Attention Deficit and Hyperactivity in a *Drosophila* Memory Mutant

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1. Abstract

The primary function of brains in all animals is to produce adaptive behavioral choices by selecting the right action at the right time. In humans, attention determines action selection as well as memory formation, while memories also guide which external stimuli should be attended to. The complex co-dependence of attention, memory, and action selection makes approaching the neurobiological basis of these interactions difficult in higher animals. Therefore, a successful reductionist approach is to turn to simpler systems for unraveling such complex biological problems. In a constantly changing environment, even simple animals have evolved attention-like processes to effectively filter incoming sensory stimuli. These processes can be studied in the fruit fly, *Drosophila melanogaster*, by a variety of behavioral and electrophysiological techniques. Recent work has shown that mutations affecting olfactory memory formation in *Drosophila* also produce distinct defects in visual attention-like behavior. In this study we extend those results to describe visual attention-like defects in the *Drosophila* memory consolidation mutant *radish*[1]. In both behavioral and brain-recording assays, *radish* mutant flies consistently displayed responses characteristic of a reduced attention span, with more frequent perceptual alternations and more random behavior compared to wild type flies. Some attention-like defects were successfully rescued by administering a drug commonly used to treat Attention-Deficit Hyperactivity Disorder (ADHD) in humans, methylphenidate (Ritalin). Our results suggest that a balance between persistence and flexibility is crucial for adaptive action selection in flies, and that this balance requires *radish* gene function during *Drosophila* brain development.

2. Mutant optomotor behavior

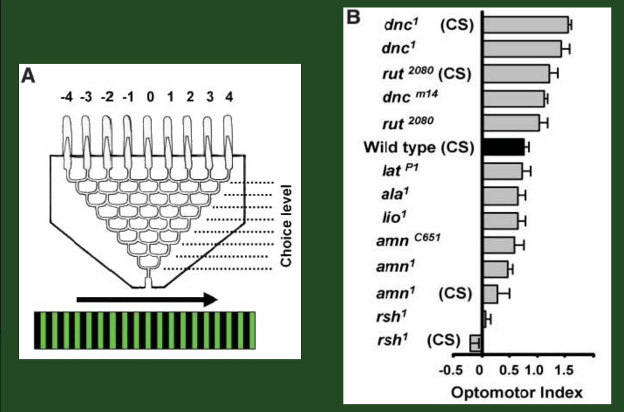

Fig. 1: ***Screening learning and memory mutants using an optomotor maze paradigm.***
A – Experimental setup without distractor. Flies are walking in a multiple Y-Maze. The maze is placed onto a horizontal screen on which a grating is displayed. The arrow denotes the direction of movement of the grating. An Optomotor Index for a population of flies is calculated from the proportion of flies collected in each vial at the end of the maze. B – Ten different learning and memory mutants compared in the optomotor maze against wildtype flies. While *dunce* and *rutabaga* show increased scores, *radish* shows reduced scores.

3. *Radish* behaves randomly in the maze

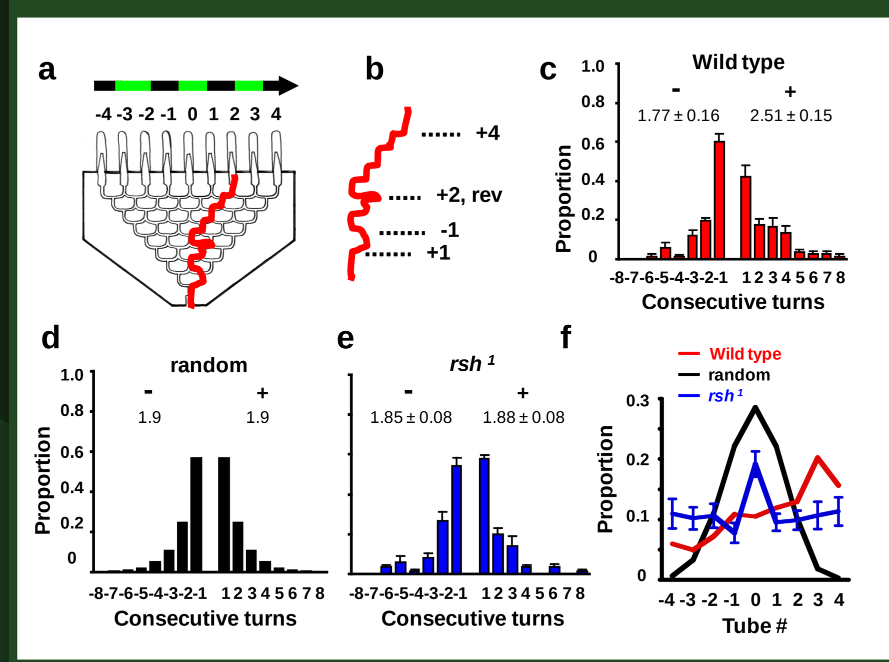

Fig. 2: ***Evaluating the behavior of individual* radish *mutant flies in the optomotor maze.***
A. Maze. Arrow: grating direction. Red trace: filmed path of an individual fly. B. Each individual fly path is quantified for optomotor stereotypy. The number of successive turns in the same direction (+, with the moving grating; -, against the grating) is tallied per fly. Reversals of direction were also counted, rev. C. The normalized frequency of consecutive turn categories is plotted as a histogram for wild type flies (n = 40 flies, +/- s.e.m.). The average value for either direction is indicated. D. Histogram for data created by a random model (50% turn probability at each choice level), with corresponding consecutive turn averages. E. Histogram and turn averages for radish mutants (n = 40 flies). F. Average distribution of flies among the 9 collection tubes (+/- s.e.m) at the end of the maze (n = 8 mazes of 25-30 flies for wild type and radish), compared to hypothetical distribution for the random model.

4. *Radish* is hyperactive

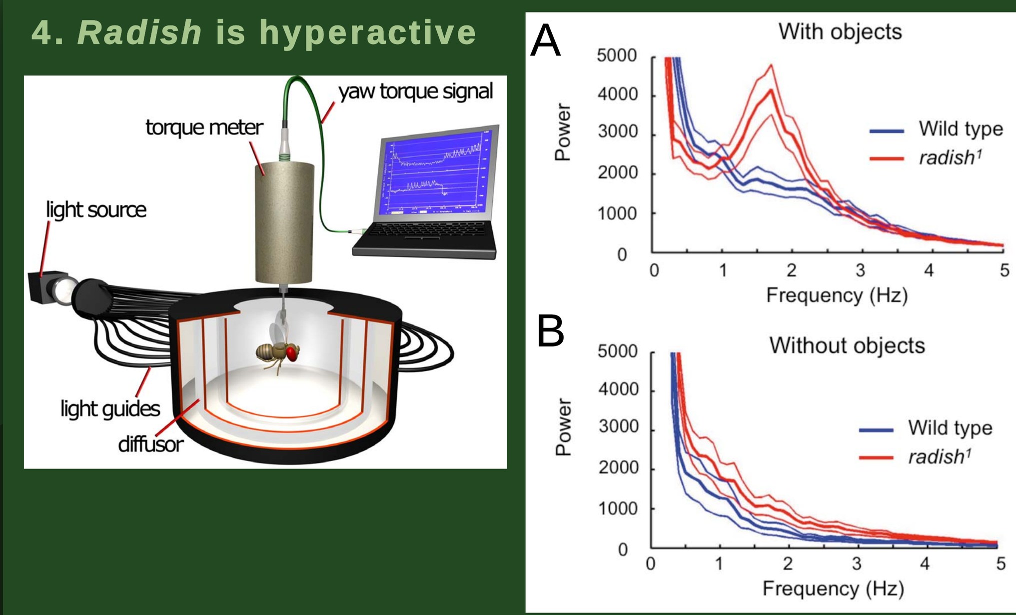

Fig. 3: ***Spontaneous turning: the behavior of individual* radish *mutant flies in tethered flight***
Left: Flies are tethered to a torque meter which measures the attempts of the fly to turn around its vertical body axis (yaw torque). Yaw torque can be made to rotate visual patterns around the fly in a flight-simulator-like situation.
Right: A. Average power spectra between 0 and 5 Hz for wild-type (blue line, n=25) and radish (red line, n=24) torque behavior in 6-minute closed-loop flights with two distinct visual objects. B. Average power spectra between 0 and 5 Hz for wild-type (blue line, n=26) and radish (red line, n=21) torque behavior in 6-minute open-loop flights without any visual landmarks.

5. Reduced fixation time in *radish*

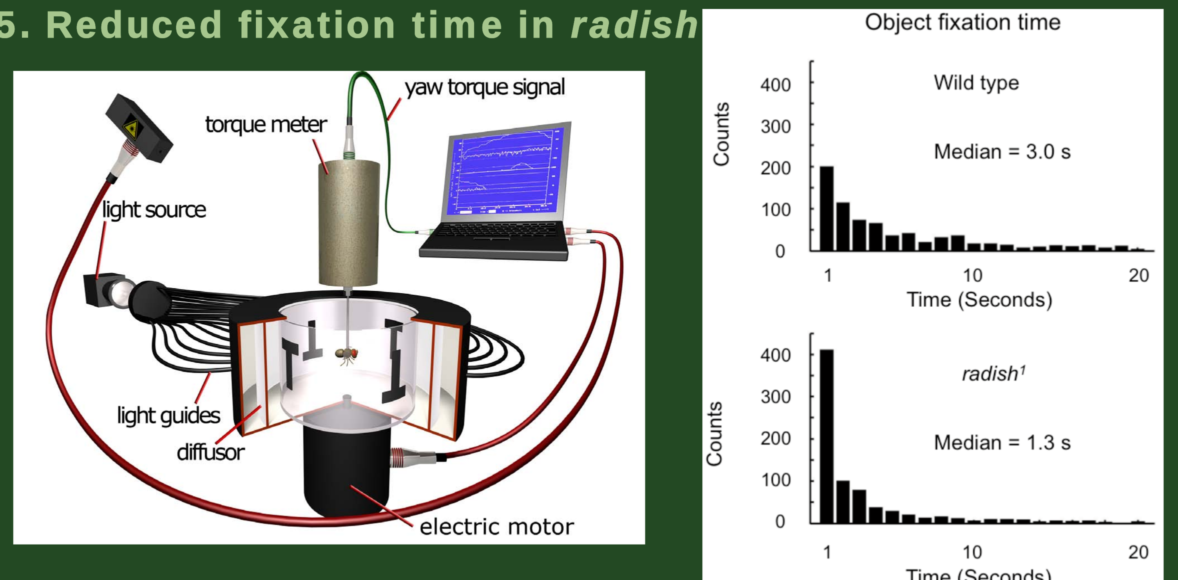

Fig. 4: ***When* radish *mutant flies are chosing flight directions between two different patterns, they fixate the patterns less than wildtype animals.***
Frequency histogram for fixation times in the tethered flight arena. Counts for each time bin are combined for all flies within a genotype; median fixation time is indicated. **A** wild type n=25 B radish[1] n=24.

6. An attention deficit in *radish*

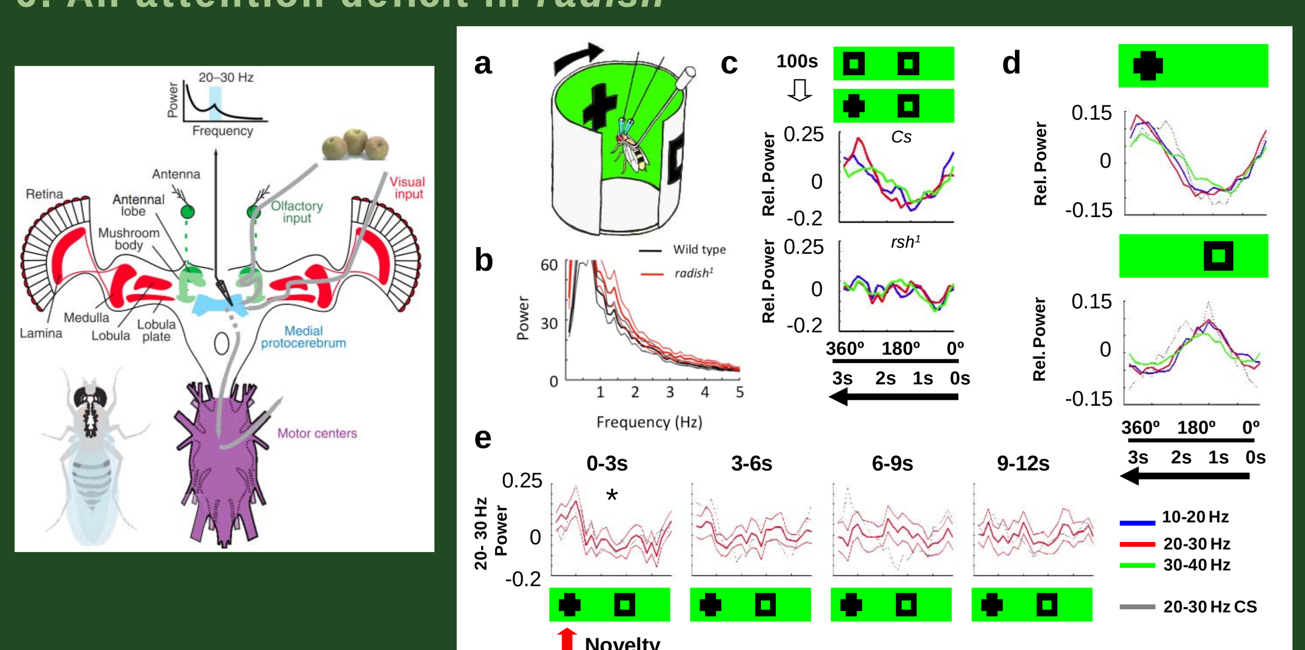

Fig. 5: ***Radish brain recordings reveal a reduction in attention span from 12s to about 3s.***
Left: Experimental setup. Extracellular electrodes in the fly's brain record local field potentials which indicate selective visual attention.
Right: A. Arena setup. Visual objects rotate around the fly counter-clockwise with a period of 3 s. B. Average power spectrum (+/- s.e.m) of radish mutant brain activity between 0 and 3 Hz (N=14 flies). The larger peak below 1 Hz (off scale) represents responses to the visual objects rotating around the fly at 0.33 Hz). C. Novelty paradigm. Flies were exposed for 100 s to two identical squares before one of the squares changed to a cross. Average Local Field Potential (LFP) activity for the 10 s following a novelty transition was calculated for three frequency domains (10-20, blue; 20-30, red, 30-40, green; Wild type, upper panel, N = 8 flies, radish, lower panel, N = 14 flies). The direction of panorama flow is indicated. D. Average LFP responses to each of the two visual objects presented individually. Wild-type 20-30 Hz responses are shown in gray for comparison. E. The same 20-30 Hz radish data as in B., above, but partitioned into successive 3 s epochs following a novelty transition (mean +/- s.e.m, n=14 flies, * = significant response, P < 0.05). Wild-type 20-30 Hz responses are shown in gray for comparison.

7. Attention-like bias switches randomly in *radish* mutant flies

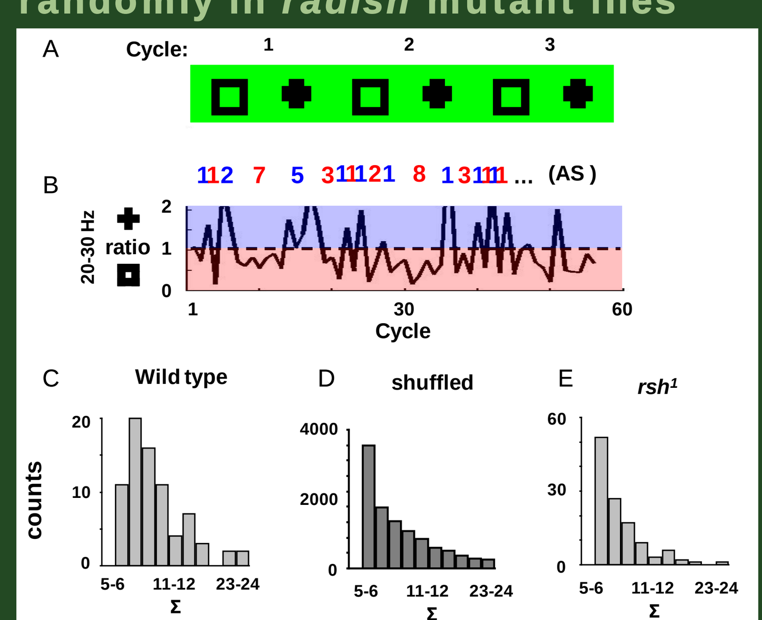

Fig. 6: ***23-30Hz alternations between competing stimuli reveal attention-like bias.***
A. Opposing visual stimuli (a square and a cross, 180° apart) rotate around the fly at 3s per cycle. Each object is thus in front of the fly for 1.5s, for which 20-30 Hz activity is separately calculated (blue and red bars). B. Log ratio of 20-30 Hz activity plotted for successive cycles of image rotation in a sample wild-type fly. AS: attention Span, or the duration (in cycles) when the ratio is biased in succession for one of the objects before alternating, indicated numerically above the graph. C. Frequency distribution of clump size data for 8 wild-type flies exposed to the two competing objects. D. Frequency distribution for 8 sets of shuffled wild-type data. E. Frequency distribution for clump size data from 14 radish flies.

8. Ritalin rescues *radish*

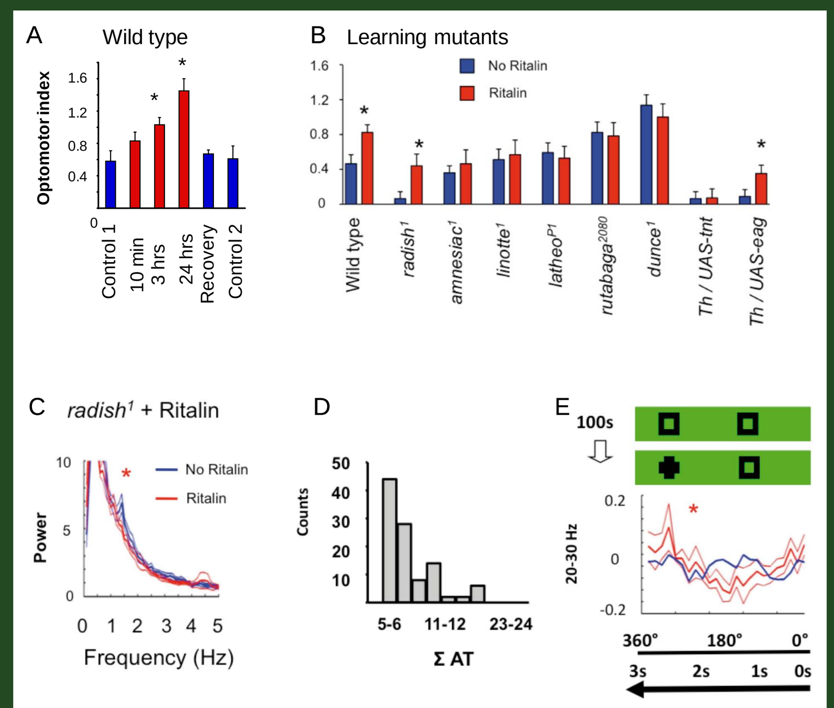

Fig. 7: ***Methylphenidate (Ritalin) rescues* radish *attention-like phenotypes and brain hyperactivity.***
A Optomotor responsiveness in wild-type flies treated with 0.5 mg/ml Ritalin (red histograms). Starved flies were transferred to drug-laced food and allowed to feed for 10 min, 3 hours, or 24 hours. Control flies were similarly transferred, but to food without drug and tested 10 min later (Control1) or the next day (Control2). Flies chronically exposed to drug for 24 hours were transferred back to normal food for 1-2 hours and tested (Recovery). * = significantly different than controls, P < 0.05 by t-test. N=8 runs of 25-30 flies for each experiment. **B** 0.5 mg/ml Ritalin was administered acutely (2-4hrs feeding on drug-laced food) to a panel of learning and memory mutants, as well as to flies with altered dopamine function (Th-Gal4 x UAS-tnt wherein dopaminergic neurons are silenced or x UAS-eag$^{\Delta932}$, where dopaminergic neurons are activated. * = significantly different than controls (red versus blue bars), P < 0.05 by t-test. N=8 runs of 25-30 flies for each experiment. **C** Spectral analysis (0-5 Hz) of LFP activity in the brains of radish mutants treated to acute Ritalin, red line. Blue line: the same flies before treatment. Data are averages of z-scored spectrograms (N=5 flies). * = significantly different (P < 0.05, by t-test). **D**. Distribution of attention-like bias in radish[1] flies treated with Ritalin (N=5 flies, 800s of data each, the same individuals as in C). The distribution was not significantly different (by Kolmogorov-Smirnov test for distributions) than for radish[1] flies fed regular food (Figure 8C). Σ AT, sum of alternation tempos. E 20-30 Hz response to novelty (± s.e.m) following 100s training, averaged for the 10s following a novelty transition, for radish[1] flies fed on 0.5 mg/ml Ritalin–laced food (red line, N = 5 flies, the same individuals as in C). * = Significantly different 20-30 Hz activity between the sectors of the arena comprising either object (P < 0.05). Blue line, 20-30 Hz response for radish[1] flies fed food without Ritalin.

9. Conclusions

It becomes increasingly apparent that many classical *Drosophila* learning and memory mutants are also defective in short-term processes relevant to selective attention. Previous studies have shown that short-term memory as well as long-term memory mutants display attention-like defects, and the current study reveals *radish* mutants to be defective as well, albeit with distinctly different symptoms. The *Drosophila* mutants *dunce*[1], *rutabaga*[2080] and *radish*[1] share olfactory memory defects but differ conspicuously for short-term processes relevant to visual attention. While the more persistent optomotor behavior of *dunce*[1] and *rutabaga*[2080] – both affecting the cyclic AMP–associated pathways – are reminiscent of the persistent preoccupation of some patients afflicted with autism, the phenotype of *radish* mutant flies described here is similar to some of the symptoms of patients with attention-deficit, hyperactivity disorder (ADHD).
Paralleling results with human patients, some of the ADHD-like phenotypes of *radish* mutants are rescued by methylphenidate (Ritalin) administration, while Ritalin treatmetn has no effect on *dunce* or *rutabaga* mutants.
Our behavioral and electrophysiological results suggest that alternation processes in *radish* mutants are defective: whereas wild-type flies showed some persistence or hysteresis between alternations in behavior or brain activity, *radish* mutants alternated too quickly or even in an oscillatory manner when presented with competing visual stimuli. In contrast, at least behaviorally, *dunce* and *rutabaga* mutants exhibit an opposite phenotype; both display persistent choice behavior in the optomotor maze and both are less distracted by competing visuals than wild type.
Attaining the right balance between persistence and flexibility is a crucial feature of adaptive behavior, as it reflects the balance between exploration and exploitation of natural resources. It is tempting to speculate that *radish* and *dunce*/*rutabaga* may constitute the two respective extremes of this balance.

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