Sabuncuoglu Serefeddin Health Sciences (SSHS)

ISSN: 2667-6338, 2021/Vol.3:3/1-14

THE EFFECT OF STRESS ON LEARNING IN MALE AND FEMALE INDIVIDUALS IN *DROSOPHILA MELANOGASTER* IN T AND Y MAZE MODELS

***[1]Mehmet FİDAN**

[1]Institute of Science, Amasya University, Amasya, Turkey

Research Article

Received: 06/11/2021; Accepted: 25/12/2021

*Corresponding author: mfidan1980@hotmail.com

Abstract

Many complex human-like behaviors are observed in *Drosophila*, exhibiting a detailed display of inter-male aggression and female courtship, self-medication with alcohol in response to stress, and even cultural transmission of social information. However, generally rodents, and especially bees and ants from among insects due to their visual place learning ability, are the living creatures that have been studied extensively in this regard in learning studies.

In this study, the learning performance *of Drosophila melanogaster* in T and Y maze models and the change in learning level due to stress were examined. The performance of stressed and unstressed male and female *Drosophila* individuals in reaching the food and finding the exit point in T and Y mazes was investigated. The vestigial mutant strain of *D. melanogaster* was used in the study. The wings of the vestigial mutant type of *Drosophila* are genetically atrophied forms.

When the success of female and male flies in both reaching the food and finding the exit point in T and Y mazes was examined, an increase was observed depending on the increase in the number of experiments. In both tests, an increase in learning performance was observed depending on the number of experiments in the flies that were not exposed to stress as a control group. However, this rate was lower when compared to individuals exposed to stress. These results show us that stress may have a positive effect on learning and that *Drosophila* can also be used in learning, stress, and short-term memory studies.

Key Words: T maze, Y maze, *Drosophila melanogaster*, Learning.

Özet

Erkekler arası saldırganlık ve kadın kurunun ayrıntılı gösterilmesi, strese tepki olarak alkolle kendi kendine ilaç tedavisi ve hatta sosyal bilgilerin kültürel aktarımını sergileyen *Drosophila*'da birçok karmaşık insan benzeri davranış gözlenmektedir. Ayrıca pek çok fizyolojik ve nörolojik olayın temel biyolojik mekanizması ve moleküler yolakları *Drosophila* ve memeliler arasına korunduğu için *Drosophila melanogaster* özellikle biyoloji ve tıp alanında çalışan bilim insanları tarafından oldukça takdir toplamıştır ve bu nedenle *Drosophila* memeli olmayan harika bir model organizma haline gelmiştir.

Fakat öğrenme çalışmalarında genel olarak kemirgenler, böceklerden ise görsel olarak yer öğrenimi yapabilmeleri nedeniyle özellikle arılar ve karıncalar bu konuda oldukça fazla çalışılan canlılardır.

Bu çalışmada *D. melanogaster*'in T ve Y labirent modellerinde öğrenme performansı ve strese bağlı olarak öğrenme düzeyinde meydana gelen değişim incelenmiştir. Strese maruz bırakılan ve strese maruz bırakılmayan dişi ve erkek *Drosophila* bireylerinin T ve Y labirentlerde besine ulaşma ve çıkış yolunu bulma performansları araştırılmıştır. Çalışmada *D. melanogaster*'in vestigial mutant soyu kullanılmıştır. Vestigial mutant tip *Drosophila*'nın kanatları genetik olarak körelmiş olan formudur.

Dişi ve erkek sineklerin T ve Y labirentte hem besine ulaşma hem de çıkış yolunu bulma başarısı incelendiğinde deneme sayısı artışına bağlı olarak yükselme gözlemlenmiştir. Her iki testte de kontrol grubu olarak strese maruz bırakılmayan sineklerde de deneme sayısına bağlı olarak öğrenme performanslarında artış görülürken bu oran strese maruz bırakılan bireyler ile karşılaştırıldığında daha düşük olduğu görülmektedir. Bu sonuçlar bize stresin öğrenme üzerinde olumlu etkisi olabileceğini ayrıca *Drosophila*'nın öğrenme, stres, kısa süreli bellek çalışmalarında da kullanılabileceğini göstermektedir.

Anahtar Kelimeler: T labirent, Y labirent, *Drosophila melanogaster*, Öğrenme.

1. Introduction

Stress has become a commonly used concept in recent years. However, the meanings attributed to this word vary from person to person. It is difficult to explain the meaning of stress since it refers to an emotional state. Nevertheless, some people define stress based on diseases it causes, such as migraine, hypertension, and ulcers. Some people focus on variables that increase stress, such as lack of communication among people, overwork, job changes, and rapid changes (Balaban, 2000).

Although there are various definitions of stress according to different fields, it can be generally expressed as "the automatic response of the organism to various internal and external stimuli that it perceives as danger" (Akpınar, 2008). Considering the issue in the context of job stress, stress is "the state of mental or physical fatigue that causes a physiological and psychological imbalance in people." Here, the remarkable point is that there is always stress in teaching and it is not possible or correct to avoid it entirely. Graham (1999) describes this situation as "an unhealthy condition." A low level of stress can motivate teachers to search for new solutions, and therefore, it is beneficial, not harmful. A high level of negative stress is harmful (Nagel & Brown, 2003).

The symptoms of stress in children can be generally listed as follows: decrease in school success, problems in friend relationships, lack of interest in social activities, decrease in self-confidence, impatience, getting angry easily, sleeping too much or insomnia, increase or decrease in appetite, negative evaluation of events, the tendency to loneliness, increase in touchiness, disobedience, risky behaviors, the tendency to substance abuse, getting bored of everything quickly, fear that something will happen to loved ones, withdrawal and preferring to be quiet, reluctance to go to school, reluctance to talk and interact, overstimulation to sound and events, outbursts of anger, and hyperactivity. Psychiatric conditions for children are depression, post-traumatic stress disorder, bedwetting (a child controlling the toilet may have a loss of control again, or inability to provide age-appropriate toilet control) and soiling (fecal soiling day or night), tic disorders, stuttering, speech disorders, behavioral changes (starting thumb sucking, childish behaviors, etc.), reactive attachment disorder, dissociative disorders, anxiety disorders, and sleep disorders (Motavalli, 1997). All of them show that student stress is an important factor to consider for effective education.

The study investigated the effect of stress on learning in *Drosophila melanogaster*, which is not commonly used as model organisms in psychology despite their widespread use in biomedical studies. Many complex human-like behaviors are observed in *Drosophila*, exhibiting detailed displays of inter-male aggression and female courtship, self-medication with alcohol in response to stress, and even cultural transmission of social information (Lewis et al., 2017).

D. melanogaster is the most important non-mammalian model organism used in the fields of medicine and biology in the last century. Many recent studies have supported the use of D. *melanogaster* as a model organism in human diseases. *D. melanogaster*, also known as the vinegar or fruit fly, is the most commonly used model organism for some of the tests used to determine the genotoxic effect of a chemical in an organism. *D. melanogaster* began to be used for these tests in 1927 when Müller introduced the sex-linked recessive lethal (SLRL) test to the scientific world (Müller, 1927). *D. melanogaster*, a holometabolous insect from the Diptera order, was identified by sequencing the entire genome of *Drosoph*ila carrying four pairs of chromosomes with a diploid number of chromosomes (Adams et al., 2000), and approximately 13 600 genes were identified (Rand, 2010). *D. melanogaster* used in laboratory studies is a good model organism for genetic studies.

The reasons for the use of *D. melanogaster* as a model organism for genetic studies by T.H. Morgan in the early 1900s and later by other researchers for genotoxicity tests are that it is a eukaryotic organism, it is large enough to live in a small habitat in laboratory conditions, it has a short generation time of approximately 9-11 days at 25°C, it produces a large number of offspring, its care and cultivation (in medium containing corn, sugar, agar, and yeast) is very easy and low cost (Vecchio, 2015), has a small number of chromosomes (one of the X/Y pairs and three autosomes), its enzyme systems responsible for bioactivation are very similar to the enzyme systems of mammals, and it allows for in vivo study in a eukaryotic organism (Rand, 2010). When they first emerge from the pupa, the body is long and light-colored, the wings are short and curved, and the newly emerged individuals become normal-looking adults within a few hours. Although the life expectancy of adult individuals is between 40-50 days, individuals living 80-90 days were also observed (Graff et al., 1992). Individuals in the third larval stage, which usually weigh 2.1-2.2 mg, find a dry place in their living environment, and pass into the pupal stage (Ashburner, 1998). Adult individuals formed by metamorphosis after the division and proliferation of imaginal disc cells emerge by tearing the pupal sheath from the upper part. Detailed information about the position of the imaginal disc cells in the larva was schematically detailed by Morgan (Morgan, 2007). Another important reason for using Drosophila as a model organism is that ethics committee approvals required for the vertebrates used in other biomedical studies are not required to comply with Drosophila (Alaraby et al., 2015).

The standards of the European Center were combined to validate alternative methods for the use of *D. melanogaster* in experimental studies (Abolaji, 2013), and a model for toxicology is already used to maintain the mechanistic studies of many environmental contaminants and toxicants. While it provides significant convenience for scientists, it also helps to implement the 3R rule (Replacement, Refinement, and Reduction), which is one of the animal research principles (Flecknell, 2002).

Half of the fly proteins have sequence similarity with mammalian proteins. The fact that Drosophila has great genetic similarities with humans, for example, it is 75-77% in terms of disease genes (Lloyd & Taylor, 2010), provides an opportunity to conduct studies with a healthy model system (Schneider, 2000). Developmental and neurological diseases, Parkinson's, Alzheimer's disease, cancer, cardiovascular diseases, intestinal infections, metabolism and storage diseases, and the genetic basis of visual, auditory, and immune system functions can be listed among these diseases (Bier, 2005). The similarity of *Drosophila* and human cell cycles and regulatory pathways serves as a model in studies on the reproduction process during tumorigenesis (Potter et al., 2000). Furthermore, *Drosophila melanogaster* has been greatly appreciated by scientists working especially in the fields of biology and medicine since the main biological mechanism and molecular pathways of many physiological and neurological events are preserved between *Drosophila* and mammals, and thus, *Drosophila* has become a great non-mammalian model organism (Pandey & Nichols, 2011). Moreover, *Drosophila* has begun to be commonly used even in areas where mammalian model organisms are considered irreplaceable, for example, in pharmacology and genotoxicology (Pandey & Nichols, 2011). When it is considered from all these perspectives, we think that *Drosophila* can be used in studies such as learning, short-term memory, long-term memory, stress, and response to stress, which are important research subjects for humans.

Stress has become a commonly used concept in recent years. However, the meanings attributed to this word vary from person to person. Although there are various definitions of stress according to different fields, it can be generally expressed as "the automatic response of the organism to various internal and external stimuli that it perceives as danger." In this study, the relationship between stress and learning was investigated in *Drosophila melanogaster*. In this context, the change in learning levels resulting from exposure to stress in *Drosophila melanogaster* female and male individuals in T and Y maze models was examined.

2. Method

Drosophila melanogaster used in the study is a popular organism that is frequently used in biological studies and has approximately 200 different mutant types (Figure 2.1). The vestigial mutant strain of *Drosophila melanogaster* was used in the study. The flies used in the study self-reproduce in the Biological Research Laboratory of Amasya University Faculty of Science and Letters under standard living conditions (25°C, 60% humidity, 12 hours daylight: 12 hours dark). Vestigial mutant type flies have genetically atrophied wings (Figure 2.2). The reason for using this mutant type in the study is that the T maze and Y maze, which were prepared for the learning test of flies, were prepared from glass tubes with a diameter of 1 cm. The primary aim here was that the fly could find the exit point and the food. In the study, no evaluation was made about the flight characteristics of the fly.

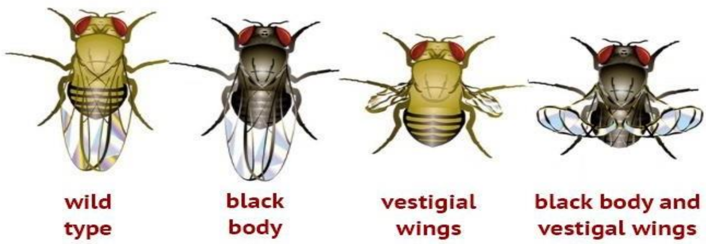

Figure 2.1. Different mutant types of *Drosophila melanogaster*

Figure 2.2*.* Vestigial (atrophied wings) mutant type of *Drosophila melanogaster*

T and Y mazes were used to measure the response of flies to learning and stress (Figure 2.3). The glass tubes used in both mazes are 1 cm in diameter and 20 cm x 18 cm x 18 cm in size.

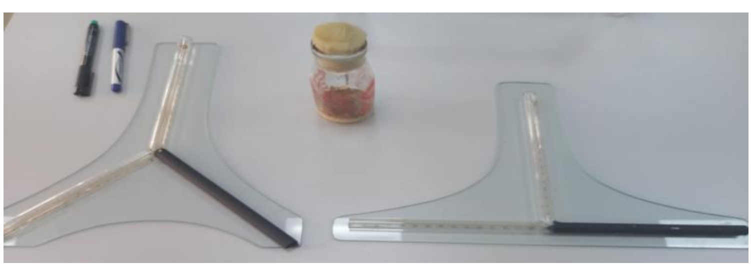

Figure 2.3. T and Y mazes used in the study

T maze was designed by Graeff et al. in 1993 as a derivative of the elevated plus or X-maze. It is a 3-arm experimental setup at a certain height from the ground, prepared from plexiglass in different sizes for different animals. It can be used to obtain both conditioned fear and unconditioned fear responses in the same animal. While inhibitory avoidance responses that reflect the learned fear are evaluated by the time taken for the animal to leave the closed arm in every three consecutive experiments, the escape response, which reflects the unconditioned fear, is evaluated by the time it takes to leave the open arm (Ramos et al., 2002).

Y maze is a behavioral test that measures rodents' willingness to explore new environments. The test is performed in a Y-shaped maze with three white opaque plastic arms at an angle of 120° from each other. After entering the center of the maze, the animal is allowed to explore the three arms freely. The number of arm entries and the number of triads are recorded to calculate the percentage of alternation (Standford, 2019).

Adult individuals obtained from previously crossed female and male vestigial mutant strains were used in the study. Attention was paid to ensure that male and female flies were of the same age. The female and male flies were separated by examining some sex characteristics under a stereomicroscope (Figure 2.4).

Figure 2.4. Separation of Vestigial mutant strains as female and male to be used in the study

During the study, ether was not used for placing the flies in mazes and then collecting them. An aspirator was used to avoid the negative effects of ether on learning and behavior. In the T and Y mazes, the standard *Drosophila* medium was used to attract the attention of male and female flies for reaching the food and reward them. This medium was placed in the Eppendorf tube and placed at the exit points of the mazes. No food was placed in the control group (Figure 2.5).

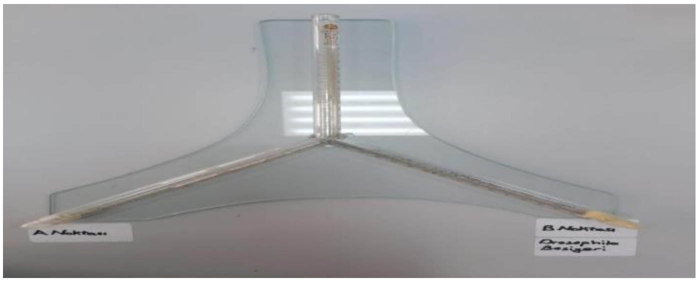

Figure 2.5. *Drosophila* medium used to determine the time it took for vestigial female and male flies to reach food, and the control group

The experimental protocol determined by Ofstad (2011) was used in our study. In this context, the flies were tested 10 times, each of which lasted 5 minutes. During this process, the same flies were used to determine the learning levels in flies. In the first test, the time it took for female and male flies to reach food in both the T maze and the Y maze was detected under normal conditions and stress. This test was performed in the 11th experiment. However, the location of the food was changed in this experiment. In the second test, the number of female and male flies that found the exit point under normal conditions and stress was determined in T and Y mazes, one arm of which was painted black (Figure 2.6). This test was also performed in the 11th experiment, and the point of exit was changed in this experiment. A sound of 250-300 Hz, which is the hearing range of the flies, was given for 30 seconds to expose the flies to stress (Albert & Göpfert, 2025). Control groups were not exposed to any sound. In the control groups, the experiments were conducted in a sound-isolated environment to avoid the sound stimulus. Furthermore, the experiments lasted for 11 days. The application was made at the same time each day. In the test of reaching the food, the flies to be tested were taken into empty bottles 6 hours before the test, and they were made hungry.

Figure 2.6. T and Y mazes to be used for the tests

3. Results

First, the standard *Drosophila* medium was placed at a point in the T and Y mazes to determine the effect of stress on learning levels in female and male flies and the difference in learning in female and male individuals. No food was placed to the other point. The experiment was repeated 10 times both in individuals exposed to sound stress and in individuals that were not exposed to stress, and 10 flies were placed in the system through aspirators in each experiment and waited for 5 minutes. The place of the food was changed in the 11th experiment. The data obtained are presented in Table 4.1, Table 4.2, Table 4.3, and Table 4.4.

Table 4.1. Number of flies that reached the food and showed learning performance as a result of vestigial female and male flies' exposure to sound (noise) stress in the T and Y mazes

	T MAZE		**Y MAZE**	
	Female Individuals (10)	**Male Individuals (10)**	**Female Individuals (10)**	**Female Individuals (10)**
Experiment 1	4	4	5	4
Experiment 2	6	5	4	6
Experiment 3	6	5	6	6
Experiment 4	7	6	8	7
Experiment 5	7	5	7	8
Experiment 6	6	8	6	7
Experiment 7	8	7	8	8
Experiment 8	8	6	7	8
Experiment 9	8	7	8	8
Experiment 10	9	8	8	8
Experiment 11	9	9	8	9

Table 4.2. Number of flies that reached the food and showed learning performance without vestigial female and male flies' exposure to sound (noise) stress in the T and Y mazes

	T MAZE		**Y MAZE**	
	Female Individuals (10)	**Male Individuals (10)**	**Female Individuals (10)**	**Female Individuals (10)**
Experiment 1	5	4	4	4
Experiment 2	5	5	4	5
Experiment 3	6	6	6	6
Experiment 4	6	7	7	7
Experiment 5	6	6	6	7
Experiment 6	6	8	6	7
Experiment 7	7	8	6	8
Experiment 8	6	8	8	7
Experiment 9	8	8	7	8
Experiment 10	7	8	8	6
Experiment 11	7	7	8	7

Table 4.3. Number of flies that found the exit point and showed learning performance as a result of vestigial female and male flies' exposure to sound (noise) stress in the T and Y mazes

	T MAZE		Y MAZE	
	Female Individuals (10)	Male Individuals (10)	Female Individuals (10)	Female Individuals (10)
Experiment 1	8	6	6	7
Experiment 2	7	8	7	6
Experiment 3	7	5	6	5
Experiment 4	7	6	8	7
Experiment 5	7	5	7	8
Experiment 6	6	8	6	7
Experiment 7	8	7	8	8
Experiment 8	10	9	9	8
Experiment 9	10	10	8	8
Experiment 10	10	10	9	8
Experiment 11	10	10	10	9

Table 4.4. Number of flies that found the exit point and showed learning performance without vestigial female and male flies' exposure to sound (noise) stress in the T and Y mazes

	T MAZE		Y MAZE	
	Female Individuals (10)	Male Individuals (10)	Female Individuals (10)	Female Individuals (10)
Experiment 1	7	6	6	6
Experiment 2	7	8	7	6
Experiment 3	7	5	6	5
Experiment 4	7	6	8	7
Experiment 5	7	5	7	8
Experiment 6	6	8	6	7
Experiment 7	8	7	8	8
Experiment 8	8	7	8	7
Experiment 9	7	8	8	9
Experiment 10	8	8	8	8
Experiment 11	8	7	8	8

The data on the performance of finding the exit point of vestigial female and male flies in the T and Y mazes are presented in Table 4.2 and Table 4.3. Ten separate experiments were conducted on both individuals exposed to sound stress and individuals that were not exposed to sound stress, and the number of flies that found their exit point within 5 minutes was recorded. Furthermore, the 11th experiment was conducted, and the learning level of the flies was determined by changing the exit point in this experiment (Table 4.2 and Table 4.3).

4. Discussion and Conclusion

When the results obtained were examined, it was also observed in Table 4.1, Table 4.2, Table 4.3, and Table 4.4 that female and male flies obtained more successful results depending on the increase in the number of experiments.

When the number of vestigial female and male flies reaching the food in the T and Y mazes was examined, t was also observed in Table 4.1 that the number of individuals that reached the food by being exposed to sound stress was even higher as the number of experiments increased. In the T maze, it was observed that the number of individuals that reached the food in the 10th experiment was 9 in female individuals and 8 in male individuals. In the Y maze, it was observed that 8 flies in females and males reached the food in the 10th experiment, which indicated an increase in the learning level in flies depending on the number of experiments. In the 11th experiment related to this test, the place of the food was changed.

The food that had been previously at point B was placed at point A, and the flies were retested (Image 2.5). It was observed that 9 of each male and female flies went to the previously learned point B. It was observed that the data obtained in Table 4.1 were higher compared to the flies whose performance of reaching the food and learning was determined without being exposed to sound stress. It was observed in Table 4.2 that there was an increase depending on the number of experiments. However, the number of successful individuals among female and male flies exposed to sound stress in the T maze in the 10th experiment was 9 and 8, respectively, and there were 8 successful individuals in both females and males in the Y maze.

In the second test, the rate of finding the exit point of the flies in the T and Y mazes was determined. When Table 4.3 was examined, among the individuals exposed to sound stress, while 8 female and 6 male individuals found the exit point in the T maze in the first experiment, 6 female and 7 male individuals found the exit point in the Y maze. In the 10th experiment, all flies found the exit point in both the T and Y mazes. It was observed that these results were higher compared to the experiments conducted without exposure to sound stress (Table 4.4).

When the results were examined in general, an increase was observed in the learning performance of flies in both tests, which indicates that *Drosophila melanogaster* individuals can be used in learning studies. Vestigial female and male flies exposed to stress had higher learning performance, as seen in Tables 4.1 and 4.3. When both results were compared with the individuals that were not exposed to stress, stress had a positive effect on learning, as seen in the 10th experiment and the 11th experiment. We thought that this situation was caused by the excessive movement of the flies from the moment the sound stress was given and the naturally occurring seeking behavior. No significant difference was found between the learning performance of the female and male flies used in the study. Like the results we obtained in the study, in the study conducted by Graham (1999), it is indicated that there is always stress in the nature of teaching and it is not possible or correct to avoid it entirely, which is described as an "an unhealthy condition." In fact, it is stated that a low level of stress can motivate teachers to search for new solutions, and therefore, it is beneficial, not harmful. A high level of negative stress is harmful (Nagel & Brown, 2003).

5. Recommendations

Many complex human-like behaviors are observed in *Drosophila*, exhibiting detailed displays of inter-male aggression and female courtship, self-medication with alcohol in response to stress, and even cultural transmission of social information, which indicates that this living creature can also be used in studies on understanding human behaviors, as seen in our study. Due to its many advantages, such as not requiring an ethical committee and being able to be grown economically, *Drosophila melanogaster* can be used in such studies as an alternative to animals such as mice, rats, and hamsters.

Acknowledgement

None.

Conflicts of interest

The authors declare that there are no potential conflicts of interest relevant to this article.

References

Abolaji, A. O., Kamdem, J. P., Farombi, E. O., & Rocha, J. B. T. (2013). *Drosophila melanogaster* as a promising model organism in toxicological studies. *Arch Bas App Med*, 1, 33-38.

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., Scherer, S.E., Li, P.W., Hoskins, R.A., Galle, R.F. (2000). The genome sequence of *Drosophila melanogaster*. *Science*, 287:2185-2195.

Akpınar, B. (2008). Eğitim sürecinde öğretmenlerde strese yol açan nedenlere yönelik öğretmen görüşleri. *Kastamonu Eğitim Dergisi*, 16(2), 359-366.

Alaraby, M., Hernández, A., Annangi, B., Demir, E., Bach, J., Rubio, L., ... & Marcos, R. (2015). Antioxidant and antigenotoxic properties of CeO_2 NPs and cerium sulphate: Studies with *Drosophila melanogaster* as a promising in vivo model. *Nanotoxicology*, 9(6), 749-759.

Albert, J. T., & Göpfert, M. C. (2015). Hearing in Drosophila. Current opinion in neurobiology, 34, 79-85.

Ashburner, A. (1989). Drosophila: A Laboratory Manual. Cold Spring Harbor, Cold Spring Harbor Laboratory Press. 1331 pp. New York.

Balaban, J. (2000). Temel eğitimde öğretmenlerin stres kaynakları ve başa çıkma teknikleri. *Pamukkale Ünv. Eğitim Fakültesi Dergisi*, Sayı:7, Özel Sayı.

Bier, E. (2005). *Drosophila,* the golden bug, emerges as a tool for human genetics. *Nature Reviews Genetics*, 6:9- 23.

Flecknell, P. (2002). Replacement, reduction and refinement. *Altex*, 19:73-78.

Graeff, F. G., Viana, M.B. and Tomaz, C. (1993). The elevated T maze, a new experimental model of anxiety and memory: effect of diazepam. *Brazilian Journal of Medical and BiologicalResearch*, 26: 67-70.

Graham, H. (1999). Stresi Kendi Yararınıza Kullanın. (Transl.: M. Sağlam and T. Tezcan). İstanbul: Alfa Basım Yayım.

Stanford, (2019). (cited: 2019, September 09) Retrieved from: https :// med.stanford.edu / sbfnl /service/bm/lm/y-maze.html.

Lewis, S. A., Negelspach, D. C., Kaladchibachi, S., Cowen, S. L., & Fernandez, F. (2017). Spontaneous alternation: a potential gateway to spatial working memory in Drosophila. *Neurobiology of learning and memory*, 142, 230-235.

Lloyd, T. E., & Taylor, J. P. (2010). Flightless flies: *Drosophila* models of neuromuscular disease. *Annals of the New York Academy of Sciences*, 1184, e1.

Morgan, D.O. (2007). The Cell Cycle: Principles of Control. New Science Press Ltd; 2007:297.ISBN: 978-0- 9539181-2-6, London.

Motavallı, N. (1997). Çocukluk Çağında Görülen "Travma Sonrası Stres Bozukluğunun" Klinik Özellikleri ve Seyri. *Yeni Sempozyum*, 35(2-3):92-95.

Müller, H. J. (1927). Artificial transmutation of the gene. *Science*, 66:84-87.

Nagel, L. And Brown, S. (2003). "The Abcs of Managing Teacher Stress." EducationalResearch, Controversy and Practices. http://educatingnm.coe.unm.edu/2003fall Date of Access: 22.07.2012.

Ofstad, T. A., Zuker, C. S., & Reiser, M. B. (2011). Visual place learning in *Drosophila melanogaster*. *Nature*, 474(7350), 204-207.

Pandey, U. B., Nichols, C. D. (2011). Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. *Pharmacological Reviews*, 63:411-436.

Potter, C. J., Turenchalk, G. S., & Xu, T. (2000). Drosophila in cancer research: an expandingrole. *Trends in genetics*, 16(1), 33-39.

Ramos, A., Kangerski, A. L., Basso, P. F., Santos, J. E. D. S., Assreuy, J., Vendruscolo, L. F.,& Takahashi, R. N. (2002). Evaluation of Lewis and SHR rat strains as a genetic model for thestudy of anxiety and pain. *Behavioural Brain Research*, 129(1-2), 113-123.

Rand, M. D. (2010). Drosophotoxicology: the growing potential for Drosophila in neurotoxicology. *Neurotoxicology and Teratology*. 32:74-83.

Schneider, D. (2000). Using *Drosophila* as a model insect. *Nature Reviews Genetics*, 1:218- 226.

Vecchio, G. (2015). A fruit fly in the nanoworld: once again Drosophila contributes to environment and human health. *Nanotoxicology*, 9:135-137.