Science Advances

Supplementary Materials for

Retinoic acid inhibitors mitigate vision loss in a mouse model of retinal degeneration

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The PDF file includes:

Figs. S1 to S3
Legends for tables S1 to S5

Other Supplementary Material for this manuscript includes the following:
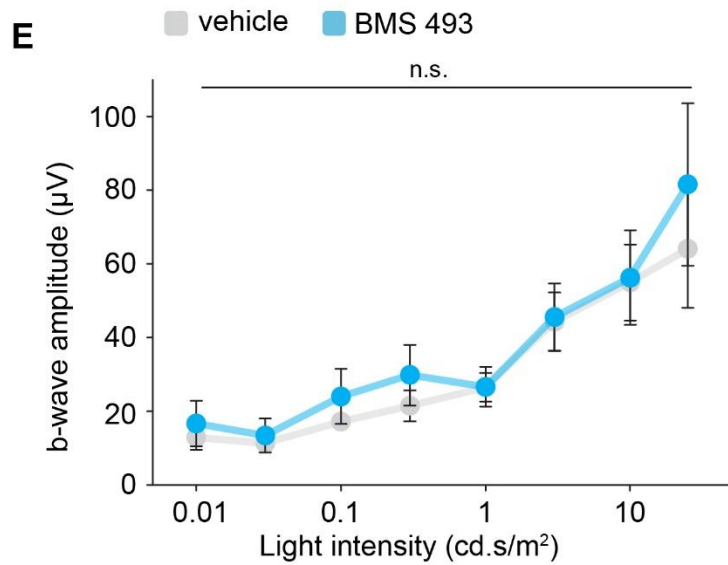
Tables S1 to S5

A control disulfram

B control disulfram

n.s.

C vehicle BMS 493
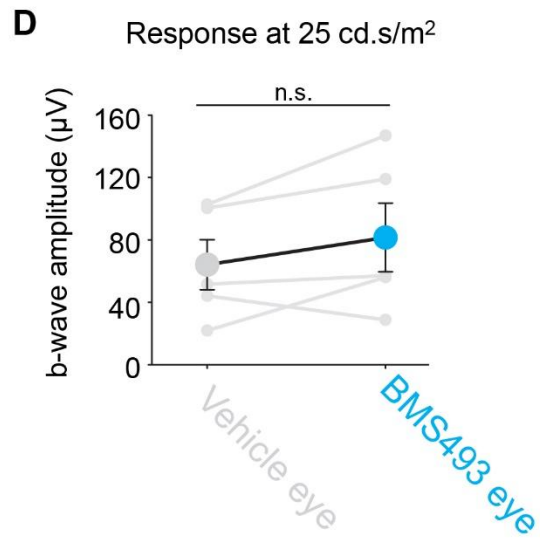
D Response at 25 cd.s/m² n.s.
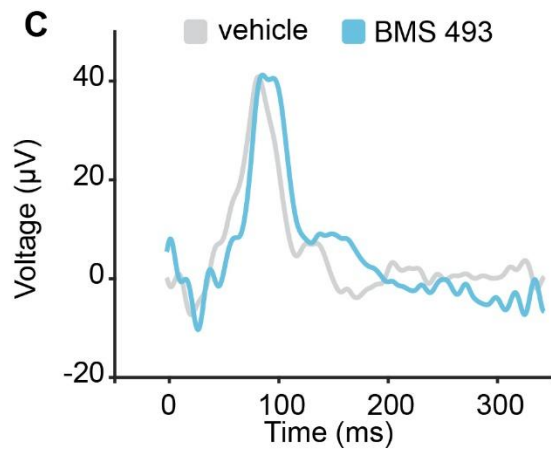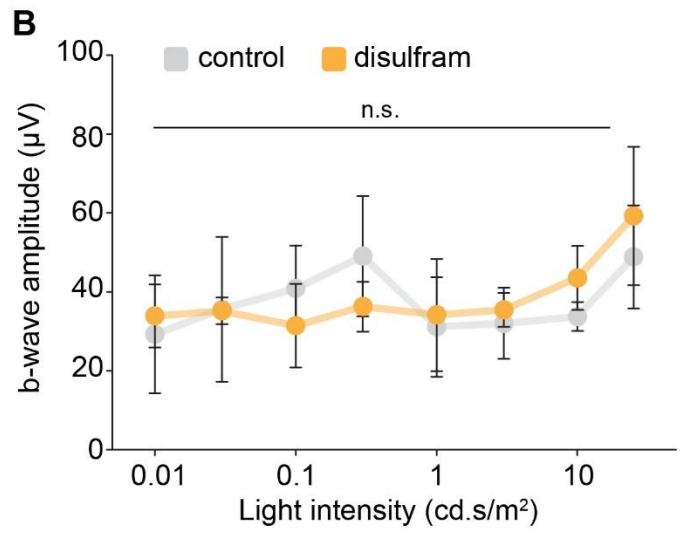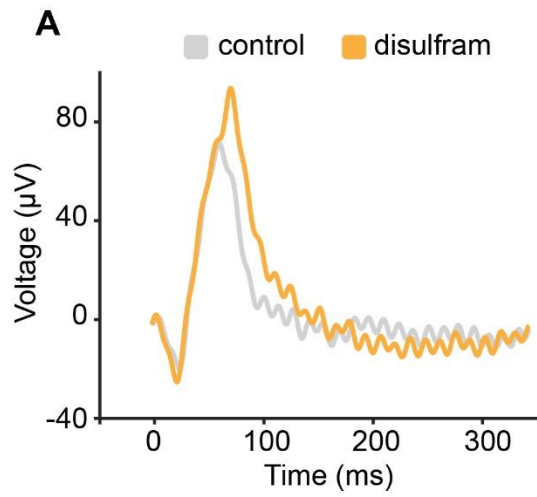
E vehicle BMS 493

n.s.

Fig. S1. The ERG b-wave is unchanged by treatment with disulfiram or BMS 493.

A) Electroretinogram (ERG) recordings of P80-90 rd10 mice treated for 40 days with disulfiram (orange) vs control (grey). Traces are the mean response to 32 flashes of light (25 cd.s/m^2 light for 20 msec) presented at a frequency of 2 Hz. **B)** Intensity-response curves showing no significant effect of disulfiram. The amplitude of the b-wave was measured from the peak of the first inward voltage deflection (a-wave) to the peak of the first outward voltage deflection. Recordings were obtained from 3 control mice (6 eyes) and 4 disulfiram mice (8 eyes) and are shown as mean ± SEM. n.s. - non-significant difference, p>0.05, 2-tailed t-test (**Table S3**). **C)** ERG recordings 4-6 days after injecting vehicle (PBS x1, grey) into one eye and BMS 493 (blue) into the contralateral eye. **D)** Responses from BMS 493-injected and vehicle-injected eyes in all 5 mice tested. **E)** Intensity-response curves showing no significant effect of BMS 493. Recordings were obtained from 5 mice (10 eyes), one eye injected with BMS 493 and the contralateral eye with vehicle. Injections were randomized across eyes and mice. n.s. - non-significant difference, p>0.05, 2-tailed paired t-test (**Table S3**).
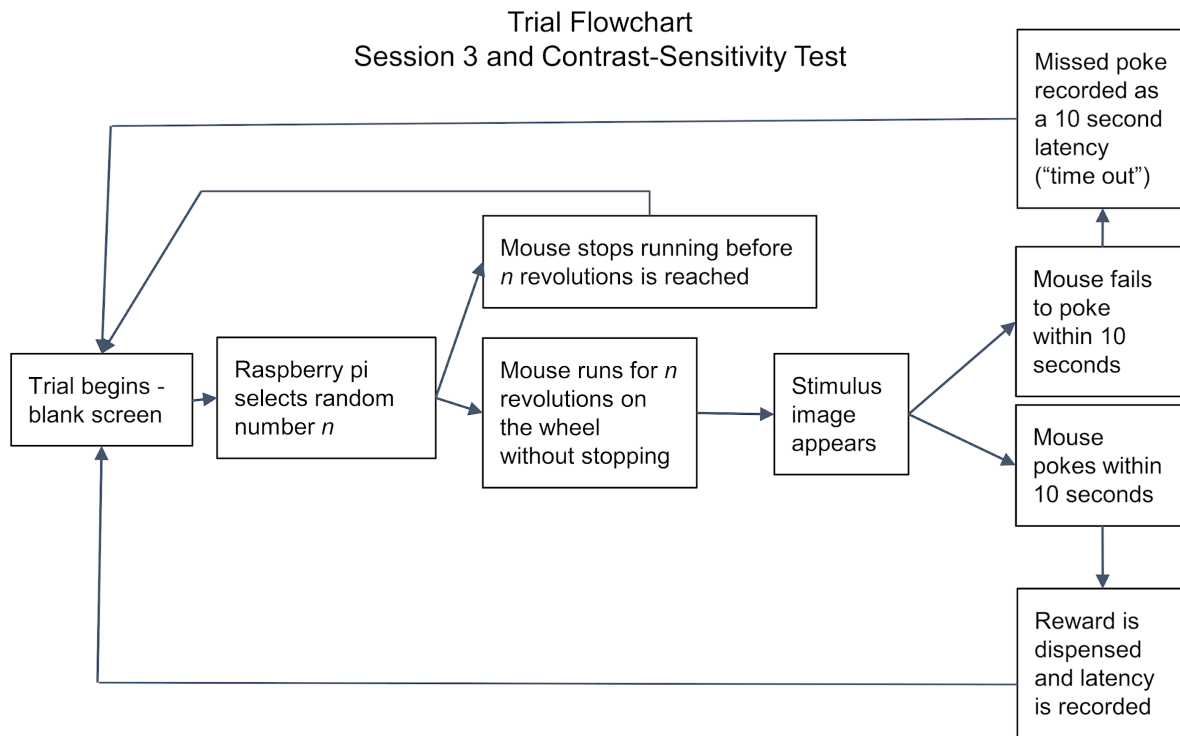
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Trial Flowchart
Session 3 and Contrast-Sensitivity Test

```
                                                    ┌──────────────┐
                                                    │ Missed poke  │
                                                    │ recorded as  │
                                                    │ a 10 second  │
                                                    │ latency      │
                                                    │ ("time out") │
                                                    └──────────────┘
          ┌────────────────────────────┐
          │  ┌──────────────────────┐   │           ┌──────────────┐
          │  │ Mouse stops running  │   │           │ Mouse fails  │
          │  │ before n revolutions │   │           │ to poke      │
          │  │ is reached           │   │           │ within 10    │
          │  └──────────────────────┘   │           │ seconds      │
          │                             │           └──────────────┘
 ┌────────────┐  ┌────────────┐  ┌──────────────┐  ┌──────────┐  ┌──────────────┐
 │ Trial      │  │ Raspberry  │  │ Mouse runs   │  │ Stimulus │  │ Mouse        │
 │ begins -   │→ │ pi selects │→ │ for n        │→ │ image    │→ │ pokes within │
 │ blank      │  │ random     │  │ revolutions  │  │ appears  │  │ 10 seconds   │
 │ screen     │  │ number n   │  │ on the wheel │  │          │  └──────────────┘
 └────────────┘  └────────────┘  │ without      │  └──────────┘
                                 │ stopping     │            ┌──────────────┐
                                 └──────────────┘            │ Reward is    │
                                                             │ dispensed    │
                                                             │ and latency  │
                                                             │ is recorded  │
                                                             └──────────────┘
```

Fig. S2. Parameters defining a single trial in a trained mouse.

During session 3 and contrast sensitivity test (**Fig. 3B-C**), the stimulus is displayed for a maximum of 10 seconds after it is triggered by wheel revolutions. Each trial begins with a blank screen displaying a 0% contrast version of the stimulus image ('blank screen'). The computer (i.e.: raspberry pi) chooses a random number of wheel revolutions (see **Table S4**) necessary to display the stimulus image. Stimulus image is always 100% contrast in sessions 1-3, or 1 out of 9 different contrasts, randomly chosen, during contrast sensitivity test. The response of the mouse (i.e. nose poke) is registered, and the delivery of reward is activated if the mouse pokes before "time out" (10 seconds for training session 3 and contrast sensitivity test, see **Table S4**).

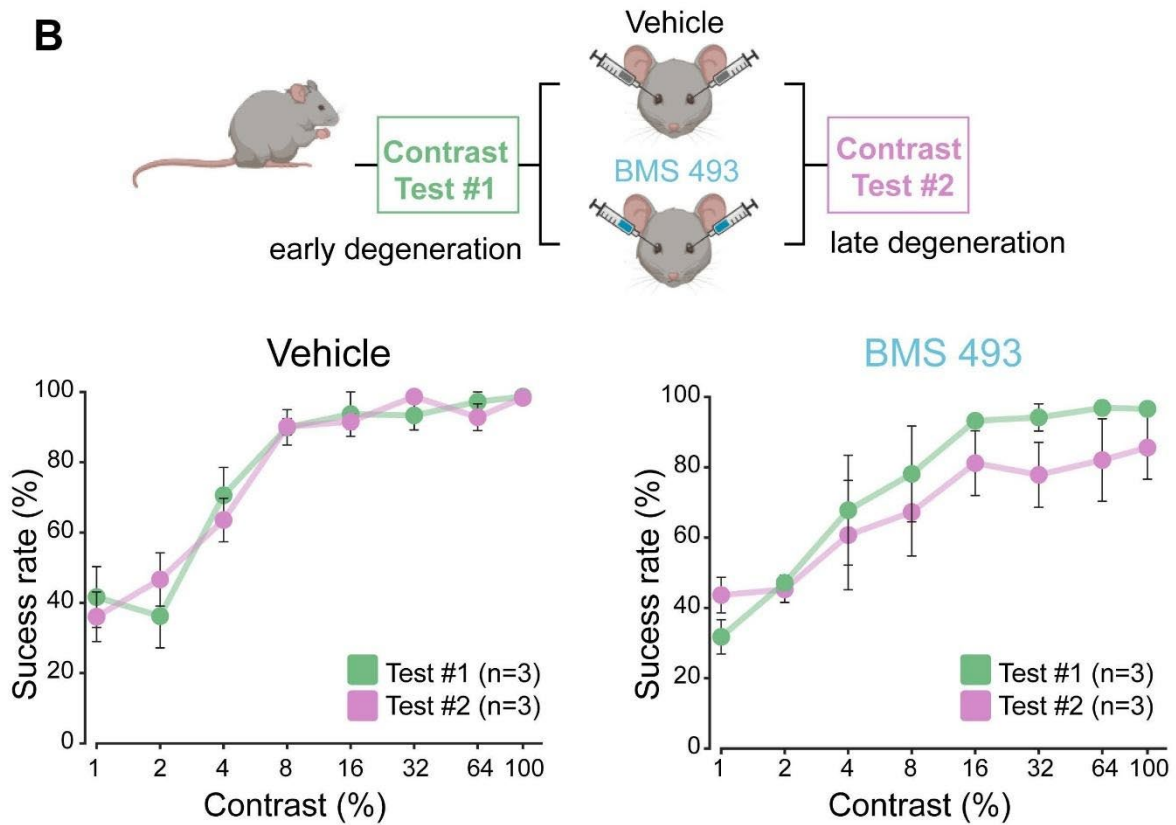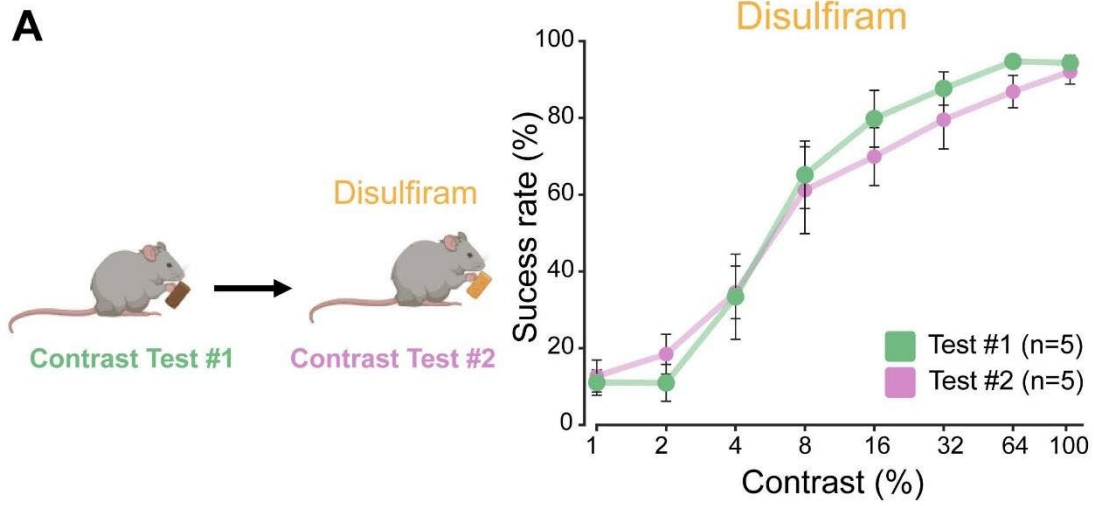Type or paste caption here. Create a page break and paste in the Figure above the caption.

A

Disulfiram

Contrast Test #1 → Disulfiram / Contrast Test #2

Disulfiram

Success rate (%) vs Contrast (%)

Test #1 (n=5)
Test #2 (n=5)

B

Vehicle

Contrast Test #1 — early degeneration

BMS 493

Contrast Test #2 — late degeneration

Vehicle

Success rate (%) vs Contrast (%)

Test #1 (n=3)
Test #2 (n=3)

BMS 493

Success rate (%) vs Contrast (%)

Test #1 (n=3)
Test #2 (n=3)

Fig. S3. Testing disulfiram and BMS 493 in wild-type mice with normal vision.

A) Left: experimental design. C57/Black male mice were trained and tested at ~P60, using the same behavioral paradigm as shown in **Fig. 3A-C**. Mice were fed a regular diet without disulfiram and tested for contrast sensitivity (test #1), after which their food was switched to that containing disulfiram (2 mg/Kg) for 40 days. At ~P100 they were tested for the second time (test #2). Right: success rate for each contrast tested before (green) and after (pink) disulfiram treatment. Values are shown as mean ± SEM, p>0.05 in all contrasts, Mann-Whitney test. **B)** Similar to **A**, above. Six C57/Black male mice were trained and tested at P60, and then injected intravitreally in both eyes with 1 µl of vehicle (PBS x1) or BMS 493 (5 µM). At 4-6 days post-injection, they were tested for a second time. The success rate in test #1 was not significantly different from the success rate during test #2 in either of the two groups. Values are shown as mean ± SEM, p>0.05 in all contrasts, Mann-Whitney test. Type or paste caption here. Create a page break and paste in the Figure above the caption.

TABLE S1   Corresponds to Figure 2

Change in # active units per retina

	control	disulfiram	
# active units	568	443	
pieces of retina	11	10	
mean # of units	51.6	44.3	
			Delta
normalized %	100	85.8	14.2

Change in # active units per retina

	vehicle	BMS	
# active units	271	135	
pieces of retina	7	6	
mean # of units	38.7	22.5	
			Delta
normalized %	100	58.1	41.9

Table S1. Data on number of active units in MEA recordings (corresponds to Figure 2).

Individual data points for mean firing frequency (**Fig. 2C**), and cumulative probability plot (**Fig. 2D**) in control and disulfiram-treated mice, as well as in vehicle and BMS 493-injected mice (**Fig. 2G,H**). In addition the table provides the data for the total and relative number of active units in retinas in all four conditions. Data obtained using ex-vivo MEA recordings in ACSF (see **Materials and Methods**).

TABLE S2 Supplemental Figure 1 - ERG in Control vs. Disulfiram, BMS 493 / Vehicle injected rd10 mice

(All data passed Normality Test Shapiro-Wilk)

Fig. S1B Control — b-wave Amplitude (μV)

Light intensity	Mouse 1		Mouse 2		Mouse 3			Light intensity	Mouse 1	Mouse 2	Mouse 3		b-wave Amplitude (μV)		2-tailed T-test
	Eye 1	Eye 2	Eye 1	Eye 2	Eye 1	Eye 2		cd.s/m^2	Mouse 1	Mouse 2	Mouse 3		mean	sem	p value
cd.s/m^2	μV	μV	μV	μV	μV	μV									
0.01	60	48.3	54.15	4.3	0.8	2.55	30	31.9	30.95	0.01	54.15	2.55	30.95	29.2167	14.9208	0.662753248
0.03	92.7	51.1	71.9	7.5	18.5	13	21	22.7	21.85	0.03	71.9	13	21.85	35.5833	18.3372	0.969682258
0.1	69.9	48.7	59.3	5.3	37.8	21.55	38.1	45	41.55	0.1	59.3	21.55	41.55	40.8	10.9039	0.383820624
0.3	86.4	71.1	78.75	14.2	42.4	28.3	27.5	52.4	39.95	0.3	78.75	28.3	39.95	49	15.2504	0.202035364
1	44.9	63.7	54.3	15.9	5.8	10.85	12.4	43.8	28.1	1	54.3	10.85	28.1	31.0833	12.6313	0.783167955
3	29.7	69.2	49.45	17.3	21.6	19.45	27.1	-	27.1	3	49.45	19.45	27.1	32	9.00014	0.728966639
10	23.2	46.5	34.85	22.8	31	26.9	32.9	46	39.45	10	34.85	26.9	39.45	33.7333	3.66564	0.171284096
25	45.9	63.2	54.55	13.3	34.5	23.9	69.7	66.3	68	25	54.55	23.9	68	48.8167	13.0493	0.441101807

(All data passed Normality Test Shapiro-Wilk)

Disulfiram — b-wave Amplitude (μV)

Light inten	Eye 1		Eye 2		Eye 1	Eye 2	Eye 1	Eye 2	Eye 1	Eye 2			b-wave Amplitude (μV)	
					Mouse 2		Mouse 3		Mouse 4					
cd.s/m^2	μV		μV		μV	μV	μV	μV	μV	μV		Light intensity	Mouse 1	Mouse 2
0.01	53.9	26.1	40	34.4	17.1	25.75	10.9	22.3	16.6	8.1	98.1	53.1	0.01	40	25.75	16.6	53.1	33.8625	8.0185	0.662753248
0.03	21	62.7	41.85	25.7	-		25.7	24.9	47.1	36	5.1	69.1	37.1	0.03	41.85	25.7	36	37.1	35.1625	3.39986	0.969682258
0.1	81.4	40.5	60.95	4	22.5	13.25	18.5	44.2	31.35	20.1	-	20.1	0.1	60.95	13.25	31.35	20.1	31.4125	10.529	0.383820624
0.3	52.5	56.6	54.55	39.2	14.5	26.85	33.1	37.3	35.2	6.5	50	28.25	0.3	54.55	26.85	35.2	28.25	36.2125	6.37931	0.202035364
1	61.5	88.6	75.05	24.1	11.5	17.8	4.8	19.6	12.2	6.5	56.4	31.45	1	75.05	17.8	12.2	31.45	34.125	14.228	0.783167955
3	52.2	4.9	28.55	31.5	23.3	27.4	28.7	56.2	42.45	24.3	62	43.15	3	28.55	27.4	42.45	43.15	35.3875	4.28842	0.728966639
10	9.5	63.1	36.3	59.3	20.7	40	26.6	34.7	30.65	52.8	81.5	67.15	10	36.3	40	30.65	67.15	43.525	8.10621	0.171284096
25	90.8	5.8	48.3	20.1	10.4	15.25	62	95.2	78.6	67.8	121.8	94.8	25	48.3	15.25	78.6	94.8	59.2375	17.5455	0.441101807

(All data passed Normality Test Shapiro-Wilk)

Fig. S1D,E — b-wave Amplitude (μV)

Light intensity	Mouse 1		Mouse 2		Mouse 3		Mouse 4		Mouse 5				Vehicle				
cd.s/m^2	Eye 1	Eye 2	Eye 1	Eye 2	Eye 1	Eye 2	Eye 1	Eye 2	Eye 1	Eye 2							
0.01	4.1	6.1	17.2	15.6	6.7	12.8	10.4	10.5	40.2	23.6		Eye 2	Eye 1	Eye 1	Eye 2	Eye 2	
0.03	31	16.2	10.4	4	11.6	10.5	10.4	6.6	11.1	11.7		6.1	17.2	6.7	10.5	23.6	
0.1	17.5	15.2	16	7.9	12	35.9	11.6	20	47	22.7		16.2	10.4	11.6	6.6	11.7	
0.3	21.8	20.2	16	16.9	32	41	12.8	9.3	56.5	29.6		15.2	16	12	20	22.7	
1	14.5	24.1	29.5	41.7	29.4	20.9	18.7	13.2	37.2	36.4		20.2	16	32	9.3	29.6	
3	19.7	43.8	72.5	73.2	47.1	36.9	40.7	27.8	57.2	30.1		24.1	29.5	29.4	13.2	36.4	
10	18.2	35.6	82.7	91.7	75.8	68.8	37.5	48.2	65.1	32.5		43.8	72.5	47.1	27.8	30.1	
25	28.7	44.2	100.3	119	102.6	146.9	57.1	51.6	56	21.9		35.6	82.7	75.8	48.2	32.5	

Vehicle — b-wave Amplitude (μV)

Light Inten	mean	sem	Eye 1	Eye 2	Eye 2	Eye 1	Eye 1	Light Inte	mean	sem	p value
0.01	12.82	3.342065231	4.1	15.6	12.8	10.4	40.2	0.01	16.62	6.19309	0.60391
0.03	11.3	1.535578067	31	4	10.5	10.4	11.1	0.03	13.4	4.58705	0.67566
0.1	17.18	1.878403578	17.5	7.9	35.9	11.6	47	0.1	23.98	7.50276	0.40493
0.3	21.42	4.222605831	21.8	16.9	41	12.8	56.5	0.3	29.8	8.23916	0.39184
1	26.52	3.860492197	14.5	41.7	20.9	18.7	37.2	1	26.6	5.39296	0.99067
3	44.26	7.991282751	19.7	73.2	36.9	40.7	57.2	3	45.54	9.12911	0.91858
10	54.96	10.31690845	18.2	91.7	68.8	37.5	65.1	10	56.26	12.8294	0.999
25	64.12	16.0090412	28.7	119	146.9	57.1	56	25	81.54	22.0418	0.5404

Table S2. Data and statistical analysis for Supplemental Figure 1.

Individual data points and statistical analysis for the b-wave's amplitude (in μV), used for the plots shown in **Fig. S1B,D,E**). Data obtained using in-vivo ERG recordings of anesthetized mice (see **Materials and Methods**).

TABLE S3	**Corresponds to Figure 3B-C**		Parameters of training and testing protocols in the behavioral paradigm			

Protocol	Required Wheel Revolutions	Reward Pump Duration	Maximum Rest	Reward Availability	Blank Image	Stimulus Images
Habituation	None	3 seconds	None	Constant	None	100% contrast
Session 1	25	3 seconds	None	Until poke	0% contrast	100% contrast
Session 2	25	1 second	10 seconds	30 seconds	0% contrast	100% contrast
Session 3	10 - 50	1 second	10 seconds	10 seconds	0% contrast	100% contrast
Contrast Sensitivity Test	10 - 25	1 second	5 seconds	10 seconds	0% contrast	0,1,2,4,8,16,32,64,100% contrast

Table S3. Parameters of training and testing protocols in the behavioral paradigm (corresponds to Figure 3).

Detailed description of the parameters employed during each protocol for habituation, training and testing, in the mouse behavioral paradigm (**Fig. 3B,C**). Each protocol lasted 12 hours from 6 PM to 6 AM. Protocols were encoded in python and manually loaded onto each raspberry pie using signature dongles, each corresponding to a specific cage.

| TABLE S4 | Figure 4 - Visual Behavior Disulfiram Treatment | | | | | | | | | | | | |

Fig 4D — Success Rate

	All mice n = 13				TEST 1	*** Before any treatment was given to any mouse					Mann-Whitney U Test	
TEST 1						Control (n=7)		Disulfiram (n=6)				
Contrast %	mean	sem				mean	sem	mean	sem		U value	P value
8	22.77	4.78				19.98	4.86	26.02	9.06		21.00	P = 0.998
16	35.00	7.74				28.97	6.89	42.02	15.05		20.00	P = 0.156
32	57.31	6.46				47.18	6.88	69.13	9.94		10.00	P = 0.486
64	85.62	3.83				82.26	6.12	89.54	4.25		15.00	P = 0.163
100	93.90	1.02				94.08	1.69	93.69	1.17		19.00	P = 0.186

Fig 4E — Success Rate

TEST 2	Control (n=7)		Disulfiram (n=6)			Mann-Whitney U Test				T-Test		
Contrast %	mean	sem	mean	sem		U value	P value		Normality Test	t	P value	
8	16.18	3.78	18.86	5.66		19.0	P = 0.836					
16	20.69	4.95	31.09	13.50		18.50	P = 0.731					
32	23.83	4.56	50.75	13.52		10.00	P = 0.138		Passed	-2.012	P = 0.0535	
64	26.26	6.37	76.65	8.28		2.00	P = 0.005		Passed	4.901	P = <0.001	
100	31.33	7.93	81.69	6.72		1.00	P = 0.002		Passed	-4.75	P = <0.001	

Inclusion and exclusion of mice in Control / Disulfiram experiments

Control Mice	Status	Reason			Disulfiram	Status	Reason	
RD10-N2	included	ratio SD/mean = 0.3			RD10-N1	included	ratio SD/mean = 0.3	
RD10-N4	excluded	<80% success Session 3			RD10-N3	included	ratio SD/mean > 0.4	
RD10-N6	included	ratio SD/mean = 0.2			RD10-N5	excluded	did not perform on Test 2	
RD10-P1	included	ratio SD/mean = 0.3			RD10-P2	excluded	ratio SD/mean > 0.4	
RD10-P3	included	ratio SD/mean = 0.35			RD10-P4	excluded	ratio SD/mean > 0.4	
RD10-P5	included	ratio SD/mean = 0.2			RD10-P6	included	ratio SD/mean = 0.2	
RD10-S2	included	ratio SD/mean = 0.15			RD10-S1	excluded	ratio SD/mean > 0.4	
RD10-S4	excluded	did not perform on Test 2			RD10-S3	included	ratio SD/mean = 0.2	
RD10-S6	included	ratio SD/mean = 0.3			RD10-S5	excluded	<80% success Session 3	
RD10-T1	excluded	ratio SD/mean > 0.4			RD10-T2	included	ratio SD/mean = 0.35	
RTD10-T3	excluded	did not perform on Test 2			RD10-T4	excluded	ratio SD/mean > 0.4	
RD10-T5	excluded	ratio SD/mean > 0.4			RD10-T6	included	ratio SD/mean = 0.2	
total trained/tested		12			total trained/tested		12	
total included		7			total included		6	

Table S4. **Data for contrast-sensitivity test in rd10 mice in control vs disulfiram conditions (corresponds to Figure 4).**

Top: Data and statistical analysis of success rates in rd10 mice during test #1 and test #2, in control and in disulfiram-treated mice (**Fig. 4D,E**). Bottom: data on the inclusion and exclusion of mice in the behavioral paradigm experiment for Figure 4.

| TABLE S5 | Figure 5 - Visual Bhehavior BMS 493 Treatment | | | | | | | | | | | |

Fig. 5D — Success Rate

TEST 1					TEST 1	*** Before any treatment was given to any mouse					
	All mice n = 12					Vehicle (n=6)		BMS 493 (n=6)		Mann-Whitney U Test	
Contrast %	mean	sem				mean	sem	mean	sem	U value	P value
8	25.82	6.36				31.37	10.96	20.27	6.73	11.00	P = 0.297
16	48.02	8.44				51.72	13.93	44.31	10.67	16.00	P = 0.818
32	70.04	6.17				70.65	10.73	69.44	7.23	17.00	P = 0.937
64	88.94	1.98				88.27	3.42	89.61	2.32	18.00	P = 1.000
100	93.70	0.68				92.87	1.22	94.54	0.54	11.00	P = 0.310

Fig. 5E — Success Rate

TEST 2	Vehicle (n=6)		BMS 493 (n=6)			Mann Whitney Rank Sum Test			T-Test		
Contrast %	mean	sem	mean	sem		U value	P value	Normality Test	t	P value	
8	20.33	6.79	20.49	4.15		16.00	P = 0.818				
16	27.85	11.44	24.26	5.75		18.00	P = 1.000				
32	29.96	12.43	28.52	7.21		15.00	P = 0.699				
64	31.37	14.40	59.28	9.60		8.00	P = 0.132	Passed	1.612	P = 0.069	
100	36.04	13.32	76.92	6.76		5.00	P = 0.041	Passed	2.736	P = 0.021	

Inclusion and exclusion of mice in Vehicle / BMS 493 experiments

Vehicle Mice	Status	Reason		BMS 493 I	Status	Reason
RD10-B6-2	included	ratio SD/mean = 0.2		RD10-B6-	excluded	ratio SD/mean > 0.4
RD10-B6-4	excluded	ratio SD/mean > 0.4		RD10-B6-	excluded	<80% success Session 3
RD10-B6-6	included	ratio SD/mean = 0.26		RD10-B6-	excluded	ratio SD/mean > 0.4
RD10-B7-1	included	ratio SD/mean = 0.12		RD10-B7-	included	ratio SD/mean = 0.25
RD10-B7-3	excluded	ratio SD/mean > 0.4		RD10-B7-	included	ratio SD/mean = 0.14
RD10-B7-5	included	ratio SD/mean = 0.2		RD10-B7-	included	ratio SD/mean = 0.28
RD10-B8-2	included	ratio SD/mean = 0.14		RD10-B8-	included	ratio SD/mean = 0.35
RD10-B8-4	excluded	did not perform on Test 2		RD10-B8-	included	ratio SD/mean = 0.32
RD10-B8-6	excluded	ratio SD/mean > 0.4		RD10-B8-	excluded	did not perform on Test 2
RD10-B9-2	excluded	ratio SD/mean > 0.4		RD10-B9-	included	ratio SD/mean = 0.2
RD10-B9-4	excluded	<80% success Session 3		RD10-B9-	excluded	found dead in cage before Test 2
RD10-B9-5	included	ratio SD/mean = 0.3		RD10-B9-	excluded	ratio SD/mean > 0.4
total trained/tested	12			total trained/tested	12	
total included	6			total included	6	

Table S5. **Data for contrast-sensitivity test in rd10 mice in vehicle vs BMS 493 conditions (corresponds to Figure 5).**

Top: Data and statistical analysis of success rates in rd10 mice during test #1 and test #2, in vehicle and in BMS 493-injected mice (**Fig. 5D,E**). Bottom: data on the inclusion and exclusion of mice in the behavioral paradigm experiment for Figure 5.