The influence of dopamine on personality in the Mediterranean field cricket (*Gryllus bimaculatus*)

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Denna rapport är ett examensarbete på kandidatnivå (16 hp) som har genomförts i samarbete med två studentkollegor, Louise Franzen och Simon Björklund Aksoy. Samarbetet har omfattat projektetplanering samt insamling och bearbetning av data, medan studenterna var för sig har författat och strukturerat rapporten i alla dess delar.
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1 Abstract

For some behavior there are consistent differences between individuals within a population, which is called animal personality. Across species, ranging from insects to mammals, personality has been described along behavioral gradients like activity, exploration, boldness and aggression. Monoamines such as dopamine have been shown to be essential for modulating animal behavior and could therefore be important also in explaining variation in animal personality. Supporting this, the dopaminergic system affect activity (in Confused flour beetles), and aggression (in Mediterranean field crickets). However, the causality and effect of dopamine on these behaviors, and also other behavioral traits used to describe personality is currently less explored. This study experimentally investigated how increased level of dopamine affects activity, boldness, exploration and aggression in Mediterranean field crickets (Gryllus bimaculatus). I show that dopamine manipulation had no effects on measured behavior. These results indicate that increased dopamine levels do not affect the scored personality traits in Mediterranean field crickets. The causality and generality of the relationship between dopamine and behavior used to score variation in personality is thus not clear in this species.

2 Introduction

Animal personality is defined as consistent differences in behavior between individuals within a population (Dall et al., 2004, Sih et al., 2004, Réale et al., 2007). Five major categories of personality traits have been put forward to describe animal personality: shyness-boldness, exploration-avoidance, activity-passivity, and variation in sociability, and aggressiveness (Réale et al., 2007). Activity and exploration can both be measured as distance moved, but activity is measured in a familiar environment and exploration in an unfamiliar environment (Réale et al., 2007). How an individual respond to a risky but familiar situation can be used as a measure of boldness (Réale et al., 2007). The observation that animals have personality is a bit of an evolutionary puzzle. This is because, from an adaptive point of view, flexible behavior should be favored compared to more set behavioral responses, i.e. personality (Wolf et al., 2007). Even though there has been an increased interest in animal personality recently, little is known about the underlying mechanisms behind it. When investigating proximate explanations to personality, studies have shown that monoamines such as dopamine, serotonin and noradrenaline are essential in modulation of animal behavior (Rillich and Stevenson, 2014, Swallow et al., 2016). In Great tits, Parus major, polymorphism of the dopamine receptor Drd4 gene is associated with
variation in animal personality (Fidler et al., 2007). That indicates that neuroendocrine mechanisms can play a role in explaining variation in animal personality. In Confused flour beetles, Tribolium confusum, the dopaminergic system plays an important part in activity (Nakayama et al., 2012). Genetic variation of the dopaminergic system also affects action/inhibition learning in humans (Richter et al., 2014). Dopamine has also been shown to play an important part in aggressive traits, such as recovery after social defeat in Mediterranean field crickets, Gryllus bimaculatus (Rillich & Stevenson, 2014). Nevertheless, there are currently not many studies that have investigated the causality of observed relationships between dopamine and personality traits (but see Fidler et al., 2007, Nakayama et al., 2012, Holtmann et al., 2016), nor effects of dopamine on several behavioral traits. Therefore, the aim of this study is to experimentally examine how dopamine affects several behavioral traits (activity, boldness, exploration and aggression) used to describe personality. For this I used Mediterranean field crickets; they are easy to keep and are regarded as a model species for neuroethological studies (Horch et al., 2017). Manipulation of dopamine levels was done by stimulating the dopamine receptors using the dopamine receptor agonist ropinirole. Ropinirole is a drug used on humans to treat Parkinson’s disease, comorbid depression and restless legs syndrome (Adler et al., 1997, Benes et al., 2011). Injecting this drug was expected to raise dopamine levels by mimicking the effects of naturally occurring dopamine, by binding to the dopamine receptors.

3 Material & Methods

3.1 Subjects

Sexually mature, adult male Mediterranean field crickets were bought from a local zoo shop. Only males were used in this study to reduce variation and to allow measure of aggression, which is only displayed by male crickets. Crickets were individually housed in plastic containers (9 cm × 16 cm × 10.5 cm) covered by a plastic net lid. Each container had paper towels at the bottom, and a shelter (in the form of a cardboard tube). Crickets were held at temperature of 23±2 °C, with a 12 h:12 h light:dark (light on from 7am to 7pm) cycle with ad libitum access to food and water (consisting of apple slices and agar water cubes, placed in small plastic dishes). All containers were visually isolated from each other using cardboard dividers. All crickets were kept isolated for at least 12h prior to all experiments, to minimize the aggression lowering effects of group living (Stevenson and Rillich, 2013).
3.2 Application of drug

Ropinirole hydrochloride (Sigma-Aldrich) was diluted in phosphate-buffered saline (PBS, Sigma-Aldrich) to yield a final concentration of 33 µM, based on concentrations used on zebrafish (Waugh et al., 2014). Experimental animals (n = 36) received a 10µl injection of ropinirole solution between the 4-5th segment of the abdominal cavity using a micro-syringe (Hamilton, Bonaduz, Switzerland). Control animals (n = 36) were given a sham injection with 10µl PBS. All injections were carried out by the same person.

3.3 Recording setup

To investigate variation in activity and exploration, an automatic recording setup consisting of four identical cabinets with black interior were used. The roof of each cabinet contained a camera and lights. The cameras were connected to a computer running Ethovision XT (version 10; Noldus, 2013), that was used to analyze produced video recordings.

3.4 Behavioral trials and scoring

Each cricket was singly assayed for the behaviors: activity, boldness, exploration and aggression. Trials were performed in sequence and in the order listed above. Crickets were weighed and divided into groups of four individuals of similar weight (±0.05g). They were of similar weight so that size differences would not be a factor in the aggression trial. Each cricket within the group was marked with different color combinations on the pronotum, so individuals could be distinguished from one another during the aggression trial (markings used: none, red, white, red and white). Assays were done simultaneously on groups of four since that was the maximum amount that could be run in the recording setup at the same time. Groups of four also allowed the forming of two dyads per group for the aggression trial, each consisting of one experimental cricket and one control. The first trial started approximately 30 minutes after injection, allowing the drug to become effective (as suggested by Stevenson et al., 2005, Rillich et al., 2011, Rillich and Stevenson, 2011).

3.4.1 Activity

The general level of activity in a familiar environment was recorded in the individual’s home containers (as according to Réale et al., 2007). Prior to the onset of activity trials, individuals in their home containers were moved to the recording setup and given 10 minutes to acclimatize. To optimize the automatic video tracking, the lid, shelter and food/water dishes were removed from the home container. Activity was recorded automatically for 15 minutes, as total amount of distance moved (in cm).
Pilot studies suggested that this was sufficient amount of time to record the activity.

### 3.4.2 Boldness and exploration

Individual boldness and exploration of a novel environment was recorded in a novel arena (as according to Réale et al., 2007). The novel arena was a clear plastic container (36 cm × 21.5 cm × 22 cm), with white sand. Prior to the onset of the boldness and exploration trials, individuals were moved while in their shelters from their home containers to individual novel arenas placed in the recording setup. Shelters were placed in the corner of the arena and secured with sticky tack. Boldness was measured as the time (s) it took for the cricket to emerge from its shelter after being moved (Réale et al., 2007, Niemelä et al., 2012). Ethovision was used to create a virtual zone at the far end of the arena opposite the shelters, so that latency for crickets to reach the other side of the arena could be recorded. Exploration was recorded automatically for 15 minutes, as the total amount of distance moved (cm) and how long (s) it took the crickets to reach the end zone. Recording started once the crickets emerged from their shelters. Pilot studies suggested that 15 minutes after emerging, was a sufficient to record exploration. Crickets that did not emerge within 20 minutes, were given the max value of 1200 (s) in boldness and a 0 (cm) in exploration.

### 3.4.3 Aggression

The general level of aggression was measured by placing two individuals of similar weight and different treatments (control vs experimental), on each side of the exploration arena divided by an opaque divider. The crickets were given 10 minutes to acclimatize before the divider was raised and their behavior was observed for 10 minutes (Santostefano et al., 2016). How many interactions a cricket won during the trial was recorded. Interactions between the crickets were defined as starting when any part of one cricket came in contact with any part of the other cricket, and ending when that contact was aborted for more than 2 seconds (Bertram et al., 2011). An interaction was deemed as won by the cricket that produced a victory song, whilst the other cricket fled (Rillich and Stevenson, 2011). At the end of the trial, the cricket with the most interactions won, was defined as the winner, and the other as the loser. If both crickets won the same amount of interactions it was counted as a draw, and if they did not interact they were not included in the analysis.
3.5 Statistics

All statistical analyses were conducted using the R software (version 3.4; R Development Core Team, 2017).

The data was not normally distributed therefore non-parametric statistics were used throughout.

A Mann-Whitney U-test was used to compare the control individuals with the ropinirole hydrochloride treated, for activity (distance moved in cm), boldness (latency to emerge from shelter in s), exploration (distance moved in cm and latency to end zone in s), aggression (number of interactions won per fight). During the exploration trial, some individuals never reached the end zone ($N_{\text{control}} = 13, N_{\text{treated}} = 9$) and were therefore excluded in the latency to end zone comparison.

A fisher’s exact test was used to compare control individuals with treated for the number of fights that ended as won/lost. All draws ($N_{\text{control}} = N_{\text{treated}} = 10$) and all no interactions ($N_{\text{control}} = N_{\text{treated}} = 6$) were excluded from the won/lost comparison.

4 Results

There were no significant differences between the control and treated group in their behavioral responses (activity, control $165.01 \pm 47.83$ cm, treated $212.56 \pm 45.66$ cm, $U = 536$, $p = 0.20$, $N_{\text{control}} = N_{\text{treated}} = 32$; fig. 1; boldness, control $716.28 \pm 84.11$ s, treated $574.72 \pm 82.05$ cm, $U = 733$, $p = 0.32$, $N_{\text{control}} = N_{\text{treated}} = 36$; fig. 2).

Fig. 1. Manipulation of dopamine and activity levels of crickets. The total distance moved (cm) per individual in an activity assay, for the control and treated crickets. Columns show mean ± SE.
Manipulation of dopamine did not alter exploration in crickets (distance moved in novel arena, control $176.16 \pm 36.48$ cm, treated $201.95 \pm 41.09$ cm, $U = 601.5$, $p = 0.59$, $N_{\text{control}} = N_{\text{treated}} = 36$; fig. 3; latency to last zone, control $409.70 \pm 47.50$ s, treated $350.90 \pm 43.72$ s, $U = 249$, $p = 0.45$, $N_{\text{control}} = 23$, $N_{\text{treated}} = 27$; fig. 4).
Fig. 4. Manipulation of dopamine and exploration levels of crickets. The amount time (s) it took the crickets to reach the end zone in the arena in an exploration assay, for the control and treated crickets. Columns show mean ± SE.

Aggression was not altered by manipulated dopamine levels (number of fights won, control 8, treated 12, \( p = 0.34 \), \( N_{\text{control}} = N_{\text{treated}} = 20 \); table 1; number of interactions won, control 1.70 ± 0.52, treated 2.15 ± 0.60, \( U = 175.5 \), \( p = 0.50 \), \( N_{\text{control}} = N_{\text{treated}} = 20 \); table 1).

Table 1. Manipulation of dopamine and aggression in crickets. Amount of fights ended as won/lost, and the mean (± SE) amount of interactions won per fight in an aggression assay, for the control and treated crickets.

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<thead>
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<th>Control</th>
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<td>Won</td>
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<tr>
<td>Lost</td>
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<tr>
<td>Interactions won</td>
<td>1.70 ± 0.52</td>
<td>2.15 ± 0.60</td>
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5 Discussion

In this study I have experimentally investigated how pharmacologically manipulated levels of dopamine influences variation in activity, boldness, exploration and aggression in Mediterranean field crickets. Although dopamine is suggested to be of importance for explaining variation in behavior and personality (Fidler et al., 2007, Nakayama et al., 2012, Holtmann et al., 2016), few studies have investigated the causal relationship, and studies investigating several personality traits simultaneously are rarely conducted. Earlier studies on dopamine and personality have shown that activity levels in Confused flour beetles, can be increased by stimulating the dopamine receptors (Nakayama et al., 2012). Although raw data may have suggested a similar response in my experiment, the statistics show that dopamine did not have any effect on cricket activity. Variation in both boldness and exploration have been linked to dopamine (Fidler et al., 2007, Holtmann et al., 2016). Similar to the activity trial, manipulation of the dopamine levels had no effect on cricket boldness or exploration. Dopamine has previously been shown to restore aggression in crickets after social defeat (Rillich & Stevenson, 2014). Here I did not look at aggression after the fights but instead focused on the effect of manipulation of dopamine levels on who won/lost the fight and how many interactions they won during the aggression trail. Results showed that manipulated dopamine levels had no effect on crickets winning/losing the fight or how many interactions that were won. There are several explanations for these results.

Firstly, I chose to manipulate dopamine levels, whilst other studies have looked at a more naturally occurring link between dopamine and personality, such as polymorphism in the Drd4 gene (Fidler et al., 2007, Holtmann et al., 2016). Just experimentally manipulating the levels of dopamine might not have been enough to give similar responses as previous experiments. I only looked at the acute effects of manipulated dopamine levels on personality, and it might require chronic conditions for personality to form.

Secondly, the dopamine receptor agonist ropinirole which I used to manipulate the dopamine levels, has not been tested on crickets before. Therefore, the concentration used was calculated from concentrations used on zebrafish, and related concentrations likely found in run off from human waste (Waugh et al., 2014). That concentration might not have been high enough for crickets. Another study was successful in using the dopamine receptor agonist homovanillyl alcohol on crickets, with a concentration of 1 mM, which is higher than the 33 µM of ropinirole that I used (Rillich & Stevenson, 2014). Since both my study and the other
injected the same amount of solution (10 µl) I suggest future studies try with a higher concentration of ropinirole, to explore if higher concentration can have an effect on cricket personality. Ropinirole is also a human drug, so it might not have the same effect on the dopaminergic system in crickets as in humans. No tests were run to see if ropinirole actually altered the dopamine levels of the crickets, so it might not have worked as intended. Therefore, I also suggest that future studies look at other ways of manipulating the dopamine levels, to explore if increased dopamine levels can have an effect on cricket behavior.

5.1 Social & ethical aspects

Since manipulation of dopamine levels is used to treat some diseases in humans it is of relevance to know what effects in can have on personality. Medical waste often end up in nature, so knowing its effect on personality in other species than humans is relevant for a range of species. Gaining more knowledge about the underlying mechanisms of animal behavior in crickets might increase the use of insects as lab animals in ethological studies.

Even though there is no need for an ethical permit when experimenting on insects; care was taken when planning and preforming the experiments, so that the crickets were not subjected to unnecessary stress.

5.2 Conclusion

As a conclusion, in an experimental study where dopamine levels were pharmacologically manipulated, altered dopamine levels had no influence on personality traits in crickets. This is probably due to the concentration of ropinirole used being too low or that ropinirole affects crickets in a different way than humans. To confirm if increased levels of dopamine does affect cricket personality, follow-up studies using a higher concentration of ropinirole, and using another dopamine receptor agonist are needed.

6 Acknowledgements

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7 References


