Attention Deficit and Hyperactivity in a *Drosophila* Memory Mutant

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1. Abstract

Action selection is modulated by external stimuli either directly or via memory retrieval. In a constantly changing environment, animals have evolved attention-like processes to effectively filter the incoming sensory stream. These attention-like processes, in turn, are modulated by memory. The neurobiological nature of how attention, action selection and memory are inter-connected is unknown. We describe here new phenotypes of the memory mutant radish in the fruit fly Drosophila. In several different behavioral and electrophysiological assays, radish mutant flies revealed a reduced attention span, more frequent and more random alternations in choice behavior, as well as a well-defined oscillatory hyperactivity in both brain activity and behavior. Specifically, radish mutants showed impaired optomotor behavior in a walking maze, despite showing optomotor behavior in flight. In the maze, radish mutant flies exhibited more random alternations in choice behavior at each branch point than wildtype flies. Furthermore, recordings of local field potentials in the fly brain revealed a shorter attention span when the flies were presented with two competing visual patterns, as well as a more random alternation of brain activity in response to these patterns. These brain recordings also revealed a peak at ~1.6Hz in the power spectrum of the local field potentials, where no such peak could be observed in the wildtype animals. The same oscillatory hyperactivity at ~1.6Hz could be observed in turning behavior measured in tethered flight, both with and without visual patterns surrounding the fly. These phenotypes were rescued by transgenically expressing the Radish protein in a mutant background during fly development, but not in the adult. In addition, administration of a drug commonly used to treat Attention-Deficit Hyperactivity Disorder (ADHD) in humans, methylphenidate (Ritalin) also rescued the optomotor behavior, the reduced attention span and abolished the ~1.6Hz hyperactivity in treated flies. We conclude that the circuits defined by radish expression in the fly brain are involved in modulating the tempo of stimulus selection and suppression. It remains to be found out if methylphenidate also rescues the radish memory defect. A failed rescue would indicate that the phenotype discovered here is not the cause for radish's memory defect. Our findings allow for the first time to study how action selection is modulated by the interplay of external stimuli, attention and memory in a genetically tractable model organism.

2. Mutant optomotor behavior

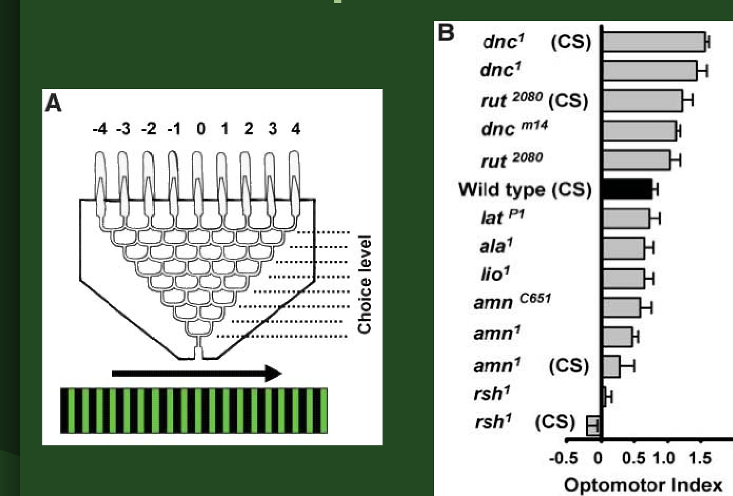

Fig. 1: ***Screening learning and memory mutants using an optomotor maze paradigm.***
A – Experimental setup without distractor. Flies are walking in a multiple Y-Maze. The maze is placed onto a horizontal screen on which a grating is displayed. The arrow denotes the direction of movement of the grating. 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

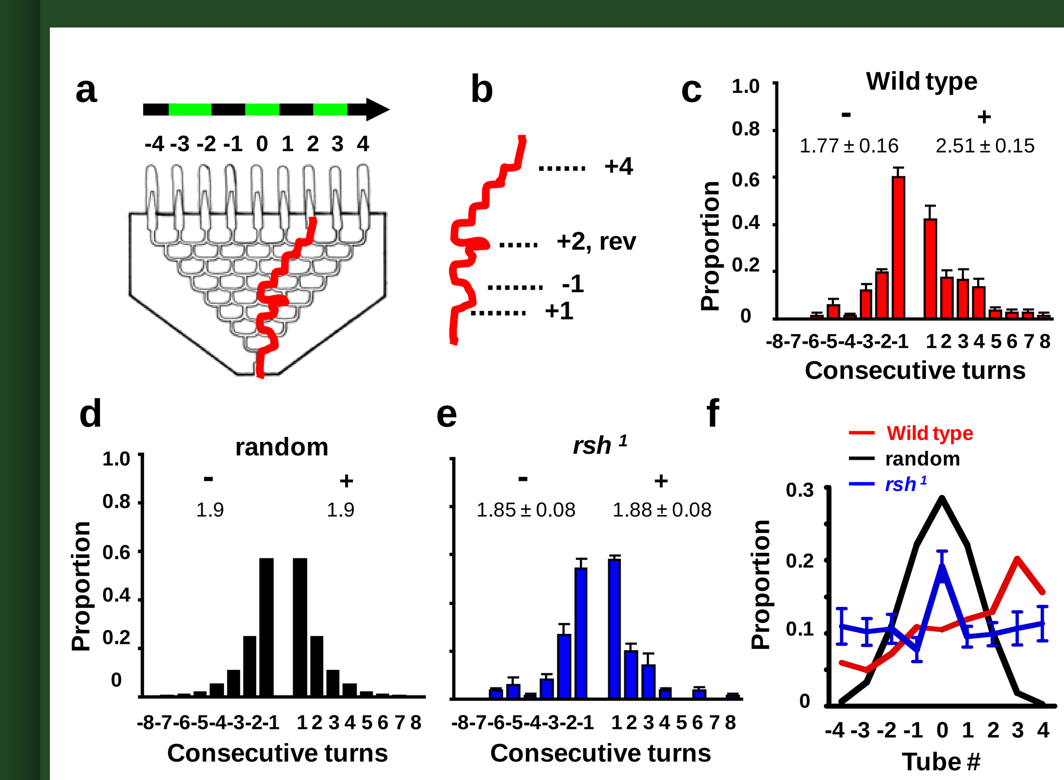

Fig. 2: ***Evaluating the behavior of individual*** **radish** ***mutant flies in the optomotor maze.***
A. Maze. Arrow: grating direction. Red trace: filmed path of an individual fly. B. Each individual fly path is quantified for optomotor stereotypy. The number of successive turns in the same direction (+, 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

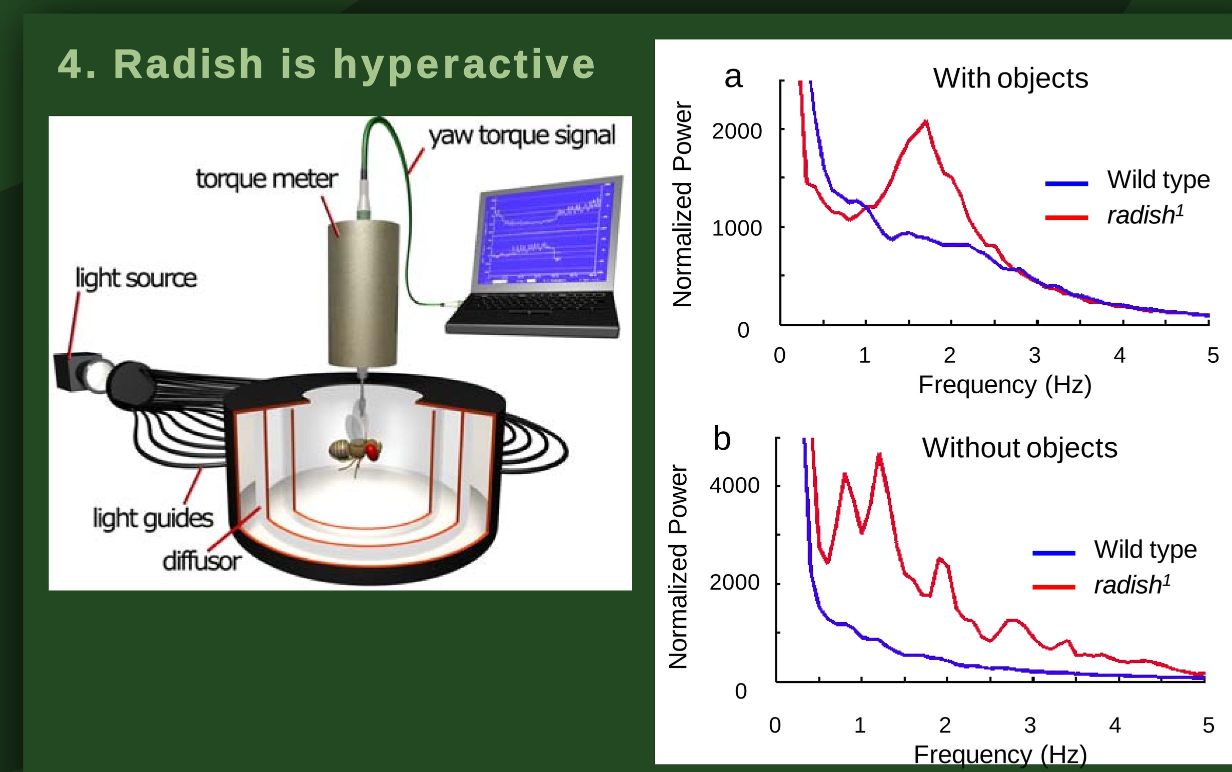

Fig. 3: ***Spontaneous turningthe behavior of individual*** **radish** ***mutant flies in tethered flight***
Left: Flies are tethered to a torque meter which measures the attempts of the fly to turn around its vertical body axis (yaw torque). Yaw torque can be made to rotate visual patterns around the 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. An attention deficit in *radish*

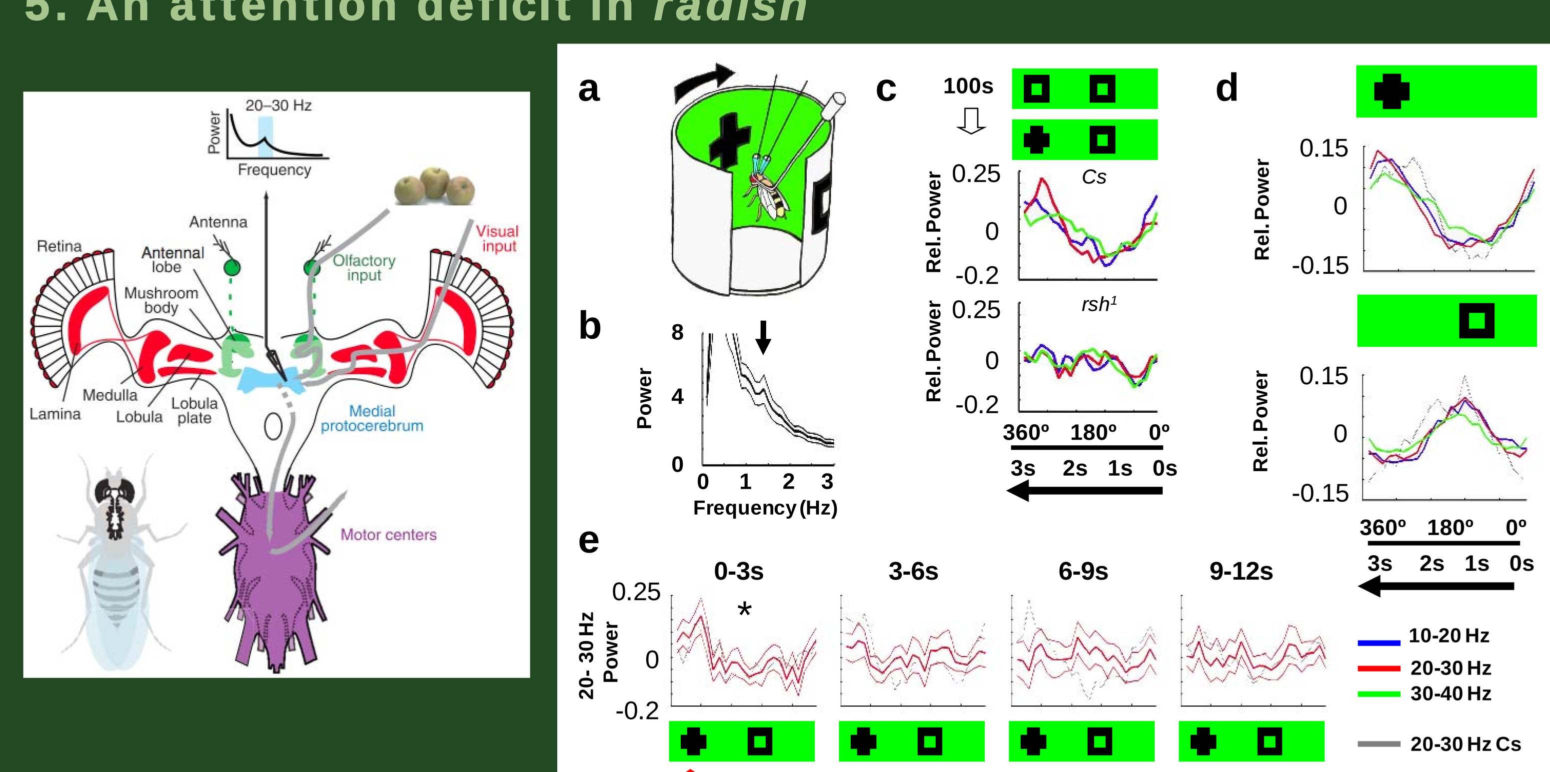

Fig. 4: ***Radish brain recordings reveal a reduction in attention span from 12s to about 3s.***
Left: Experimental setup. Extracellular electrodes in the fly's brain record local field potentials which indicate selective visual attention.
Right: A. Arena setup. Visual objects rotate around the fly counter-clockwise 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). Arrow, peak at ~1.5 Hz. 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.

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

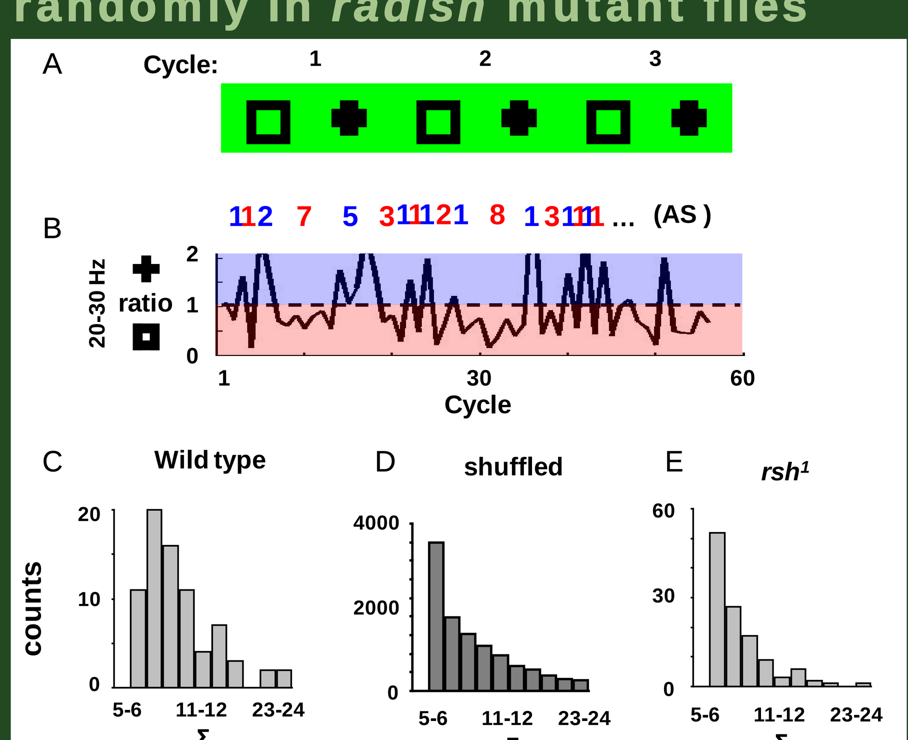

Fig. 5: ***23-30Hz alternations between competing stimuli reveal attention-like bias.***
A. Opposing visual stimuli (a square and a cross, 180° apart) rotate around the fly at 3s per cycle. Each object is thus in front of the fly for 1.5s, for which 20-30 Hz activity is separately calculated (blue and 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.

7. Ritalin rescues *radish*

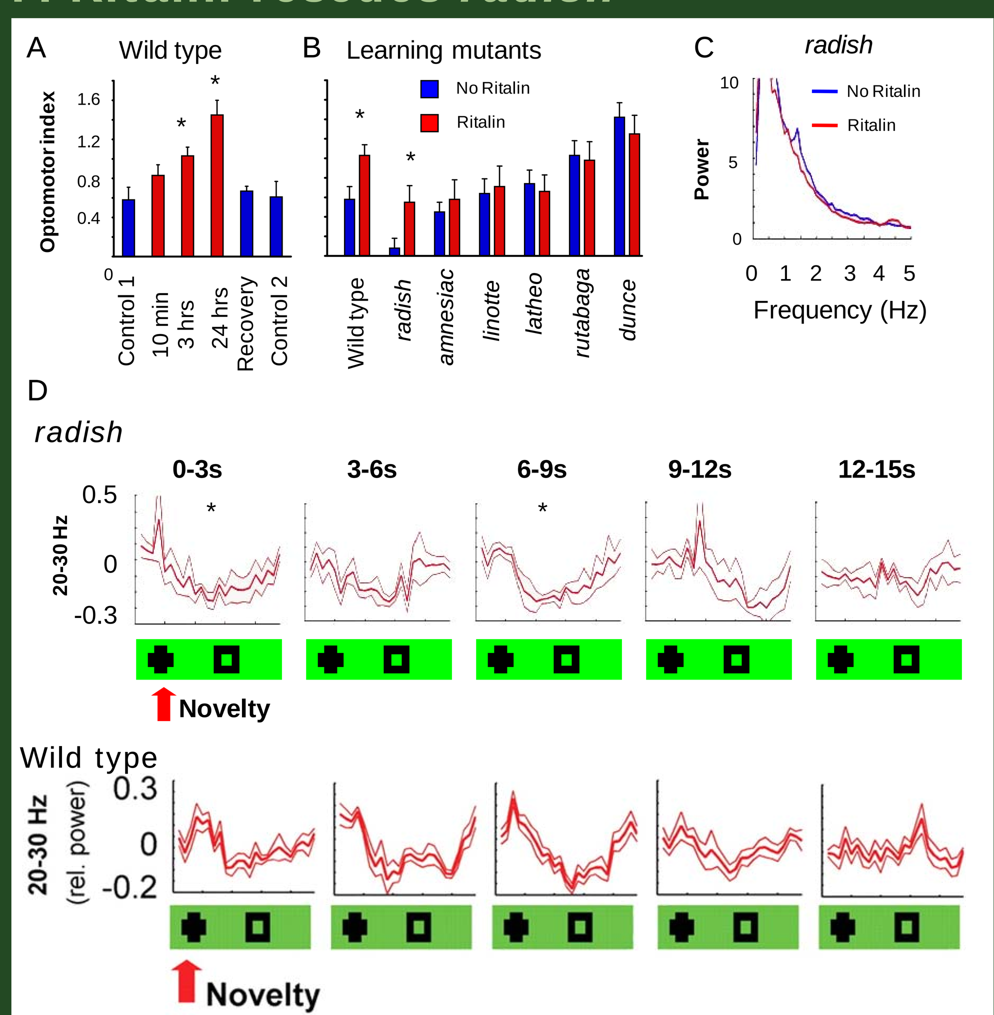

Fig. 6: ***Methylphenidate (Ritalin) rescues*** **radish** ***attention-like phenotypes and brain hyperactivity.***
A. Optomotor responsiveness in wild-type flies treated with 0.5 mg/ml methylphenidate (red histograms). Starved flies were transferred to drug-laced food and allowed to feed for 10 min, 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 methylphenidate was administered acutely (2-4hrs feeding on drug-laced food) to a panel of learning and memory mutants. * = significantly different than controls (red versus grey bars), P < 0.05 by t-test. N=8 runs of 25-30 flies for each experiment. C. Spectral analysis of LFP activity in the brains of *radish* mutants treated to acute methylphenidate (Ritalin, red line). Blue line: the same flies before treatment. Data are averages of z-scored spectrograms (N=5 flies). D top. 20-30 Hz response to novelty (+/- s.e.m) following 100s training (as in Fig. 4), plotted as successive 3s average, for *radish* flies fed on 0.5 mg/ml methylphenidate –laced food. N = 5 flies. * = Significantly different 20-30 Hz activity between the sectors of the arena comprising either object (P < 0.05). D bottom. Previously published analogous experiment in wild type flies.