EFFECT OF FLUOXETINE ON THE SEXUAL BEHAVIOUR OF DROSOPHILA MELANOGASTER

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ABSTRACT

Objective: *Antidepressant drugs are administered in appropriate doses to get rid of depression. Most of these drugs have adverse effects on sexual function. Selective serotonin reuptake inhibitors (SSRIs) are antidepressants that cause sexual dysfunction. The effect of this drug on the sexual behaviour of Drosophila melanogaster is studied here.*

Methodology: *The male, female and both treated groups were used. The effect of fluoxetine (FLX) on the sexual behavioural elements such as, orientation, tapping, wing vibration, scissoring, licking, circling, extruding and decamping were observed. The F1 and F2 progeny obtained from inbreeding of the above combinations were also used for observation. Three sub lethal concentrations (0.02, 0.04 and 0.06%) were administered by larval feeding method.*

Results: *Most of the male courtship acts and the non-receptive behaviour of females are affected by treatment of antidepressant drug in parents' generation. Sexual behaviour elements of male and female were insignificant in F1 and F2 progeny.*

Conclusion*: The LC_{50} and concentrations used are very high. Furthermore, these drugs have no effect on the progeny. Therefore, the author is of the opinion that low concentrations of these drugs could be used by man to get rid of depression.*

Key words*: Fluoxetine, Antidepressant drugs, Behaviour, Drosophila melanogaster*

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INTRODUCTION

Antidepressant drugs have been available about 40 years (since 1973) as treatment against depression. Clinical reports have suggested that antidepressant medication may contribute to the sexual dysfunction experienced by some depressed patients[1]. Sexual dysfunction has long been noted as both a symptom of depressive illness and as a side effect of many of the medications used to treat depression. Although most people suffering from a major depressive illness would like to be sexually active, half experience a decrease in desire or sexual performance. Antidepressant medications often interfere with several parts of the sexual response[2]. Depression may also be associated with sexual disturbances, especially reduced libido[3]. According to Benelli et al. impairment of sexual activity is one of the most frequent side effects of antidepressant drugs[4]. Selective serotonin reuptake inhibitors (SSRIs) are antidepressants that cause sexual dysfunction. The SSRI fluoxetine (FLX) appears to particularly affect the ejaculatory response. Ejaculation is regulated both at brain and spinal levels. The increase in the synaptic concentrations of serotonin seems to be mainly responsible. Cantor et al. has reported that fluoxetine decreased ejaculatory responses of male rats in a dose- and time-dependent fashion[5].

Relatively little is known about the sexual side-effects of psychotropic drugs on animal and human, probably due to the taboos surrounding discussion of sexual matters.

Drosophila serves as the most suitable test system for such studies. This species has already been extensively used for mutagenic and toxicological studies. As the courtship of Drosophila involves a series of sequential stereotyped elem-

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ents of behaviour, it can also be used to study the effect of such chemicals on sexual behaviour. Further, until now no such tests have been conducted to study the effects of antidepressant drugs on sexual behaviour of any species including Drosophila. Therefore, present work has been undertaken. The objective of this research is to study the effect of fluoxetine on sexual behaviour of D. Melanogaster and to give recommendation if any for its judicious use.

METHODOLOGY

The effect of fluoxetine was studied by larval feeding method. Wheat cream agar medium containing sub lethal concentrations of fluoxetine (0.02, 0.04 and 0.06%) was prepared and distributed to food vials[6]. The newly hatched larvae were continuously fed on the above food medium. When adults emerged, virgin females and bachelor males were isolated within 3 hours of eclosion and maintained separately in normal media for 5 days for observation of the courtship behaviour. These flies were used to study the effect of fluoxetine through larval feeding experiments. The normal medium was used as control.

Following combination of crosses in treated parents were made for observing the effect of antidepressant drug on courtship behaviour.

Control crosses (Untreated ♀ x Untreated ♂).

Male treated crosses (Treated ♂x Untreated ♀).

Female treated crosses (Treated♀x Untreated♂).

Both treated crosses (Treated ♂ x Treated ♀).

After observation of the behavioural parameters, the pairs were kept in individual vials with normal media to obtain the progeny. When progeny appeared the virgins and bachelors were collected from each group and aged for 5 days. One male and one female from each group were again crossed together and their behaviour was observed. These crosses were comparable to the F1 inbreeding. This procedure is intended to know the long-term effect of these antidepressant drugs. Subsequently the F2 progeny was also obtained for each of the above crosses and their courtship behaviour were observed.

For observing sexual behaviour of D. Melanogaster in control and different crosses of treated groups, a single virgin female was aspirated out gently and introduced into Ellen's Wattiaux mating chamber (Rectangular glass boxes of 55x55x20 mm diameter with a glass lid). A bachelor male was added to it and allowed to acclimatize to the chamber for 30 seconds. The details of courtship and mating were directly observed through hand lens of 10x magnification. Male and female behaviour was recorded separately and simultaneously by two observers. Continuous observations were made for 60 minutes, and then if there had been no copulation; the pair was replaced by a fresh pair. The sequence of activities performed by both males and females in control and treated groups was recorded from 5 pairs. The number of times a behavioral element performed by a successful pairs was recorded. 10 pairs were observed for each cross. All experiments were made during morning (7-11 A.M.) in a room with a temperature of 24±2º C under normal laboratory light condition.

Following is the description of behavioral elements which were observed in the control and treated groups.

Orientation: Courtship in D. Melanogaster is initiated by male orienting itself towards female. Orientation is the movement of male through which he approaches the female.

Foreleg tapping: The male initiates courtship with foreleg tapping. The male partly extends and simultaneously elevates one or both forelegs and then strikes downwards thus bringing the ventral surface of the tarsus in contact with the female.

Wing Vibration: The wing movement of male involves expanding of one wing laterally from the resting position to an angle of 45° and moving it rapidly up and down. Since the movement is quite rapid, it is not possible to count each movement. Usually bouts of vibrations are counted. A bout means a unit of vibrations starting from expanding wings to closing them back to original position.

Scissoring: A courting male, during the interval between wing vibrations, will sometimes open and close both wings with a scissors-like movement.

Licking: Courting male positions himself closely behind the female extends his proboscis, and licks her body parts mostly her genitalia.

Circling: The male, after posturing at the side or rear end of a non-receptive female, will leave his position and circle around the female, facing towards her as she moves. Sometimes, he moves until he is in front of her and then retracts his path to the rear; at other times he moves completely about her in a circle of 360°.

Extruding: Non-receptive female contracts muscles of the vaginal plates and also contracts certain abdominal muscles, to deny position to

males by forming temporary tube.

Decamping: Non-receptive females often attempt to escape the male's overtures by running, jumping or flying away from the immediate vicinity of the courting male.

For the purpose of statistical analysis of the effect of antidepressant drug the above combinations were divided into four groups and four treatments in the treated parents, F1 and F2 progeny. The groups included, control, male treated, female treated and both treated; while the treatments included control, first (0.02%), second (0.04%) and third (0.06%) concentrations.

The data were compiled; means and standard errors were calculated. Two-way ANOVA was also carried out for each of the parameters analyzed by using SPSS software version 10.

RESULTS

Most of the male courtship acts were affected by treatment of antidepressant drug in parents' generation (Table 1). In this survey mean value for number of orientation of control (24.30±4.33) was lesser than their corresponding fluoxetine treated batches. The highest number of orientation (84.90±19.41) was noticed in the highest concentrations of both treated group of larval feeding method. Further, this antidepressant drug had effect on orientation in all treated groups (male, female and both treated) of parents, when compared with control. The acts such as tapping, vibration, scissoring and circling were also affected in the treated batches. The treated males had to perform these acts more number of times to appease the females. The licking act was not affected in most of treated batches with fluoxetine. The variations in the acts of males in the F1 and F2 were insignificant (Table 2, 3 and 4).

In the present studies the number of bouts of wing vibrations had increased in the highest concentrations of fluoxetine in all treated groups of parents compared to their respective controls (Table 1). Also this antidepressant drug used, had no effect on wing vibration of F1 and F2 progeny (Table 4). Through wing vibration Drosophila male produces species-specific auditory signals, which are often referred to as “courtship song”. In the present studies, the frequency of wing vibration could not be counted hence; it is not possible to predict whether the sound frequency is affected due to treatment of fluoxetine. However, significant variation in the number of bouts of wing vibration was noticed indicating the production of song for longer duration is required for treated flies.

Among the non-receptive behaviour of females both extruding and decamping were affected by treatment of antidepressant drug. During extruding, non-receptive female contracts and relaxes certain muscles in abdominal region and vaginal plate and is allowed to form a slender tube to deny copulatory position to male. In the present experiments, extruding by females in most of treated batches with fluoxetine were more than their controls (Table 1). Present studies have shown that treatments of fluoxetine have resulted significant increase of decamping display, compared to their control (Table 4). Thus it is evident from these data that the treatment of antidepressant drug particularly fluoxetine make the females non-receptive. In Drosophila, mate selection is made by the female. Because of the non-

Table 1: Effect of Fluoxetine on Courtship Elements of D. Melanogaster in Parents

	Concentration (%)	Orientation	Tapping	Wing vibration	Scissoring	Licking	Circling	Extruding	Decamping
Control		24.30±4.33	3.30±0.62	5.70±2.25	1.90±0.38	2.20±0.70	0.90±0.23	1.30±0.21	4.70±1.43
Male treated	0.02	38.10±7.35	6.50±1.54	7.10±1.50	4.70±0.99	1.40±0.31	1.10±0.38	1.60±0.31	5.70±1.58
	0.04	61.90±9.13	5.70±1.28	5.30±1.36	6.80±1.83	1.20±0.42	2.00±0.45	2.90±0.67	4.90±1.43
	0.06	83.40±14.39	4.80±1.27	11.00±2.96	5.20±1.21	2.00±0.45	2.10±0.57	2.80±0.49	7.00±2.15
Female treated	0.02	58.80±9.22	3.00±0.56	5.30±1.53	3.00±0.56	1.00±0.37	1.40±0.43	2.30±1.20	3.80±1.36
	0.04	53.10±9.83	4.10±0.95	5.60±2.00	3.70±0.99	2.10±0.48	2.20±0.33	3.10±0.60	6.40±3.43
	0.06	61.40±14.51	8.40±1.91	17.30±3.44	11.90±2.39	2.40±0.43	2.80±0.51	3.50±0.85	18.00±4.25
Both treated	0.02	47.50±10.79	2.30±0.65	5.00±0.94	2.10±0.38	1.20±0.36	0.70±0.21	1.30±0.26	1.50±0.34
	0.04	73.00±17.93	3.90±0.67	10.30±3.38	5.20±1.42	1.90±0.55	3.10±0.92	2.50±0.48	7.30±2.47
	0.06	84.90±19.41	11.80±3.79	28.00±5.01	23.30±5.65	4.10±1.34	3.40±0.56	3.30±0.72	29.30±5.95

Values represent mean and their standard errors.

Table 2: Effect of fluoxetine on courtship elements of *D. melanogaster* in F1 progeny

	Concentration (%)	Orientation	Tapping	Wing vibration	Scissoring	Licking	Circling	Extruding	Decamping
Control		25.00±3.08	2.50±0.34	5.20±1.13	2.90±0.48	2.60±0.56	0.60±0.22	1.50±0.27	4.20±1.13
Male treated	0.02	23.40±3.00	3.80±0.49	4.40±0.82	2.70±0.45	1.90±0.35	0.80±0.25	2.20±0.36	4.40±1.19
	0.04	27.50±4.43	4.30±0.62	3.90±0.84	3.50±0.75	2.60±0.54	1.60±0.52	2.10±0.35	3.70±0.99
	0.06	28.50±3.21	3.90±0.85	5.90±1.05	3.80±0.51	2.60±0.50	0.90±0.23	2.80±0.55	4.50±1.21
Female treated	0.02	25.80±3.42	3.00±0.71	4.00±1.32	2.70±0.80	1.40±0.52	0.90±0.18	1.50±0.31	4.90±1.31
	0.04	28.80±3.43	3.40±0.72	4.80±1.01	3.00±0.58	2.00±0.47	1.70±0.56	2.00±0.60	6.00±1.04
	0.06	29.50±4.15	3.40±0.60	5.10±1.56	3.60±0.60	1.80±0.79	1.40±0.16	1.60±0.22	6.50±1.49
Both treated	0.02	24.50±2.83	2.70±0.60	4.60±1.00	2.50±0.48	2.70±0.60	1.30±0.26	1.90±0.43	4.50±0.76
	0.04	27.70±4.01	3.80±0.71	3.30±1.01	3.00±0.70	3.10±0.50	1.50±0.34	1.60±0.43	3.10±0.69
	0.06	26.70±3.11	3.70±0.67	5.70±0.87	5.20±1.08	2.90±0.57	1.90±0.59	1.80±0.36	4.40±1.11

Values represent mean and their standard errors.

Table 3: Effect of Fluoxetine on Courtship Elements of *D. Melanogaster* In F2 Progeny

	Concentration (%)	Orientation	Tapping	Wing vibration	Scissoring	Licking	Circling	Extruding	Decamping
Control		29.90±3.86	2.90±0.50	3.70±1.07	2.80±0.63	1.70±0.45	1.00±0.30	1.20±0.13	2.90±1.20
Male treated	0.02	25.90±3.97	3.70±0.80	5.10±2.16	1.70±0.40	2.10±0.48	0.90±0.23	1.40±0.27	2.40±0.67
	0.04	18.20±3.50	3.50±0.45	6.40±1.39	3.20±0.63	2.30±0.52	1.00±0.21	1.70±0.26	4.50±1.15
	0.06	23.80±3.23	3.10±0.69	4.60±1.56	2.80±0.81	1.30±0.52	1.20±0.20	1.60±0.27	3.40±1.02
Female treated	0.02	24.10±2.25	4.40±0.99	5.70±1.44	3.20±0.66	3.20±0.68	0.90±0.31	1.40±0.31	4.30±1.13
	0.04	22.50±3.32	3.40±0.54	4.40±1.82	2.20±0.57	2.00±0.67	0.70±0.21	1.50±0.22	3.80±1.08
	0.06	20.80±2.69	3.60±0.67	4.80±0.98	2.50±0.54	2.30±0.56	1.20±0.36	1.30±0.21	3.50±0.50
Both treated	0.02	25.70±4.15	3.60±0.54	4.30±1.93	2.70±0.68	1.40±0.52	1.10±0.23	1.00±0.26	3.20±1.05
	0.04	23.70±3.57	2.90±0.50	4.90±1.03	3.10±0.50	2.60±0.56	1.20±0.51	1.80±0.29	2.90±0.38
	0.06	21.90±4.22	2.60±0.64	5.20±2.18	4.00±0.75	2.40±0.98	0.70±0.21	2.00±0.33	4.20±1.17

Values represent mean and their standard errors.

Table 4: ANOVA for Courtship Elements of D. Melanogasterin Parents Fed with Fluoxetine, F1 and F2 Progeny

	Concentration (%)	Orientation	Tapping	Wing vibration	Scissoring	Licking	Circling	Extruding	Decamping
Parents	Group	0.572	0.202	4.889*	4.031*	1.564	1.359	0.778	4.141*
	Treatment	3.863*	6.500*	20.850*	18.701*	5.603*	9.794*	4.192*	21.061*
F1	Group	0.223	1.102	0.026	0.364	3.386*	1.223	2.669*	2.343
	Treatment	1.029	0.870	1.743	4.243*	0.871	2.097	0.227	0.460
F2	Group	0.120	1.036	0.098	1.133	0.735	0.090	0.507	0.227
	Treatment	0.966	1.255	0.039	0.610	0.199	0.051	2.177	0.211

Mean difference is significant at 0.05 level by two way ANOVA.

receptivity of treated females, the males have to perform their courtship components such as orientation, tapping, vibration, scissoring, licking and circling more times than the normal flies. Furthermore, extruding and decamping behaviours of females were insignificant in F1 and F2 progeny (Table 4).

DISCUSSION

The present observation of sexual behaviour of both control and treated was similar to that described by Spieth[7]. In other words fluoxetine treatment did not affect the sexual behaviour qualitatively.

According to Spieth , the male engages in a series of actions that include orienting towards and following the female, tapping her with his forelegs, singing a species-specific courtship song produced by extending one of his wings and vibrating it, licking the female's genitalia, and curling his abdomen to attempt copulation[8]. The forelegs, wings and mouthparts of the males serve as signaling structures. The only parts of the body that are not involved in courtship signaling in this species are the metathoracic legs. The female's signals are fewer in number and less diverse than those of male and are produced by the wings, legs, genitalia and movements of the abdomen. These signals are produced in response to the courtship acts of a male and include rejection and acceptance responses[9]. However, the author was able to record quantitative differences. Foregoing is an account on such differences and probable reasons for such differences.

The courtship is always first initiated by males in Drosophila by orienting himself to females[10-12]. In eye color mutants, because of defects in vision which tend to affect orientation in relation to female in turn in the termination of tracking and consequently the whole act of courtship is affected[12,13].

In Drosophila, successful mating depends on male's activity and female's, receptivity. Female is usually the discriminating partner in the mating act, i.e., she actively accepts or rejects a courting male[14].

The male partly extends and simultaneously elevates one or both forelegs and then strikes downwards thus bringing the ventral surface of the tarsus in contact with female[8,15].

The wing movement of male involves expanding of one wing laterally from the resting position to an angle of 45° and moving it rapidly up and down. Usually bouts of vibrations are counted but not vibrations themselves. A bout means a unit of vibrations starting from expanding wings to closing them back to original position[8,16].

A courting male, during the interval between wing vibrations, will sometimes open and close both wings with a scissor-like movement referred to as "scissoring"[16]. In the present studies, treatment fluoxetine to flies has resulted in dose dependent increase in scissoring and hence all treated batches have performed more number of scissoring in the feeding method.

When a male Drosophila is courting the female, he extends his proboscis, and licks genitalia of female. The duration of contact may be short or prolonged but always involves labellar surface[10,12,17]. Through licking the male tends to excite the female[18].

There is distinct relationship between locomotor activity and courtship in D. melanogaster species group[12]. The increased circling activity may be originated as a manoeuvre strategy to prevent non-receptive female escaping from activities of males[19].

During extruding, non-receptive female contracts and relaxes certain muscles in abdominal region and vaginal plate and is allowed to form a slender tube to deny copulatory position to male[20]. In the present experiments, extruding by females in most of treated batches with fluoxetine were more than their control in larval method. Thus treated flies show more extruding compared to non-treated, indicating influence of the drug used. It is interesting to note that number of extruding was highest in most of female and both (female and male) treated groups.

Decamping is the bahaviour of non-receptive female during, which she attempts to escape male's overtures by running, jumping or flying away from the vicinity of male[18]. The frequency of decamping depends on the degree of receptivity of female[19]. Present studies have shown that treatments of fluoxetine have resulted in significant increase in the number of decampings, compared to their controls. Thus it is evident from these data that the treatment of antidepressant drugs particularly fluoxetine make the females non-receptive. In Drosophila, mate selection is made by the female[21-23]. Because of the non-receptivity of females, the males have to perform their courtship components such as: orientation, tapping, vibration, scissoring, licking and circling

more times than the normal flies.

Furthermore, in the present studies, fluoxetine has only temporary effect, which would be best only in the treated generation. As the F1 and F2 progeny were not affected, it is obvious that, application of these drugs, do not seem have any effect genetical aspect in Drosophila.

CONCLUSION

Thus the results obtained show that the antidepressant drug used here affects the sexual bahaviour of Drosophila. However, the LC_{50} and concentrations used are very high. Furthermore, fluoxetine has no effect on the progeny. Therefore, the author is of the opinion that man to get rid of depression could use low concentrations of this drug.

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REFERENCES

1. Kowalski A, Stanley RO, Dennertein L, Burrows G, Maguire KP. The sexual side effects of antidepressant medication: a double blind comparison of two antidepressants in a non-psychiatric population. Br J Psychiatry 1985;147:413-8.
2. Ferguson JM. The effects of antidepressants on sexual functioning in depressed patients: a review. J Clin Psychiatry 2001;62:22-34.
3. Waldinger MD, Olivier B. Selective serotonin reuptake inhibitor-induced sexual dysfunction: clinical and research considerations. Int Clin Psychopharmacol 1998;13:27-33.
4. Benelli A, Frigeri C, Bertolini A, Genedani S. Influence of mitazapine on the sexual behaviour of male rats. Psychopharmacology (Berl) 2004;171:250-8.
5. Cantor JM, Binik YM, Pfaus JG. Chronic fluoxetine inhibits sexual behaviour in the male rat: reversal with oxytocine. Psychopharmacology (Berl) 1999;144:355-62.
6. Nazari M, Hegde SN. Toxic effect of flouxetine: an antidepressant drug on the rate of development and viability of drosophila melanogaster. Dros Inf Serv 2003;86:55-7.
7. Spieth HT. Evolutionary implications of sexual bahaviour in drosophila. Evol Biol 1968; 2:157-93.
8. Spieth HT. Mating behaviour of drosophila ananassae and ananassae-like flies from the Pacific. Univ Tex Publ 1966;3:133-46.
9. Spieth HT, Ringo JM. Mating behaviour and sexual isolation in d*rosophila. I*n: Ashburner M, Carson HI, Thompson Jr, editors. The Genetics and biology of d*rosophila. London:* Academic press; 1983. p. 223-84.
10. Spieth HT. Mating behavior within the genus drosophila (Diptera). Bull Am Museum Nat Hist 1952;99:395-74.
11. Manning A. Drosophila and the evolution of behaviour. In: Carthy JD, Duddington CL, editors. Viewpoints in biology. London: Butterwarths; 1965. p. 125-69.
12. Patterson HE. A comment on mate recognition systems. Evolution 1980;34:330-1.
13. Markow TA. Reproductive behaviour of drosophila melanogaster and D. nigrospiracula in the field and in the laboratory. J Comp Psychol 1988;102:169-73.
14. Ehrman L, Parsons PA. Behaviour genetics and evolution. New York: McGraw-Hill; 1981. p. 45.
15. Hegde SN, Krishna MS. Size assortative mating in drosophila malerkotliana. Anim Behav 1997;1.54:419-26.
16. Hegde SN, Krishnamurthy NB. Studies on mating behaviour in the drosophila bipectinata complex. Aust J Zool 1979;27:421-31.
17. Markow TA. Male mating experience and competitive courtship success in drosophila melanogaster. London: Nature; 1978. p. 821-2.
18. Scott D, Richmond RC, Carlson DA. Pheremones exchanged during mating: a mechanism for mate assessment in drosophila. Anim Behav 1988;36:1164-73.
19. Tompkins L, Gross AC, Hall JC, Gailey DA, Siegel R. The role of female movement in the sexual behavior of drosophila melanogaster. Behav Genet 1982;12:295-307.
20. Spieth HT. Courtship patterns and evolution of the drosophila adiastola and Plantibia species subgroups. Evolution1978;32:435-51.
21. Fisher RA. The genetical theory of natural selection. Oxford: Clarendon Press; 1930. p. 291.
22. Taylor CE, Kekic V. Sexual selection in a natural population of drosophila melanogaster. Evolution 1988;42:197-9.
23. Markow TA, Bustoz D, Pitnick S. Sexual selection and a secondary sexual character in two drosophila species. Anim Behav 1996;52:759–766

GRANT SUPPORT, FINANCIAL DISCLOSURE AND CONFLICT OF INTEREST

None Declared