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REVIEW

Maggot learning and Synapsin function

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Summary

***Drosophila* larvae are focused on feeding and have few neurons. Within these bounds, however, there still are behavioural degrees of freedom. This review is devoted to what these elements of flexibility are, and how they come about. Regarding odour–food associative learning, the emerging working hypothesis is that when a mushroom body neuron is activated as a part of an odour-specific set of mushroom body neurons, and coincidently receives a reinforcement signal carried by aminergic neurons, the AC-cAMP-PKA cascade is triggered. One substrate of this cascade is Synapsin, and therefore this review features a general and comparative discussion of Synapsin function. Phosphorylation of Synapsin ensures an alteration of synaptic strength between this mushroom body neuron and its target neuron(s). If the trained odour is encountered again, the pattern of mushroom body neurons coding this odour is activated, such that their modified output now allows conditioned behaviour. However, such an activated memory trace does not automatically cause conditioned behaviour. Rather, in a process that remains off-line from behaviour, the larvae compare the value of the testing situation (based on gustatory input) with the value of the odour-activated memory trace (based on mushroom body output). The circuit towards appetitive conditioned behaviour is closed only if the memory trace suggests that tracking down the learned odour will lead to a place better than the current one. It is this expectation of a positive outcome that is the immediate cause of appetitive conditioned behaviour. Such conditioned search for reward corresponds to a view of aversive conditioned behaviour as conditioned escape from punishment, which is enabled only if there is something to escape from – much in the same way as we only search for things that are not there, and run for the emergency exit only when there is an emergency. One may now ask whether beyond 'value' additional information about reinforcement is contained in the memory trace, such as information about the kind and intensity of the reinforcer used. The *Drosophila* larva may allow us to develop satisfyingly detailed accounts of such mnemonic richness – if it exists.**

Key words: cognition, *Drosophila melanogaster*, gustation, retrieval, memory, olfaction, PKA.

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Behavioural paradigms for associative learning in larval *Drosophila*

The brain is not the maggot's most impressive organ (Fig. 1). It contains an estimated order of magnitude fewer neurons than that of adult *Drosophila*, and is correspondingly smaller: the entire antennal lobe of a stage 3 larva fits into a single glomerulus of an adult fly (and a fly brain fits into the antennal lobe of a bee, we hesitate to add). Indeed, a larva's brain is considerably smaller than its salivary glands, betraying that one way or another much of brain function is devoted to food uptake, while a number of other occupations, such as flying, courtship, copulation and egg-laying, obviously are of no concern to a larva. Thus, with the evolutionary 'outsourcing' of the feeding stage into a separate, larval form of life in the holometabolous insects, not only did various external structures such as wings, legs and sex organs become dispensable, but a number of brain structures did as well, such as the central complex. Given the relatively slow locomotion of larvae, sensory systems can operate at massively reduced cell numbers. This is particularly striking in the visual system, which in the adult is comprised of approximately 13,000 neurons (2×6000 photoreceptor neurons in the eyes, 3×80 in the ocelli and 2×6 in the Hofbauer–Buchner eyelet) as compared with 24 in larvae [2×12 (Steller et al., 1987)]; we note that in line with our above comment the gustatory, cephalic contact chemosensory system is relatively well equipped in the larva with 80 (Python and Stocker, 2002) to 90 (Colomb et al., 2007) neurons per body side. Therefore, the maggot brain is impressive – in both numerical simplicity and functional 'focus'. Notably, within these bounds there still are behavioural degrees of freedom for the larvae. This review is devoted to what these elements of flexibility are, and how they come about. It aims at two goals: providing an overview of maggot learning as well as a detailed and comparative account of Synapsin function. This is because Synapsin provides an evolutionarily conserved experimental handle for unbroken chains of explanation between the behavioural and the molecular levels of mnemonic processing.

We restrict ourselves to Pavlovian conditioning. In this form of associative learning, animals learn the predictive relationship between two events, allowing them to behave according to the causal texture of the world (Dickinson, 2001). Regarding adult *Drosophila*, this research was pioneered by Benzer and colleagues (Quinn et al., 1974). In the presently used form (Tully and Quinn,

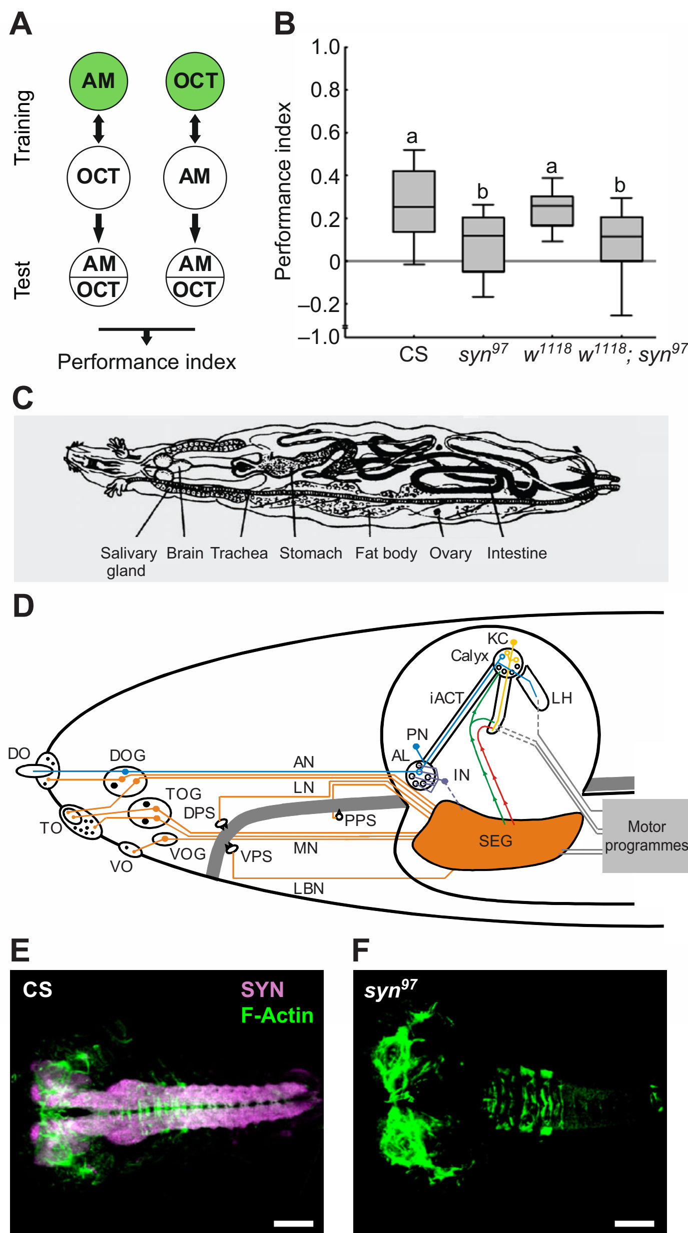

Fig. 1. (A) The odour–sugar associative learning paradigm. Circles represent Petri dishes containing a sugar reward (fructose, green) or plain agarose (white). Groups of 30 larvae each are either trained such that *n*-amylacetate is rewarded and 1-octanol is not (AM+/OCT), or are trained reciprocally (OCT+/AM) (CAS numbers for AM and OCT: 628-63-7, 111-87-5). After three such training cycles, larvae are tested for their choice between AM *versus* OCT (for half of the cases, the sequence of training trials is reversed: OCT/AM+ and AM/OCT+). From the difference in preference between the reciprocally trained groups the performance index is calculated to quantify associative learning (for details, see Gerber et al., 2010). (B) Associative function is reduced by ~50% in syn^{97} mutant larvae relative to CS wild-type larvae; this effect is also seen in the w^{1118} background. Note that associative function is equal in CS wild-type and w^{1118} mutant larvae; this is important as one typically uses w^{1118} as genetic background for keeping track of transgenes when employing the Gal4-UAS technique. Box plots present the median as the horizontal line; box boundaries and whiskers present the 25th and 75th, and 10th and 90th quantiles, respectively. Different lettering indicates significant differences in Mann–Whitney *U*-tests corrected for multiple comparisons. For behavioural controls, see the Synapsin function, *Drosophila* section. (C) Body plan of larval *Drosophila* (modified from Demerec and Kaufmann, 1972). (D) Overview of the cephalic chemosensory pathways of the larva (modified from Stocker, 2008). Olfactory sensory neurons (blue) project towards the brain hemispheres into the antennal lobe and then, *via* projection neurons (blue), towards both the mushroom body (yellow) calyx region and the lateral horn. Gustatory sensory neurons (orange) bypass the brain hemispheres and are collected in various regions of the subesophageal ganglion. The green and red arrows indicate pathways to short-circuit taste-driven aminergic reinforcement signals from the subesophageal ganglion towards the mushroom body. Abbreviations: AL, antennal lobe; AN, antennal nerve; DO/DOG, dorsal organ/dorsal organ ganglion; DPS, dorsal pharyngeal sensillae; iACT, inner antenno-cerebral tract; IN, antennal lobe interneurons; KC, Kenyon cells comprising the mushroom body; LBN, labial nerve; LH, lateral horn; LN, labral nerve; MN, maxillary nerve; PN, projection neurons; PPS: posterior pharyngeal sensillae; SEG: suboesophageal ganglion; TO/TOG, terminal organ/terminal organ ganglion; VO/VOG, ventral organ/ventral organ ganglion; VPS, ventral pharyngeal sensillae. (E,F) Immunoreactivity against Synapsin (magenta) and F-Actin (green) in third instar larval brains of (E) a wild-type (CS) larva and (F) a syn^{97} mutant larva. Synapsin immunoreactivity is found throughout the neuropil area of the brain and ventral nerve cord in the wild type, but is absent in the syn^{97} mutant. Scale bars, 50 μm. Panels B, E and F are based on published data (Michels et al., 2011).

1985), the paradigm presents flies with an odour (A) together with electric shock (–), while another odour is presented without shock (B). After such A–/B training, the animals are offered a choice between A and B (A *versus* B). Because A signals upcoming shock, the flies escape from A and/or move towards the previously unpunished, safe odour B. In order to average out a possible innate preference for B, it is essential to run the experiment in a reciprocal manner (A/B–, test A *versus* B) in a second set of animals. Given that the two experimental groups differ in only the target parameter, namely the association between odours and shock, this allows one to calculate an associative performance index by comparing the A *versus* B choice after A–/B training to the A *versus* B choice after the reciprocal A/B– training (Tully, 1984) (paradigms and findings not using such reciprocal design and thus potentially confounded by non-associative effects are not considered here). Approximately a decade later this paradigm was supplemented with an appetitive version (A+/B, test A *versus* B as well as the reciprocal A/B+, test A *versus* B), where a sugar reward instead of shock punishment is used to induce a conditioned search for the sugar at the previously rewarded odour (Tempel et al., 1983). These two forms of learning attracted much experimental attention, and are, owing to the joint efforts of the *Drosophila* community, among the better understood study cases in the field of learning and memory to date (reviewed in Heisenberg, 2003; Gerber et al., 2004b; Margulies et al., 2005; McGuire et al., 2005; Schwärzel and Müller, 2006; Keene and Waddell, 2007; Heisenberg and Gerber, 2008; Zars, 2010; Davis, 2011).

Five years after the seminal paper on adult *Drosophila*, a corresponding paradigm for odour–shock associative learning in larvae was introduced (Aceves-Piña and Quinn, 1979), but compared with adult *Drosophila* the progress in understanding this form of learning has been slow (Tully et al., 1994; Khurana et al., 2009; Pauls et al., 2010a). Notably, it was as much as 25 years later that Scherer et al. (Scherer et al., 2003) introduced an appetitive version of this assay, using fructose as food reward (Gerber et al., 2010). Maybe thanks to the ecological validity of odour–food

associations, this low-tech assay turned out to be robust and was adopted both in a number of research programmes and in teaching at graduate, undergraduate as well as high school levels. Subsequently, low concentrations of salt [~0.5 $mol\,l^{-1}$ (Niewalda et al., 2008; Russell et al., 2011)] were likewise found to be effective as a reward, whereas high concentrations of salt as well as quinine (Gerber and Hendel, 2006; Niewalda et al., 2008; Selcho et al., 2009; Russell et al., 2011; Schleyer et al., 2011) and mechanosensory disturbances (Eschbach et al., 2011) can work as punishment. Last, but not least, an associative light–tastant learning paradigm is available (Gerber et al., 2004a), but it yields a less favourable signal-to-noise ratio as compared with odour–tastant learning (possibly because it is less ecologically valid), and therefore has received less experimental attention (Kaun et al., 2007; von Essen et al., 2011). In the following sections, we sketch the circuitry and molecular mechanisms for the acquisition and storage of odour–reward memory traces, and will discuss how these memory traces, once established, are used for behavioural organization.

Chemobehavioural circuitry of larval *Drosophila*

In general, the behavioural repertoire towards odours is largely restricted to orienting movements and subsequent search or escape behaviour, dependent on the presumed nature of the odour source. In other words, animals use odours 'to guess what's over there', to decide whether to search for or escape from the odour source, and to prepare for action in case the odour source is successfully tracked, or should escape fail. In order for this 'guesswork' to be as informed as possible, the olfactory sensory-motor loop features stages of processing with an enormous potential to discriminate odours, as well as to attach acquired meaning to them.

In contrast, contact chemosensation serves to behave in situations of immediate physical contact. Indeed, contact chemosensation is closely entangled with mechanosensation. Typically, contact chemosensory sensilla in addition to chemosensory receptor neurons also harbour one mechanosensory neuron (Falk et al., 1976; Awasaki and Kimura, 1997; Ishimoto and Tanimura, 2004), serving to integrate the chemosensory 'what' with the mechanosensory 'where'. This may allow for fairly direct and often local, i.e. body-site specific, sensory-motor loops in diverse contexts such as predation and defence, kin and/or nestmate recognition, aggression, the pursuit of courtship and copulation, oviposition and pupariation. Furthermore, cephalic and oral contact chemosensation organizes eating and drinking behaviour, and it is this 'taste' system that is of relevance in the present review.

The cellular architecture of the chemobehavioural system in *Drosophila* was revealed by the systematic analyses of the Stocker group over the past 30 years (reviewed in Stocker, 1994; Stocker, 2001; Vosshall and Stocker, 2007; Stocker, 2008), providing a basis for the ongoing work at the molecular and behavioural levels. It turned out that this architecture in the larva is similar to the one in adults, but with reduced cell numbers. In simplified terms, odours activate specific combinations of the 21 olfactory sensory neurons housed in the dome of the dorsal organ of each body side, largely dependent on the ligand profile of the receptor expressed in the respective cell [for detailed reviews and reference to the relevant original literature, as well as for exceptions to the 'rules of thumb' presented here, please consult the following papers (Stocker 1994; Cobb, 1999; Tissot and Stocker, 2000; Gerber and Stocker, 2007; Melcher et al., 2007; Vosshall and Stocker, 2007; Stocker, 2008; Cobb et al., 2009; Gerber et al., 2009)]. Each of these cells expresses one member of the *Or* receptor gene family [plus the *Orco* (CG10609) gene product (Vosshall and Hansson, 2011) that is necessary for proper receptor trafficking and function], and projects to one glomerulus in the larval antennal lobe; these glomeruli can be identified by position. In turn, each receptor gene is expressed by one olfactory sensory neuron, and each glomerulus receives input from one of these sensory neurons. Whether the receptors of the *Ir* gene family (Benton et al., 2009) play a role for larval chemosensation remains to be investigated.

In the antennal lobe, lateral connections between the glomeruli [(Thum et al., 2011); this study also contains the description of hitherto unrecognized neurons connecting the antennal lobe and the subesophageal ganglion] shape the pattern of activation that then is carried forward by the output elements of the antennal lobe, the projection neurons. The projection neurons typically receive input in one glomerulus; correspondingly, a given antennal lobe glomerulus harbours the dendrites from typically one projection neuron. Thus up to this level in the circuit, the olfactory system lacks cellular redundancy. Notably, the axons of the projection neurons are branched: one axon collateral conveys odour information directly towards the lateral horn and presumably onto pre-motor circuits; the other collateral establishes a detour of olfactory information flow *via* the mushroom bodies. In the input region of the mushroom bodies, the calyx, the coding space is massively expanded, and then is massively reduced. That is, each of the approximately 21 projection neurons typically innervates one of the approximately 35 mushroom body glomeruli (thus, some mushroom body glomeruli apparently receive input from neurons other than the olfactory projection neurons). Different from the situation in adults, many of the mushroom body glomeruli can be identified by position, revealing a reliable relationship between the antennal lobe glomerulus, in which a projection neuron receives input, and the mushroom body glomerulus, to which it provides output. This allows the majority of the projection neurons to be individually identified. Each of the strikingly many mature mushroom body neurons [~600, of which 250–300 are embryonic-born and may be of particular relevance for associative function (Pauls et al., 2010b)] receives convergent input in an apparently random set of one to six mushroom body glomeruli and hence from one to six projection neurons (Fig. 2C). Depending on how many of these inputs need to be activated to drive the mushroom body neuron, it thus may sample up to a third of the larval odour space. In turn, each projection neuron diverges to 30–180 mushroom body neurons [for details of this approximation, see Gerber and Stocker (Gerber and Stocker, 2007)], thus reporting to a third of the mushroom body coding space (Fig. 2D). Assuming that mushroom body neurons need coincident excitatory input from more than one projection neuron to fire, this combined convergent–divergent architecture allows for combinatorial coding and can enhance small differences in input [for a review of temporal aspects of olfactory coding, see Laurent et al. (Laurent et al., 2001)]. Output from the mushroom bodies is drawn by relatively few neurons [a reasonable guess based on Pauls et al. (Pauls et al., 2010b) is that these may be an order of magnitude fewer neurons than mushroom body neurons], which each sample many, if not all, mushroom body neurons, and which project onto pre-motor circuitry.

It is important to note that the pre-motor systems, ill-characterized as they are, thus receive two kinds of olfactory signal, one from the direct pathway and one *via* the mushroom body detour. As will be argued below, the direct pathway mediates innate

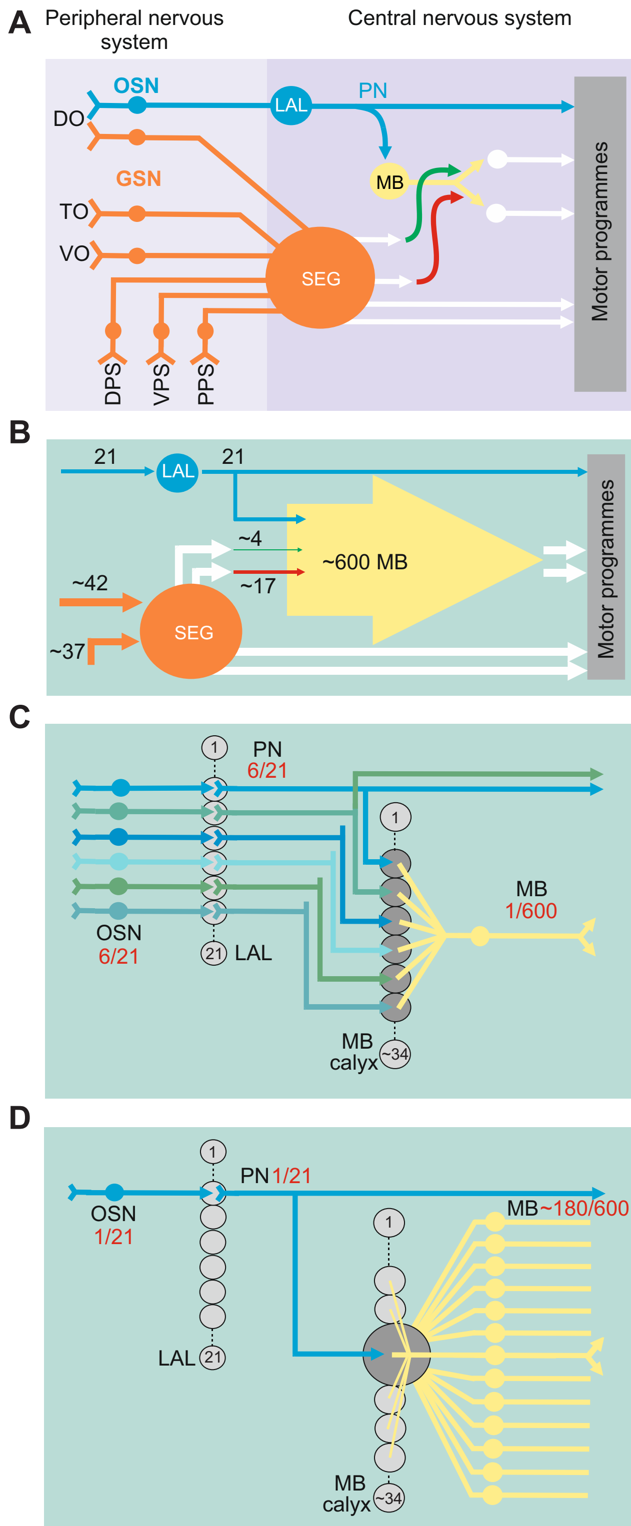

Fig. 2. Schematic representation of the cephalic olfactory (blue) and gustatory (orange) pathways of the *Drosophila* larva, and their downstream elements, in particular the mushroom body (MB) neurons (yellow). White fill indicates unknown or relatively ill-characterized parts of the circuit. (A) Olfactory sensory neurons (OSN) housed in the dome of the dorsal organ (DO) terminate in the larval antennal lobes (LAL). From the LAL, projection neurons (PN) deliver olfactory information towards (pre)motor circuits *via* two routes, directly *via* the lateral horn (not shown) sufficient for innate olfactory behaviour, and through a detour *via* the MB. Gustatory sensory neurons (GSN) from the bulge of the DO, the terminal and ventral organs (TO, VO), and the internal dorsal, ventral and posterior pharyngeal sense organs (DPS, VPS, PPS) terminate in the subesophageal ganglion (SEG); note that DO, TO, VPS and likely the DPS in addition contain non-gustatory neurons. From the SEG, gustatory processing follows two routes. One route connects towards innate gustatory behaviour. The second route consists of modulatory octopaminergic/tyraminergic (OA, green) and dopaminergic (DA, red) neurons. These have a number of targets (Selcho et al., 2009; Selcho et al., 2012); here we show those that likely relay reinforcement information towards the MB neurons; note that OA and DA innervate different subcompartments of the MB. The net effect of the OA (as covered by TDC-Gal4) system is rewarding, and the net effect of the DA system (as covered by TH-Gal4) is punishing (see last paragraph of Chemobehavioural circuitry of larval *Drosophila* for important caveats concerning this simplified dichotomy). Thus, the MB neurons are a likely cellular site of convergence of olfactory processing and reinforcement signalling. Downstream of the MB, conditioned olfactory behaviour is organized (for details of the organization of conditioned behaviour, see Fig. 6). Note that the LAL also harbours intrinsic neurons and neurons to connect LAL and SEG (not shown). (B) Simplified version of A, with the size of the elements drawn according to the number of known involved cells; numbers are per hemisphere. (C,D) Convergence–divergence relationships of the PN–MB interface at the calyx of the MB. Up to six of the 21 OSN–PN input lines converge onto a given MB neuron (C). In turn, within its cognate MB glomerulus (enlarged for clarity), a given PN diverges to up to 180 of the 600 MBs (D) (Gerber and Stocker, 2007); note that this number game is based on the simplified assumption that MB neurons receive input from six PNs (these six connections are drawn only for one MB neuron) although MBs actually are heterogeneous, connecting to one to six PNs; also note that it is unknown how many PNs need to be active to drive a MB neuron.

behaviour such as olfactory choice in experimentally naive animals, while the mushroom body detour mediates learned behavioural tendencies towards odours.

Compared with the olfactory system, the taste system is less well understood (for reviews, see Gerber and Stocker, 2007; Melcher et al., 2007; Cobb et al., 2009; Gerber et al., 2009; and references therein). It comprises approximately 2×80–90 gustatory sensory neurons, located in six paired sensory structures, three of them external (terminal organ, ventral organ and the bulge of the dorsal organ surrounding the central dome) and three internal (ventral, dorsal and posterior pharyngeal sense organs). The gustatory sensory neurons bypass the brain proper and project to the subesophageal ganglion in a way that depends on the receptor gene(s) expressed and their sense organ of origin (Colomb et al., 2007). Taste information then is relayed to modulatory neurons, which detour towards the brain and/or to the ventral nerve cord, as well as to (pre-)motor circuitry presumably in the ventral nerve cord to mediate taste-related behaviours. Such behaviours include preference for sugars and low salt concentrations, as well as avoidance of high salt concentrations and ‘bitter’ substances; both sugars and salt stimulate feeding at relatively low, but suppress feeding at higher concentrations. However, despite significant recent progress (e.g. Kwon et al., 2011), the principles of circuit organization underlying taste-mediated behaviours in the larva remains unresolved. In particular, we lack a comprehensive view of the entanglement of the taste system with mechanosensation and thus of the ‘what?’ and ‘where?’ of contact chemosensory processing, and of the role of chemosensory input from the gut and/or of metabolic feedback. Also, one can at present only suspect that, in analogy to the situation in adult flies, the ability of the larvae to discriminate between, for example, different kinds of sweetness or bitterness is limited, and that some of these limits are established

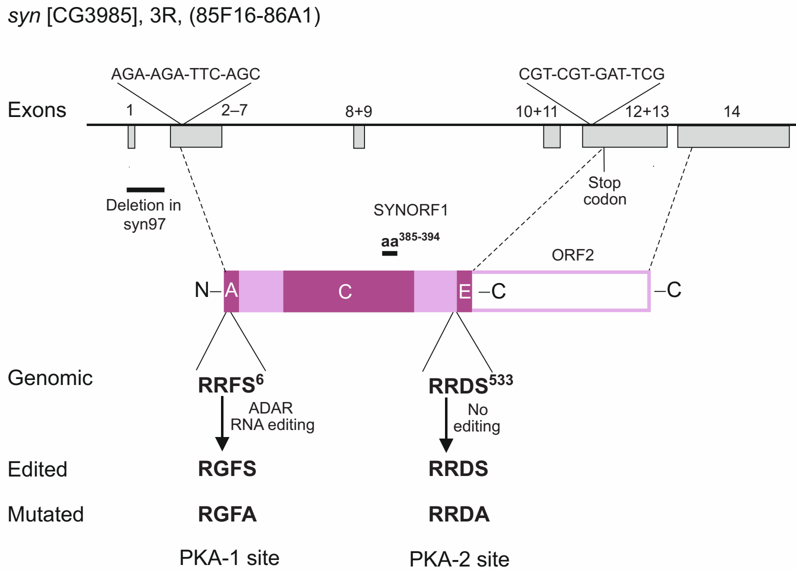

Fig. 3. Genomic organization of the *synapsin* locus and organization of the Synapsin protein. Position and size of the *syn*[97] deletion at the regulatory region up to the first intron is indicated. The exons are translated into several protein isoforms [two short isoforms of 70–80 kDA (magenta-filled boxes) and a 143 kDa full-length isoform (ORF2)]. In these proteins, the N-terminal A domain and the centrally located C domain are highly conserved between vertebrates and flies. The C terminus contains a prolin-rich region and the less conserved E domain; amino acids 385–394 feature the LFGGMEVCGL epitope of the SYNORF1 mouse monoclonal antibody. Superimposed are the locations of consensus sites (RRXS) for PKA and CaMK I/IV and their amino acid sequences based on the genomic codons. RNA editing at the PKA-1 site leads to a change from RRFS to RGFS on the protein level. The rescue construct used in the two leftmost panels of Fig. 4A uses a UAS construct to express the 'edited' motif, whereas the rightmost panel uses the 'mutated' motifs (Diegelmann et al., 2006).

already at the level of the sensory neurons. In other words, in the gustatory system the sensory-motor 'watershed' appears to be pushed relatively far out towards the sensory periphery, categorizing inputs early on into behavioural categories. This organization contrasts with the olfactory system, which features stages with an enormous capacity to discriminate odours, and the flexibility to tell apart or categorize between odours, depending on the task (Mishra et al., 2010).

In any event, from the discussion above it appears as if there is no connection between olfactory and taste pathways (but see Thum et al., 2011). How, then, can odours be associated with tastants? In a seminal paper, Martin Hammer (Hammer, 1993) identified the octopaminergic VUMmx1 neuron in the honeybee, which receives gustatory input likely in the subesophageal ganglion and provides output to the antennal lobe, the mushroom body and the lateral horn. This neuron thus 'short-circuits' taste with olfactory pathways, and is sufficient to mediate the rewarding function of sugar in honeybee olfactory learning. This neuron exists in adult (Busch et al., 2009) and larval *Drosophila* as well (Selcho et al., 2012). In larvae, the net effect of driving subsets of octopaminergic/tyraminergic neurons (TDC-Gal4) can substitute for reward in olfactory learning, while driving subsets of dopaminergic neurons (TH-Gal4) can substitute for punishment (Schroll et al., 2006). However, current research suggests that the mnemonic function of the neurons contained in these sets is not restricted to such reinforcement processing during training. For example, in adult *Drosophila*, the so-called dopaminergic MB-MP neurons seem to carry visceral and/or metabolic feedback information ('satiety state') towards the mushroom body to effectively inhibit mushroom body output (Krashes et al., 2009). Also, it turned out that some dopaminergic and conceivably also octopaminergic/tyraminergic neurons transgress a simple appetitive–aversive dichotomy (Schroll et al., 2006; Honjo and Furukubo-Tokunaga, 2009; Selcho et al., 2009; Kravitz et al., 2012; Liu et al., 2012). What is important for the present discussion is that the convergence of the olfactory pathway is not with the gustatory pathway itself, but with a modulatory signal branching off of the gustatory sensory-motor loop. At which point(s) in the circuit does such memory trace-inducing convergence take place? Or in other words, where in the circuit are odour–reward memory traces formed?

Synapsin function

Background

We used a three-step strategy towards memory trace localization (Gerber et al., 2004b; Heisenberg and Gerber, 2008): (1) find a gene that, when mutated, impairs neuronal plasticity and odour–reward learning; (2) test in which parts of the chemobehavioural circuits restoring this gene is sufficient to restore associative function; and (3) test whether these sites of sufficiency also are sites where the expression of that gene is necessary. As a candidate gene for such an analysis, we chose to focus on *synapsin* (Fig. 3 displays the *synapsin* gene of *Drosophila*).

Synapsins are highly abundant soluble neuronal phosphoproteins, located in nerve terminals and associated with the cytoplasmic surface of synaptic vesicle membranes [for reviews of the original literature, please see the following papers (Südhof et al., 1989; DeCamilli et al., 1990; Greengard et al., 1993; Südhof, 1995; Hilfiker et al., 1999; Kao et al., 1999; Ferreira and Rapoport, 2002; Fdez and Hilfiker, 2006; Cesca et al., 2010; Fornasiero et al., 2010; Benfenati, 2011)]. Vertebrates posses a three-member family of *synapsin* genes, while in invertebrates such as *Drosophila* only one *synapsin* gene is found (Klagges et al., 1996); from each gene, typically multiple protein isoforms are produced by alternative splicing. Synapsins are part of the presynaptic molecular network to fine-tune synaptic output, in particular to regulate the balance between the different presynaptic vesicle pools in a phosphorylation-dependent manner. That is, there are likely at least three functionally distinct (yet not necessarily spatially segregated) presynaptic vesicle pools (Rizzoli and Betz, 2004) [for a novel perspective regarding these pools, see the following papers (Denker et al., 2011a; Denker et al., 2011b)]: (1) a reserve pool bound to Actin filaments and not immediately available for release; (2) a readily releasable pool of docked vesicles at the presynaptic membrane primed for immediate exocytosis; and (3) the cycling pool, comprised of those vesicles engaged in the exo-endocytic cycle. Synapsins can bind to synaptic vesicles and to Actin, the latter in a phosphorylation-dependent way. Seminal work of Greengard and colleagues suggested a role of Synapsin in non-associative plasticity (see Benfenati, 2011). Their analyses indicate that phosphorylation of Synapsin reduces its affinity to Actin and thus recruits vesicles from the reserve pool for subsequent rounds

of exocytosis. From a comparative perspective, it is noteworthy that domains A and C at the N-terminal side of the protein are shared by all known Synapsin isoforms, whereas additional domains (D to J) at the C-terminal side are less conserved. Phosphorylation sites in the A domain include an evolutionarily highly conserved PKA/CaM I/IV kinase consensus site; also, a candidate membrane-binding region was identified within this A domain. The C domain likewise contains such a candidate membrane-binding region, while other sites within the C domain appear responsible for binding to Actin filaments. These insights into Synapsin function were subsequently enriched in a number of ways (Benfenati, 2011):

(1) Concomitant with the discovery of the requirement of Synapsin in non-associative short-term plasticity in *Aplysia* (Humeau et al., 2001), it was shown that serotonin induces Synapsin phosphorylation in *Aplysia* (Angers et al., 2002), likely *via* the cAMP-PKA pathway (Fiumara et al., 2004; see also Dolphin and Greengard, 1981) and/or the MAP kinase pathway (Giachello et al., 2010), and that these processes impact non-associative short-term plasticity (Fioravante et al., 2007; Doussau et al., 2010; Giachello et al., 2010; Valente et al., 2012). Strikingly, it was recently reported that the *synapsin* gene of *Aplysia* features a CRE-recognition site, that serotonin induces *de novo* synthesis of Synapsin *via* the CREB1 pathway, and that this is required for non-associative long-term plasticity (Hart et al., 2011).

(2) The description of the crystal structure of a recombinant Synapsin C domain revealed a similarity to ATP-binding enzymes (Esser et al., 1998) and it was then shown that recombinant C domains of Synapsin I, II and III bind ATP *in vitro* (Hosaka and Südhof, 1998a; Hosaka and Südhof, 1998b). Interestingly, ATP binding by Synapsin I but not by Synapsin II is Ca^{2+}-dependent, while Synapsin III is inhibited by Ca^{2+}.

(3) Additionally, the crystal structure of the C domain suggested a possible dimerization of Synapsins (Esser et al., 1998), which was subsequently confirmed (Hosaka and Südhof, 1999). This process is promoted by ATP and Ca^{2+} (Brautigam et al., 2004). Given that Actin depolymerization apparently does not disrupt vesicle clusters (Sankaranarayanan et al., 2003), whereas Synapsin 'neutralization' by antibodies does (Pieribone et al., 1995), it is speculated that Synapsins may bind vesicles not only to the Actin cytoskeleton but to each other as well (Bykhovskaia, 2011; Shupliakov et al., 2011).

(4) Recently, an ALPS (amphipathic lipid packing sensor) motif in the B domain was discovered (Krabben et al., 2011) that, when mutated, affects the association of Synapsin with curved membranes, including synaptic vesicles, suggesting a novel way of how Synapsin acts in regulating vesicle function.

(5) Additional implications of Synapsins, partially mediated by MAP kinases, include developmentally relevant aspects such as neurite elongation, synaptogenesis and synapse maturation (Fornasiero et al., 2010; Valtorta et al., 2011).

(6) Last but not least, the mosaic expression pattern of different Synapsin isoforms in the vertebrate brain early on suggested a functional heterogeneity not only between domains and phosphorylation sites of Synapsin, but also between isoforms. Indeed, *synapsin II*, but not *synapsin I*, mutant mice were later reported to be impaired in associative learning (Silva et al., 1996). Mice lacking all three *synapsin* genes performed poorly in a number of tested reflexes and their ability to hang from a suspended wire; they also showed seizures upon disturbance, reduced piloerection and difficulties maintaining balance (Gitler et al., 2004). Regarding mnemonic function, these animals also performed poorly in a test for spatial memory, without reported task-relevant disturbances in motivation or motor ability. In humans, mutations in *synapsin* genes can apparently entail severe neurological, behavioural and/or psychiatric phenotypes (Garcia et al., 2004; Fassio et al., 2011; Porton et al., 2011).

Thus, the *synapsin* genes contribute to bewilderingly many functions, dependent on which particular species and gene is looked at, which isoform, domain or site is considered, how the respective protein is embedded into the local molecular network at a given presynapse, and the way the respective neuron is integrated into a functional circuit and ultimately into behavioural task(s). In other words, there does not seem to be anything like 'the' role of Synapsin. Assigning one particular behavioural role for a particular gene, isoform and molecular interaction, at a particular synapse of a given circuit obviously does not rule out other roles in other contexts. In turn, the discussion below will show that for a precisely defined behavioural task, a cellular site and a molecular mode of Synapsin action can be assigned with satisfying precision.

Drosophila

The presence of Synapsin-like immunoreactivity in invertebrates was known since the mid-1980s, but the first invertebrate gene coding for Synapsin (*synapsin*, *syn*, CG3985) was identified from *Drosophila* only in 1996 in a seminal project of the Buchner group to identify brain-specific proteins (Fig. 3) (Klagges et al., 1996; Hofbauer et al., 2009). The intron/exon structure of this gene exhibits similarity to *synapsin* genes of vertebrates (Klagges et al., 1996; Godenschwege et al., 2004) and, as in many of these genes, the gene for the tissue inhibitor of metalloproteinase (*timp*, CG6281) is nested within the *syn* gene on the antiparallel DNA strand (Pohar et al., 1999). Alternative splicing of the primary transcript produces two *synapsin* transcripts, leading to two protein isoforms with relatively low molecular mass (70–80 kDa); additionally, a full-length protein (~143 kDa) can be generated *via* read-through of an *amber* stop codon into the second open reading frame (Klagges et al., 1996; Nuwal et al., 2011); indeed, polyclonal mouse sera against this ORF2 specifically detect the 143 kDa band (Klagges et al., 1996). The mouse monoclonal antibody SYNORF1 binds in the conserved C domain of Synapsin (epitope LFGGMEVCGL, Fig. 3) (Godenschwege et al., 2004); it has turned out to be a particularly useful tool, because of its high-affinity binding, its suitability in histology and western blotting, and the fact that it can be used across species. Notably, the mentioned antisera against the full-length protein reveal staining patterns not obviously different from that of SYNORF1.

The *Drosophila* Synapsin protein contains domains homologous to the N-terminal A domain and the central, Actin- and vesicle-binding C domain (50% identity, 89% similarity of amino acids); additionally, the prolin-rich C terminus shows similarity to the E domain found in a-type vertebrate synapsins. *Drosophila* Synapsin contains a number of predicted phosphorylation sites, including the evolutionarily conserved PKA/CamK I/IV consensus motif RRFS at Ser^{6} (henceforth called the PKA-1 site), the evolutionarily non-conserved PKA/CamK I/IV consensus motif RRDS at Ser^{533} (henceforth called the PKA-2 site) and additionally suspect sites for other kinases, i.e. CamK II, prolin-dependent kinase and PKC (Nuwal et al., 2011). *Drosophila* Synapsin is expressed exclusively in most if not all neurons, starting at mid-embryogenesis. On a subcellular level, the SYNORF1 antibody reveals expression in type-Ib and type-Is boutons of larval motorneurons, but not in type-II and -III boutons (Klagges et al., 1996; Godenschwege et al., 2004). This situation, together with the role of Synapsins in

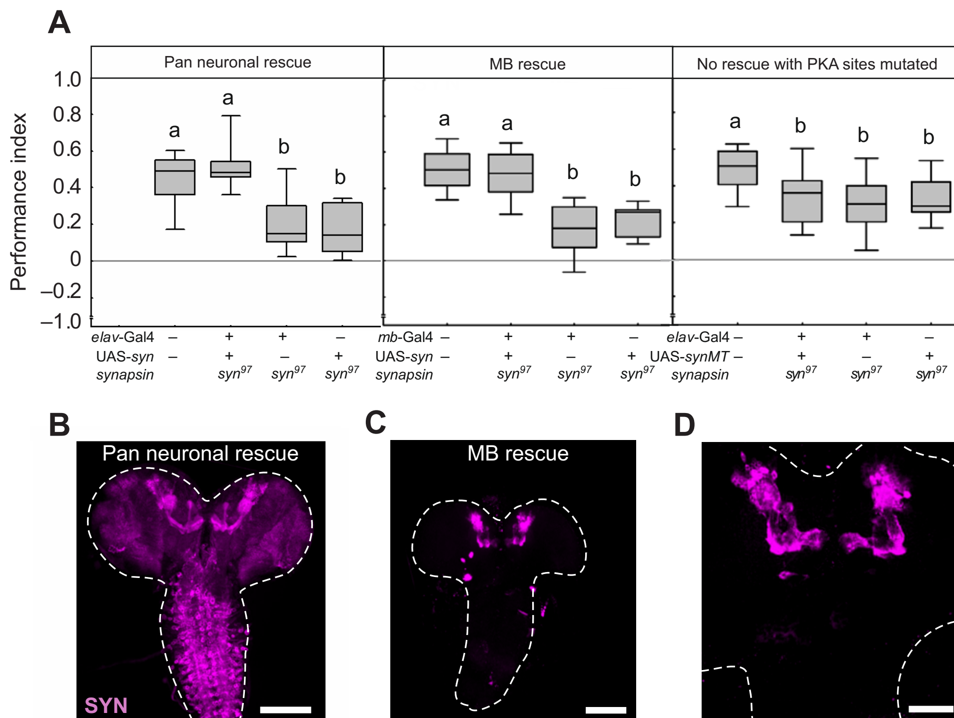

Fig. 4. (A) The defect of the *syn*[97] mutant in associative function is fully restored using a pan neuronal driver line in combination with a normal UAS-syn-cDNA construct (left panel, corresponding to the 'edited' site in Fig. 4); the same is observed with acutely induced Synapsin expression (see the Synapsin function, *Drosophila* section). A rescue is also achieved if Synapsin expression is restored only within the mushroom bodies (MB) (middle panel), but not when Synapsin is restored in the projection neurons or outside the MBs (see the Synapsin function, *Drosophila* section). No rescue is seen, either, by using the same MB-driver line but in combination with a UAS strain to express a version of Synapsin with both PKA consensus sites mutationally inactivated (right panel, corresponding to the 'mutated' sites in Fig. 4). Other details as in Fig. 1. (B–D) Immunreactivity against Synapsin (magenta) in the brains of the indicated genotypes. In D, a close-up of C is shown. Scale bars, 50 µm (B,C), 25 µm (D). Based on published data (Michels et al., 2011).

synaptic function and plasticity in mammals and molluscs (see above), prompted the question of the function of Synapsin in *Drosophila*. To this end, the *syn*[97] mutation was used, which shows a deletion including promoter regions, the first exon and part of the first intron of the *synapsin* gene (Fig. 3). Using SYNORF1 as well as a mouse polyclonal anti-synapsin antiserum, it was shown that the deletion leads to a complete lack of the Synapsin protein [adults (Godenschwege, 1999; Godenschwege et al., 2004); larvae (Michels et al., 2005; Michels et al., 2011)]. After removing the *white*[1118] mutation (*white*, CG2759, which is used as genetic background to keep track of transgenes) by repeated outcrossing to wild type, *syn*[97] mutant flies were found to be viable, fertile and normal in a number of simple behavioural tasks (initial olfactory jump response, basic optomotor response, basic walking parameters), as well as in presynaptic structure at the larval neuromuscular junction (i.e. the number of synaptic boutons, integrity of the T-shaped synaptic ribbon, number of vesicles immediately close to it, structure of the subsynaptic reticulum) and in basic transmission at this synapse (Godenschwege et al., 2004). Interestingly, the notion of Synapsin maintaining vesicles in place is consistent with the finding that the synaptic vesicles in the boutons of motoneurons are distributed over larger areas in *syn*[97] mutant larvae than in wild-type larvae; this effect can be phenocopied by motoneuron stimulation in wild-type larvae (Angers et al., 2002; Akbergenova and Bykhovskaia, 2010). Fittingly, it was found that vesicles in *syn*[97] mutants are more likely to be part of the non-reserve pools (Denker et al., 2011a), in turn implying that Synapsin maintains the reserve pool.

Importantly in the current context, it was found that in arguably more demanding and/or complex behaviour assays (Godenschwege et al., 2004) in adult *Drosophila* [ethanol tolerance, visual object fixation, conditioned courtship suppression, wing beat frequency during sustained flight, habituation of the olfactory jump response, performance in the heat-box conditioning paradigm, and in a paradigm where flies learn about the nutritional value of food (Fujita and Tanimura, 2011)], the *syn*[97] mutant does show phenotypes (Godenschwege et al., 2004). Critically, the *syn*[97] mutation entails an approximately 25% decrease in odour–shock associative function, but leaves intact task-relevant odour processing, shock processing and the respective behavioural faculties to behave towards these stimuli [the mutants also behave normally to odours after training-like exposure to either odours alone or to shock alone (Knapek et al., 2010)]. This prompted us to test the *syn*[97] mutant for associative function at the larval stage, too.

In larval *syn*[97] mutant animals, odour–sugar associative function is reduced by approximately half (Fig. 1B, Fig. 4) (Michels et al., 2005; Michels et al., 2011), an effect that can be phenocopied by reducing Synapsin levels throughout development by transgenically expressing RNAi with elav-Gal4 as the driver (Michels et al., 2011), while all behavioural control experiments (olfactory preference, sugar preference, susceptibility to training-like odour or sugar exposure) did not reveal a phenotype (Michels et al., 2005). As the *syn*[97] mutation thus specifically affects associative function, we wondered when, in which cells and by which molecular mechanism Synapsin would function.

The reduced associative faculties of *syn*[97] mutant larvae in the odour–sugar paradigm can be rescued by acutely restoring Synapsin throughout the nervous system (elav-Gal4; tub-gal80^{ts}; UAS-syncDNA, *syn*[97]). Locally restoring Synapsin in the mushroom bodies is sufficient to fully restore associative ability (mb247-Gal4, UAS-syncDNA, *syn*[97]), whereas restoring Synapsin in the projection neurons is not (either GH146-Gal4 or NP225-Gal4; UAS-syncDNA, *syn*[97]) (in the two aforementioned cases and in all following cases, rescues were performed non-acutely, i.e. without tub-gal80^{ts}). We note that in cases where no rescue is observed, it is necessary to test not only (by immunohistochemistry) whether the transgene of interest is indeed properly expressed, but also whether the Gal4-driver element *per se* may have an effect in the experimental animals, such that an actually successful rescue is obscured; indeed, no such effects of the Gal4-driver elements were observed in any of the cases of lack-

of-rescue (Michels et al., 2011). In any event, if Synapsin is restored in wide areas of the brain excluding the mushroom bodies, learning ability is not restored either (elav-Gal4; mb247-Gal80; UAS-syncDNA, *syn*^{97}), suggesting that Synapsin in the mushroom body may also be necessary for proper associative function. This latter conclusion of necessity remains tentative, however, because the mb247-Gal80 construct leads to Gal4 suppression also outside the mb247-Gal4 expression pattern and even outside of the mushroom bodies. Also, blocking synaptic output of mb247-Gal4 positive neurons with *shibire*^{ts} (Kitamoto, 2001) reportedly does not impair odour–sugar mnemonic processing (Pauls et al., 2010b), while broader mushroom body expression, arguably in embryonic-born mushroom body neurons in particular, does (201Y-Gal4, NP1131-Gal4). Thus, a Synapsin-dependent memory trace is located in the mushroom bodies, and this may turn out to be the only (i.e. necessary) site where such a trace is established in this particular paradigm. These findings prompted an enquiry into the molecular mode of action of Synapsin in memory trace formation, in particular into its possible role as a target of the AC-cAMP-PKA cascade. Indeed, with respect to odour–shock associative function in adult flies, it was found that the *syn*^{97} mutation is non-additive with *rut*^{2080}, a hypomorphic mutation in the *rutabaga* gene (CG9533) coding for a type I adenylate cyclase (Knapek et al., 2010).

The role of the AC-cAMP-PKA cascade for olfactory short-term memory has been intensely studied in adult flies (Tomchik and Davis, 2009; Gervasi et al., 2010; and references therein). The *rutabaga* type I adenylyl cyclase acts as molecular coincidence detector between the reinforcer and the olfactory activation of the mushroom body neurons. On the one hand, the odour leads to presynaptic calcium influx, and hence to an activation of calmodulin, in the subset of mushroom body neurons that is activated by this odour. On the other hand, the reinforcer activates aminergic neurons and hence the respective G-protein coupled amine receptors in many if not all mushroom body neurons. In the larva, an octopaminergic/tyraminergic set of neurons, defined by TDC-Gal4, carries a net rewarding effect, whereas a dopaminergic set of neurons, defined by TH-Gal4, carries a net punishing effect (Schroll et al., 2006) (see final paragraph of Chemobehavioural circuitry of larval *Drosophila* for qualifying detail). Those subsets of aminergic neurons that target the mushroom bodies likely each connect to most if not all mushroom body neurons, with octopaminergic/tyraminergic and dopaminergic neurons innervating different domains of the mushroom bodies (Pauls et al., 2010b; Selcho et al., 2012). Critically, it is only by the simultaneous activation of the calmodulin (‘odour’) and G-protein pathways (‘reward’ or ‘punishment’, respectively) that the AC is substantially activated. Thus it is only in those mushroom body neurons that receive both odour and reinforcement activation that cAMP levels and PKA activity are boosted, and the respective protein substrates get phosphorylated. However, the behaviourally relevant substrates of PKA in flies remained obscure. Our working hypothesis was that one of these PKA substrates may be Synapsin, such that PKA-mediated phosphorylation of Synapsin recruits reserve-pool vesicles. Thus, a subsequent presentation of the learnt odour would result in enhanced output from an odour-specific set of mushroom body neurons, and ultimately in conditioned behaviour towards the learnt odour (Fig. 5). We therefore reasoned that transgenic mushroom-body expression of a Synapsin protein that carries dysfunctional PKA sites (mb247-Gal4, UAS-syncDNA-PKA$^{S6A/S533A}$, *syn*^{97}) will not be able to rescue the *syn*^{97} associative defect. This is indeed the case (Michels et al., 2011).

Interestingly, the evolutionarily conserved PKA-1 site, but not the non-conserved PKA-2 site, undergoes ADAR-dependent pre-mRNA editing (Diegelmann et al., 2006). This manifests itself in a discrepancy between the genomic sequence and the sequence of at least the majority of mRNAs, and ultimately the amino acid sequence of the protein: the amino acid motif RRFS, which should be optimal for phosphorylation by PKA, is altered *via* RNA editing into RGFS in both larval and adult flies. This edited RGFS form of Synapsin can hardly be phosphorylated, at least in an *in vitro* study using bovine PKA (Diegelmann et al., 2006). It should now be interesting to see *in vivo* whether and how RNA editing at Ser^{6} affects the efficacy of phosphorylation by PKA and/or other kinases, and how such fine regulation of phosphorylation impacts associative function. Along these lines we note that, in order to restore the wild-type situation, the rescue experiment displayed in Fig. 4A (left and middle panels) uses the edited form of Synapsin and that it does restore the defect in associative function.

As a working hypothesis, we thus propose that a Synapsin-dependent memory trace underlying attraction to the conditioned odour in the larva is localized within the mushroom body neurons. Whenever a mushroom body neuron, as a part of an odour-specific subset of mushroom body neurons, is activated coincidently with an aminergic reinforcement signal, the cAMP-PKA cascade is triggered. One substrate of this cascade is Synapsin. Phosporylation of Synapsin likely by PKA but possibly also by other kinases ensures an alteration of synaptic strength between this mushroom body neuron and its target(s). If the trained odour is encountered again at test, the same odour-specific subset of mushroom body neurons is activated again, such that now their modified collective output towards their target neuron(s) allows conditioned behaviour. In other words, the memory trace for the association of an odour with a reward consists of the pattern of changed synaptic weights across the array of the mushroom body neurons. But how, actually, is conditioned behaviour organized?

From memory trace to conditioned behaviour

At the moment of testing, the odour-reward memory trace manifests itself in a preference for the previously rewarded over the previously non-rewarded odour (Fig. 1A,B). To see how the memory-trace-to-behaviour process is organized, we manipulated the test conditions such that the larvae were tested either in the absence or in the presence of the reward (Fig. 6A,B) (Gerber and Hendel, 2006; Schleyer et al., 2011). Strikingly, despite identical training and thus in possession of the same odour–reward memory trace, the presence of the training reward prevents the larvae from showing appetitive conditioned behaviour (Fig. 6B, leftmost and middle groups). This was shown to be the case both for sugar and for low salt as reward. In turn, after aversive training, either with quinine or with high salt concentrations, larvae express aversive conditioned behaviour in the presence of the punishment, but not in its absence (Gerber and Hendel, 2006; Schleyer et al., 2011), and the same was found with respect to mechanosensory disturbance as punishment (Eschbach et al., 2011) (for a discussion of the generality of this effect see Schleyer et al., 2011). Importantly, tastants do not influence innate olfactory choice behaviour (Fig. 6C), and in turn odours do not affect innate gustatory behaviour (Fig. 6D). Thus, the gustatory situation specifically affects conditioned olfactory behaviour, while innate olfactory and innate gustatory behaviour seem to be mutually insulated.

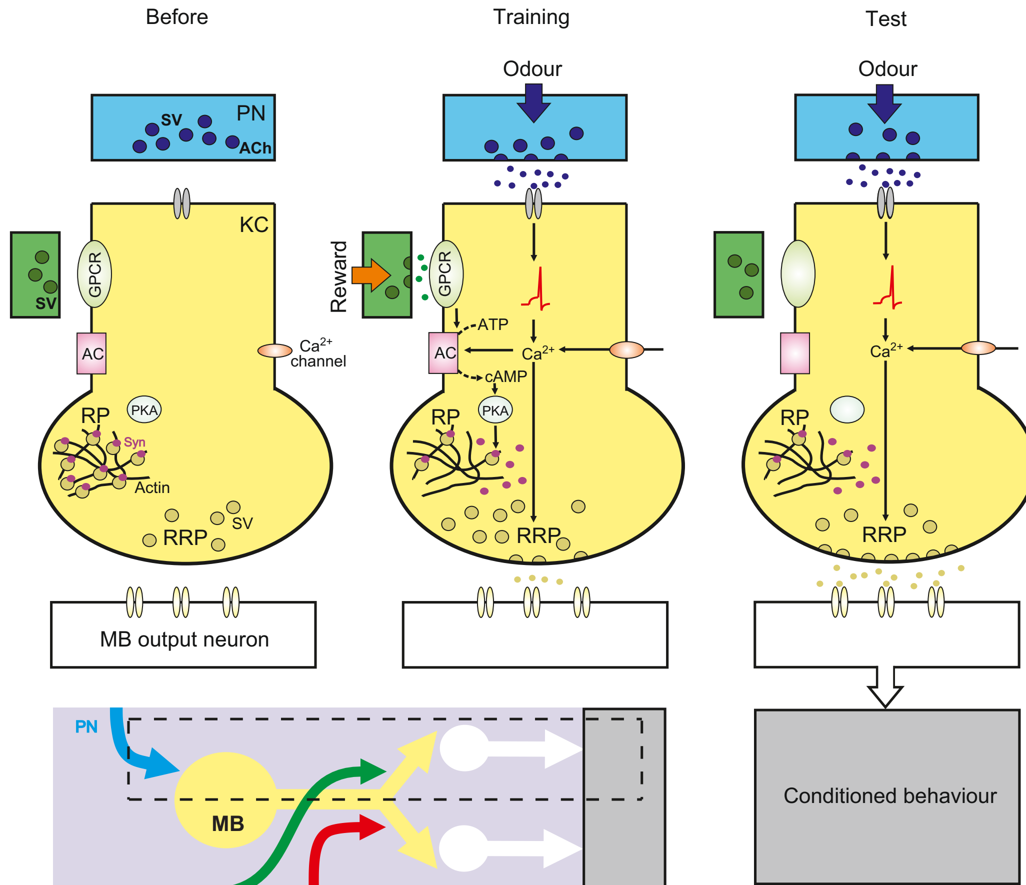

Fig. 5. Working hypothesis of the role of Synapsin in memory trace formation as a target of the AC-cAMP-PKA pathway. Sketched are the molecular events at the synapse before, during and after paired presentations of odour and sugar reward. The odour evokes action potentials in an odour-specific subset of mushroom body neurons, and hence opening of voltage-gated presynaptic Ca^{2+} channels. On the one hand, this leads to fusion of synaptic vesicles (SV) from the readily releasable pool (RRP). On the other hand, the adenylate cyclase (AC) is activated by coincident Ca^{2+} and G-protein signaling, the latter induced by sugar-evoked release of an aminergic reinforcement signal (for details, see the Synapsin function, *Drosophila* section) and hence activation of the respective G-protein coupled amine receptors (GPCR). cAMP has a number of intracellular effects including activation of protein kinase A (PKA). PKA in turn also has many targets, including Synapsin, which contains two PKA/CaMK I/IV consensus sequences, as well as consensus sequences for other kinases. Synapsin tethers vesicles to the Actin cytoskeleton, thereby maintaining a reserve pool (RP) of vesicles. Upon activation by kinases (e.g. PKA), Synapsin gets phosphorylated and lowers its affinity to the cytoskeleton, allowing for recruitment of synaptic vesicles for later release; note that RP and RRP are functionally yet not necessarily spatially segregated. If the learned odour is encountered again, the additionally recruited vesicles support potentiated output from the mushroom body neuron, and ultimately conditioned behaviour. Note that this display refers to odour–reward associations only; odour–punishment associations are likely established in functionally separated domains of the mushroom body neurons and a distinct aminergic punishment signal (Fig. 2A; lower left panel of this figure, the stippled box indicates those processes included in the main figure). Also note that the cycling pool of vesicles has been omitted for clarity. Redrawn from published work (Michels et al., 2011).

These findings can be understood when conditioned behaviour, rather than as response to the odour, is regarded as an action in pursuit of its outcome (Dickinson, 2001; Elsner and Hommel, 2001; Hoffmann, 2003; Gerber and Hendel, 2006; Schleyer et al., 2011). Accordingly, appetitive conditioned behaviour is viewed as ‘informed search’ for food – which is abolished in the presence of the sought-for food. Conversely, aversive conditioned behaviour is viewed as ‘informed escape’ from punishment – which is disabled in the absence of punishment – much in the same way as we only search for things that are not present, and run for the emergency exit in a movie theatre only when there is an emergency. Thus, conditioned behaviour is not an automatic response: associative memory traces can be behaviourally expressed, or not.

Upon further analysis, it turned out that this organization of conditioned behaviour involves a comparative step: after training larvae with a particular concentration of sugar, they express conditioned behaviour only when the sugar concentration at the moment of testing is less than the sugar concentration during training. Given that the larvae of the four leftmost groups displayed in Fig. 6B had all been trained in the same way and thus will have established the same memory trace, it is obviously not the memory trace *per se* that determines the behaviour of the animals in the test. In turn, animals that are tested under the same sugar concentration but that have been trained with different concentrations also differ in behaviour: if the sugar concentration during training was higher than it is during testing, conditioned ‘search for more’ is expressed (Fig. 6B, rightmost group). Critically, at this very same test concentration of sugar, no conditioned behaviour is seen if the training concentration had been equally high as the testing concentration (Fig. 6B, middle group). Thus, the testing situation *per se* is not a sufficient determinant of conditioned behaviour, either [this is in contrast

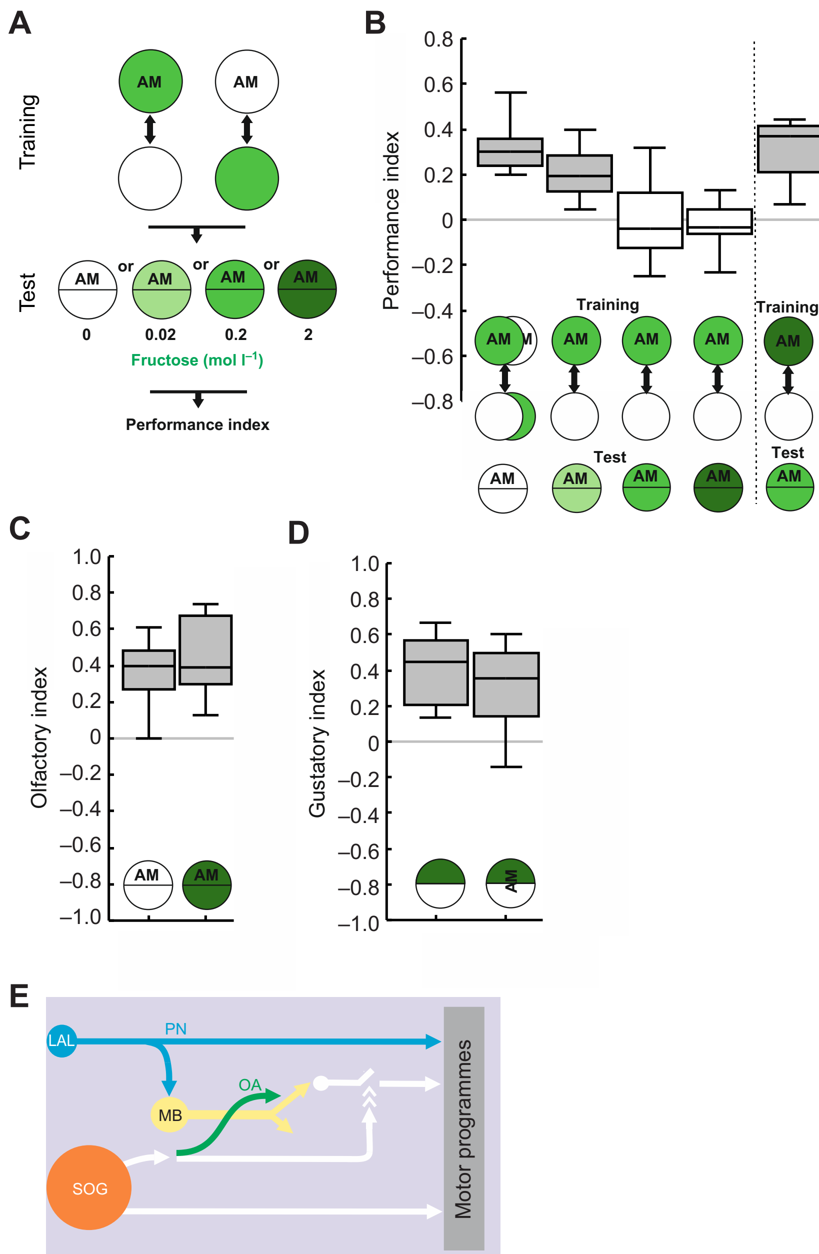

Fig. 6. (A) Experimental design. Circles represent Petri dishes containing a sugar reward (fructose, green) or plain agarose (white). Larvae are trained such that in the reciprocal groups *n*-amylacetate (AM) is either paired with or not paired with a 0.2 mol l^{-1} fructose reward. Subsequently, larvae are tested for their AM preference on either a tasteless, plain agarose substrate, or in the presence of 0.02, 0.2 or 2 mol l^{-1} fructose. From the difference of olfactory behaviour of two reciprocally trained groups (tested on the same substrate), the performance index is calculated to quantify associative learning. (B) Neither the training concentration of fructose alone, nor the testing concentration of fructose alone can account for conditioned behaviour. Rather, the comparison of both theses pieces of information determines whether conditioned behaviour is expressed. That is, appetitive conditioned behaviour is expressed only when the trained fructose concentration is higher than the fructose concentration during the test. (C) Innate, experimentally naive olfactory preference towards AM is not influenced by the presence of fructose. (D) Innate, experimentally naive gustatory preference towards fructose is not influenced by the presence of AM. (E) Circuit model of the 'decision' process to behaviourally express (or not express) an appetitive memory trace. At the moment of testing, the 'value' of the appetitive memory trace as a read-out from the MB is compared with the 'value' of the currently detected appetitive gustatory inputs. The connection to express appetitive conditioned behaviour remains interrupted (arrows) as long as these gustatory inputs are at least as 'good' as predicted by the activated memory trace. Thus, the learned odour is tracked down only 'in search for more', i.e. when doing so promises a positive outcome or 'gain'. For the aversive case (not shown) (Schleyer et al., 2011), the situation is inverse: aversive memory traces are behaviourally expressed only when the testing situation is as 'bad' as or worse than the punishment used in training. Other details as in Fig. 1. Data shown in B–D are based on published work (Schleyer et al., 2011).

to effects of visceral and/or metabolic feedback (Krashes et al., 2009), which arguably shuts down mushroom body output altogether at a site 'upstream' of the comparative process under study here]. Rather, it is the comparison between the memory trace and the testing situation that needs to be considered (Fig. 6E): the larvae compare the value of the current situation (the 'is state', based on gustatory input) with the value of the odour-activated memory trace (the 'could-get state', based on mushroom body output). Clearly, for this comparison to happen, the memory trace has to be 'read out', i.e. the mushroom bodies need to actually output – but off-line from behaviour. The circuit towards conditioned search is only closed if the memory trace suggests a place better than the current one; in other words, if there is something to search for, if there is a gain to be expected from tracking down the learned odour. It is this outcome expectation, and not the value of the memory trace, that is the immediate cause of conditioned behaviour.

This flexible, open organization of conditioned behaviour contrasts with the more rigid, closed processing stream mediating innate olfactory behaviour, which is expressed regardless of the testing situation (Fig. 6C). This separation of olfactory processing streams corresponds to the bifurcation of the projection neurons: the direct pathway towards the lateral horn mediates innate, closed kinds of olfactory tendencies, whereas the mushroom body detour corresponds to an open, flexible olfactory processing stream that can be regulated to express conditioned behaviour – or not.

Outlook

It seems that olfaction and olfactory learning in *Drosophila* are beginning to be understood satisfyingly well. Indeed, both in genuinely sensory aspects and in terms of olfactory memory trace formation, reasonable working hypotheses seem within reach. These working hypotheses are detailed enough to make the gaps in understanding obvious, with respect to, for example, the exact

organization of the aminergic system in terms of mnemonic processing; the processing of odour intensity information and the way it is included in olfactory memories (Yarali et al., 2009); the change in valence of memory upon reversing stimulus timing during associative training (Tanimoto et al., 2004); the molecular and cellular architecture of gustation and the entanglement of gustation with mechanosensory processing; the role of visceral and/or metabolic feedback in associative processing; the molecular network organization of the presynapse; the role of the postsynapse in associative plasticity; the temporal dynamics of the memory trace(s) formed and their respective content; or the mechanisms of integration across sensory modalities.

It is precisely because the situation overall is already fairly satisfying that an extension of scope is now called for. For example, the internally motivating factors of behaviour remain distressingly unclear. Likewise, one may ask whether, beyond the mere 'good' and 'bad', information about the particular kind of reinforcer and its intensity is contained in the memory trace, whether the animals 'know' when and how often they had been rewarded, or whether they experience degrees of 'certainty' about what they have learned. The combination of carefully designed and fine-grained behavioural analyses, together the cellular simplicity and genetic tractability of the *Drosophila* larva, may allow us to develop circuit-level accounts of such mnemonic richness, if it indeed exists.

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References

Aceves-Piña, E. O. and Quinn, W. G. (1979). Learning in normal and mutant *Drosophila* larvae. *Science* **206**, 93-96.
Akbergenova, Y. and Bykhovskaia, M. (2010). Synapsin regulates vesicle organization and activity-dependent recycling at *Drosophila* motor boutons. *Neuroscience* **170**, 441-452.
Angers, A., Fioravante, D., Chin, J., Cleary, L. J., Bean, A. J. and Byrne, J. H. (2002). Serotonin stimulates phosphorylation of *Aplysia* synapsin and alters its subcellular distribution in sensory neurons. *J. Neurosci.* **22**, 5412-5422.
Awasaki, T. and Kimura, K. (1997). pox-neuro is required for development of chemosensory bristles in *Drosophila. J. Neurobiol.* **32**, 707-721.
Benfenati, F. (2011). Synapsins – molecular function, development and disease. *Semin. Cell Dev. Biol.* **22**, 377-434.
Benton, R., Vannice, K. S., Gomez-Diaz, C. and Vosshall, L. B. (2009). Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila. Cell* **136**, 149-162.
Brautigam, C. A., Chelliah, Y. and Deisenhofer, J. (2004). Tetramerization and ATP binding by a protein comprising the A, B, and C domains of rat synapsin I. *J. Biol. Chem.* **279**, 11948-11956.
Busch, S., Selcho, M., Ito, K. and Tanimoto, H. (2009). A map of octopaminergic neurons in the *Drosophila* brain. *J. Comp. Neurol.* **513**, 643-667.
Bykhovskaia, M. (2011). Synapsin regulation of vesicle organization and functional pools. *Semin. Cell Dev. Biol.* **22**, 387-392.
Cesca, F., Baldelli, P., Valtorta, F. and Benfenati, F. (2010). The synapsins: key actors of synapse function and plasticity. *Prog. Neurobiol.* **91**, 313-348.
Cobb, M. (1999). What and how do maggots smell? *Biol. Rev. Camb. Philos. Soc.* **74**, 425-459.
Cobb, M., Scott, K. and Pankratz, M. (2009). Gustation in *Drosophila melanogaster. SEB Exp. Biol. Ser.* **63**, 1-38.
Colomb, J., Grillenzoni, N., Ramaekers, A. and Stocker, R. F. (2007). Architecture of the primary taste center of *Drosophila melanogaster* larvae. *J. Comp. Neurol.* **502**, 834-847.
Davis, R. L. (2011). Traces of *Drosophila* memory. *Neuron* **70**, 8-19.
De Camilli, P., Benfenati, F., Valtorta, F. and Greengard, P. (1990). The synapsins. *Annu. Rev. Cell Dev. Biol.* **6**, 433-460.
Demerec, M. and Kaufmann, B. P. (1972). *Drosophila Guide. Introduction to the Genetics and Cytology of Drosophila melanogaster.* Washington, DC: The Carnegie Institution.
Denker, A., Bethani, I., Kröhnert, K., Körber, C., Horstmann, H., Wilhelm, B. G., Barysch, S. V., Kuner, T., Neher, E. and Rizzoli, S. O. (2011a). A small pool of vesicles maintains synaptic activity *in vivo. Proc. Natl. Acad. Sci. USA* **108**, 17177-17182.
Denker, A., Kröhnert, K., Bückers, J., Neher, E. and Rizzoli, S. O. (2011b). The reserve pool of synaptic vesicles acts as a buffer for proteins involved in synaptic vesicle recycling. *Proc. Natl. Acad. Sci. USA* **108**, 17183-17188.
Dickinson, A. (2001). The 28th Bartlett Memorial Lecture. Causal learning: an associative analysis. *Q. J. Exp. Psychol. B* **54**, 3-25.
Diegelmann, S., Nieratschker, V., Werner, U., Hoppe, J., Zars, T. and Buchner, E. (2006). The conserved protein kinase-A target motif in synapsin of *Drosophila* is effectively modified by pre-mRNA editing. *BMC Neurosci.* **7**, 76.
Dolphin, A. C. and Greengard, P. (1981). Serotonin stimulates phosphorylation of protein I in the facial motor nucleus of rat brain. *Nature* **289**, 76-79.
Doussau, F., Humeau, Y., Benfenati, F. and Poulain, B. (2010). A novel form of presynaptic plasticity based on the fast reactivation of release sites switched off during low-frequency depression. *J. Neurosci.* **30**, 16679-16691.
Elsner, B. and Hommel, B. (2001). Effect anticipation and action control. *J. Exp. Psychol. Hum. Percept. Perform.* **27**, 229-240.
Eschbach, C., Cano, C., Haberkern, H., Schraut, K., Guan, C., Triphan, T. and Gerber, B. (2011). Associative learning between odorants and mechanosensory punishment in larval *Drosophila. J. Exp. Biol.* **214**, 3897-3905.
Esser, L., Wang, C. R., Hosaka, M., Smagula, C. S., Südhof, T. C. and Deisenhofer, J. (1998). Synapsin I is structurally similar to ATP-utilizing enzymes. *EMBO J.* **17**, 977-984.
Falk, R., Bleiseravivi, N. and Atidia, J. (1976). Labellar taste organs of *Drosophila melanogaster. J. Morphol.* **150**, 327-341.
Fassio, A., Raimondi, A., Lignani, G., Benfenati, F. and Baldelli, P. (2011). Synapsins: from synapse to network hyperexcitability and epilepsy. *Semin. Cell Dev. Biol.* **22**, 408-415.
Fdez, E. and Hilfiker, S. (2006). Vesicle pools and synapsins: new insights into old enigmas. *Brain Cell Biol.* **35**, 107-115.
Ferreira, A. and Rapoport, M. (2002). The synapsins: beyond the regulation of neurotransmitter release. *Cell. Mol. Life Sci.* **59**, 589-595.
Fioravante, D., Liu, R. Y., Netek, A. K., Cleary, L. J. and Byrne, J. H. (2007). Synapsin regulates basal synaptic strength, synaptic depression, and serotonin-induced facilitation of sensorimotor synapses in *Aplysia. J. Neurophysiol.* **98**, 3568-3580.
Fiumara, F., Giovedì, S., Menegon, A., Milanese, C., Merlo, D., Montarolo, P. G., Valtorta, F., Benfenati, F. and Ghirardi, M. (2004). Phosphorylation by cAMP-dependent protein kinase is essential for synapsin-induced enhancement of neurotransmitter release in invertebrate neurons. *J. Cell Sci.* **117**, 5145-5154.
Fornasiero, E. F., Bonanomi, D., Benfenati, F. and Valtorta, F. (2010). The role of synapsins in neuronal development. *Cell. Mol. Life Sci.* **67**, 1383-1396.
Fujita, M. and Tanimura, T. (2011). *Drosophila* evaluates and learns the nutritional value of sugars. *Curr. Biol.* **21**, 751-755.
Garcia, C. C., Blair, H. J., Seager, M., Coulthard, A., Tennant, S., Buddles, M., Curtis, A. and Goodship, J. A. (2004). Identification of a mutation in synapsin I, a synaptic vesicle protein, in a family with epilepsy. *J. Med. Genet.* **41**, 183-186.
Gerber, B. and Hendel, T. (2006). Outcome expectations drive learned behaviour in larval *Drosophila. Proc. Biol. Sci.* **273**, 2965-2968.
Gerber, B. and Stocker, R. F. (2007). The *Drosophila* larva as a model for studying chemosensation and chemosensory learning: a review. *Chem. Senses* **32**, 65-89.
Gerber, B., Scherer, S., Neuser, K., Michels, B., Hendel, T., Stocker, R. F. and Heisenberg, M. (2004a). Visual learning in individually assayed *Drosophila* larvae. *J. Exp. Biol.* **207**, 179-188.
Gerber, B., Tanimoto, H. and Heisenberg, M. (2004b). An engram found? Evaluating the evidence from fruit flies. *Curr. Opin. Neurobiol.* **14**, 737-744.
Gerber, B., Stocker, R. F., Tanimura, T. and Thum, A. S. (2009). Smelling, tasting, learning: *Drosophila* as a study case. *Results Probl. Cell Differ.* **47**, 187-202.
Gerber, B., Biernacki, R. and Thum, J. (2010). Odour-taste learning in larval *Drosophila*. In *Drosophila Neurobiology Methods: A Laboratory Manual* (ed. S. Waddell), pp. 443-455. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
Gervasi, N., Tchénio, P. and Preat, T. (2010). PKA dynamics in a *Drosophila* learning center: coincidence detection by rutabaga adenylyl cyclase and spatial regulation by dunce phosphodiesterase. *Neuron* **65**, 516-529.
Giachello, C. N., Fiumara, F., Giacomini, C., Corradi, A., Milanese, C., Ghirardi, M., Benfenati, F. and Montarolo, P. G. (2010). MAPK/Erk-dependent phosphorylation of synapsin mediates formation of functional synapses and short-term homosynaptic plasticity. *J. Cell Sci.* **123**, 881-893.
Gitler, D., Takagishi, Y., Feng, J., Ren, Y., Rodriguiz, R. M., Wetsel, W. C., Greengard, P. and Augustine, G. J. (2004). Different presynaptic roles of synapsins at excitatory and inhibitory synapses. *J. Neurosci.* **24**, 11368-11380.
Godenschwege, T. A. (1999). Untersuchungen zum Synapsin-Locus in *Drosophila melanogaster*: Herstellung und Charakterisierung von Mutanten. PhD thesis, Bayerische Julius-Maximilians Universität Würzbug.

Godenschwege, T. A., Reisch, D., Diegelmann, S., Eberle, K., Funk, N., Heisenberg, M., Hoppe, V., Hoppe, J., Klagges, B. R., Martin, J. R. et al. (2004). Flies lacking all synapsins are unexpectedly healthy but are impaired in complex behaviour. *Eur. J. Neurosci.* **20**, 611-622.

Greengard, P., Valtorta, F., Czernik, A. J. and Benfenati, F. (1993). Synaptic vesicle phosphoproteins and regulation of synaptic function. *Science* **259**, 780-785.

Hammer, M. (1993). An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. *Nature* **366**, 59-63.

Hart, A. K., Fioravante, D., Liu, R. Y., Phares, G. A., Cleary, L. J. and Byrne, J. H. (2011). Serotonin-mediated synapsin expression is necessary for long-term facilitation of the *Aplysia* sensorimotor synapse. *J. Neurosci.* **31**, 18401-18411.

Heisenberg, M. (2003). Mushroom body memoir: from maps to models. *Nat. Rev. Neurosci.* **4**, 266-275.

Heisenberg, M. and Gerber, B. (2008). Behavioral analysis of learning and memory in *Drosophila*. In *Learning and Memory: A Comprehensive Reference* (ed. J. Byrne), pp. 549-559. Oxford: Elsevier.

Hilfiker, S., Pieribone, V. A., Czernik, A. J., Kao, H.-T., Augustine, G. J. and Greengard, P. (1999). Synapsins as regulators of neurotransmitter release. *Philos. Trans. R. Soc. Lond. B* **354**, 269-279.

Hofbauer, A., Ebel, T., Waltenspiel, B., Oswald, P., Chen, Y. C., Halder, P., Biskup, S., Lewandrowski, U., Winkler, C., Sickmann, A. et al. (2009). The Wuerzburg hybridoma library against *Drosophila* brain. *J. Neurogenet.* **23**, 78-91.

Hoffmann, J. (2003). Anticipatory behavioural control. In *Anticipatory Behaviour in Adaptive Learning Systems* (ed. M. Butz, O. Sigaud and P. Gérard), pp. 44-65. Heidelberg: Springer.

Honjo, K. and Furukubo-Tokunaga, K. (2009). Distinctive neuronal networks and biochemical pathways for appetitive and aversive memory in *Drosophila* larvae. *J. Neurosci.* **29**, 852-862.

Hosaka, M. and Südhof, T. C. (1998a). Synapsins I and II are ATP-binding proteins with differential Ca^{2+} regulation. *J. Biol. Chem.* **273**, 1425-1429.

Hosaka, M. and Südhof, T. (1998b). Synapsin III, a novel synapsin with an unusual regulation by Ca^{2+}. *J. Biol. Chem.* **273**, 13371-13374.

Hosaka, M. and Südhof, T. C. (1999). Homo- and heterodimerization of synapsins. *J. Biol. Chem.* **274**, 16747-16753.

Humeau, Y., Doussau, F., Vitiello, F., Greengard, P., Benfenati, F. and Poulain, B. (2001). Synapsin controls both reserve and releasable synaptic vesicle pools during neuronal activity and short-term plasticity in *Aplysia*. *J. Neurosci.* **21**, 4195-4206.

Ishimoto, H. and Tanimura, T. (2004). Molecular neurophysiology of taste in *Drosophila*. *Cell. Mol. Life Sci.* **61**, 10-18.

Kao, H. T., Porton, B., Hilfiker, S., Stefani, G., Pieribone, V. A., DeSalle, R. and Greengard, P. (1999). Molecular evolution of the synapsin gene family. *J. Exp. Zool.* **285**, 360-377.

Kaun, K. R., Hendel, T., Gerber, B. and Sokolowski, M. B. (2007). Natural variation in *Drosophila* larval reward learning and memory due to a cGMP-dependent protein kinase. *Learn. Mem.* **14**, 342-349.

Keene, A. C. and Waddell, S. (2007). *Drosophila* olfactory memory: single genes to complex neural circuits. *Nat. Rev. Neurosci.* **8**, 341-354.

Khurana, S., Abu Baker, M. B. and Siddiqi, O. (2009). Odour avoidance learning in the larva of *Drosophila melanogaster*. *J. Biosci.* **34**, 621-631.

Kitamoto, T. (2001). Conditional modification of behavior in *Drosophila* by targeted expression of a temperature-sensitive shibire allele in defined neurons. *J. Neurobiol.* **47**, 81-92.

Klagges, B. R., Heimbeck, G., Godenschwege, T. A., Hofbauer, A., Pflugfelder, G. O., Reifegerste, R., Reisch, D., Schaupp, M., Buchner, S. and Buchner, E. (1996). Invertebrate synapsins: a single gene codes for several isoforms in *Drosophila*. *J. Neurosci.* **16**, 3154-3165.

Knapek, S., Gerber, B. and Tanimoto, H. (2010). Synapsin is selectively required for anesthesia-sensitive memory. *Learn. Mem.* **17**, 76-79.

Krabben, L., Fassio, A., Bhatia, V. K., Pechstein, A., Onofri, F., Fadda, M., Messa, M., Rao, Y., Shupliakov, O., Stamou, D. et al. (2011). Synapsin I senses membrane curvature by an amphipathic lipid packing sensor motif. *J. Neurosci.* **31**, 18149-18154.

Krashes, M. J., DasGupta, S., Vreede, A., White, B., Armstrong, J. D. and Waddell, S. (2009). A neural circuit mechanism integrating motivational state with memory expression in *Drosophila*. *Cell* **139**, 416-427.

Kravitz, A. V., Tye, L. D. and Kreitzer, A. C. (2012). Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nat. Neurosci.* **15**, 816-818.

Kwon, J. Y., Dahanukar, A., Weiss, L. A. and Carlson, J. R. (2011). Molecular and cellular organization of the taste system in the *Drosophila* larva. *J. Neurosci.* **31**, 15300-15309.

Laurent, G., Stopfer, M., Friedrich, R., Rabinovich, M., Volkovskii, A. and Abarbanel, H. (2001). Odor encoding as an active, dynamic process: experiments, computation, and theory. *Annu. Rev. Neurosci.* **24**, 263-297.

Liu, C., Plaçais, P. Y., Yamagata, N., Pfeiffer, B. D., Aso, Y., Friedrich, A. B., Siwanowicz, I., Rubin, G. M., Preat, T. and Tanimoto, H. (2012). A subset of dopamine neurons signals reward for odour memory in *Drosophila*. *Nature* **488**, 512-516.

Margulies, C., Tully, T. and Dubnau, J. (2005). Deconstructing memory in *Drosophila*. *Curr. Biol.* **15**, R700-R713.

McGuire, S. E., Deshazer, M. and Davis, R. L. (2005). Thirty years of olfactory learning and memory research in *Drosophila melanogaster*. *Prog. Neurobiol.* **76**, 328-347.

Melcher, C., Bader, R. and Pankratz, M. J. (2007). Amino acids, taste circuits, and feeding behavior in *Drosophila*: towards understanding the psychology of feeding in flies and man. *J. Endocrinol.* **192**, 467-472.

Michels, B., Diegelmann, S., Tanimoto, H., Schwenkert, I., Buchner, E. and Gerber, B. (2005). A role for Synapsin in associative learning: the *Drosophila* larva as a study case. *Learn. Mem.* **12**, 224-231.

Michels, B., Chen, Y. C., Saumweber, T., Mishra, D., Tanimoto, H., Schmid, B., Engmann, O. and Gerber, B. (2011). Cellular site and molecular mode of synapsin action in associative learning. *Learn. Mem.* **18**, 332-344.

Mishra, D., Louis, M. and Gerber, B. (2010). Adaptive adjustment of the generalization-discrimination balance in larval *Drosophila*. *J. Neurogenet.* **24**, 168-175.

Niewalda, T., Singhal, N., Fiala, A., Saumweber, T., Wegener, S. and Gerber, B. (2008). Salt processing in larval *Drosophila*: choice, feeding, and learning shift from appetitive to aversive in a concentration-dependent way. *Chem. Senses* **33**, 685-692.

Nuwal, T., Heo, S., Lubec, G. and Buchner, E. (2011). Mass spectrometric analysis of synapsins in *Drosophila melanogaster* and identification of novel phosphorylation sites. *J. Proteome Res.* **10**, 541-550.

Pauls, D., Pfitzenmaier, J. E., Krebs-Wheaton, R., Selcho, M., Stocker, R. F. and Thum, A. S. (2010a). Electric shock-induced associative olfactory learning in Drosophila larvae. *Chem. Senses* **35**, 335-346.

Pauls, D., Selcho, M., Gendre, N., Stocker, R. F. and Thum, A. S. (2010b). *Drosophila* larvae establish appetitive olfactory memories via mushroom body neurons of embryonic origin. *J. Neurosci.* **30**, 10655-10666.

Pieribone, V. A., Shupliakov, O., Brodin, L., Hilfiker-Rothenfluh, S., Czernik, A. J. and Greengard, P. (1995). Distinct pools of synaptic vesicles in neurotransmitter release. *Nature* **375**, 493-497.

Pohar, N., Godenschwege, T. A. and Buchner, E. (1999). Invertebrate tissue inhibitor of metalloproteinase: structure and nested gene organization within the synapsin locus is conserved from *Drosophila* to human. *Genomics* **57**, 293-296.

Porton, B., Wetsel, W. C. and Kao, H. T. (2011). Synapsin III: role in neuronal plasticity and disease. *Semin. Cell Dev. Biol.* **22**, 416-424.

Python, F. and Stocker, R. F. (2002). Adult-like complexity of the larval antennal lobe of *D. melanogaster* despite markedly low numbers of odorant receptor neurons. *J. Comp. Neurol.* **445**, 374-387.

Quinn, W. G., Harris, W. A. and Benzer, S. (1974). Conditioned behavior in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **71**, 708-712.

Rizzoli, S. O. and Betz, W. J. (2004). The structural organization of the readily releasable pool of synaptic vesicles. *Science* **303**, 2037-2039.

Russell, C., Wessnitzer, J., Young, J. M., Armstrong, J. D. and Webb, B. (2011). Dietary salt levels affect salt preference and learning in larval *Drosophila*. *PLoS ONE* **6**, e20100.

Sankaranarayanan, S., Atluri, P. P. and Ryan, T. A. (2003). Actin has a molecular scaffolding, not propulsive, role in presynaptic function. *Nat. Neurosci.* **6**, 127-135.

Scherer, S., Stocker, R. F. and Gerber, B. (2003). Olfactory learning in individually assayed *Drosophila* larvae. *Learn. Mem.* **10**, 217-225.

Schleyer, M., Saumweber, T., Nahrendorf, W., Fischer, B., von Alpen, D., Pauls, D., Thum, A. and Gerber, B. (2011). A behavior-based circuit model of how outcome expectations organize learned behavior in larval *Drosophila*. *Learn. Mem.* **18**, 639-653.

Schroll, C., Riemensperger, T., Bucher, D., Ehmer, J., Völler, T., Erbguth, K., Gerber, B., Hendel, T., Nagel, G., Buchner, E. et al. (2006). Light-induced activation of distinct modulatory neurons triggers appetitive or aversive learning in *Drosophila* larvae. *Curr. Biol.* **16**, 1741-1747.

Schwärzel, M. and Müller, U. (2006). Dynamic memory networks: dissecting molecular mechanisms underlying associative memory in the temporal domain. *Cell. Mol. Life Sci.* **63**, 989-998.

Selcho, M., Pauls, D., Han, K. A., Stocker, R. F. and Thum, A. S. (2009). The role of dopamine in *Drosophila* larval classical olfactory conditioning. *PLoS ONE* **4**, e5897.

Selcho, M., Pauls, D., El Jundi, B., Stocker, R. F. and Thum, A. S. (2012). The role of octopamine and tyramine in *Drosophila* larval locomotion. *J. Comp. Neurol.* **520**, 3764-3785.

Shupliakov, O., Haucke, V. and Pechstein, A. (2011). How synapsin I may cluster synaptic vesicles. *Semin. Cell Dev. Biol.* **22**, 393-399.

Silva, A. J., Rosahl, T. W., Chapman, P. F., Marowitz, Z., Friedman, E., Frankland, P. W., Cestari, V., Cioffi, D., Südhof, T. C. and Bourtchuladze, R. (1996). Impaired learning in mice with abnormal short-lived plasticity. *Curr. Biol.* **6**, 1509-1518.

Steller, H., Fischbach, K. F. and Rubin, G. M. (1987). Disconnected: a locus required for neuronal pathway formation in the visual system of *Drosophila*. *Cell* **50**, 1139-1153.

Stocker, R. F. (1994). The organization of the chemosensory system in *Drosophila melanogaster*: a review. *Cell Tissue Res.* **275**, 3-26.

Stocker, R. F. (2001). Drosophila as a focus in olfactory research: mapping of olfactory sensilla by fine structure, odor specificity, odorant receptor expression, and central connectivity. *Microsc. Res. Tech.* **55**, 284-296.

Stocker, R. F. (2008). Design of the larval chemosensory system. In *Brain Development in Drosophila* (ed. G. M. Technau), pp. 69-81. New York: Springer Science and Business Media.

Südhof, T. C. (1995). The synaptic vesicle cycle: a cascade of protein–protein interactions. *Nature* **375**, 645-653.

Südhof, T. C., Czernik, A. J., Kao, H. T., Takei, K., Johnston, P. A., Horiuchi, A., Kanazir, S. D., Wagner, M. A., Perin, M. S., De Camilli, P. et al. (1989). Synapsins: mosaics of shared and individual domains in a family of synaptic vesicle phosphoproteins. *Science* **245**, 1474-1480.

Tanimoto, H., Heisenberg, M. and Gerber, B. (2004). Experimental psychology: event timing turns punishment to reward. *Nature* **430**, 983.

Tempel, B. L., Bonini, N., Dawson, D. R. and Quinn, W. G. (1983). Reward learning in normal and mutant *Drosophila*. *Proc. Natl. Acad. Sci. USA* **80**, 1482-1486.

Thum, A. S., Leisibach, B., Gendre, N., Selcho, M. and Stocker, R. F. (2011). Diversity, variability, and suboesophageal connectivity of antennal lobe neurons in *D. melanogaster* larvae. *J. Comp. Neurol.* **519**, 3415-3432.

Tissot, M. and Stocker, R. F. (2000). Metamorphosis in *Drosophila* and other insects: the fate of neurons throughout the stages. *Prog. Neurobiol.* **62**, 89-111.

Tomchik, S. M. and Davis, R. L. (2009). Dynamics of learning-related cAMP signaling and stimulus integration in the *Drosophila* olfactory pathway. *Neuron* **64**, 510-521.
Tully, T. (1984). *Drosophila* learning: behavior and biochemistry. *Behav. Genet.* **14**, 527-557.
Tully, T. and Quinn, W. G. (1985). Classical conditioning and retention in normal and mutant *Drosophila melanogaster*. *J. Comp. Physiol. A* **157**, 263-277.
Tully, T., Cambiazo, V. and Kruse, L. (1994). Memory through metamorphosis in normal and mutant *Drosophila*. *J. Neurosci.* **14**, 68-74.
Valente, P., Casagrande, S., Nieus, T., Verstegen, A. M., Valtorta, F., Benfenati, F. and Baldelli, P. (2012). Site-specific synapsin I phosphorylation participates in the expression of post-tetanic potentiation and its enhancement by BDNF. *J. Neurosci.* **32**, 5868-5879.
Valtorta, F., Pozzi, D., Benfenati, F. and Fornasiero, E. F. (2011). The synapsins: multitask modulators of neuronal development. *Semin. Cell Dev. Biol.* **22**, 378-386.
von Essen, A. M., Pauls, D., Thum, A. S. and Sprecher, S. G. (2011). Capacity of visual classical conditioning in *Drosophila* larvae. *Behav. Neurosci.* **125**, 921-929.
Vosshall, L. B. and Hansson, B. S. (2011). A unified nomenclature system for the insect olfactory coreceptor. *Chem. Senses* **36**, 497-498.
Vosshall, L. B. and Stocker, R. F. (2007). Molecular architecture of smell and taste in *Drosophila*. *Annu. Rev. Neurosci.* **30**, 505-533.
Yarali, A., Ehser, S., Hapil, F. Z., Huang, J. and Gerber, B. (2009). Odour intensity learning in fruit flies. *Proc. Biol. Sci.* **276**, 3413-3420.
Zars, T. (2010). Short-term memories in *Drosophila* are governed by general and specific genetic systems. *Learn. Mem.* **17**, 246-251.