

The effect of sleep quantity on
neurological behavior of
Drosophila melanogaster in open-field
assays.

Titilayo Kuloyo, Spring 2021

Background

- Sleep quantity has been linked to gene expression and cognitive response, negative cognitive response has been associated with sleep deprivation (Mackiewicz et al., 2007).
- The periods of wakefulness in humans and animals are largely spent on responding to external stimuli using all sensory organs. Increase in habituation during wakefulness results in reduced attention and memory deficit (Vyazovski et al., 2017).
- The effect of sleep quantity has also been observed in rodents and humans in studies related to memory reactivation, suggesting that sensory cues and sounds recognized during wake periods can be replicated during sleep thereby increasing memory replay and memory retrieval (Rasch, B., & Born, J., 2013).

Background

- Humans and drosophila exhibit a circadian rhythm sleep pattern regulated by genetic markers and biochemicals in both organisms. Similarities in molecular markers, easy breeding and production of offspring make drosophila an ideal model organism for neurobehavioral analysis (Dubowy, C., & Sehgal, A., 2017).
- Previous experiments used genetically modified flies (Canton-S, $w^+;krz^1$, w^{118}) to demonstrate that the *krz* arrestin gene is responsible for high activity during period of exploration in *Drosophila* (Liu et al., 2007).

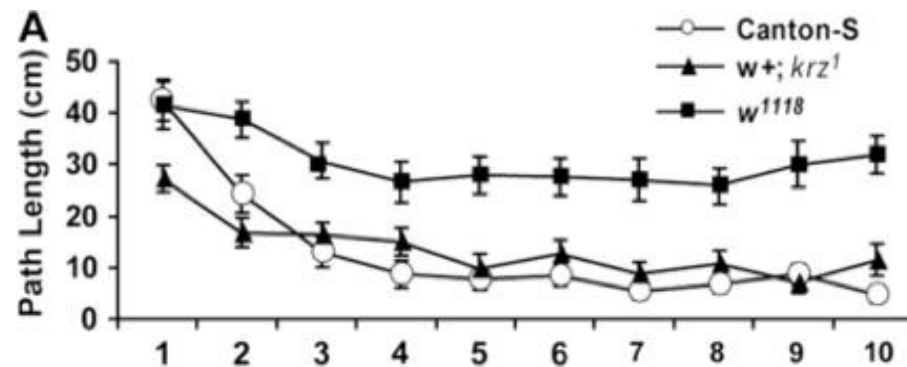


Fig 1. Path length of modified genotypes with 10-minute interval.

- **Purpose:** To investigate the correlation between sleep quantity and neurological behaviors in *Drosophila melanogaster* how it is reciprocated in humans.
- **Research Question:** is there a correlation between sleep and neurological behavior?

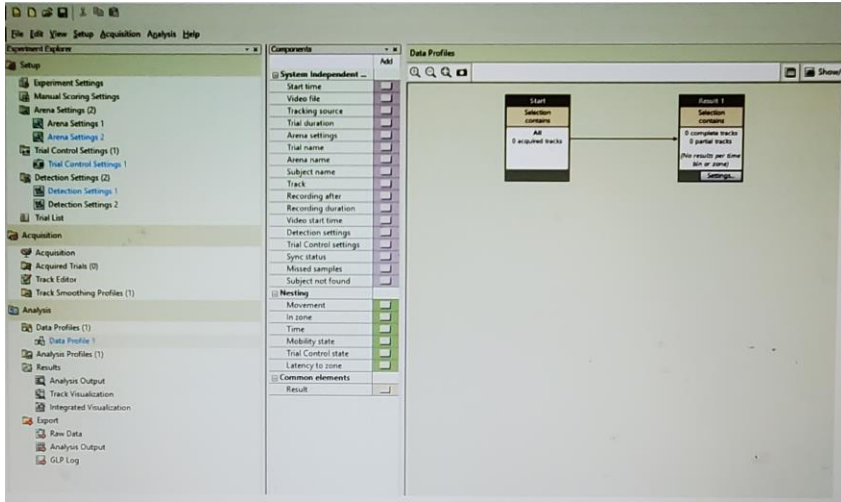


Fig 2. Ethovision software setting for data acquisition.

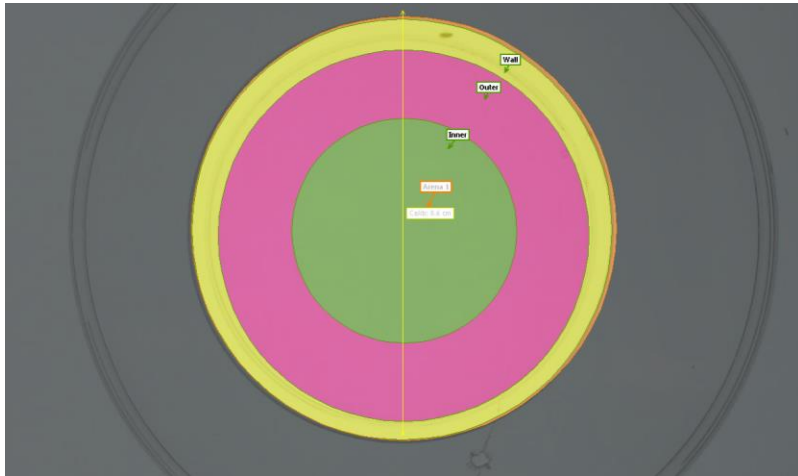


Fig 3. Arena setting and zone colors.

Methods

- The research method used in this study is the open-field arena automated and analyzed by a video tracking software: **Ethovision XT**
- Arena Setting and Zones: Scale is calibrated to 8.6cm. Wall is set to 8.6cm, Outer arena is set to 7.60cm, and inner arena is set 4.30cm.
- Detection Setting – Automated setup with detection in all zones, ensuring fly is detected throughout the video.
- Acquisition: Numerous planned trials can be selected using same arena and detection setting.
- Analysis: Result setting (results per time bin). Dependent variables are Distance moved, velocity, Mobility state (mobile-immobile) frequency, cumulative duration.

Experimental Design

- Average distance moved of each trial was calculated for 10 minutes.
- Average distance moved during exploration; average time spent in outer zone.
- $SEM = \frac{\text{standard deviation (trial)}}{\text{sqrt}(n)}$
- Average time spent in outer zone for 1st & 2nd minute for each trial of sleep line was compiled and calculated.
- Flies used : Drosophila Genetic Reference Panel (DGRP), Long sleep flies, short sleep flies.
- N=18
- Exploratory activity is predicted to be higher in long sleep lines than in short sleep and DGRP. There should be segregation between sleep lines.

U2	f_x	=STDEV(B2:S2)/SQRT(18)																				
1	DISTANCE MOVED (PER MIN.)	DGRP 1A	DGRP 1B	DGRP 1C	DGRP 2A	DGRP 2B	DGRP 2C	DGRP 3A	DGRP 3B	DGRP 3C	DGRP 4A	DGRP 4B	DGRP 4C	DGRP 5A	DGRP 5B	DGRP 5C	DGRP 6A	DGRP 6B	DGRP 6C	AVERAGE	SEM	
2		1	42.6152	18.3317	44.43	78.4907	34.2361	63.8521	46.4393	34.4809	60.4776	14.0493	123.471	114.659	55.9342	29.277	75.2559	35.1441	44.2566	28.5871	52.4445444	7.06618276
3		2	29.286	23.7461	14.7153	55.5203	0.85945	37.8852	35.7914	25.4928	47.2054	4.31003	106.555	80.9206	29.9348	15.0099	45.4835	16.4924	5.97469	9.80382	32.4936214	6.4716772
4		3	4.81664	16.7039	36.3657	26.7252	0.991886	41.3054	34.8808	21.8486	35.815	12.4075	58.4297	86.3251	42.8128	3.66386	48.3161	38.4571	2.00432	4.49613	28.689853	5.3972261
5		4	9.64223	4.17396	40.1145	27.517	1.03576	34.5455	43.099	19.6406	45.6142	4.86056	9.69919	36.4762	54.8664	5.8722	67.8524	29.6698	2.99758	12.2116	14.9944267	4.7437985
6		5	12.4921	4.00181	6.10449	31.4509	1.04049	28.6405	29.3487	13.3937	37.0799	17.8251	1.85513	72.3114	18.832	0.682219	66.0248	21.5574	2.28627	4.62375	20.5650366	4.99272329
7		6	3.9759	1.79988	13.0126	39.3272	2.06196	46.072	14.204	6.89369	36.8747	14.9183	5.39883	75.9346	18.9546	1.67534	49.716	14.3254	2.11571	3.76567	19.5144767	5.91350451
8		7	4.29267	2.88186	18.0579	36.1251	1.0908	26.571	26.6752	8.26689	43.0229	13.612	52.222	28.626	7.92154	2.07776	58.6539	16.9292	3.12882	8.05929	19.9008299	4.22709789
9		8	8.14565	2.71182	11.1521	30.0879	1.12402	23.108	13.4824	24.4901	25.6967	3.93303	29.3679	9.73083	16.6996	2.69665	41.9175	3.05074	2.40762	4.05254	14.1030611	2.88545466
10		9	24.9132	2.15378	23.4742	34.1938	3.05347	24.2098	0.713831	5.63682	46.3059	2.45125	1.79833	43.1516	5.84223	3.48052	66.9489	8.44406	7.83483	4.21158	16.9376723	4.60535197
11		10	18.1446	2.62322	16.8847	47.2404	0.386201	28.23	0.484314	4.98662	38.1347	2.45323	2.29667	41.934	31.7056	0.970456	30.1925	4.37697	3.75231	13.6491	16.0247551	3.79804198
12																						
13																						
14																						
15																						
16	DISTANCE MOVED (PER MIN.)	L1-1_1A	L1-1_1B	L1-1_1C	L1-1_2A	L1-1_2B	L1-1_2C	L1-1_3A	L1-1_3B	L1-1_3C	L1-1_4A	L1-1_4B	L1-1_4C	L1-1_5A	L1-1_5B	L1-1_5C	L1-1_6A	L1-1_6B	L1-1_6C			
17		1	64.5907	34.2638	24.0518	103.055	45.5316	49.8211	18.4504	67.7709	51.8042	43.059	66.2802	60.9249	114.703	62.7557	56.3269	40.2921	38.3356	35.4305	54.3014944	5.77773986
18		2	53.2285	43.0531	42.0484	100.624	18.8659	19.0889	1.331519	51.4247	57.0275	25.6599	31.1369	38.6095	83.7034	36.3842	62.5143	18.7483	9.80324	40.4758	40.6506517	5.94786205
19		3	73.4744	21.4153	21.7845	12.9803	30.2575	7.28617	0.305755	64.2691	25.1654	23.9113	28.9199	33.2868	48.7689	40.9719	52.9568	2.28566	5.1152	33.3568	28.0286158	5.02011911
20		4	85.138	6.4692	2.36084	1.00047	5.0236	1.41896	0.431519	4.70426	18.445	12.3351	23.6344	43.5494	33.9795	15.5848	45.712	1.91124	12.5895	30.6232	19.7186105	5.28009498
21		5	79.9839	3.02382	4.64997	1.45047	2.08996	7.23775	0.780058	1.37628	6.07627	11.6509	30.4974	25.6181	49.2693	35.7032	50.64444	2.16117	23.862	30.2593	19.2422311	5.27693102
22		6	51.57	2.03561	2.34865	1.47101	2.14979	7.72833	0.621177	3.25727	3.27701	14.4872	10.0613	34.16	90.9962	28.7254	20.6913	1.86641	21.3112	29.1894	18.1648487	5.49615103
23		7	3.96465	3.49	3.80325	1.12085	3.64146	1.54164	0.3594	5.05821	3.23434	21.3545	32.6059	37.7791	56.6872	13.0014	6.41013	1.85022	34.3747	26.1024	14.1167417	3.9397897
24		8	46.0328	2.11794	12.0396	0.772972	2.11564	7.80395	2.7244	1.04779	1.24466	7.51465	25.3214	18.1566	71.7029	30.5216	47.3079	21.15	26.019	38.8426	20.1464668	4.78875191
25		9	34.3461	3.46663	8.43406	0.813816	0.625958	13.1887	3.61598	0.81729	2.59644	8.90539	10.9799	57.0244	63.1403	32.1142	47.3014	2.28566	19.6541	44.1368	19.6333902	4.97255008
26		10	6.77995	1.66115	2.11049	0.837731	1.19963	32.1556	3.11206	1.20748	2.32385	2.16145	21.158	50.3227	66.3278	29.8018	54.1784	1.91224	16.5823	26.3693	17.9059906	5.09950764
27																						
28																						
29	DISTANCE MOVED (PER MIN.)	L1-2_1A	L1-2_1B	L1-2_1C	L1-2_2A	L1-2_2B	L1-2_2C	L1-2_3A	L1-2_3B	L1-2_3C	L1-2_4A	L1-2_4B	L1-2_4C	L1-2_5A	L1-2_5B	L1-2_5C	L1-2_6A	L1-2_6B	L1-2_6C			
30		1	37.2293	123.667	29.1064	107.795	97.382	91.7786	119.996	60.5284	40.2481	87.9507	16.0487	2.62337	39.8905	4.67442	3.12334	73.8757	5.27182	3.98206	52.3646339	10.2799638
31		2	1.23106	74.6043	20.8005	60.0332	64.519	88.0017	56.5794	112.263	19.3301	78.1607	4.31009	2.1293	17.5875	3.42140	4.95269	4.5876	7.43648	37.0132011	8.48121735	
32		3	1.32363	63.2585	2.34325	40.0946	66.265	68.7337	50.8278	57.1518	17.6068	77.6249	32.4075	2.16027	18.2713	1.47875	1.79445	55.0065	2.95189	4.76019	30.4533681	6.72085526
33		4	1.92148	80.798	13.7942	74.502	40.8367	53.6156	41.6146	17.9919	15.7912	53.238	4.86066	2.69222	29.6208	3.18479	4.65128	51.3472	3.26286	9.14474	27.7062794	6.1390863
34		5	2.06106	53.6598	32.4962	29.4543	46.5365	80.225	9.75141	11.3038	33.1284	65.2495	14.9149	37.2427	16.0653	3.23952	2.34865	26.8554	3.05765	6.87453	24.3628976	5.2105485
35		6	0.841323	41.0179	21.6604	5.6001	38.7338	80.225	9.75141	11.3038	33.1284	65.2495	14.9149	37.2427	16.0653	3.23952	2.34865	26.8554	3.05765	6.87453	24.3628976	5.2105485
36		7	1.02557	37.8821	1.41243	2.25562	69.8152	52.333	7.45648	35.5691	18.7318	67.723	13.612	8.51951	20.5332	5.8621	2.1276	45.9105	1.84013	11.4772	20.9130911	5.43938042
37		8	0.767068	47.9343	4.09614	1.19225	57.5536	44.1381	3.69404	42.7114	2.17998	52.8808	1.93303	22.1448	0.35897	6.34732	13.0419	52.6752	1.32085	2.48508	19.2251597	5.2764068
38		9	0.697149	60.7281	1.13277	1.83982	19.9367	65.3754	26.7267	46.5861	0.429256	52.0848	2.45125	16.092	18.6876	3.56433	0.863039	40.9935	3.25312	0.61805	20.1155269	5.42257919
39		10	1.26261	63.1286	0.874685	0.615815	15.9969	63.3145	26.9547	32.7094	1.60373	66.5002	2.45323	26.4253	29.5755	3.17994	3.92459	53.7316	4.38765	12.7403	22.7429583	5.6696458
40																						
41																						
42																						
43	DISTANCE MOVED (PER MIN.)	L1-3_1A	L1-3_1B	L1-3_1C	L1-3_2A	L1-3_2B	L1-3_2C	L1-3_3A	L1-3_3B	L1-3_3C	L1-3_4A	L1-3_4B	L1-3_4C	L1-3_5A	L1-3_5B	L1-3_5C	L1-3_6A	L1-3_6B	L1-3_6C			
44		1	14.5102	30.995	40.134	40.134	40.134	40.134	40.134	40.134	40.134	40.134	40.134	40.134	40.134	40.134	40.134	40.134	40.134	40.134	40.134	40.134
45																						

Fig. 4. Data acquisition of trials on excel sheet

Fig 4. Data acquisition of trials on excel sheet

Results

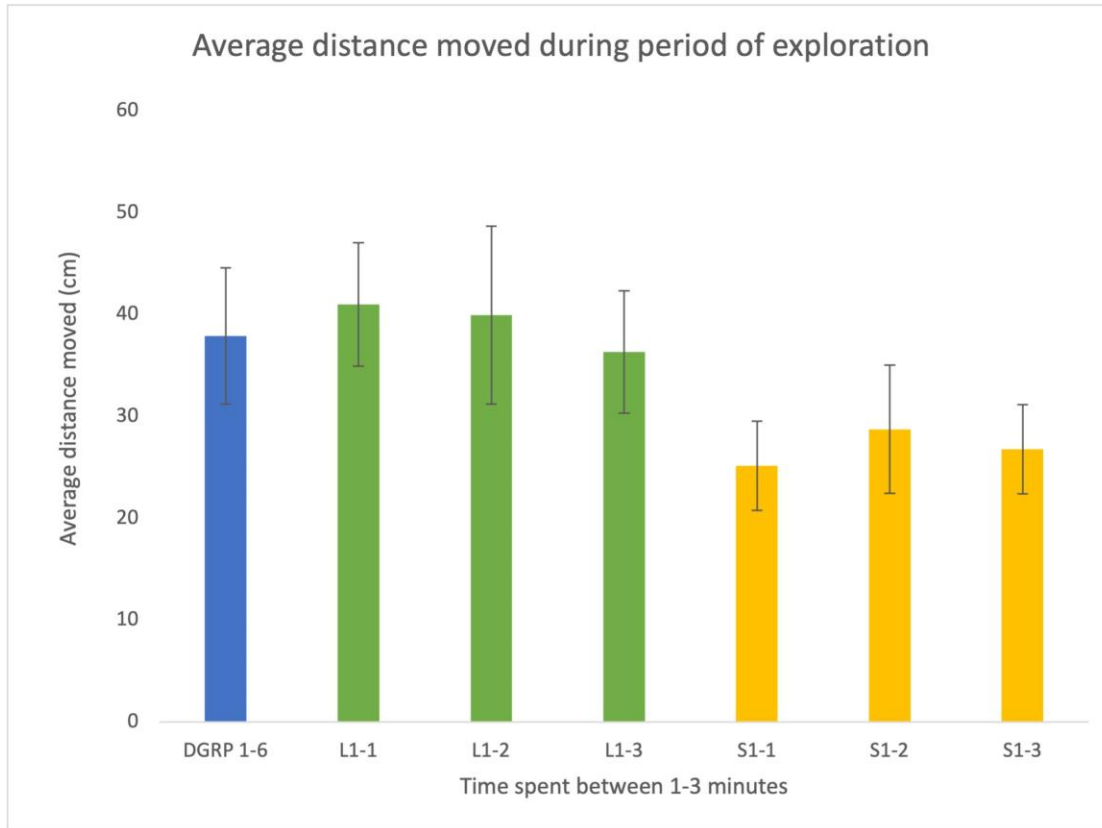


Fig. 5: Average distance moved during period of exploration.

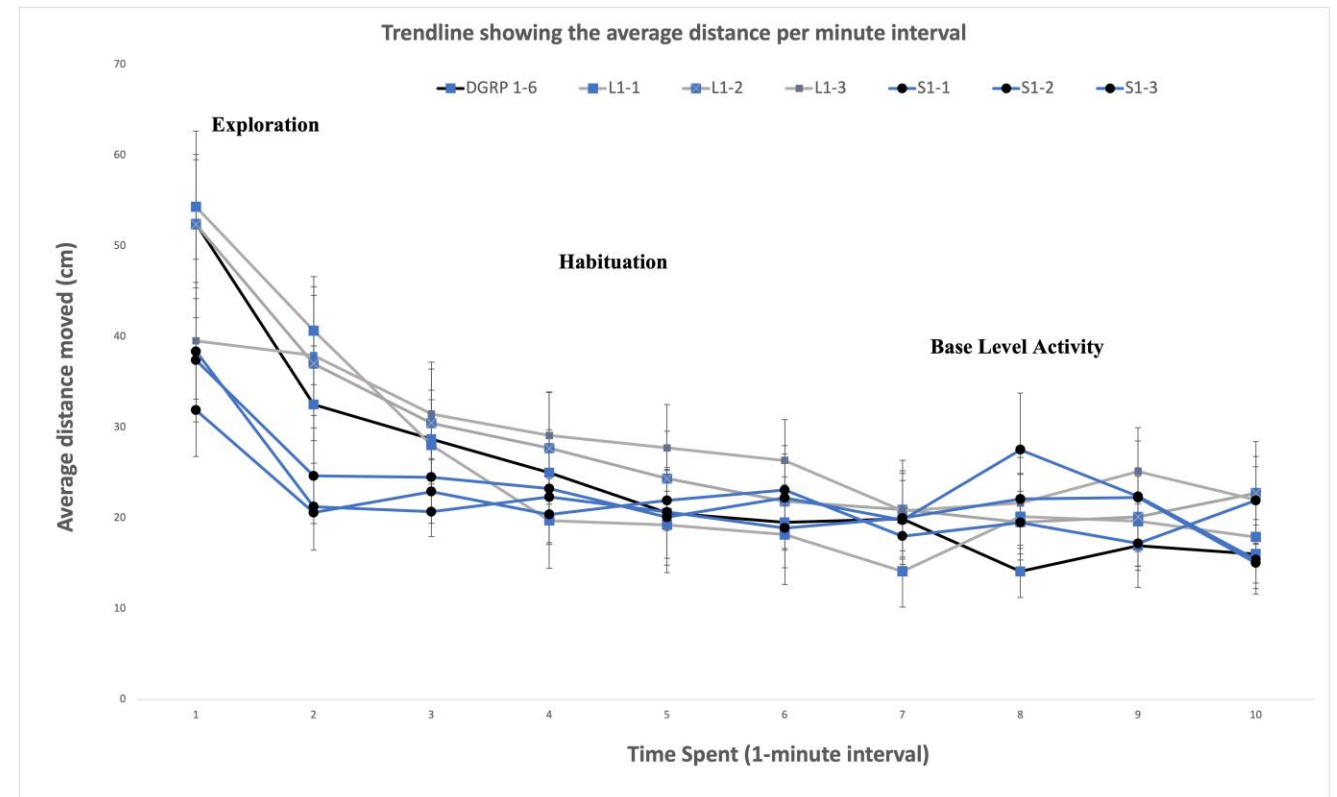
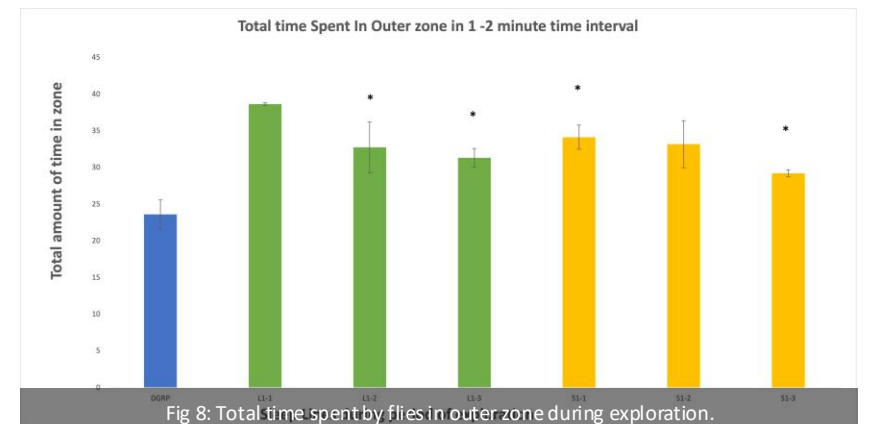
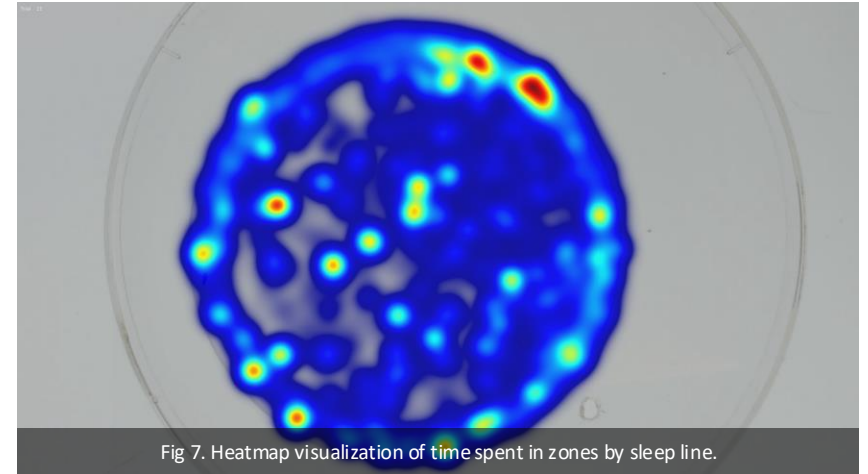


Fig 6: Average distance moved by flies in 10-minute interval.

Results

- $P < 0.05$ **
- Trendlines show significant difference in time spent in outer zone during exploration by sleep lines.
- Steady decline in exploration by DGRP, variation in decline of short sleep line and steady exploration in long sleep line.
- The use of data analysis software allows for reliable data to be acquired and evaluated.



Conclusion And Discussion

- There is a correlation between sleep and neurological behaviors.
- Segregation is observed between long sleep lines and short sleep lines.
- Higher levels of activity are observed during exploration in long sleep lines than in short sleep lines.
- Future experiment would include the use of more sleep lines as well as mutations in inbred lines to understand specific genes responsible for sleep variation.

Learning Reflection

- Difficulties during project implementation were resolved by contacting former researcher(s) and researching articles on proposed topic.
- The most important thing I have learned as a researcher is that research requires time management. It takes motivation to constantly try different methods to achieve adequate results.
- Data analysis and data visualization were most learned and utilized skills in this research experience.

References

- Mackiewicz, M., Shockley, K. R., Romer, M. A., Galante, R. J., Zimmerman, J. E., Naidoo, N., Baldwin, D. A., Jensen, S. T., Churchill, G. A., & Pack, A. I. (2007). Macromolecule biosynthesis: a key function of sleep. *Physiological genomics*, 31(3), 441–457. <https://doi.org/10.1152/physiolgenomics.00275.2006>
- Vyazovskiy, V. V., Walton, M. E., Peirson, S. N., & Bannerman, D. M. (2017). Sleep homeostasis, habits and habituation. *Current opinion in neurobiology*, 44, 202–211. <https://doi.org/10.1016/j.conb.2017.05.002>
- Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiological reviews*, 93(2), 681–766. <https://doi.org/10.1152/physrev.00032.2012>
- Liu, L., Davis, R. L., & Roman, G. (2007). Exploratory activity in *Drosophila* requires the kurtz nonvisual arrestin. *Genetics*, 175(3), 1197–1212. <https://doi.org/10.1534/genetics.106.068411>
- Dubowy, C., & Sehgal, A. (2017). Circadian Rhythms and Sleep in *Drosophila melanogaster*. *Genetics*, 205(4), 1373–1397. <https://doi.org/10.1534/genetics.115.185157>
- Liu, L., Davis, R. L., & Roman, G. (2007). Exploratory activity in *Drosophila* requires the kurtz nonvisual arrestin. *Genetics*, 175(3), 1197–1212. <https://doi.org/10.1534/genetics.106.068411>