done with Quantity ONE 4.6.8 (BioRad) according to the manufacturer's instructions.

RT-PCR and analysis of alternative splicing

The sequence of oligonucleotides used in this study are listed in [Supplementary Table 1](#). RNA extraction from eggs or dissected central brains and RT-PCR was done as described (Koushika et al., 1999). For RT, AM GSP exon 13 RT1 was used at concentration of 10 nM spiked into oligo dT (10 μ M) (Haussmann et al., 2011).

For high resolution analysis of *Dscam* alternative splicing primers AM *Dscam* 3F1MH and AM *Dscam* 5R1 were used to amplify exon 4 variables, and AM *Dscam* 9F2 and AM *Dscam* 11R2 for exon 10 variables from cDNA by PCR with Taq according to the manufacturer's instructions for 40 cycles as described (Soller and White, 2005). One of the primers was labeled using gamma³²P-ATP (NEN). PCR products for exon 4 variables were digested with a combination of restriction enzymes *Sau3AI*, *HaeIII*, and *MboI*, and PCR products for exon 10 variables with a combination of restriction enzymes *RsaI*, *PvuII*, and *BsaJI*. Then, PCR products were separated on 5% sequencing type denaturing polyacrylamide gels. Polyacrylamide gels were dried, exposed to phosphorimager screens (BioRad) and quantified with QuantityOne (BioRad).

Amplicon sequencing and sequence analysis

For amplicon sequencing, primers in constant exons flanking the variable parts were used for amplification from cDNA generated from dissected central brains as described above. For amplification of variable exon 6 from three groups of three individual honey bees AM *Dscam* 5F1A, B and C were used for the different groups and AM *Dscam* 7R1A, B and C were used for the three individual honey bees. Amplicons were then quantified on ethidium bromide stained agarose gels against a DNA marker

(100 bp ladder, NEB), pooled and then Illumina sequenced (paired end, read-length 126) by GATC (Eurofins, Germany). Sequences were demultiplexed according to barcodes for exon 6 variables or by sequence cluster identity for exon 4 and 10 variables and barcodes removed using an in-house script. The demultiplexed data was mapped to the Honey Bee reference genome (version Amel_HAv3.1) using STAR (STAR version 272B, parameters: –alignIntronMin 20 –alignIntronMax 200000 –alignMatesGapMax 200000 –twopassMode Basic). Junction read coverage matching *Dscam* (AY686596) (Graveley et al., 2004) have been used to compute inclusion levels of splicing events, following which junction reads were manually checked.

Statistics and reproducibility

Multiple planned pairwise comparisons of expression levels were done by ANOVA followed by Fisher's protected least significance difference *post-hoc* test using Prism. To compare proportions of conditioned responses between groups, a repeated-measure analysis of variance (ANOVA) was run for the acquisition data (one factor, *treatment*, with *trial* as the repeated measure) (Mota and Giurfa, 2010). Comparisons of responses at each memory test were run using McNemar and Fischer's exact tests, for intra- and inter-group comparisons, respectively.

Data availability statement

All data are available in the main text or the **Supplementary Data 1**. Reagents are available upon reasonable request from the corresponding author. Amplicon-Seq data have been deposited at GEO (GSE244051).

Ethics statement

Ethical review and approval was not required for this study because this study was conducted with an invertebrate model – honey bee (*Apis mellifera*). Experiments with invertebrates are not regulated by law.

Author contributions

PU: Formal analysis, Investigation, Writing – review & editing. DM: Data curation, Formal analysis, Investigation, Writing – review & editing. AR: Formal analysis, Investigation, Writing – review & editing. TD: Formal analysis, Investigation, Writing – review & editing. IH: Conceptualization, Formal

analysis, Investigation, Writing – review & editing. RA: Data curation, Formal analysis, Supervision, Writing – review & editing. J-MD: Conceptualization, Formal analysis, Investigation, Validation, Writing – review & editing. MS: Conceptualization, Funding acquisition, Investigation, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnmol.2023.1322808/full#supplementary-material>

References

- Alavi, M., Song, M., King, G. L., Gillis, T., Propst, R., Lamanuzzi, M., et al. (2016). Dscam1 forms a complex with robo1 and the N-terminal fragment of slit to promote the growth of longitudinal axons. *PLoS Biol.* 14:e1002560. doi: 10.1371/journal.pbio.1002560

- Alberini, C. M. (2009). Transcription factors in long-term memory and synaptic plasticity. *Physiol. Rev.* 89, 121–145.
- Alberini, C. M., Ghirardi, M., Metz, R., and Kandel, E. R. (1994). C/EBP is an immediate-early gene required for the consolidation of long-term facilitation in *Aplysia*. *Cell* 76, 1099–1114.
- Andrews, G. L., Tanglao, S., Farmer, W. T., Morin, S., Brotman, S., Berberoglu, M. A., et al. (2008). Dscam guides embryonic axons by Netrin-dependent and -independent functions. *Development* 135, 3839–3848. doi: 10.1242/dev.023739
- Anreiter, I., Tian, Y. W., and Soller, M. (2023). The cap epitranscriptome: Early directions to a complex life as mRNA. *Bioessays* 45:e2200198. doi: 10.1002/bies.202200198
- Beffert, U., Weeber, E. J., Durudas, A., Qiu, S., Masiulis, I., Sweatt, J. D., et al. (2005). Modulation of synaptic plasticity and memory by Reelin involves differential splicing of the lipoprotein receptor Apoer2. *Neuron* 47, 567–579. doi: 10.1016/j.neuron.2005.07.007
- Chen, B. E., Kondo, M., Garnier, A., Watson, F. L., Puettmann-Holgado, R., Lamar, D. R., et al. (2006). The molecular diversity of Dscam is functionally required for neuronal wiring specificity in *Drosophila*. *Cell* 125, 607–620.
- Clayton, D. F. (2000). The genomic action potential. *Neurobiol. Learn. Mem.* 74, 185–216.
- Crayton, M. E. III, Powell, B. C., Vision, T. J., and Giddings, M. C. (2006). Tracking the evolution of alternatively spliced exons within the Dscam family. *BMC Evol. Biol.* 6:16. doi: 10.1186/1471-2148-6-16
- Cvetkovska, V., Hibbert, A. D., Emran, F., and Chen, B. E. (2013). Overexpression of Down syndrome cell adhesion molecule impairs precise synaptic targeting. *Nat. Neurosci.* 16, 677–682. doi: 10.1038/nn.3396
- Decio, P., Ustaoglu, P., Derecka, K., Hardy, I. C. W., Roat, T. C., Malaspina, O., et al. (2021). Thiamethoxam exposure deregulates short ORF gene expression in the honey bee and compromises immune response to bacteria. *Sci. Rep.* 11:1489. doi: 10.1038/s41598-020-80620-7
- Decio, P., Ustaoglu, P., Roat, T. C., Malaspina, O., Devaud, J. M., Stoger, R., et al. (2019). Acute thiamethoxam toxicity in honeybees is not enhanced by common fungicide and herbicide and lacks stress-induced changes in mRNA splicing. *Sci. Rep.* 9:19196.
- Demares, F., Drouard, F., Massou, I., Crattelet, C., Loeuillet, A., Bettiol, C., et al. (2014). Differential involvement of glutamate-gated chloride channel splice variants in the olfactory memory processes of the honeybee *Apis mellifera*. *Pharmacol. Biochem. Behav.* 124, 137–144. doi: 10.1016/j.pbb.2014.05.025
- Demares, F., Raymond, V., and Armengaud, C. (2013). Expression and localization of glutamate-gated chloride channel variants in honeybee brain (*Apis mellifera*). *Insect Biochem. Mol. Biol.* 43, 115–124.
- Dong, Y., Taylor, H. E., and Dimopoulos, G. (2006). AgDscam, a hypervariable immunoglobulin domain-containing receptor of the Anopheles gambiae innate immune system. *PLoS Biol.* 4:e229. doi: 10.1371/journal.pbio.0040229
- Graveley, B. R. (2005). Mutually exclusive splicing of the insect Dscam pre-mRNA directed by competing intronic RNA secondary structures. *Cell* 123, 65–73. doi: 10.1016/j.cell.2005.07.028
- Graveley, B. R., Kaur, A., Gunning, D., Zipursky, S. L., Rowen, L., and Clemens, J. C. (2004). The organization and evolution of the dipteran and hymenopteran Down syndrome cell adhesion molecule (Dscam) genes. *RNA* 10, 1499–1506. doi: 10.1261/rna.7105504
- Haussmann, I. U., Li, M., and Soller, M. (2011). ELAV-mediated 3'-end processing of ewg transcripts is evolutionarily conserved despite sequence degeneration of the ELAV-binding site. *Genetics* 189, 97–107. doi: 10.1534/genetics.111.131383
- Haussmann, I. U., Ustaoglu, P., Brauer, U., Hemani, Y., Dix, T. C., and Soller, M. (2019). Plasmid-based gap-repair recombined transgenes reveal a central role for introns in mutually exclusive alternative splicing in Down Syndrome Cell Adhesion Molecule exon 4. *Nucleic Acids Res.* 47, 1389–1403. doi: 10.1093/nar/gky1254
- Haussmann, I. U., Wu, Y., Nallasivan, M. P., Archer, N., Bodi, Z., Hebenstreit, D., et al. (2022). CMT α cap-adjacent 2'-O-ribose methyltransferases are required for reward learning and mRNA localization to synapses. *Nat. Commun.* 13:1209. doi: 10.1038/s41467-022-28549-5
- Hemani, Y., and Soller, M. (2012). Mechanisms of *Drosophila* Dscam mutually exclusive splicing regulation. *Biochem. Soc. Trans.* 40, 804–809.
- Hernández, K., Godoy, L., Newquist, G., Kellermeyer, R., Alavi, M., Mathew, D., et al. (2023). Dscam1 overexpression impairs the function of the gut nervous system in *Drosophila*. *Dev. Dyn.* 252, 156–171. doi: 10.1002/dvdy.554
- Hourcade, B., Muenz, T. S., Sandoz, J. C., Rossler, W., and Devaud, J. M. (2010). Long-term memory leads to synaptic reorganization in the mushroom bodies: A memory trace in the insect brain? *J. Neurosci.* 30, 6461–6465. doi: 10.1523/JNEUROSCI.0841-10.2010
- Hu, Y., Flockhart, I., Vinayagam, A., Bergwitz, C., Berger, B., Perrimon, N., et al. (2011). An integrative approach to ortholog prediction for disease-focused and other functional studies. *BMC Bioinformatics* 12:357. doi: 10.1186/1471-2105-12-357
- Hughes, M. E., Bortnick, R., Tsubouchi, A., Baumer, P., Kondo, M., Uemura, T., et al. (2007). Homophilic Dscam interactions control complex dendrite morphogenesis. *Neuron* 54, 417–427. doi: 10.1016/j.neuron.2007.04.013
- Kandel, E. R., Dudai, Y., and Mayford, M. R. (2014). The molecular and systems biology of memory. *Cell* 157, 163–186.
- Koushika, S. P., Soller, M., Desimone, S. M., Daub, D. M., and White, K. (1999). Differential and inefficient splicing of a broadly expressed *Drosophila* erect wing transcript results in tissue-specific enrichment of the vital EWG protein isoform. *Mol. Cell Biol.* 19, 3998–4007. doi: 10.1128/MCB.19.6.3998
- Lefer, D., Perisse, E., Hourcade, B., Sandoz, J., and Devaud, J. M. (2012). Two waves of transcription are required for long-term memory in the honeybee. *Learn. Mem.* 20, 29–33.
- Liu, G., Li, W., Wang, L., Kar, A., Guan, K. L., Rao, Y., et al. (2009). DSCAM functions as a netrin receptor in commissural axon pathfinding. *Proc. Natl. Acad. Sci. U. S. A.* 106, 2951–2956.
- Liu, H., Caballero-Florán, R. N., Hergenreder, T., Yang, T., Hull, J. M., Pan, G., et al. (2023). DSCAM gene triplication causes excessive GABAergic synapses in the neocortex in Down syndrome mouse models. *PLoS Biol.* 21:e3002078. doi: 10.1371/journal.pbio.3002078
- Lowe, S. A., Hodge, J. J. L., and Usowicz, M. M. (2018). A third copy of the Down syndrome cell adhesion molecule (Dscam) causes synaptic and locomotor dysfunction in *Drosophila*. *Neurobiol. Dis.* 110, 93–101. doi: 10.1016/j.nbd.2017.11.013
- Matthews, B. J., Kim, M. E., Flanagan, J. J., Hattori, D., Clemens, J. C., Zipursky, S. L., et al. (2007). Dendrite self-avoidance is controlled by Dscam. *Cell* 129, 593–604. doi: 10.1016/j.cell.2007.04.013
- Miura, S. K., Martins, A., Zhang, K. X., Graveley, B. R., and Zipursky, S. L. (2013). Probabilistic splicing of Dscam1 establishes identity at the level of single neurons. *Cell* 155, 1166–1177. doi: 10.1016/j.cell.2013.10.018
- Mota, T., and Giurfa, M. (2010). Multiple reversal olfactory learning in honeybees. *Front. Behav. Neurosci.* 4:48. doi: 10.3389/fnbeh.2010.00048
- Neves, G., Zucker, J., Daly, M., and Chess, A. (2004). Stochastic yet biased expression of multiple Dscam splice variants by individual cells. *Nat. Genet.* 36, 240–246. doi: 10.1038/ng1299
- Noyes, N. C., Phan, A., and Davis, R. L. (2021). Memory suppressor genes: Modulating acquisition, consolidation, and forgetting. *Neuron* 109, 3211–3227. doi: 10.1016/j.neuron.2021.08.001
- Palavalli, A., Tizón-Escamilla, N., Rupprecht, J. F., and Lecuit, T. (2021). Deterministic and stochastic rules of branching govern dendrite morphogenesis of sensory neurons. *Curr. Biol.* 31, 459–472.e4. doi: 10.1016/j.cub.2020.10.054
- Poplawski, S. G., Peixoto, L., Porcari, G. S., Wimmer, M. E., McNally, A. G., Mizuno, K., et al. (2016). Contextual fear conditioning induces differential alternative splicing. *Neurobiol. Learn. Mem.* 134(Pt B), 221–235. doi: 10.1016/j.nlm.2016.07.018
- Schmucker, D., and Chen, B. (2009). Dscam and DSCAM: Complex genes in simple animals, complex animals yet simple genes. *Genes Dev.* 23, 147–156.
- Schmucker, D., Clemens, J. C., Shu, H., Worby, C. A., Xiao, J., Muda, M., et al. (2000). *Drosophila* Dscam is an axon guidance receptor exhibiting extraordinary molecular diversity. *Cell* 101, 671–684. doi: 10.1016/S0092-8674(00)80878-8
- Sengar, A. S., Li, H., Zhang, W., Leung, C., Ramani, A. K., Saw, N. M., et al. (2019). Control of long-term synaptic potentiation and learning by alternative splicing of the NMDA receptor subunit GluN1. *Cell Rep.* 29:e5. doi: 10.1016/j.celrep.2019.11.087
- Soba, P., Zhu, S., Emoto, K., Younger, S., Yang, S. J., Yu, H. H., et al. (2007). *Drosophila* sensory neurons require Dscam for dendritic self-avoidance and proper dendritic field organization. *Neuron* 54, 403–416. doi: 10.1016/j.neuron.2007.03.029
- Soller, M. (2006). Pre-messenger RNA processing and its regulation: A genomic perspective. *Cell Mol Life Sci* 63, 796–819.
- Soller, M., and White, K. (2005). ELAV multimerizes on conserved AU4-6 motifs important for ewg splicing regulation. *Mol. Cell Biol.* 25, 7580–7591. doi: 10.1128/MCB.25.17.7580-7591.2005
- Stern, S. A., and Alberini, C. M. (2013). Mechanisms of memory enhancement. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 5, 37–53.
- Torres-Mendez, A., Bonnal, S., Marquez, Y., Roth, J., Iglesias, M., Permanyer, J., et al. (2019). A novel protein domain in an ancestral splicing factor drove the evolution of neural microexons. *Nat. Ecol. Evol.* 3, 691–701. doi: 10.1038/s41559-019-0813-6
- Torres-Méndez, A., Pop, S., Bonnal, S., Almudi, I., Avola, A., Roberts, R. J. V., et al. (2022). Parallel evolution of a splicing program controlling neuronal excitability in flies and mammals. *Sci. Adv.* 8:eabk0445. doi: 10.1126/sciadv.abk0445
- Ule, J., and Blencowe, B. J. (2019). Alternative splicing regulatory networks: Functions, mechanisms, and evolution. *Mol. Cell* 76, 329–345.
- Ustaoglu, P., Gill, J. K., Doubovetzky, N., Haussmann, I. U., Dix, T. C., Arnold, R., et al. (2021). Dynamically expressed single ELAV/Hu orthologue elavl2 of bees is required for learning and memory. *Commun. Biol.* 4:1234. doi: 10.1038/s42003-021-02763-1

- Ustaoglu, P., Haussmann, I. U., Liao, H., Torres-Mendez, A., Arnold, R., Irimia, M., et al. (2019). Srrm234, but not canonical SR and hnRNP proteins, drive inclusion of Dscam exon 9 variable exons. *RNA* 25, 1353–1365. doi: 10.1261/rna.071316.119
- Vacca, R. A., Bawari, S., Valenti, D., Tewari, D., Nabavi, S. F., Shirooie, S., et al. (2019). Down syndrome: Neurobiological alterations and therapeutic targets. *Neurosci. Biobehav. Rev.* 98, 234–255.
- Villar, M. E., Marchal, P., Viola, H., and Giurfa, M. (2020). Redefining single-trial memories in the honeybee. *Cell Rep.* 30:e3. doi: 10.1016/j.celrep.2020.01.086
- Vuong, C. K., Black, D. L., and Zheng, S. (2016). The neurogenetics of alternative splicing. *Nat. Rev. Neurosci.* 17, 265–281.
- Wang, J., Zugates, C. T., Liang, I. H., Lee, C. H., and Lee, T. (2002). Drosophila Dscam is required for divergent segregation of sister branches and suppresses ectopic bifurcation of axons. *Neuron* 33, 559–571. doi: 10.1016/s0896-6273(02)00570-6
- Watson, F. L., Puttmann-Holgado, R., Thomas, F., Lamar, D. L., Hughes, M., Kondo, M., et al. (2005). Extensive diversity of Ig-superfamily proteins in the immune system of insects. *Science* 309, 1874–1878. doi: 10.1126/science.1116887
- Wojtowicz, W. M., Flanagan, J. J., Millard, S. S., Zipursky, S. L., and Clemens, J. C. (2004). Alternative splicing of Drosophila Dscam generates axon guidance receptors that exhibit isoform-specific homophilic binding. *Cell* 118, 619–633. doi: 10.1016/j.cell.2004.08.021
- Wojtowicz, W. M., Wu, W., Andre, I., Qian, B., Baker, D., and Zipursky, S. L. (2007). A vast repertoire of Dscam binding specificities arises from modular interactions of variable Ig domains. *Cell* 130, 1134–1145. doi: 10.1016/j.cell.2007.08.026
- Zaharieva, E., Chipman, J. K., and Soller, M. (2012). Alternative splicing interference by xenobiotics. *Toxicology* 296, 1–12.
- Zhang, Z., So, K., Peterson, R., Bauer, M., Ng, H., Zhang, Y., et al. (2019). Elav-mediated exon skipping and alternative polyadenylation of the dscam1 gene are required for axon outgrowth. *Cell Rep.* 27, 3808–3817.e7. doi: 10.1016/j.celrep.2019.05.083