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Effects of serotonin on personality in field crickets (*Gryllus bimaculatus*)

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Sammanfattning
Abstract

Animal personality can be defined as a set of physiological and behavioral characteristics that differ between individuals, but are consistent over time and across situations. The evolution of individual differences in behavior that are consistent over time and situations is still not clear. Our understanding of why animals have personality can be improved by investigating the underlying physiological mechanisms of animal behavior. Serotonin is a key monoamine that serves as a physiological modulator of animal behavior. Selective serotonin reuptake inhibitors are a group of chemicals that increase levels of serotonin in the brain. Fluoxetine is one such chemical and is used to treat depression in humans. In the field cricket (*Gryllus bimaculatus*), increased levels of serotonin have been linked to higher activity and boldness, which are both personality traits. In the current study, the effects of induced serotonin on activity, exploration, boldness and aggression was investigated. My results show that injecting fluoxetine causes substantial changes in behavioral traits used to describe personality in field crickets. This result is opposite to previous studies, as serotonin induced individuals were less active, less explorative, and won less fights, compared to control individuals. This could be due to serotonin existing naturally within the circulatory system of the field cricket, whereas fluoxetine is a manufactured chemical intended for human receptors, or that fluoxetine has a similar effect in modulating personality in field crickets as in humans. Since fluoxetine acts similarly in field crickets as in humans, an increased understanding of the effects of induced serotonin on different behaviors in field crickets could be beneficial for treating psychological illnesses.

Nyckelord
Keyword
Activity, Aggression, Boldness, Exploration, Fluoxetin, *Gryllus bimaculatus*, Personality, Selective serotonin reuptake inhibitors

[1] This report is a bachelor thesis (16 hp) that has been implemented in collaboration with two student colleagues, Kristoffer Lundgren and Louize Franzén. This cooperation has included project planning, data collecting and data processing, while the students have each written and structured reports individually.

Table of contents

1 Abstract

Animal personality can be defined as a set of physiological and behavioral characteristics that differ between individuals, but are consistent over time and across situations. The evolution of individual differences in behavior that are consistent over time and situations is still not clear. Our understanding of why animals have personality can be improved by investigating the underlying physiological mechanisms of animal behavior. Serotonin is a key monoamine that serves as a physiological modulator of animal behavior. Selective serotonin reuptake inhibitors are a group of chemicals that increase levels of serotonin in the brain. Fluoxetine is one such chemical and is used to treat depression in humans. In the field cricket (*Gryllus bimaculatus*), increased levels of serotonin have been linked to higher activity and boldness, which are both personality traits. In the current study, the effects of induced serotonin on activity, exploration, boldness and aggression was investigated. My results show that injecting fluoxetine causes substantial changes in behavioral traits used to describe personality in field crickets. This result is opposite to previous studies, as serotonin induced individuals were less active, less explorative, and won less fights, compared to control individuals. This could be due to serotonin existing naturally within the circulatory system of the field cricket, whereas fluoxetine is a manufactured chemical intended for human receptors, or that fluoxetine has a similar effect in modulating personality in field crickets as in humans. Since fluoxetine acts similarly in field crickets as in humans, an increased understanding of the effects of induced serotonin on different behaviors in field crickets could be beneficial for treating psychological illnesses.

2 Introduction

Animal personality can be defined as a set of physiological and behavioral characteristics that differ between individuals, and are consistent over time and across situations (Dall et al. 2004; Coppens et al. 2010). Five personality traits are commonly used when investigating animal personality; boldness, exploration, activity, sociability and aggressiveness (Réale et al. 2007). Boldness is typically measured by examining the subjects risk-taking abilities, for example in the presence of a predator. Exploration is often measured as the subject's distance moved in a novel area, while activity is measured in a familiar environment (Réale et al. 2007). Sociability can be measured by investigating the effects of separation from conspecifics, and aggression can be scored by looking at aggressive displays or at the subject's reaction to contact (Réale et al. 2007). It makes sense for an animal to

adjust its behavior to fit its current conditions, such as environmental differences, since it could favor the survival and fitness of the individual. However, researchers find that individuals do not adopt this flexible strategy, but rather, show personality (Dall et al. 2004). Why animals have personality may be better understood by investigating the underlying physiological mechanisms of these behavioral traits. Monoamines are a group of chemical modulators that serve as physiological modulators of animal behavior and can thus affect personality (Swallow et al. 2016). Since monoamines can affect personality, it is possible that manipulating the levels of monoamines can result in alteration of personality (Swallow et al. 2016). One key monoamine is serotonin. Levels of extracellular serotonin have been suggested to be a major mechanism behind modulating escape behavior in animals (Dyakonova and Schürmann 1999). Serotonin influences the expression of aggressive behaviors in a wide number of animal species such as the domestic chicken (*Gallus gallus domesticus*, Buchanan et al. 1994), Rhesus macaque (*Macaca mulatta*, Mehlman et al. 1994; Raleigh et al. 1991) and is also involved in social behaviors in the vervet monkey (*Chlorocebus pygerythrus*, Raleigh et al. 1991) and the American lobster (*Homarus americanus*, Huber et al. 1997a). This means that by increasing serotonin levels, it may be possible to examine if variation in a behavior of interest is caused by variation in serotonin levels. Among the serotonin increasing substances there is a group of chemicals called selective serotonin reuptake inhibitors (SSRIs). This chemical group has a mechanism that revolves around either blocking or stalling the reabsorption of serotonin in the presynaptic cell, its releaser, thus increasing the levels of serotonin in the synapse and in turn the brain (Invernizzi et al. 1996). Fluoxetine is a SSRI drug commonly used to treat depression and anxiety in humans, since these are commonly the result of serotonin imbalances in the brain (Invernizzi et al. 1996; drugs.com). In the Chinook salmon (*Oncorhynchus tshawytscha)*, increased levels of fluoxetine have resulted in reduction of aggression, activity and exploring, which are all personality traits (Réale et al. 2007; Dzieweczynski et al. 2016). In mussels (*Lampsilis fasciola*) however, fluoxetine treated individuals expressed higher activity levels than non-treated (Hazelton et al. 2014). This suggests that the mechanism of fluoxetine does not induce a fixed behavior pattern in animals, which means that the effects of fluoxetine could differ between species (Hazelton et al. 2014).

The Mediterranean field cricket (*Gryllus bimaculatus*) is a model organism for investigating neurobiology and behavior, with particular focus on aggression (Alexander 1961; Stevenson et al. 2000). This is mainly due to males of this species are very aggressive towards each other, which often results in physical confrontations (Alexander 1961; Stevenson et al. 2000). When male field crickets interact, it often results in violent duels that includes grappling, biting, mandible fencing and antennae fencing (Stevenson et al 2000). The fight continues until a clear winner can be determined, which upon winning sings a short song by vibrating its wings (Stevenson and Rilich 2012; Santostefano et al. 2016). These aggressive interactions are easily distinguished in males, which makes it easy to study aggressive interactions in field crickets (Santostefano et al. 2016). The field cricket is also used as a model organism since its small body size is suitable for investigating the effects of neuropharmacological experiments in modulating behavior (Yanoa et al. 2011). Serotonin increases activity and aggressive singing in field crickets, traits that are predominantly manifested when two males are asserting dominance among each other (Dyakonova and Schürmann 1999; Dyakonova and Krushinsky 2013). Field crickets injected with serotonin explore more, show no avoidance when faced with a potential opponent, and fight length increases (Dyakonova and Krushinsky 2013). Escape responsiveness in field crickets following aggressive interactions can be manipulated by increasing or inhibiting serotonin, where inhibited individuals express higher escape responsiveness to physical contact than non-inhibited individuals. This suggest that serotonin affects boldness (Dyakonova and Schürmann 1999; Stevensson et al. 2000). Inhibiting serotonin in field crickets has resulted in individuals becoming more hyperactive and being more cautious to physical contact, however no difference in aggression has been noted between serotonin inhibited individuals and non-treated individuals (Stevensson et al. 2000; Stevensson and Rillich 2012).

In the present study, the effects of fluoxetine on field cricket personality was examined by measuring exploration, boldness, activity and aggression in field crickets. By investigating the effect of fluoxetine on multiple behaviors used to describe variation in personality, it will be possible to determine if fluoxetine acts on specific behaviors or multiple behavior in the field cricket. Previously, serotonin has been increased in field crickets by injecting serotonin, or its precursors, directly into the circulatory system (Dyakonova and Krushinsky 2013). Fluoxetine however increases the level of natural serotonin in the circulatory system (Invernizzi et al. 1996. By comparing the effects of increased serotonin artificially versus increasing natural serotonin levels with fluoxetine, the

two methods can be compared. Since fluoxetine is given to humans to prevent depression, an increased understanding on the behavioral modulations of serotonin could be beneficial in treating psychological illnesses

I predict that increasing serotonin levels via fluoxetine injection will result in higher activity, aggression, exploration and boldness in field crickets. This is based on previous studies showing that increased serotonin via other manipulations resulted in individuals becoming more active and more prone to fighting (Dyakonova and Schürmann 1999; Dyakonova and Krushinsky 2013).

3 Material & methods

3.1 Housing of animals

To investigate the effects of fluoxetine on activity, exploration, boldness and aggression in field crickets, 72 adult male Mediterranean field crickets were obtained from a local zoo shop. To ensure that all individuals were kept in the same conditions, each individual was housed in a closed laboratory environment solitarily in a plastic container (10.5 x 17.5 x 10.5 cm) furnished with food and water and a cardboard roll acting as a shelter. A perforated lid was used to enable air flow. Food was given ad libitum in the form of peeled apple slices and oat meal, while water cubes were given by mixing agar and water. Food and water were replaced every three to four days. All containers were placed on a table under a light setup with opaque barriers between crickets and the rest of the room, to both visually isolate crickets from each other and to avoid unnecessary disturbance caused by people entering the lab. The crickets were held at a temperature of 23 °C with a 12/12-hour day-night cycle, with light being available from 7am to 7pm.

3.2 Experimental treatment

To ensure that size would not be a factor during the duels in the aggression trial (see below), individuals were weighted and split into groups of four containing individuals of similar weights with maximum weight difference being 0.05 grams, for a total of 18 groups. Half of the individuals in each group were given a 10 µl injection containing 10 µM fluoxetine, while the control group was given an injection of 10 µl phosphate buffer saline, to control for injection of the fluid. The phosphate buffer saline was used since it contained similar salt concentrations as a field cricket. To avoid bias during the experiment, each individual was given a letter depending on its treatment, only known by a pre-determined injector and not the observers. Solutions were

injected into the gastrointestinal body cavity between the ventral third and fourth segment, since it is an accessible method to reach the circulatory system in crickets (Dyakonova and Schürmann 1999). Each group was tested in four trials: activity, exploration, boldness, and aggression (see below).

3.3 Behavioral testing

All individuals were tested individually during the experimental trials, except in the aggression test.

3.3.1 Activity trial

The general level of activity in a familiar environment was recorded in the individuals' home containers by using the data program Ethovision. Prior to the onset of activity trials, selected individuals within their home containers were moved from the housing area to the recording setup, and given 10 minutes to acclimate. To optimize automatic video tracking, the lid, shelter and food/water dishes were removed from the home container for the duration of the assay. The activity test was recorded automatically for 15 minutes, and scored as total amount of distance moved by the subject (in cm).

3.3.2 Exploration and boldness trial

Individual exploration was recorded in a novel area. Prior to the onset of exploration trials, individuals were transferred from their home container and placed individually in a novel area (22 x 36 x 21 cm) in their familiar shelter. The novel area had its floor covered in white sand, which made the tracking of the crickets easier. To optimize for the automatic video tracking, the arenas were again empty except for the shelters. The exploration test was scored as the total distance the moved (in cm) in 15 minutes, starting when the crickets emerge from their shelters. To measure for the individuals' boldness, individuals were scored for the time they emerged from their shelter (since they willingly leave their familiar shelter to explore). Crickets that did not emerge within 20 minutes of the test were given a score of 20 x 60 seconds, while individuals that emerged directly were given the score of 1 second.

3.3.3 Aggression trial

An aggression test was performed immediately after the exploration and boldness test. To measure the effect of fluoxetine on aggression in field crickets, two individuals of the same group but with a different treatment, were placed on each side of an area divided by an opaque divider. The

crickets were then given 10 minutes to acclimate before the divider was raised, to avoid stress between handling. Duels were observed for 10 minutes visually. The aggression test was scored as the number of times an individual performed a song after winning a physical interaction, called the victory song (Stevenson and Rilich 2012; Santostefano et al. 2016). Some males sang prior to and during physical interactions, however these behaviors were ignored as they not necessarily signals winning (Santostefano et al. 2016). The male that performed the most victory songs was scored as the winner of the duel.

3.3.4 Statistical analyses

Statistical analyses were carried out in SPSS. Since the data from the activity, exploration and boldness trials were non-normal distributed, a Mann-Whitney U test was performed for each. To investigate if winning a duel was more apparent in either the experimental group or control group, the aggression trial was analyzed using a Pearson Chi-Square test for unpaired data, since data was normal distributed.

4 Results

4.1 Activity

Fluoxetine reduced activity of male field crickets (Experimental: 96.27 ± 38.23 cm, Control: 420.31 ± 101.41 cm, U = 418, $N_1 = N_2 = 36$, $p = 0.00$).

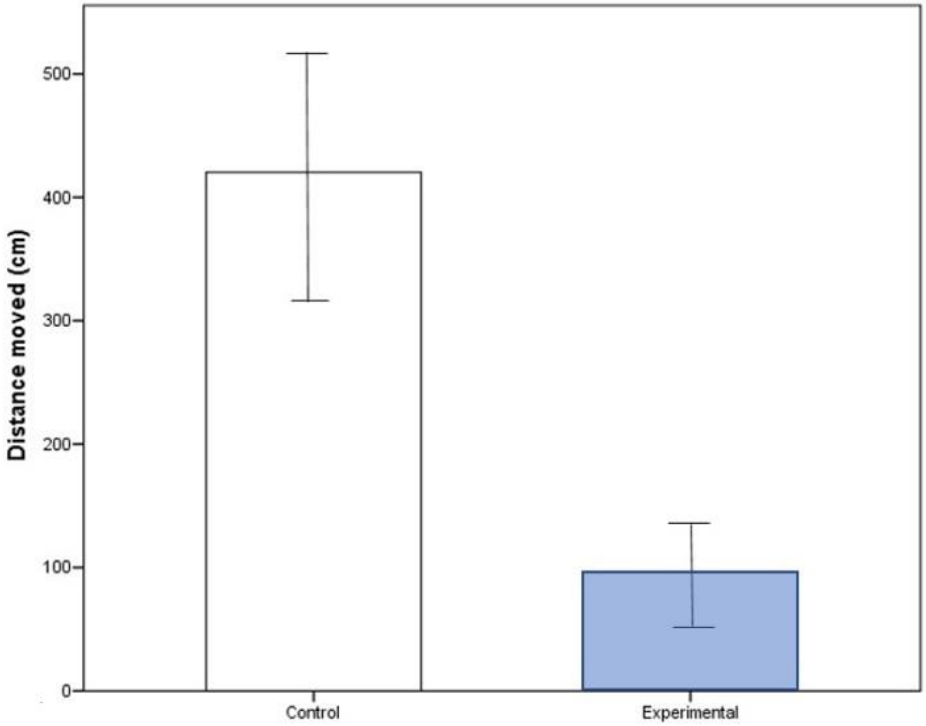

Figure 1. Influence of fluoxetine on cricket activity. Males treated with fluoxetine (blue) moved

shorter distance than control males (white) in their home containers. Columns show mean ± SE.

4.2 Exploration

Fluoxetine reduced exploration of male field crickets (Experimental: 36.04 ± 16.12 cm, Control: 219.08 ± 48.90 cm, U = 386, $N_1 = N_2 = 36$, $p = 0.001$).

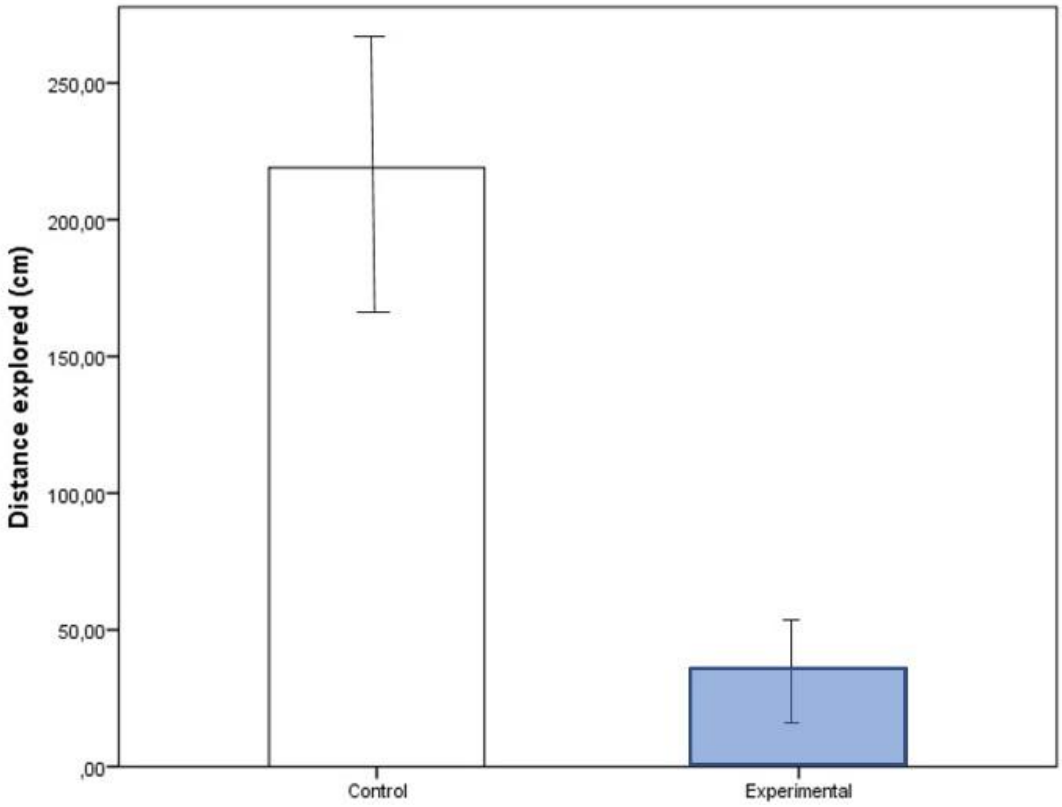

Figure 2. Influence of fluoxetine on cricket exploration. Males treated with fluoxetine (blue) explored shorter distances than control males (white) in a novel area. Columns show mean ± SE.

4.3 Boldness

Fluoxetine reduced boldness of male field crickets (Experimental:1024.22 ± 62.42 seconds, Control: 723.31 ± 82.69 seconds, U = 430.5, $N_1 = N_2 = 36$, $p = 0.004$).

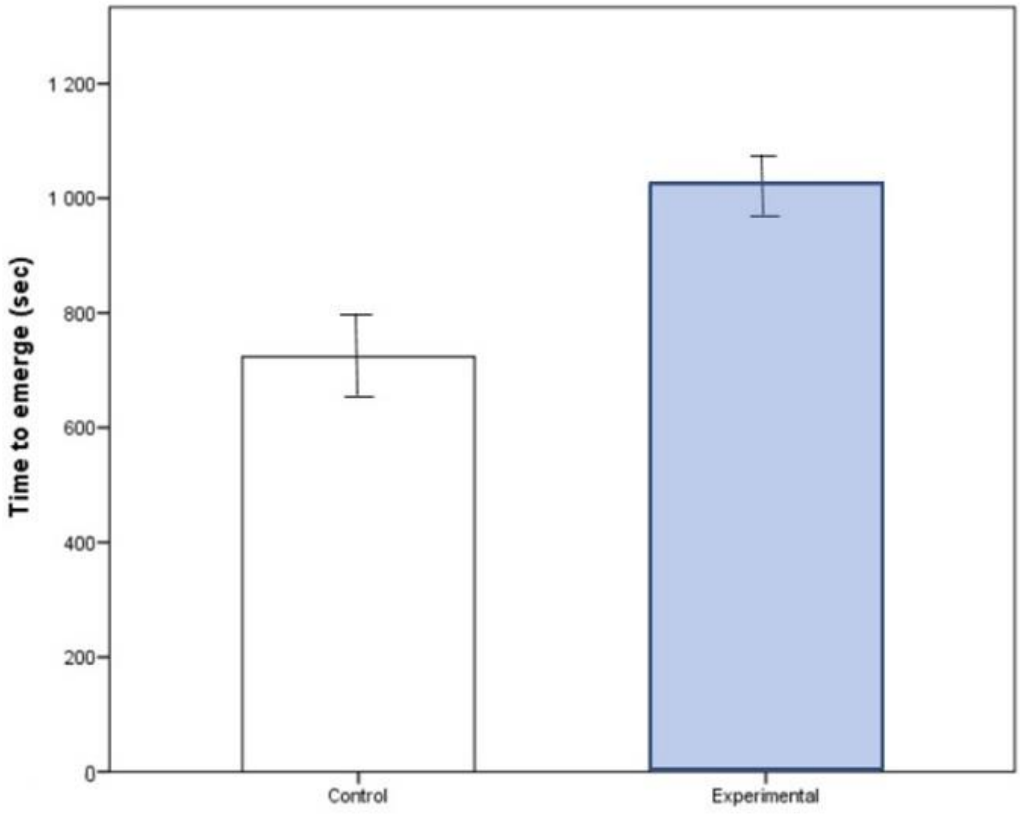

Figure 3. Influence of fluoxetine on boldness in crickets. Males treated with fluoxetine (blue) emerged later from their shelters than control males (white) in a novel area. Columns show mean ± SE.

4.4 Wins and losses of the experimental group and the control group

Fluoxetine reduced aggression in male field crickets ($X^2_2 = 45.5$, n = 18, $p < 0.001$)

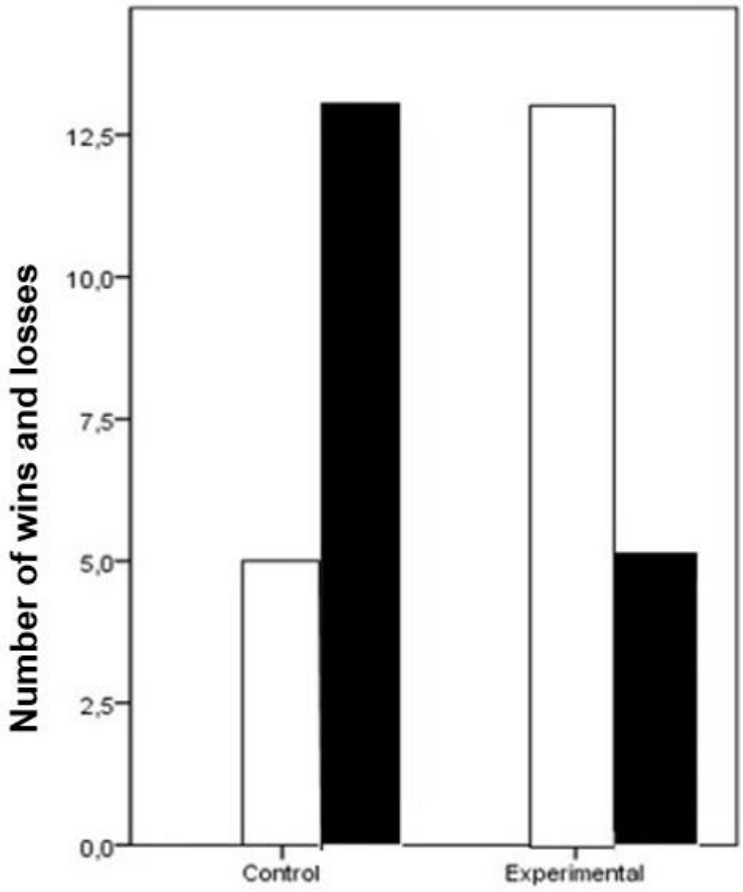

Figure 4. Influence of fluoxetine on the number of wins and losses in crickets. Males treated with fluoxetine lost more duels than control males. Columns show wins, presented as a black bar, and loss as a white bar.

5 Discussion

This study demonstrates that the injection of fluoxetine, with the aim to increase serotonin levels, causes substantial changes in multiple behavioral traits describing personality in the field cricket (*Gryllus bimaculatus*). Crickets injected with fluoxetine were less active in their home containers, explored less and emerged later in a novel area, and won fewer duels than the control group. Directly injecting serotonin in field crickets have reportedly resulted in higher aggression in the form of longer fight durations and more aggressive singing (Dyakonova and Krushinsky 2013). Lowering the level of circulatory serotonin have instead reduced the expression of aggressive behaviors in crickets, suggesting that serotonin may be involved in the modulation of aggressive behaviors (Stevenson et al. 2000). Although my study on fluoxetine in altering field cricket behavior supports the prediction that serotonin is involved in modulating aggressive behaviors in the field cricket, my result does not support that serotonin increases aggression in the field cricket. My results yielded the opposite result, inducing serotonin levels lowered aggression. Injecting serotonin has previously demonstrated an increase in both activity and exploration in field crickets (Dyakonova and Schürmann 1999; Dyakonova and Krushinsky 2013). This is not supported by my results, which show that induced serotonin levels through injection of fluoxetine yielded lower exploration and activity. It has also previously been reported that boldness in field crickets following aggressive interactions can be manipulated by increasing or inhibiting serotonin, where inhibited individuals express higher escape responsiveness to physical contact than non-inhibited individuals (Dyakonova and Schürmann 1999; Stevensson et al. 2000). I found that increasing serotonin levels with fluoxetine decreased boldness, therefore opposing previous studies on boldness in crickets (Dyakonova and Schürmann 1999; Stevensson et al. 2000). A potential cause for the effect of fluoxetine on the investigated behaviors could be due to serotonin existing naturally within the circulatory system of the field cricket, whereas fluoxetine is a manufactured chemical intended for human receptors (Dyakonova and Krushinsky 2013; Invernizzi et al. 1996). This is supported by that circulatory levels of serotonin are dependent on the regulation of serotonin transport proteins, which makes the system sensitive to alterations (Ojeda-Gómez et al. 2004). However, since my results match human responses to fluoxetine (less activity, boldness and aggressiveness in response to fluoxetine), fluoxetine may have a similar mechanism in field crickets as in humans. This is

supported by that serotonin has an identical biosynthesis in invertebrates as in vertebrates (Blenau and Baumann 2001).

5.1 Society & Ethical aspects

This study could enable an increased understanding of the role of serotonin in influencing personality. Due to fluoxetine being widely used in humans to treat many types of psychological illnesses, an increased understanding of the effects of fluoxetine on different behaviors could be beneficial when treating psychological illnesses. Although altering behaviors of animals could be regarded as unethical, there are no legislations in Sweden regarding the keeping of insects. Thus, an increased understanding of the complexity of insect behavior could therefore assist future legislations regarding the keeping of insects, and in turn better animal welfare.

5.2 Conclusion

In the current study, the effects of the serotonin inducer fluoxetine on personality in the Mediterranean field cricket (*Gryllus bimaculatus*) was investigated. In conclusion, this study demonstrates that injecting fluoxetine causes substantial changes in behavioral traits related to personality in field crickets. This result is opposite to previously reported data on the effects of induced serotonin on field cricket behavior, as serotonin induced individuals were less active, less explorative, and won less fights, compared to control individuals. This is possibly due to serotonin existing naturally within the circulatory system of the field cricket, while fluoxetine is manufactured for human receptors, or that fluoxetine has a similar effect in modulating personality in field crickets as in humans.

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7 References

Alekseyenko OV, Lee C, Kravitz EA (2010) Targeted manipulation of serotonergic neurotransmission affects the escalation of aggression in adult male Drosophila melanogaster. PLOS ONE 5:e 10806 doi: 10.1371/journal.pone.0010806.

Blenau W, Baumann A (2001) Molecular and Pharmacological Properties of Insect Biogenic Amine Receptors: lessons from Drosophila melanogaster and Apis mellifera. Archives of Insect Biochemistry and Physiology 48, 13-38.

Buchanan CC, Shrier EM, Hill WL (1994) Time-dependent effects of PCPA on social aggression in chicks. Pharmacology Biochemistry and Behavior 49, 483-488.

Coppens MC, de Boer FS, Koolhaas MJ (2010) Coping styles and behavioural flexibility: towards underlying mechanisms. Philosophical Transactions of the Royal Society B 365, 4021-4028.

Dall XRS, Houston IA, McNamara MJ (2004) The behavioural ecology of personality: consisitent individual differences from an adaptive perspective. Ecology Letters 7, 734-739.

Dyakonova EV, Schürmann WF (1999) Effects of serotonergic and opioidergic Drugs on Escape Behaviors and Social Status of Male Crickets. Naturwisswnschaften 86, 435-437.

Dyakonova EV, Krushinsky LA (2013) Serotonin precursor (5-hydroxytryptophan) causes substantial changes in the fighting behavior of male crickets, *Gryllus bimaculatus*. Journal of Comparative Physiology 199, 601-609.

Dzieweczynski TL, Kane JL, Camobell BA, Lavin LE (2016) Fluoxetine exposure impacts boldness in female Siamese fighting fish, Betta splendens. Ecotoxicology 25, 69-79.

Hazelton PD, Du B, Haddad SP, Fritts AK, Chambliss CK, Brooks BW, Bringolf RB (2014) Chronic fluoxetine exposure alters movement and burrowing in freshwater mussels. Aquatic Toxicology 151, 27-35.

Huber R, Orzeszyna M, Pokorny M, Kravitz EA (1997) Biogenic amines and aggression: experimental approaches in crustaceans. Brain, Behavior and Evolution 50, 60-68.

Huber R, Smiths K, Delago Antonia, Isaksson K, Kravitz A (1997) Serotonin and aggressive motivation in crustaceans: Altering the descision to retreat.Proceedings of the National Academy of Sciences 94, 5939-5942.

Invernizzi R, Bramante M, Samanin R (1996) Role of 5-HT_{1A} receptors in the effects of acute and chronic fluoxetine on extracellular serotonin in the frontal cortex. Pharmacology Biochemistry and Behavior 54, 143-147.

Kravitz EA (1988) Hormonal control of behavior: amines and the biasing of behavioural output lobsters. Science 241, 1775-1781.

Mehlman PT, Higley JD, Faucher I, Lilly AA, Taub DM, Vickers J, Suomi SJ, Linnoila M (1994) Low CSF 5-HIAA concentrations and severe aggression and impaired impulse control in nonhuman primates. American Journal of Psychiatry 151, 1485-1491.

Murakami S, Itoh TM (2001) Effects of aggression and wing removal on brain serotonin levels in male crickets, *Gryllus bimiculatus*. Journal of Insect Physiology 47, 1309-1312.

Ojeda-Gómez C, Pessoa-Mahana H, Iturriaga- Vasguez P, Pessoa-Mahana C.D, Recabarren-Gajardo G, Mendes-Rojas C (2014) Synthesis and Biological Screening of Novel Indolalkyl Arenes Targeting the Serotonine Transporter. Archiv der Pharmazie -Chemistry in Life Sciences 347, 174-184.

Prescription drug information and news for professionals and consumers (2000). https://www.drugs.com/ (accessed 14 May 2017).

Raleigh MJ, McGuire MT, Brammer GL, Pollack DB, Yuwiler A (1991) Serotonergic mechanisms promote dominance acquisition in adult male vervet monkeys. Brain Research 559, 181-190.

Réale D, Reader MS, Sol D, McDougall TP, Dingemanse JN (2007) Integrating animal temperament within ecology and evolution. Biological Reviews 2, 291-318.

Santostefano F, Wilson JA, Araya-Ajoy GY, Dingemanse JN (2016) Interacting with the enemy: indirect effects of personality on conspecific aggression in crickets. Behavioral Ecology 27, 1235-1246.

Stevenson AP, Hofmann AH, Schoch K, Schildberger K (2000) The Fight and Flight Responses of Crickets Depleted of Biogenic Amines. Journal of Neurobiology 43, 107-120.

Stevenson A, Rillich J (2012) The descision to fight or flee- insights into underlying mechanism in crickets. Frontiers in Neuroscience 118, 1-12.

Swallow GJ, Bubak NA, Grace, LJ (2016) The role of monoamines in modulating behavior. Current Zoology 62, 263-255.

Yanoa S, Ikemoto Y, Aonuma H, Asama H (2011) Forgetting curve of cricket, *Gryllus bimaculatus*, derived by using serotonin hypothesis. Robotics and Autonomous Systems 60, 722-728.