The decision to approach or avoid: Influence of social experiences during development on the establishment of consistent inter-individual differences and the role of neuromodulators in *Gryllus bimaculatus*

Der Fakultät für Lebenswissenschaften

der Universität Leipzig

genehmigte

DISSERTATION

zur Erlangung des akademischen Grades

doctor rerum naturalium
(Dr. rer. nat.)

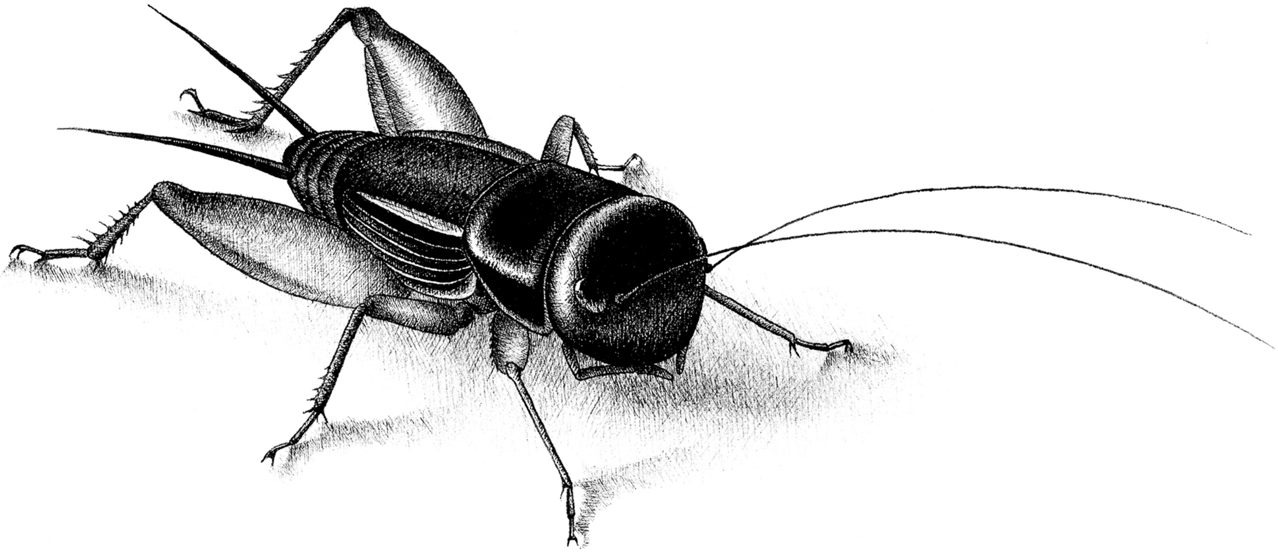
vorgelegt

von Master of Science Julia Sophie Balsam

geboren am 11.08.1988 in Suhl

Dekan:	Prof. Dr. Marc Schönwiesner
Gutachter:	Prof. Dr. Robert J. Kittel
	Prof. Dr. Hans A. Hofmann

Tag der Verteidigung: 11. März 2022, Leipzig



BIBLIOGRAPHISCHE DARSTELLUNG

Julia Sophie Balsam

The decision to approach or avoid: Influence of social experiences during development on the establishment of consistent inter-individual differences and the role of neuromodulators in *Gryllus bimaculatus*

Fakultät für Lebenswissenschaften
Universität Leipzig
Dissertation
123 Seiten, 125 Literaturangaben, 22 Abbildungen, 9 Tabellen

Intraspecific aggression is a wildly distributed, highly plastic behaviour throughout the animal kingdom and serves to secure resources, as members of the same species compete for identical ecological niches. But the costs can rapidly exceed the advantages. Over the past years, the two-spotted Mediterranean field cricket, *Gryllus bimaculatus*, has emerged to a model organism for studying the mechanisms underlying aggressive behaviour. Crickets implement seemingly complex decisions *via* the action of well-known neuromodulators with analogues in vertebrates including humans. This study shows that an individual´s decision to approach or avoid an agonistic stimulus is mainly shaped by social experiences gathered during nymphal development and early adult life. In particular, the chronic subjugation of nymphs by adult males in the breeding colony and the absence thereof lead to the establishment of distinct behavioural ethotypes shifting the answer to the question of whether inter-individual differences are nature or nurture in favour of nurture. Individuality in adult behaviour can thus result from social experiences during development alone. Moreover, the decision to approach or avoid a potentially agonistic stimulus is differentially modulated by the actions of the neuromodulators octopamine, serotonin and nitric oxide, which are released in response to social interactions. Interestingly, the social status dependent predisposed response to an antennal stimulus can be altered by octopamine alone. Furthermore, the present study reveals that the nitridergic and serotonergic system play a major role in the assessment of agonistic signals.

Statement of authorship

I, Julia Sophie Balsam, hereby confirm that I have written this dissertation independently and that I have not used any references other than those listed in the bibliography.

All passages taken verbatim or in spirit from published or as yet unpublished sources are marked as such.

The drawings or illustrations in this thesis have been created by myself or have been provided with an appropriate source reference.

Furthermore, I confirm that this thesis has not been submitted in identical or a similar form as a part of another examination process.

This dissertation is partly published and elements are taken verbatim or in spirit from Balsam & Stevenson, 2020 and 2021. The corresponding figures are indicated as such.

Selbstständigkeitserklärung

Hiermit versichere ich, Julia Sophie Balsam, dass ich die vorliegende Dissertation selbständig und ohne fremde Hilfe verfasst und keine anderen als die im Literaturverzeichnis angegebenen Quellen benutzt habe.

Alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten oder noch nicht veröffentlichten Quellen entnommen sind, sind als solche gekennzeichnet.

Die Zeichnungen oder Abbildungen in dieser Arbeit sind von mir selbst erstellt worden oder mit einem entsprechenden Quellennachweis versehen.

Diese Arbeit ist in gleicher oder ähnlicher Form noch bei keiner anderen Prüfungsbehörde eingereicht worden.

Diese Dissertation ist teilweise veröffentlicht und Elemente sind wörtlich oder sinngemäß aus Balsam & Stevenson, 2020 und 2021 übernommen. Die korrespondierenden Abbildungen sind als solche gekennzeichnet

Leipzig, 20.10.2021

____

Julia S. Balsam

Abbreviations

°	degree, angle
°C	degree Celsius
5HT	serotonin
5HTP	5-Hydroxy-L-tryptophan
5HTR	serotonin receptor
8-OH-DPAT	(R)-(+)-8-Hydroxy-DPAT hydrobromide
95% CI	95% confidence interval
$CaCl_2$	calcium chloride
CDM	chlordimeform hydrochloride
cm	centimetre
cm/s	centimetre per second
CO_2	carbon dioxide
d	day, days
DMSO	dimethylsulfoxide
D-NAME	Nω-Nitro-D-arginine methyl ester hydrochloride
DOI	(R)(-)-DOI hydrochloride
e.g.	exempli gratia, for example
Fig./ Figs.	figure, figures
g/mol	grams per mole
h	hour, hours
Hz	hertz
IQR	interquartile range
KCl	potassium chloride
L-NAME	Nω-Nitro-L-arginine methyl ester hydrochloride
LTI	long term isolated crickets, long term isolates
mg	milligram
$MgCl_2$	magnesium chloride
min	minute, minutes
mm	millimetre
mM	millimolar

mmol/l	millimoles per litre
ms	milliseconds
MW	molar weight
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
NO	nitric oxide
NOS	nitric oxide synthase
OA	octopamine
OAR	octopamine receptor
pr-loser	prospective loser
pr-winner	prospective winner
s	second, seconds
SNAP	S-Nitroso-N-acetyl-DL- penicillamine
STI	short term isolated crickets, short term isolates
Tab./ Tabs.	table, tables
w/	with
w/o	without
w/f	with females
µl	microlitre
-ve	negative
+ve	positive

Table of Contents

1 Introduction

Aggression among conspecifics is a widely distributed, highly plastic behaviour throughout the animal kingdom. Aggressive interactions between members of the same species serves to secure resources, as they compete for the same ecological niches in mating partners, food, burrows and territory. Arthropods, including insects and crustaceans, have been particularly successful during evolution, illustrated by the large amount of various species and their wide distributions. Many concepts of neuroscience were achieved with the use of arthropod model systems. At the same time, research has discovered multiple examples for unique neuronal adaptations to environmental changes with no parallels in vertebrates. Over the past years, the two-spotted Mediterranean field cricket, *Gryllus bimaculatus*, has emerged to a model organism for studying the mechanisms underlying aggressive behaviour (reviews: Simpson & Stevenson, 2015, Stevenson & Rillich, 2012; 2019). Adult male crickets exhibit a highly stereotypic, impressive, but easily quantifiable fighting behaviour, whereas their nervous system is comparatively simple containing relatively few, but individually identifiable neurones. Nonetheless, they can implement seemingly complex decisions *via* the action of well-known neuromodulators with analogues in vertebrates including humans. As in mammals, aggression in crickets is enhanced by physical activity, winning a preceding contest, the presence of resources, and suppressed by defeat, particularly chronic subjugation with long-lasting, potentially life-long consequences (Stevenson & Rillich, 2017; 2019). Various cricket species show aggressive behaviour and, kept under laboratory conditions, exhibit a hierarchy which is established as a result of intense fighting in the breeding colony (Alexander, 1961). The natural aggression releasing stimulus in adult male crickets in considered to be antennal fencing where individuals lash each other's antennae for at least 2 s at 20 Hz (Hofmann & Schildberger, 2001). It occurs on first contact with conspecifics and comprises mechanical and olfactory information (Adamo & Hoy, 1995; Iwasaki & Katagiri, 2008; Nagamoto *et al*., 2005; Rillich & Stevenson, 2015; Sakura & Aonuma, 2013; Thomas & Simmons, 2008). The decision to engage in fighting or start courting depends on the transmitted pheromonal information. Crickets also recognise the structure, texture, size and shape of an object or conspecific through antennal contact (Staudacher *et al*., 2005) and adapt their behaviour accordingly (Okada & Akamine, 2012). The present study provides evidence that nymphs neither produce nor recognise adult pheromonal signatures which are essential for initiating aggression. Furthermore, results reveal that nymph-nymph interactions do not have any long-lasting influence on adult behavioural profiles, but chronic subjugation by adult males during nymphal development and dominance or subjugation during early adult life shape their behaviour. Experiencing aggression, particularly chronic social subjugation and dominant-subordinate relationships, is recognised as a major factor inducing depression and depression-like symptoms in humans and animals alike (de Boer *et al*., 2016) and thus thought to have a major impact on the development of consistent inter-individual behavioural differences in mammals, commonly referred to as *personality*. One of the most consequential recent findings in the field of animal behaviour is that many invertebrate species also exhibit consistent inter-individual differences in specific behavioural traits (Bell *et al*., 2009; Carter *et al*., 2013; Sih *et al*., 2014; Wilson *et al*., 2019), here referred to as *individuality*, a less anthropomorphic term. Moreover, suites of different behavioural traits exhibited by individual animals are often found to correlate across time and different contexts forming *behavioural syndromes* (Sih *et al*., 2004a; 2014). However, there is still no consensus explaining the proximate mechanisms that generate individuality and distinct behavioural phenotypes (Wilson *et al*., 2019). A key question regarding the establishment of behavioural ethotypes is whether those differences between individuals of the same species are inherited or acquired from social experiences - *nature* or *nurture*. Individual differences in behaviour are reported to be nearly always driven by genetic factors in a wide variety of animals (Kendler & Greenspan 2006). However, the occurrence of consistent behavioural differences in genetically homogeneous humans (Polderman *et al*., 2015) and invertebrates (Takagi & Benton, 2020) highlights social experience during development as being at least as important as genetics. The factors driving animal individuality are thus currently considered to include potentially all aspects of an individual's physiology and environment (Sih *et al*., 2014), including immune challenge (Adamo *et al*., 2015; Niemelä *et al*., 2012), metabolic rate (Briffa & Sneddon, 2007), nutrient supply and diet (Bertram *et al*., 2009; Han & Dingemanse, 2017), general body condition and fitness (Bertram & Rook, 2012) as well as aggressive experiences (Briffa *et al*., 2015), which can vary across species, developmental stages, context and situation (Briffa & Sneddon, 2016). Adult male crickets are reported to show consistent inter-individual differences in aggression, general motility and exploratory behaviour (*Acheta domesticus:* Dochtermann & Nelson, 2014; Wilson *et al*., 2010; *Gryllus bimaculatus:* the present study and Rose *et al*., 2017a; *G. campestris:* Fisher *et al*., 2015; Niemelä *et al*., 2015; Rodríguez-Muñoz, 2010; Santostefano *et al*., 2016; *G. integer:* Hedrick & Kortet, 2012; Niemelä *et al*., 2012). Furthermore, dominant adult males exhibit increased aggressiveness, heightened motility and are more likely to approach a novel stimulus than subordinates (*Gryllus bimaculatus:* Rose *et al*., 2017a; *G. campestris:* Santostefano *et al*., 2016; *Gryllus integer:* Kortet & Hedrick, 2007; *Teleogryllus oceanicus:* Rudin *et al*., 2017). Surprisingly, the same behavioural traits were found in the prospective winners and losers before dominance and subordinate hierarchy is established (Rose *et al*., 2017a), although crickets were socially isolated for 48 h prior experimentation and all known effects of earlier aggressive encounters were known to last little more than 3 h in crickets (Rillich & Stevenson, 2011; Stevenson & Rillich, 2013). This supports the hypothesis that inter-individual differences arise due to events with long-lasting consequences during nymphal development and early adult life. Other work and the present study show that chronic social subjugation lead to potentially life-long depression of agonistic behaviour in adult crickets (Rose *et al*., 2017b; Rillich & Stevenson 2018). Together this implies that distinct ethotypes in adult male crickets can arise from defeat stress during development, as also reported in rodent models for human depression (Hammels *et al*., 2015; Laman-Maharg & Trainor, 2017).

This study employed a video-tracking analysis to evaluate differences in general behavioural traits of adult male crickets raised in various social environments and isolated socially for different lengths of time. Furthermore, the decision to approach or avoid an agonist stimulus directed at one antenna using a severed antenna from another male was examined. A main aim of this study is, therefore, to determine to which extent pre-adult agonistic experiences shape future adult behaviour. Furthermore, the hypothesis is tested that multiple winning and losing experiences during development and/or early adult life establish inter-individual differences which could account for different behavioural ethotypes in adult male crickets and the underlying role of neuromodulators therein. Crickets implement the decision to retreat or to persist in fighting by modulation of the behavioural threshold to flee (review: Stevenson & Rillich, 2019).

Potentially rewarding experiences like physical activity (e.g. flying: Hofmann & Stevenson, 2000; Stevenson *et al*., 2005), winning a preceding contest (Rillich & Stevenson, 2011; 2015), and resource possession such as shelter (Rillich *et al*., 2007; Rodriguez-Munoz *et al*., 2008), food (Nosil, 2002) or the accessibility of a female conspecific (Rillich *et al*., 2019) raise this threshold *via* the action of octopamine, so that the animal is less susceptible to defeat and persists longer in fighting (Rillich & Stevenson, 2015). Octopamine is a biogenic amine homologous to noradrenaline in vertebrates. It acts as a neurohormone, neurotransmitter and neuromodulator in invertebrates and is present in neuronal and non-neuronal tissues in high concentrations (Roeder, 1999). In contrast, this threshold to flee is lowered by the gaseous neurotransmitter nitric oxide, released in response to adverse experiences (e. g. the opponent's agonistic actions), and is thus increasing the tendency to retreat (Rillich & Stevenson, 2017; Stevenson & Rillich, 2012; 2015). A cricket retreats, when the sum of the opponent's actions exceeds the inherent threshold and, therefore, the motivation to persist in fighting (*cumulative assessment model*: Payne 1998; see also Rillich *et al*.; 2007; Stevenson & Rillich, 2019). The neuromodulator serotonin is still widely discussed. While modulating appetite, sleep, learning and social behaviour across a variety of insect examples (Bubak *et al*., 2014; Vleugels *et al*., 2015; Westwick & Ritschoff, 2021), there is still a major discrepancy on the role of serotonin in aggression in vertebrates and invertebrates (Johnson *et al*., 2009; Kravitz, 2000; Oliver, 2015; Rillich & Stevenson, 2018; Stevenson & Rillich, 2019).

5HT in vertebrates is mainly reported to decrease aggression e.g. by promoting withdrawal *via* $5HT_{1A-}$ and $5HT_{1B}$-receptors in the *Raphe nucleus* (Olivier, 2015). In invertebrates, the neuromodulator is commonly associated with the opposing effect: 5HT, the precursor 5HTP, $5HT_{1A}$-agonists and the activation of specific 5HT-neurones are reported to increase aggression and win chances, while raising the threshold to flee in lobsters (Kravitz, 2000) and Drosophila (Alekseyenko *et al*., 2014; Johnson *et al*., 2009). The influence of 5HT on initial fighting behaviour of socially naive crickets remains unclear (Dyakonova & Kruschinsky, 2013; Stevenson & Rillich, 2017). After chronic defeat, however, $5HTR_2$-blocker ketanserin accelerates loser recovery and inhibits the establishment of submissiveness (Rillich & Stevenson, 2018). Together with the finding of the present study that DOI, as a $5HTR_2$-agonist, inhibits recovery after defeat and the establishment of dominance after multiple winning experiences, it is likely that 5HT is released specifically after defeat to maintain the low threshold to retreat and modulates the assessment of agonistic signals.

Overall this study provides experimental evidence that an individual´s decision to approach or avoid an agonistic stimulus is shaped by social experiences, particularly chronic subjugation during development and dominant-subordinate relationships in early adult life, which together results in differing behavioural ethotypes of adult male crickets. This decision can be differentially modulated by the biogenic amine octopamine and the assessment of a potentially agonistic stimulus is largely dependent the actions of serotonin and nitric oxide, which are all released in response to social experiences.

2 Methods

2.1 Experimental animals

All experiments were performed on last instar nymphs or sexually mature adult Mediterranean field crickets, *Gryllus bimaculatus* (De Geer) of both sexes (Fig. 1) that were bred and maintained at the Leipzig University animal housing facility since 26 years. All animals were kept under the same standard conditions (temperature 22 - 24 °C, relative humidity 40 - 60%, 12 h : 12 h artificial light : dark regime) in groups of 30 to 40 individuals with varying social compositions (see below) in transparent perspex containers (l.w.h: 40 x 23 x 27 cm), with a sand covered floor and egg cartons for shelter. The animals were fed regularly on protein flakes (Tetra GmbH, Melle, Germany), fresh apples, carrots and water *ad libitum*. All experiments were performed between January 2018 and September 2021 at room temperature (20 - 24 °C) during daylight hours, excluding midday, extremely hot periods during summer or rainy, overcast days when the general behaviour of the crickets tends to be subdued (Dixon & Cade, 1986; Stevenson *et al.*, 2000). The animals were taken from the colony prior experimentation and kept isolated in individual glass jars (height: 7 cm, 400 cm^3) with perforated lids under the same ambient conditions for varying lengths of time (Fig. 2). On the day of the experiment, focal crickets were placed individually in different observation arenas (details see below) and left undisturbed to adapt to the new surroundings for at least 1 min. When the same animals were tested on consecutive days this was performed at approximately the same time of day (+/- 1 h). The analyses are based on observations of 2200 crickets, none of which were used more than once for an experiment or again in other experiments unless explicitly stated otherwise. All treatments are in accordance with the Principles of Laboratory Animal Care and German Animal Welfare Act (*Deutsches Tierschutzgesetz*).

Gryllus bimaculatus are hemimetabolous insects and go through 8 nymphal stages, each of which lasting 4 to 5 days before finally moulting to the adult, which are identified visually by the possession of functional hind-wings (Donoughe & Extavour, 2016). Adults reach maturity and achieve their peak dominance around 10 to 14 days after the final moult (Alexander, 1961; Dixon & Cade, 1986).

Fig. 1: Last instar nymphal and adult crickets of both sexes. A Male last instar nymph. **B** Male adult. **C** Female last instar nymph. **D** Female adult. Scale for size reference is indicated in D. Nymphs and adults are discriminated by the possession of functional hind-wings. Male and female individuals by ovipositor possession and, in adults, structure of the hind-wings.

2.2 Experimental groups based on social experiences and isolation time

Animals with different social experiences during post-embryonic, nymphal development and early adult life were generated by varying the composition of the colony and the duration of complete social isolation, when animals were kept individually for different lengths of time until experimentation (Fig. 2).

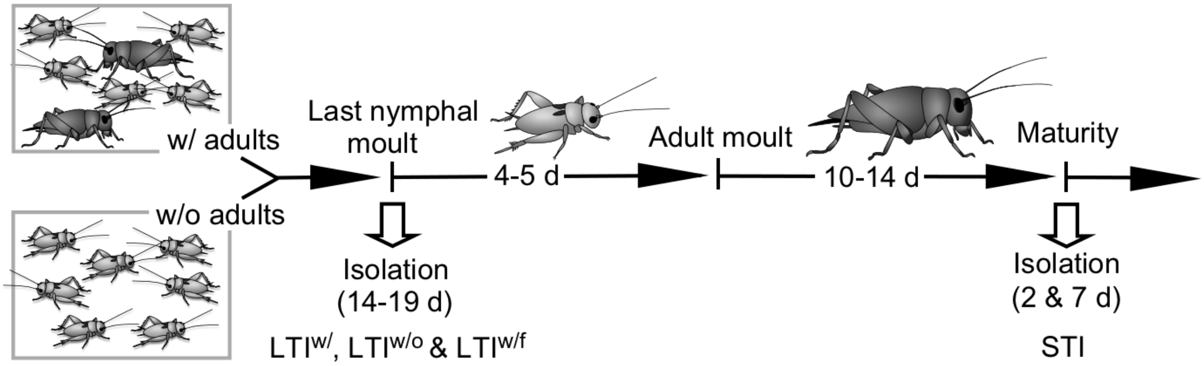


Fig. 2: Schema illustrating key events of cricket life together with culture conditions and periods of social isolation for the different experimental groups of adult male crickets. LTI$^{w/}$: long term isolates with prior contact to adult males during nymphal life; LTI$^{w/o}$: long term isolates without prior contact to adult males during nymphal life; LTI$^{w/f}$: long term isolates with prior contact to adult females during nymphal life, all separated on the day to the last nymphal instar until fully mature adult (14 to 19 days); STI: short term isolates, separated at full maturity for a period of 2 or 7 days. (Adapted from Balsam & Stevenson, 2021).

The following focal animals with different social experiences were used for experimentation:

Nymphs

Male and female last instar nymphs with prior contact to other nymphs and mature adults throughout all nymphal stages from hatching onwards. These animals were taken from the standard, age- and sex-mixed breeding colony on the day of the moult to the last nymphal stage and kept socially isolated for 48 h until experimentation.

STI - short term isolates

Adult male crickets with prior contact to nymphs and mature adults of both sexes throughout all nymphal stages from hatching onwards and as young adults. Adult males were taken from the standard breeding colony and kept isolated for 48 h until experimentation. In one experiment (mentioned explicitly in the results) adult males were taken from the same colony composition, but kept socially isolated for a period of 7 days.

To test for possible long term behavioural effects from aggressive subjugation of nymphs by adults, 3 cohorts of long term isolated adult males reared in various colony compositions were generated:

LTI$^{w/}$ - long term isolates „with“

Adult male crickets with prior contact to nymphs of both sexes and adult males during nymphal development only. Last instar nymphs were taken from the colony directly after the moult to the last nymphal stage, when still pale in colour, and identified by general body size and shape of the wing-patches of the not yet fully developed hind-wings. They were kept in social isolation throughout the last nymphal instar (4 - 5 days) and early adult life until reaching sexual maturity when they achieve their peak dominance and start singing (10 - 14 days after the final moult; Alexander, 1961).

LTI$^{w/o}$ - long term isolates „without“

Adult male crickets without any prior contact to adults of both sexes but to other nymphs during nymphal development. Last instar nymphs were taken from the pure nymphal colony directly after the moult to the last nymphal stage and kept individually until adult maturity.

LTI$^{w/f}$ - long term isolates „with females“

Adult male crickets with prior contact to nymphs of both sexes and adult females during nymphal development only. As for LTI$^{w/}$ and LTI$^{w/o}$, these were taken on the day of the moult to the last nymphal stage and kept socially isolated until fully mature.

As soon as all nymphs in the 3 different colonies were between the 5th and 6th nymphal instar, the number of either nymph-nymph or adult-nymph attacks (physical engagement involving pushing and/or biting) was visually recorded for a total of ten 1 h observation periods, spaced 2 per day (09:00 - 10:00 and 14:00 - 15:00), over 5 consecutive days. Before each 1 h observation period, the colony sizes were temporarily adjusted to 30 nymphs and 10 adult males or females by randomly removing nymphs and adults if necessary, but without changing the general colony composition. From the visual data gathered, the mean number of attacks (and 95% CI) experienced per nymph per hour was calculated (see Fig. 10B).

2.3 Evaluation of dominance and subordination

Adult male crickets exhibit a highly ritualised, stereotypic fighting pattern which can be categorised in distinct levels with increasing intensity (0 to 6, Fig. 3) commonly referred to as *levels of aggression* (Alexander, 1961; Hofmann & Schildberger, 2001; Stevenson & Rillich, 2012; Stevenson *et al.*, 2005).

Level 0: mutual avoidance, no aggressive interaction.
Level 1: pre-stablished dominance, one cricket attacks, the other retreats immediately.
Level 2: antennal fencing, individuals lash each other's antennae (also shown at the beginning of courtship between adults) at a frequency of approximately 20 Hz (Hofmann & Schildberger, 2001), first contact between the conspecifics.
Level 3: aggressive unilateral mandible threat display (exhibited by one cricket).
Level 4: aggressive bilateral mandible threat displays (exhibited by both crickets).
Level 5: mandible engagement, interlocking mandibles, first actual physical interaction between conspecifics, attacks and lunging.
Level 6: wrestling/grappling, cricket turn opponents on the back (attack, all-out fight).

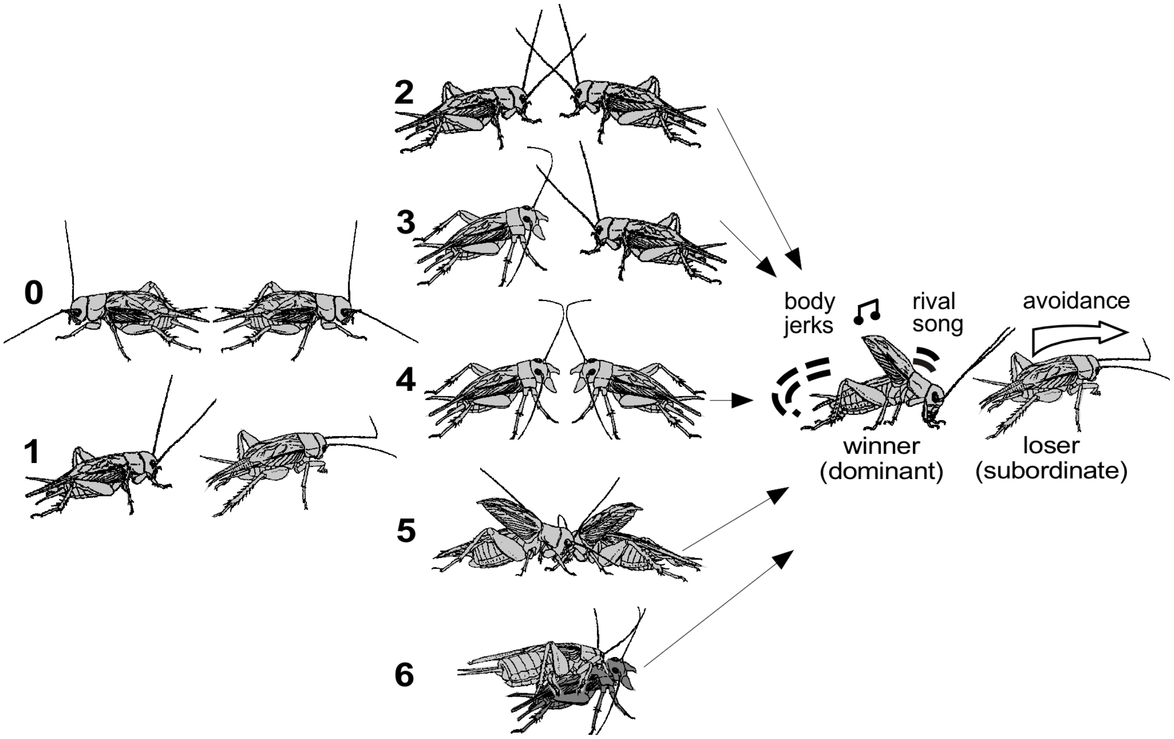


Fig. 3: Levels of increasing aggression in fights between adult male crickets. 0: Mutual avoidance (both); **1**: avoidance (one), pre-established dominance; **2**: antennal fencing, first physical contact; **3**: mandible threat display (one); **4**: mandible threat display (both); **5**: mandible interlocking; **6**: wrestling/ grappling, all out fight. Fights can end at any level with the retreat of the subordinate. Winners (dominants) usually exhibit body jerk movements and the typical rival song. (Adapted from Stevenson *et al*., 2005).

Fights can end at any level with the retreat of the then established loser (subordinate). The winner (dominant) usually displays body jerk movements and the rival song, while chasing the opponent. Aggressive behaviour towards conspecifics was evaluated in staged dyadic contests between two equally sized males (< 5% weight difference) in a clear Perspex-glass rectangular observation arena (l.w.h.: 16 × 9 × 7 cm) with a sand-covered floor after various isolation periods (see above and explicitly stated in results). For this, the opponents were placed at opposite ends of the arena and separated by a removable door in the middle. Before starting the experiment, the animals were allowed to acclimatise for at least 1 min in the arena. The animals' interactions were then noted after the door was removed until clear winners and losers were established. Losers retreat, become submissive and avoid other males (Rose *et al*., 2017; Stevenson & Rillich, 2013), whereas winners generate the characteristic rival song and remain highly aggressive towards other males (Hsu *et al*., 2005; Rillich & Stevenson, 2011). This hierarchy was verified after 1 min using the *loser test* in which the loser was confronted again with the designated winner and subsequently avoided any further interaction and retreated immediately (Iwasaki *et al*., 2006; Stevenson & Rillich, 2013). Contest duration or the time focal crickets persisted in fighting (persistence) was measured to the nearest second as the time from initial contact up until establishment of a clear loser and winner with a stopwatch. On a few occasions the animals briefly lost vision and contact to each other during the encounters, then the stopwatch was paused, so only the durations of their actual interactions were recorded. Additionally, in one set of experiments individual male nymphs and adults were presented with a conspecific nymph or adult of both sexes (Fig. 5). The individuals' responses to the presence of a single male or female, adult or nymph conspecific were evaluated and compared. In each case, the number of occurrence (frequency) of the following elements of aggressive behaviour was observed and recorded: *antennal fencing, mandible threat displays* and *attack* (one individual lunged and physically engaged the opponent).

2.4 Multiple wins and defeats

Since winners of a preceding contest become hyper-aggressive and losers highly submissive (*winner & loser effect*: Hsu *et al*., 2005; Rillich & Stevenson, 2011; Rose *et al*., 2017b; Stevenson & Rillich, 2012; 2013; 2019), 2-fold winners, respectively 2-fold losers were matched against each other maximally 10 min after the first fight to confirm the winner- and loser effect reported in STI adult male crickets. This procedure was also attempted for male nymphs. However, fights between nymphs do not always generate clear dominants and subordinates, therefore only those interactions were evaluated where this seemed apparent and could be confirmed with a loser test 1 min after the initial interaction. In other sets of experiments pairs of adult male crickets of similar weights, reared in different colony compositions and after various times of isolation (explicitly mentioned in results), were matched against each other 6 times in succession with an inter-fight interval of 30 min to subsequently yield 6-fold winners and 6-fold losers. In most cases, the winners of the first fight won all consecutive fights, if not the pair of animals was excluded from further analyses.

2.5 Contests against a hyper-aggressive opponent

Adult male crickets were matched against a standard, hyper-aggressive male generated by flying in a wind stream for 1 min (Stevenson *et al.*, 2005). All focal animals engaged the hyper-aggressive opponent, but retreated after 1 - 15 s and subsequently avoided further contact with the opponent, which signalled its dominance by generating the rival song and body jerks and subsequently chased the subordinates. Since the focal animals always lost these contests, their individual aggressive motivation could be measured by recording the level of aggression visually and the persistence (fight duration in s) of their interaction with the hyper-aggressive opponent from initial antennal contact to retreat, deducting the time when the animals lost contact, by using a stopwatch.

2.6 The mandible threat display in response to antennal stimulation and feeding

Since adult male crickets distinguish between the sexes at the first antennal contact with a conspecific (*Gryllus bimaculatus*: Hofmann & Schildberger, 2001; Iwasaki & Katagiri, 2008; Rillich *et al.*, 2019; *Teleogryllus oceanicus*: Thomas & Simmons, 2008) to engage in fighting (with males) or exhibit mating behaviour (with females) afterwards, the agonistic responses as the frequency of aggressive mandible threat displays of male nymphs and adults to manual antennal stimulation with severed antennae from male and female adults and male nymphs were compared (Fig. 6). In one set of experiments, an adult male´s antenna was washed twice for 10 min with n-hexane (Sigma Aldrich, Deisenhofen, Germany) to remove all cuticular pheromones (Iwasaki & Katagiri, 2008; Nagamoto *et al.*, 2005). In addition, the angular extent of the resulting mandible spreading in response to antennal stimulation and during feeding was measured (Fig. 6B). The spread angle was evaluated post event by analysing the filmed responses (4K Video, Sony Alpha 6300 with Zeiss macro-zoom objective) on a computer (Dell Precision 3620, Round Rock, Texas, USA) with the software MB-Ruler (version 5.3, MB Softwaresolutions, Iffezheim, Germany).

2.7 The priming effect

Stimulation of a cricket’s antenna by manually stroking it with a freshly excised antenna of another adult donor male for approximately 20 s (*priming*) is known to increase aggression in adult males after losing a preceding contest (Rillich & Stevenson, 2015). The influence of prior antennal stimulation on aggression in adults and nymphs was evaluated by staging weight-matched contest between pairs of adults and pairs of nymphs. Resultant losers of those interactions were either untreated as a control or the antennae of each subordinate cricket were subsequently stimulated for 20 s (5 min after defeat) with a donor antenna glued to a wooden stick excised from either a male adult or a male nymph. Each loser’s aggressiveness was then again evaluated 10 min later in a second contest staged against the same dominant opponent as in the first fight (Fig. 8).
Since previous studies indicate that the priming effect in adults increased dramatically when losers were additionally treated with the tissue permeable, irreversible octopamine receptor agonist chlordimeform hydrochloride (CDM; Rillich & Stevenson, 2015), this procedure was tested on male nymphs (Fig. 9A). Individual nymphs were injected with 20 µl of 1 mM CDM in saline solution (see pharmacological treatment for more details). Control animals received vehicle only (DMSO in saline). After waiting for 60 min, focal crickets were subjected to 20 s antennal stimulation, as described above, using a freshly excised donor antenna from an adult male. Immediately afterwards, dyadic contests were staged between pairs of weight-matched, treated nymphs.

2.8 Influence of food as a resource

It is known that the presence of a resource, such as food (Nosil, 2002), promotes aggression in adult male crickets. To test, whether its presence also influences aggression in nymphal crickets, male nymphs were isolated for 48 h and deprived of food for 24 h prior experimentation and their aggressiveness was subsequently evaluated in weight-matched, dyadic contests staged in the presence of a small piece of freshly cut carrot, not big enough to feed both crickets at the same time (Fig. 9B). Control contests were performed in the complete absence of food.

2.9 Set up and video tracking

All experimental observations of the freely moving, unrestrained, focal crickets were performed in a recording chamber (l.w.h.: 80 x 60 x 79 cm) lined on three sides with reflective card and evenly illuminated by two LED light panels (NL480, Neewer, Luo hu district, Shenzhen, Guangdong, China) arranged on the opposite sites of the observation arena to minimise interferences and shadows (Fig. 4). On the day of the experiment animals were transferred to the recording chamber. After allowing the animals to acclimatise for 5 minutes the new surroundings, they were placed in a round glass arena (height: 7 cm, diameter: 18 cm) with a filter paper-covered floor which was changed frequently. Each animal's behaviour was filmed from above with a digital USB video camera (Basler acA1920-155uc, Ahrensburg, Germany, 60 frames/s with a vari-focal lens f = 4 - 8 mm), analysed using commercial video-tracking software (EthoVision XT14, Noldus, Wageningen, Netherlands) and then stored on a computer (Dell Precision 3620, Round Rock, Texas, USA) with Windows system software (10, Microsoft, Redmond, Washington, USA). The animal´s movement was detected by using the live tracking routine provided by EthoVision which is based on an X-Y coordinate system calibrated in cm and degree. Three-point tracking was employed to detect the body centre, head and abdominal tip (*centre*, *head* and *tail-base*) of each cricket by the grey scaling method (minimum grey value 0, maximum 140) to track the position of the animal's longitudinal axis. To reduce jitter and continuous detection of minimal movements for example from ventilation, a “minimum distance moved” filter was set to 0.02 cm under which no response was registered. The raw data were provided automatically by EthoVision as a list of values for all analysed parameters and every 1/60 s frame (approximately 16.7 ms) in an Excel file (Microsoft Excel for MAC, Version 15.23, Microsoft 2016, Redmond, WA, USA) for subsequent calculations and minor editing of erroneous measurements. To avoid visual stimulation, the operator was obscured from the animals´ view by a white paper curtain in front of the recording chamber. Environmental conditions were closely monitored. Room temperature was controlled to ensure consistent conditions using an infrared thermometer (Voltcraft IR 260-85, Conrad Electronic, Hirschau, Germany) and the natural light - dark cycles of the seasons were taken into account when planning the experiments.

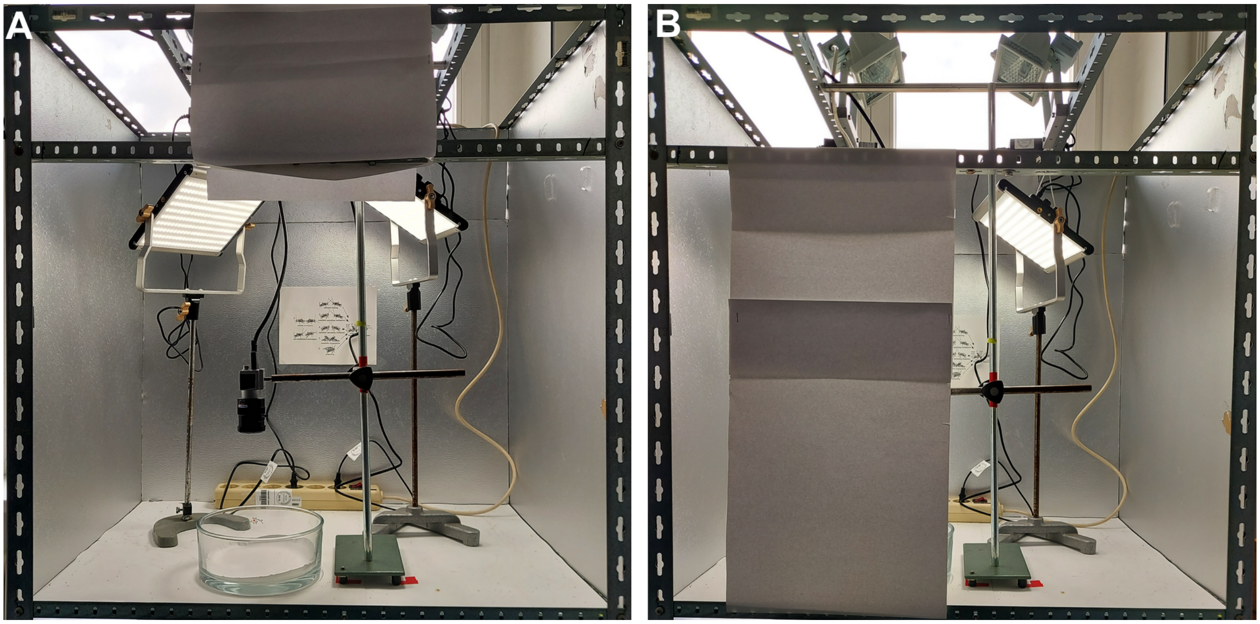


Fig. 4: Setup. Metal recording chamber lined on 3 sides with reflective card and an open front. Inside: 2 LED panels, a USB video camera and the round recording arena. Bottom lined with white paper. Paper curtain at the front (open in **A**, closed in **B**) to avoid startling the focal animal.

2.10 Evaluation of exploratory behaviour

To examine the exploratory behaviour of adult male crickets, focal animals were placed individually in the round observation arena and their activity was recorded for 3 min or, in one set of experiments, for 5 min (explicitly stated in results). The total distance moved (distance, cm) was recorded for each animal as the length of the animal´s path in cm covered in the observation period and also the mean velocity of bouts of actual walking, excluding times when the animal was stationary (velocity, cm/s). Recording was started manually as soon as the animal was placed in the arena and EthoVision started the analysis with a delay of 1 s to check for settings and recording parameters. After the scheduled time, the recording stopped automatically.

2.11 Response to a single antennal touch with an adult male´s antenna

To evaluate the responses of adult male crickets to touching one antenna briefly with a freshly excised antenna from another adult donor male, focal animals were placed individually in the observation arena after allowing them to adapt to the new surroundings by placing them into the recording chamber for 1 min. Because crickets tend to remain close to the wall of the observation arena, this touch was most easily achieved manually, using an antenna attached with glue to the distal end of a 20 cm long, white stick to minimise visual stimulation and false movement detection by the video-tracking system. The donor antenna was replaced every 10 to 15 test animals. The animals were placed in the arena 1 min before experimentation. To avoid startling the crickets, the severed antenna was positioned slowly in the vicinity of the animal's head and held still for at least 1 - 2 s before stimulation. The stimulus was applied when the animal was not excessively mobile and delivered to the most accessible antenna which was about equally on the left- and right-side throughout all experiments. Pilot experiments revealed no difference between left- and right-side antennal stimulation and further no response to sham stimulation in the vicinity of the focal animal´s antennae without a touch. The donor antenna was moved above the focal animal´s antenna and a single touch was applied with a force just sufficient to deflect the antenna visibly (average duration of stimulus stroke: 133 - 200 ms, actual touch duration with visible deflection: 33 - 55 ms, as measured from the number of video frames). Video-taped responses were analysed and occasionally occurring false detections were corrected manually with EthoVision XT. Each animal's angular turning response towards or away from the applied stimulus from the moment of touch was measured every 1/60 ms for a total of 1 s (60 frames). Raw data of each track were adjusted to set the longitudinal axis of the animal's starting position to 0° using Microsoft Excel. Angular changes > 90° in 1 frame, that occasionally occurred due to erroneous detection of head and tail points during rapid movements or jumps, were replaced by the mean of the previous and subsequent measurement.

To analyse the effects of prior social experiences, the turning responses exhibited by individual crickets were initially measured before staging their first fight just after isolation, when the social status as dominant or subordinate was still unknown. After 1 h, contests were staged between pairs of males matched by weight alone, irrespective of their previously exhibited turn angle, to generate clear winners and losers. The turning responses were then measured again after the fight, for the established winners and losers, and the turning response of each individual was compared to the performance before fighting, in the prospective winners and prospective losers.

In some set of experiments, the influence of multiple wins and defeats on turning responses was evaluated. For this, pairs of crickets of similar weights were matched against each other 6 times in succession at 30 min intervals to yield 6-fold winners and 6-fold losers (see above). Turning responses were measured before and after the 6 consecutive contest as well as 1 day later or, in one experiment (explicitly mentioned in results), 6 days later.

To test for correlation between turning and individual aggressiveness, the turn angles of STI crickets of unknown social status after 48 h of isolation were recorded. The focal crickets were subsequently matched against a standard hyper-aggressive male to measure their persistence.

2.12 Pharmacological treatment

All drugs were obtained from Sigma (Sigma Aldrich, Deisenhofen, Germany) unless stated otherwise. With whole-animal pharmacology the role of biogenic amines (released in response to social experience) on turning behaviour was evaluated using the following drugs: the selective OAR-blocker epinastine hydrochloride, the tissue permeable, irreversible OAR-agonist chlordimeform hydrochloride (CDM; for octopamine in invertebrates see also Roeder *et al.*, 1998), the competitive NOS-inhibitor Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME), the NO-donor S-Nitroso-N-acetyl-DL-penicillamine (SNAP; Tocris Bioscience, Bristol, UK), the 5HTR-blockers methiothepin mesylate salt, ketanserin (+)-tartrate salt (Tocris Bioscience) and WAY-100635 maleate (Tocris Bioscience), the 5HT-precursor 5-Hydroxy-L-tryptophan (5HTP), the 5HT reuptake-inhibitor fluoxetine hydrochloride as well as the 5HTR-agonists (R)(-)-DOI hydrochloride (DOI) and (R)-(+)-8-Hydroxy-DPAT hydrobromide (8-OH-DPAT; Tocris

Bioscience). The terms agonist (receptor activator, donor, reuptake-inhibitor, precursor) and antagonist (receptor blocker, synthesis inhibitor) are used in their broadest sense.

The listed drugs were applied in the most effective dosages which influence aggressive behavioural traits, but without any detrimental effects on general motility (selectivity and dosages of the used drugs are given in Tab. 1). These effective dosages have been determined in previous studies (Stevenson *et al*., 2005; Rillich & Stevenson, 2011; 2014; 2015; 2018; Rillich *et al*., 2011; Stevenson & Rillich, 2015) or in case of DOI, WAY and 8-OH-DPAT with a recovery analysis (see Rillich & Stevenson, 2018) for 1 mM, 5 mM and 10 mM drug solutions. For this focal animals were matched against a hyper-aggressive opponent 1 h after they received either vehicle or drug treatment and their aggressiveness was tested again 30, 60 and 180 min after defeat to check for recovery effects. Adult male crickets typically regain their aggressiveness 3 h after a single defeat (Rillich & Stevenson, 2014; Stevenson & Rillich, 2013). Effective drug concentration was then determined by the influence on recovery, but with no obvious detrimental effects on motility (1 mM and 5 mM: no obvious effects on loser recovery; 10 mM: Fig 20). All of the treated and analysed animals were monitored individually after experimentation and survived at least 24 h after the treatments. To provide the required concentration, drugs were either dissolved in insect saline (saline components in mmol/l: NaCl 140, KCl 10, $CaCl_2$ 7, $NaHCO_3$ 8, $MgCl_2$ 1, N-trismethyl-2-aminoethanesulfonic acid 5, D-trehalose dehydrate 4, pH 7.4) or in 1% DMSO and subsequently saline depending on the solubility.

The focal animals were first sorted in positive and negative turners and assigned randomly to a treatment group afterwards. General motility and turning behaviour was evaluated before (pre-drug) and 1 h after (post-drug) the test animals were injected with 20 µl of the drug solution using a 60 µl micro-syringe (Hamiliton, Bonaduz, Switzerland) inserted through the membranous cuticle underlying the pronotal shield into the haemocoel. Control animals received 20 µl of either DMSO in saline or D-NAME (Nω-Nitro-D-arginine methyl ester hydrochloride, inactive isomer of L-NAME; explicitly stated in legends and results). Subsequent aggression measured as persistence against a hyper-aggressive opponent was evaluated post-drug only, to avoid influences of defeat on further experiments. In one set of experiments combinations of 2 drugs were used (see Fig. 19). For this 20 µl of each drug was injected alternately into the haemocoel, 40 µl in total, and for the control groups 40 µl of the vehicle accordingly.

Tab. 1: Overview of drugs, actions and effective dosages. Name and action of applied drugs with molar weight (MW), dosages of active drug per injection (20 µl), mg of drug for 1 ml solution and dilution/vehicle.

		MW	**Dosage**		
Applied drug	**Action of applied drug**	**(g/mol)**	**mM/20µl**	**mg/1ml**	**Dilution/vehicle**
Epinastine hydrochloride	Selective OAR-blocker	285.77	10	2.86	Saline + 1% DMSO
Chlordimeform hydrochloride	OAR-agonist	233.14	1	0.23	Saline + 1% DMSO
N_{ω}-Nitro-L-arginine methyl ester hydrochloride	Competitive NOS-inhibitor	269.69	10	2.70	Saline
N_{ω}-Nitro-D-arginine methyl ester hydrochloride	Inactive isomer	269.69	10	2.70	Saline
S-Nitroso-N-acetyl-DL-penicillamine	NO-donor	220.25	1	0.22	Saline
Ketanserin (+)-tartrate salt	5HTR2-blocker	545.51	10	5.46	Saline + 1% DMSO
Methiothepin mesylate salt	5HTR1,6,7-blocker	452.65	10	4.53	Saline + 1% DMSO
WAY-100635 maleate	Selective 5HTR1A antagonist	538.64	10	5.39	Saline + 1% DMSO
5-Hydroxy-L-tryptophan (5HTP)	5HT-precursor	220.22	5	1.10	Saline + 1% DMSO
Fluoxetine hydrochloride	5HT-reuptake-inhibitor	345.79	1	0.35	Saline + 1% DMSO
(R)-(+)-8-Hydroxy-DPAT hydrobromide	Full 5HTR1A agonist	328.29	10	3.28	Saline + 1% DMSO
(R)(-)-DOI hydrochloride	5HTR2 agonist	357.62	10	3.58	Saline + 1% DMSO

2.13 Data analysis

All statistical analyses were performed using commercial software (GraphPad Prism 7 for MAC, Version 7.0c, GraphPad Software, Inc., La Jolla, CA, USA) running on a Macintosh computer (Apple, Cupertino, CA, USA). The Shapiro - Wilk test was used to test for data normality. For normally distributed data the means and 95% confidence intervals in preference to the standard deviation were calculated (lower to upper 95% CI of the mean; Hazra, 2017). To test for significant differences between the same groups before and after fights or treatments Student´s two-tailed paired t-test and for differences between independent groups Student´s two-tailed unpaired t-test were performed. Pearson´s correlation was used to test for correlations in parametric data sets.

For nominal data (level of aggression) the median with interquartile range (IQR) was calculated. The following non-parametric procedures were applied to test for significant differences in data distributions: the Mann-Whitney U test for unpaired data sets and the Wilcoxon signed-rank test for paired data sets. The differences in relative frequencies of behavioural elements between groups were compared with the Fisher´s exact test. The number of crickets for each analysis and test group is given in the figures, legends and/or results. For single comparisons, the significance level alpha was set to $p < 0.05$. Occasionally, the same data set was used in more than one statistical analysis. To compensate for errors due to multiple comparisons the Bonferroni correction to alpha was applied accordingly (explicitly mentioned in results and legends). All components of the figures were created with canvas DRAW 5 for MAC (Version 5.0.2, ACD Systems International Inc., Victoria, BC, Canada). This dissertation is partly published or adapted from published articles and elements of methods, results and discussion are taken verbatim or in spirit from Balsam & Stevenson, 2020 and 2021. The corresponding figures are indicated as such.

3 Results

3.1 Responses of nymphs and adults towards conspecifics

Observations of the age- and sex-mixed colony cages revealed that adult males behave differently towards nymphs and other adult males or females and that nymphs rarely exhibit aggressive interactions with conspecifics. After the initial antennal contact, adult male crickets exhibited a set of aggressive behaviours towards adult male conspecifics such as the mandible threat display, biting, pushing, body jerking and the rival song all of which signalling aggressive motivation. Contrasting this, after the first antennal contact with adult females they showed courting behaviour and exhibited the courtship song.

The first step towards a classification of aggressive behaviours in male nymphs was to evaluate their interactions with conspecifics of both sexes and different developmental stages and to compare the nymphal behaviour with the typical adult responses (Fig. 5). In a small arena, STI adult male crickets and last instar male nymphs were presented with conspecifics of both ages and sexes individually to evaluate their responses towards each other in a controlled situation apart from the colony cage. For this the 3 following responses were analysed by calculating the frequencies of occurrence in interactions with different opponents: antennal fencing, which is the first actual contact between conspecifics and it initiates either fighting or courting (Hofmann & Schildberger, 2001; Iwasaki & Katagiri, 2008; Rillich & Stevenson, 2015; Rillich *et al.*; 2019) in adult males; the mandible threat display, which signals aggressive motivation from one or both focal crickets and attack, which is with lunging and biting the first physically aggressive interaction.

As depicted in Fig. 5A, both male nymphs and male adults showed antennal fencing behaviour towards conspecifics indiscriminately of the opponent´s developmental stage or sex, but with a total of 50% the tested male nymphs exhibited significantly less antennal fencing responses compared to male adults (90 - 95%; *p* = 0.0033 for all comparisons, except for responses towards female nymphs: *p* = 0.0138). Similarly, male nymphs showed the mandible threat display (Fig. 5B) towards both male and female nymphs in almost the same frequencies (25%, 30% respectively), but it was never observed towards male or female adults (0%; differences in nymphal response towards adult females and males: $p = 0.0202$, 0.0471 respectively; latter insignificant after Bonferroni correction to alpha = 0.025). Contrasting this, male adults exhibited mandible threats significantly more frequently towards male nymphs (90%), as well as male adults (90%) and also female nymphs (75%; $p < 0.0005$, < 0.0005, 0.0104 respectively; Bonferroni correction to alpha = 0.025), but never on contact with adult females (0%, significantly different to response to female nymphs: $p < 0.0005$; Bonferroni correction to alpha = 0.025). Male nymphs occasionally attacked male and female nymphs (20% for both; Fig. 5C), but that was, again, never observed on contact with adult males and females (0%; $p = 0.0305$ for both, differences insignificant after Bonferroni correction to alpha = 0.025). Contrasting this, male adults frequently attacked male nymphs (90%), other adult males (70%) and female nymphs (80%; $p < 0.0005$ each; Bonferroni correction to alpha = 0.025). However, male adults never attacked adult females (0%; significantly different to response to female nymphs: $p < 0.0005$; Bonferroni correction to alpha = 0.025).

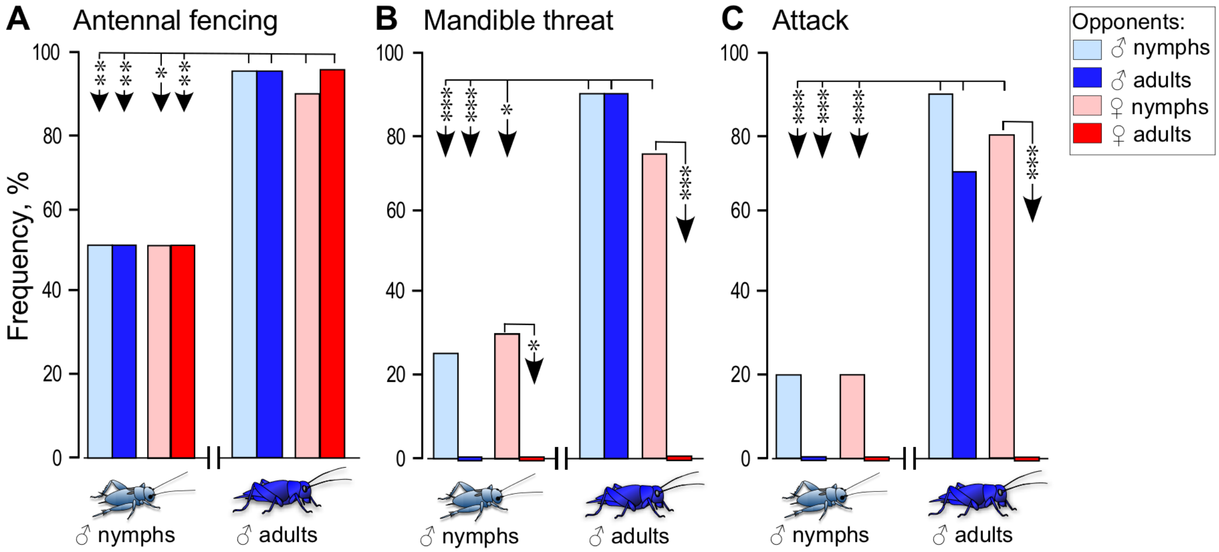


Fig. 5: Observed behaviours of male nymphs and adults towards other nymphs and adults of both sexes. Bars giving the frequency of **A** antennal fencing, **B** mandible threat display and **C** attack (lunging with physical interaction) of STI male nymphs and adults towards different opponents (see key in C): male nymphs (light blue bars), male adults (blue bars), female nymphs (light red bars) and female adults (red bars; $n = 20$ each, always comprising different animals). Significant differences between nymphal and adult reactions are indicated by arrows and given as asterisks (A * $p < 0.05$, ** $p < 0.01$; B and C * $p < 0.025$, *** $p < 0.0005$; Bonferroni correction for two comparisons). (Balsam & Stevenson, 2020).

3.2 Mandible threat display and the mandible spread angle

To further analyse the responses of male nymphs and adults, the occurrence frequency of mandible threat displays after mechanical antennal stimulation with freshly excised donor antennae was evaluated. So far, the data indicate that male nymphs neither discriminate between sexes of the opponents nor are their interactions as violent as those observed in adult males. Nymphs also showed significantly less mandible threat displays or physical attacks, particularly when confronted with an adult (Fig. 5).

To provoke mandible spreading, the antennae of male nymphs and adults were manually stroked with different donor antennae taken from male and female adults and male nymphs (Fig. 6A). Confirming earlier findings (Rillich & Stevenson, 2015), adult male crickets frequently showed mandible threats in response to mechanical stimulation of their antennae with an adult male donor antenna (80%). In contrast, male nymphs exhibited mandible threats with a significantly lower frequency (45%, $p = 0.0024$; Bonferroni correction to alpha = 0.025). To check for the influence of pheromones on the responses to antennal stimulation, a donor antenna was washed twice in n-hexane to remove all cuticular pheromones. Now adult males showed the aggressive mandible threat significantly less frequently ($p = 0.0034$), but equally as often as male nymphs confronted with the washed antenna (both 40%). Similarly, when stroked with a male nymph's antenna, nymphs and adults both exhibited an equally low mandible threat frequency (both 35%). On antennal contact with adult females, adult males almost immediately start courting. So an adult female´s antenna was severed to check again for influences and more importantly perception of pheromones. In response to stroking with an adult female´s antenna, male nymphs showed the same frequency of mandible threat displays (40%), whereas adult males responded significantly less frequently (5%, $p = 0.0197$) and in most cases started courting (15 out of 20 tested animals).

It was also observed that nymphs not only exhibited mandible threats less often in response to stimulation with an adult male´s antenna, they also spread their mandibles significantly less wide compared to adult males (nymphs: mean: 40°, 95% CI: 36 to 44°, $n = 18$; adults: mean: 71°, 95% CI: 69 to 74°, $n = 32$; $p < 0.0005$; Bonferroni correction to alpha = 0.025; Fig. 6B). To check whether this is due to some physical inability which could reflect a lower frequency of engaging in violent fights as seen in adult males, the maximum spread angle of individual nymphs during feeding was measured (Fig. 6B). This revealed that nymphs spread their mandibles significantly wider while feeding (mean: 69°, 95% CI: 66 to 72°, n = 18; p < 0,0005; Bonferroni correction to alpha = 0.025) and that they are physically able to open their mandibles as wide as adult conspecifics (mean: 72°, 95% CI: 68 to 75°, n = 18).

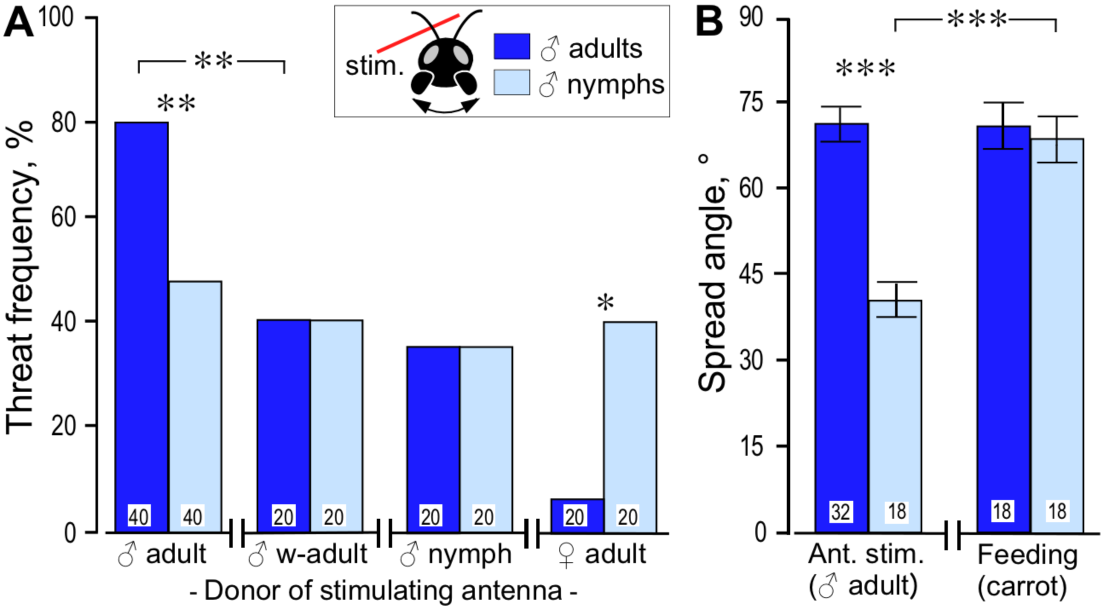


Fig. 6: Mandible threat display in response to antennal stimulation and comparison of the mandible spread angle. A Frequency of mandible threats for STI male adults and male nymphs (light and dark blue bars respectively) in response to mechanical stimulation with a freshly excised antenna from different donors (left to right): male adult, male adult and washed twice in n-hexane, male nymph and female adult. **B** Maximum angle of mandible spreading (mean ± 95% CI) observed for male adults and nymphs in response to antennal stimulation (Ant. stim.) with a freshly excised male antenna and during feeding. Significant differences between data sets are indicated (* p < 0.025, ** p < 0.005, *** p < 0.0005; Bonferroni correction for two comparisons, n for each group is given in the bars). (Balsam & Stevenson, 2020).

3.3 Influence of dominance and subordination

As shown in previous studies, adult male crickets become hyper-aggressive after winning a preceding contest and losers are highly submissive and avoid contact to conspecifics for the next hours (Hsu *et al*., 2005; Rillich & Stevenson, 2011; Stevenson & Rillich, 2013). To test whether a prior winning or losing experience has any influence on subsequent aggressiveness in male nymphs, the focal nymphs were matched against an opponent of similar weight in a staged dyadic contest. Because winner and loser of a fight between nymphs were not always apparent and the fights never escalated on a physical level, only pairs with a clearly established dominance were used for the analysis. The loser should retreat from the winner which in turn continued to chase to opponent.

As a control and confirming previous studies, STI adult males were paired weight-matched and their fighting behaviour was evaluated. The typical fights between adult males escalated to level 4 (median, IQR: 4 to 5, $n = 20$) and lasted 7 s (mean: 7.45 s, 95% CI: 5.25 to 9.65 s; $n = 20$; Fig. 7A). Contests between pairs of adult males that both won a previous fight escalated significantly higher compared to the first interaction (median: 5.5, IQR: 5 to 6; $p = 0.0023$; $n = 20$) and they also persisted longer (mean: 14.5 s, 95% CI: 12.74 to 16.26 s; $p < 0.0001$). Contrasting this, pairs of losers of a previous aggressive interaction were significantly less aggressive compared to the first fight regarding level (median: 0, IQR: 0 to 1; $p < 0.0001$; $n = 20$) and fight duration (mean: 0.4 s, 95% CI: 0.17 to 0.64 s; $p < 0.0001$).

In marked contrast, these winner and loser effects were not observed in staged fights between STI male nymphs and the contests between nymphs were far less violent (Fig. 7B). The typical initial encounters never exceeded level 3 (median: 2, IQR:1 to 2, $n = 20$) or lasted longer than 4 s (mean: 1.75 s, 95% CI: 1.35 to 2.15 s). Furthermore, pairs of winners showed no change in aggressiveness compared to their initial interaction (level: median: 2, IQR: 1 to 2; $p = 0.8770$; duration: mean: 1.9 s, 95% CI: 1.64 to 2.16 s; $p = 0.5906$) and interactions between losers were also not significantly different to those of the same animals at their first contest (level: median: 1.5, IQR: 1 to 2; $p = 0.0586$; duration: mean: 1.4 s, 95% CI: 0.96 to 1.84 s; $p = 0.2170$).

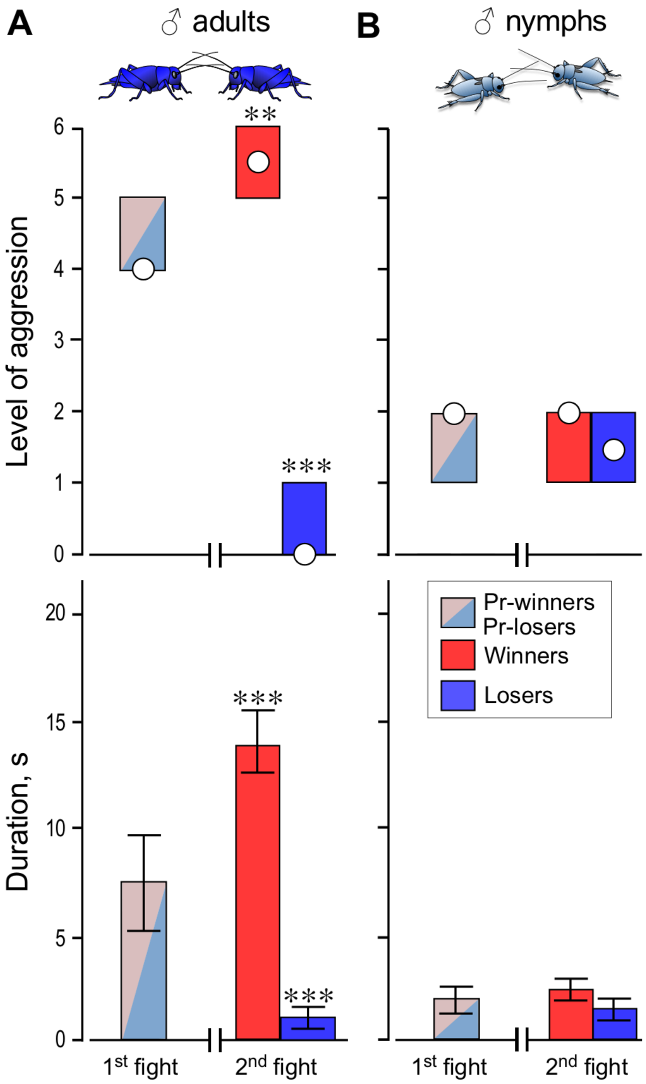


Fig. 7: Effects of aggressive experience on subsequent aggression (dominance and subordination). Bars giving the level (top, circles: median, boxes: interquartile range) and duration (bottom, mean ± 95% CI) of agonistic interactions between weight-matched pairs of **A** male adults and **B** male nymphs (both STI) at their 1st fight after isolation (light red/light blue bars, prospective winners and losers, $n = 40$) and for a 2nd fight, staged between pairs of the resultant winners and pairs of resultant losers (red and blue bars respectively, $n = 20$ each). Significant differences between the prospective and actual winners and prospective and actual losers are indicated (** $p < 0.01$, *** $p < 0.001$). Note the absence of winner and loser effects in nymphs. (Adapted from Balsam & Stevenson, 2020).

3.4 Influence of prior antennal stimulation (priming)

Since adult male crickets were found to show increasing aggression after prior antennal stimulation for 20 s with a freshly excised donor male antenna (Rillich & Stevenson, 2015), male nymphs and adults that previously lost a fight were treated with different donor antennae for 20 s and subsequently re-matched with the winner of the first interaction. As a control and confirming earlier studies, STI adult losers were re-matched 10 min after the initial fight either untreated, stroked with an adult male´s antenna or with a male nymph´s antenna (n = 20 each; Fig. 8A). Those first encounters typically escalated to level 5 (IQR: 3 to 6 for all groups) and lasted on average 7 s.

Untreated adult losers showed significantly reduced aggressiveness compared to their initial performance (level: median: 1, IQR: 0.25 to 1; $p < 0.001$; duration: mean: 1 s, 95% CI: 0.63 to 1.37 s; p < 0.001). Contrasting this, when stroked with an adult male´s antenna, the treated losers showed no difference to their first fight and the loser effect was no longer evident (priming effect; level: median: 4, IQR: 4 to 5; $p = 0.7327$; duration: mean: 6.1 s, 95% CI: 5 to 7.2 s; p = 0.1111). Interestingly, after stroking the adult losers´ antennae with a donor antenna of a male nymph for 20 s this priming effect is no longer evident and the subordinates again showed a clear loser effect when re-matched with the previous opponent 10 min after the initial fight (level: median: 1, IQR: 0.25 to 1; $p < 0.001$; duration: mean: 1.05 s, 95% CI: 0.66 to 1.44 s; p < 0.001).

In marked contrast to adults, untreated male nymphs typically fought on level 2 for 1 to 2 s in their first interactions. After losing, untreated male nymphs (Fig. 8B, see also Fig. 7B) showed no obvious loser effect compared to their first encounters with weight-matched conspecifics (level: median: 1, IQR: 1 to 2; $p = 0.0762$; duration: mean: 1 s, 95% CI: 0.6 to 1.4 s; p = 0.2321). Mechanical stimulation also failed to have any significant effect on their aggression expressed at a subsequent, second contest regardless of whether an adult male´s antenna (level: median: 2, IQR: 1 to 3; $p = 0.3398$; duration: mean: 1.9 s, 95% CI: 1.14 to 2.66 s; p = 0.5848) or male nymph´s antenna (level: median: 1, IQR: 1 to 2; $p = 0.1289$; duration: mean: 1.8 s, 95% CI: 1.1 to 2.52 s; p = 0.3733) was used as a stimulus.

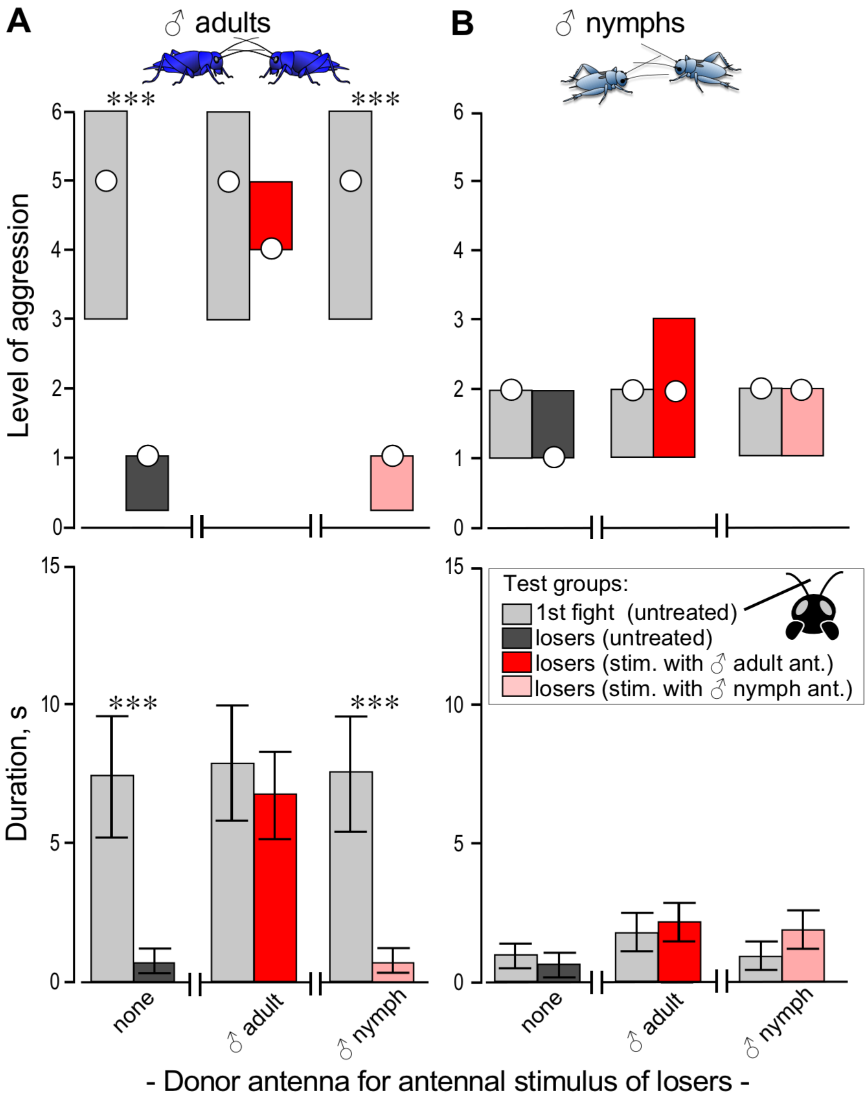


Fig. 8: Influence of prior antennal stimulation on subsequent aggression. Bars giving the level (top; circles: median, boxes: interquartile range) and duration (bottom; mean ± 95% CI) of agonistic interactions between weight-matched pairs of **A** STI male adults and **B** STI male nymphs at their 1^{st} interaction (light grey bars) and for the same animals 10 min later. Before the second fight, the losers were either untreated (dark grey), or stimulated with an antenna from a male adult (red) or male nymph (light red, *n* = 20 for each group). Significant differences between paired data sets are indicated (*** p < 0.001). (Adapted from Balsam & Stevenson, 2020).

3.5 Influence of priming coupled with CDM

So far, nymphs showed no physical fights (above level 3) when paired with conspecifics of different developmental stages and both sexes. In a further attempt to increase aggressiveness in STI male nymphs, the effect of different aggression modulators was evaluated. Since priming alone failed to have any significant effect on subsequent aggression (Fig. 8B), the priming stimulus was coupled with the OAR-agonist CDM. In STI adults, this leads to an increase in subsequent aggressiveness (Rillich & Stevenson, 2015). However, in treated nymphs no difference in escalation level was observed compared to nymphs that received the vehicle only (vehicle: median: 2, IQR: 1 to 2; CDM: median: 2, IQR: 1 to 3; *p* = 0.6267; *n* = 13; Fig. 9A), but a significant change in fight duration (CDM: mean: 2.92 s, 95% CI: 1.54 to 4.31 s; *p* = 0.0273). compared to the control group that received vehicle only (level: median: 2; IQR: 1 to 2; duration: mean: 1.31 s; 95% CI: 0.74 to 1.88 s). Notably, however, one CDM-treated pair of nymphs exhibited actual physical fighting behaviour after prior antennal stimulation, during which the individuals interlocked their mandibles to push each other (level 5) in a contest that lasted 7 s, which is within the range typically shown by untreated adult males.

3.6 Influence of food as a resource

The presence of food is described as another aggression modulator based on earlier studies (Simmons, 1987). Interactions between adult crickets can significantly increase in aggressiveness when a resource is provided (Nosil, 2002; Rillich *et al*., 2011). To check if food as a resource heightens aggression in male nymphs, the focal animals were starved for 24 h and fights between weight-matched pairs were staged (*n* = 20 each; Fig. 9B). Contrasting to previous findings in adult males, no significant differences in level (median: 2, IQR: 1 to 2) and duration (mean: 1.55 s, 95% CI: 1.08 to 2.02 s) were found for STI nymphs that interacted in the presence of food compared to the control group without food (level: median: 2, IQR: 1 to 2; *p* = 0.9081; duration: mean: 1.35 s, 95% CI: 0.89 to 1.81 s; *p* = 0.5282).

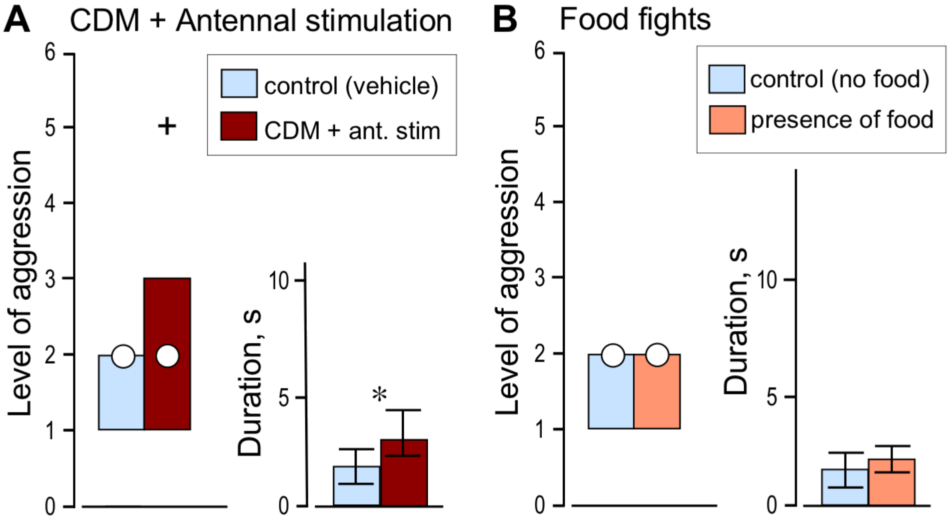


Fig. 9: Effects of aggression modulators on aggression in male nymphs. Bars giving the level (circles: median, boxes: interquartile range) and duration (mean ± 95% CI) of agonistic interactions between STI male nymphs. **A** After treatment with the OAR-agonist chlordimeform (CDM in 1% DMSO) combined with antennal stimulation (dark red bars, $n = 13$). Note the one pair of nymphs (indicated with **+**) that engaged in an actual physical fight after the treatment which is within the range typically exhibited by adults. **B** In the presence of food (orange bars, $n = 20$). Controls (light blue bars) received DMSO in saline (A, $n = 13$) or no food (B, $n = 20$). Significant differences between unpaired data sets are indicated (* $p < 0.05$). (Adapted from Balsam & Stevenson, 2020).

3.7 Effects of different social experiences during nymphal development on adult behaviour

Since nymphs were frequently attacked by adult male crickets in staged interactions and nymphs almost never engaged in physical fights, a closer look at the breeding colonies and the interactions therein was indicated. To evaluate the effects of different social experiences during post-embryonic nymphal development on later adult behaviour, 3 colonies with various social compositions were created (Fig. 10A) and the aggressive attacks between the focal animals in the cages were quantified as the number of occurrence (Fig. 10B). For this, all attacks were counted in colonies with 30 nymphs either in the complete absence of adults, in the presence of 10 adult males or the presence of 10 adult females. In the ten 1 h intervals observed, nymphs were rarely attacked by other nymphs in the grouped culture conditions (mean attacks endured per nymph per hour for n = 10 x 1 h sessions: 0.07, 95% CI: 0.06 to 0.08). Similarly, adult females also hardly ever attacked nymphs (mean attacks endured per nymph per hour: 0.07, 95% CI: 0.04 to 0.09). In marked contrast, each individual nymph in the colony was attacked by an adult almost once an hour, and with that significantly more often than by other nymphs or adult females (mean attacks endured per nymph per hour: 0.93, 95% CI: 0.84 to 1.02; $p < 0.0005$ for both; Bonferroni correction to alpha = 0.025). Next, last stage male nymphs from the 3 different colonies were isolated and kept individually until maturity (LTI). As soon as the focal animals were fully mature adults, motility, as total distance moved in a 5 min interval, and aggressiveness in weight-matched fights against each other were evaluated (Figs. 10C, D respectively). Video-tracking analysis revealed that adult males raised as nymphs without adult males and females ($\mathrm{LTI}^{w/o}$) and also those raised together with adult females only ($\mathrm{LTI}^{w/f}$) were significantly more active compared to adults housed as nymphs together with adult males ($\mathrm{LTI}^{w/}$; $n = 20$ each; Fig. 10C). $\mathrm{LTI}^{w/o}$ adults that never had any social contact to other adults walked a significantly longer distance during the observation period (mean: 189 cm, 95% CI: 174 to 204 cm), compared to those subjected to repeated subjugation by adult males as nymphs ($\mathrm{LTI}^{w/}$; mean 150 cm, 95% CI: 138 to 162 cm; $p = 0.0002$; Bonferroni correction to alpha = 0.025). Similarly, $\mathrm{LTI}^{w/f}$ adults were also significantly more active during the observation period compared to $\mathrm{LTI}^{w/}$ adult males (mean: 215 cm, 95% CI: 164 to 267 cm; $p = 0.0139$; Bonferroni correction to alpha = 0.025). Furthermore, the mean velocity of the walking episodes was significantly faster for adult males from the pure nymph colony (mean: 0.74 cm/s, 95% CI: 0.67 to 0.81 cm/s; $p < 0.0001$; Bonferroni correction to alpha = 0.025) and the mixed nymphal-adult female colony (mean: 0.78 cm/s, 95% CI: 0.59 to 0.97 cm/s; $p = 0.0139$; Bonferroni correction to alpha = 0.025) compared to adult males raised in the mixed nymphal-adult male colony (mean: 0.54 cm/s, 95% CI: 0.49 to 0.59 cm/s). Finally, an evaluation of fighting behaviour revealed that $\mathrm{LTI}^{w/o}$ were more aggressive with regards to level (median: 6, IQR: 5 to 6; $p = 0.0337$; insignificant after Bonferroni correction to alpha = 0.025; Fig. 10D) and duration (mean: 12.8 s; 95% CI: 10.62 to 14.98 s; $p = 0.0154$; Bonferroni correction to alpha = 0.025) of fights compared to adults isolated from the mixed nymphal-adult male colony (level: median: 5, IQR: 4 to 5.75; duration: mean: 9.55 s; 95% CI: 7.98 to 11.12 s). Similarly, $\mathrm{LTI}^{w/f}$ adults showed increased aggression (level: median: 6, IQR: 5 to 6; duration: mean: 14.2 s; 95% CI: 11.25 to 17.15 s; $p = 0.0470$, $p = 0.0033$ respectively;

first insignificant after Bonferroni correction to alpha = 0.025) compared to LTI$^{w/}$ adults that were frequently attacked from adult males during development.

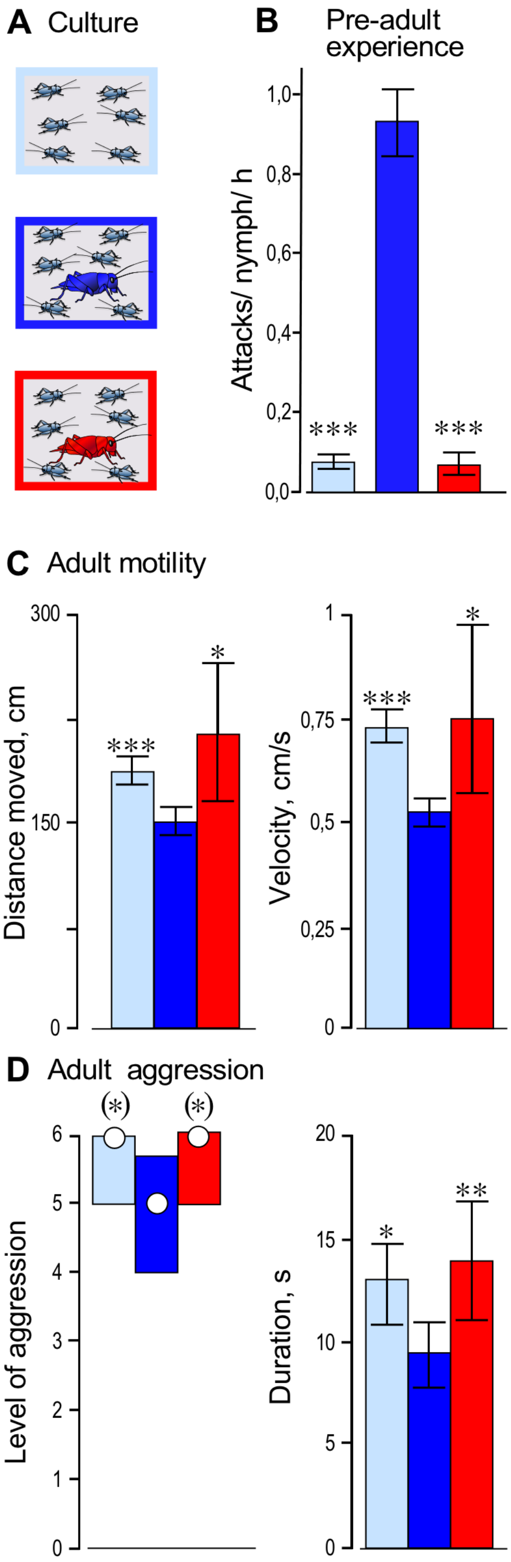


Fig. 10: Influence of social experiences during nymphal development on their adult behaviour. A Schema of culture conditions: 30 male nymphs were raised either without adults of both sexes (light blue) from hatching until the last nymphal stage, or with 10 adult males (blue) or 10 adult females (red) from the 4th to the last nymphal instar. **B** Bars giving the mean (± 95% CI) number of attacks experienced per nymph per hour observed in 10 x 1 h sessions, from either other nymphs in the pure nymph culture (light blue, number divided by 3 to adjust for the larger number of nymphs in the cage), or adult males (dark blue) or adult females (red) in the mixed adult-nymph cultures. **C** Bars giving the distance moved (left) and mean velocity (right) of 5 min walking bouts (means ± 95% CI) for LTI$^{w/o}$ (light blue), LTI$^{w/}$ (blue) and LTI$^{w/f}$ (red, n = 20 each). **D** As for C, but giving the level (left, circles: median, boxes: interquartile range) and duration (right, mean ± 95% CI) of fights between pairs of crickets from the three groups (n = 20 each). Significant differences between the mixed nymph-adult males colony and the two other housing conditions are indicated (* $p < 0.025$, *** $p < 0.0005$; (*) significant before correction). Bonferroni correction for two comparisons was applied. (Adapted from Balsam & Stevenson, 2020).

3.8 Turning responses as a reaction to a single antennal touch with an adult male´s antenna

3.8.1 Short term isolates

The data so far indicate that adult male crickets, raised under various social conditions, exhibited differences in behavioural profiles with regard to general motility and aggression towards conspecifics (Fig. 10). To further evaluate the aggressive motivation of individual crickets, a single antennal touch with a freshly excised adult male´s antenna was applied and the responses of focal animals (a turn towards or away from the stimulus) were quantified. The aggressive mandible threat (Rillich & Stevenson, 2015; Figs. 5 and 6) was never observed after the brief, single stimulus applied in the following experiments. However, positive turners (*n* = 40 observed) occasionally lunged forward (*n* = 9), exhibited body jerks (*n* = 11) or generated the rival song (*n* = 4), this set of behaviours was never exhibited by negative turners. Notably, all effects from previous interactions should have waned after the isolation period of 48 h. However, as shown in Figs. 11A and C, crickets with still unknown social status (pre-fight) turned either towards or away from the direction of the stimulated antenna (37% positive turn angle, respectively 58% negative turn angle, 5% no clear directional preference, *n* = 60). The focal animals were subsequently paired in dyadic contests according to their weight, disregarding previously exhibited angular direction. After fighting the majority of now established winners turned towards the stimulus (63%) and losers away from it (77%, *n* = 30 each). After 1 s, winners had turned on average +22° and losers -58° (means, 95% CI: 5 to 39° and -78 to -39° respectively, significantly different to winners: $p < 0.0001$; Fig. 11D). A sequential plot of the difference-probability *p* revealed a statistically significant difference between the mean turn angles for these two groups 34 ms after the touch stimulus was applied (2 video frames; $p = 0.048$). After retrospective sorting, the data revealed that the majority of prospective winners (pre-fight) also approached the stimulus (83%), whereas the prospective losers mainly showed avoidance (90%, *n* = 30 each). When measured 1 s after the touch, the prospective winners had turned +34° and the prospective losers -63° (means, 95% CI: 16 to 52° and -80 to -46° respectively, significantly different to prospective winners: *p* <

0.0001; Fig. 11C). The mean turn angles were significantly different to each other after 34 ms ($p = 0.008$). Disregarding angular direction, losers on average made turns almost twice as large as winners, but this was not quite statistically significant ($p = 0.0501$). Taken together, the entire data set revealed a linear correlation when each individual´s turn angles were plotted before and after the fight ($r = 0.517$, $p < 0.0001$, $n = 60$; Fig. 11E and Tab. 2). When analysed separately, however, prospective and actual winners as well as prospective and actual losers showed no correlation (winners: $r = 0.112$, $p = 0.557$; losers: $r = 0.087$, $p = 0.647$, $n = 30$ each; Fig. 11E and Tab. 2). Stress, e.g. from an intervening fight, could influence the measurements and correlation of the turn angles. To check for influences of this fight, the initial response to the touch stimulus in a separate cohort of STIs was measured after isolation and in the same individuals again on the following day without staging a contest in between. This revealed a linear correlation for the negative turners but again not for positive turners (positive tuners: $r = 0.032$, $p = 0.892$; negative turners: $r = 0.446$, $p = 0.049$, $n = 20$ each; Fig. 11F and Tab. 2). To test for a predisposition to win or lose depending on the turning direction as a response to an agonistic stimulus, the turning responses in another cohort of STIs were measured and positive turners were subsequently matched against negative turners. Positive turners won almost all the fights (90%, significantly different to negative turners: $p < 0.0001$ and to 50%: $p = 0.0138$, $n = 20$). For an additional cohort of STIs the pre-fight turning responses were evaluated and the total time each individual spent engaging in a fight against a hyper-aggressive opponent before retreating was subsequently measured. On average, positive turners persisted significantly longer than negative turners (positive turners: mean persistence: 9,05 s, 95% CI: 7.18 to 10.92 s; negative turners: mean: 5.7 s, 95% CI: 3.94 to 7.47 s; $p = 0.0096$, $n = 20$ each). For individuals showing avoidance in response to the touch stimulus, the turn angle correlated positively with persistence, but this was not evident for positive turners (positive tuners: $r = -0.074$, $p = 0.756$; negative turners: $r = 0.598$, $p = 0.005$, $n = 20$ each; Fig. 11G and Tab. 2). Furthermore, as given in Tab. 2, pooled data of positive and negative turners revealed that individual turning responses correlated with motility measured as the total distance moved in 3 min ($r = 0.396$, $p = 0.011$, $n = 40$) and also with individual aggressiveness in a subsequent fight against a hyper-aggressive opponent ($r = 0.363$, $p = 0.021$, $n = 40$). However, separate analyses revealed only a correlation of turn angle and motility for positive turners ($r = 0.570$, $p = 0.009$, $n = 20$).

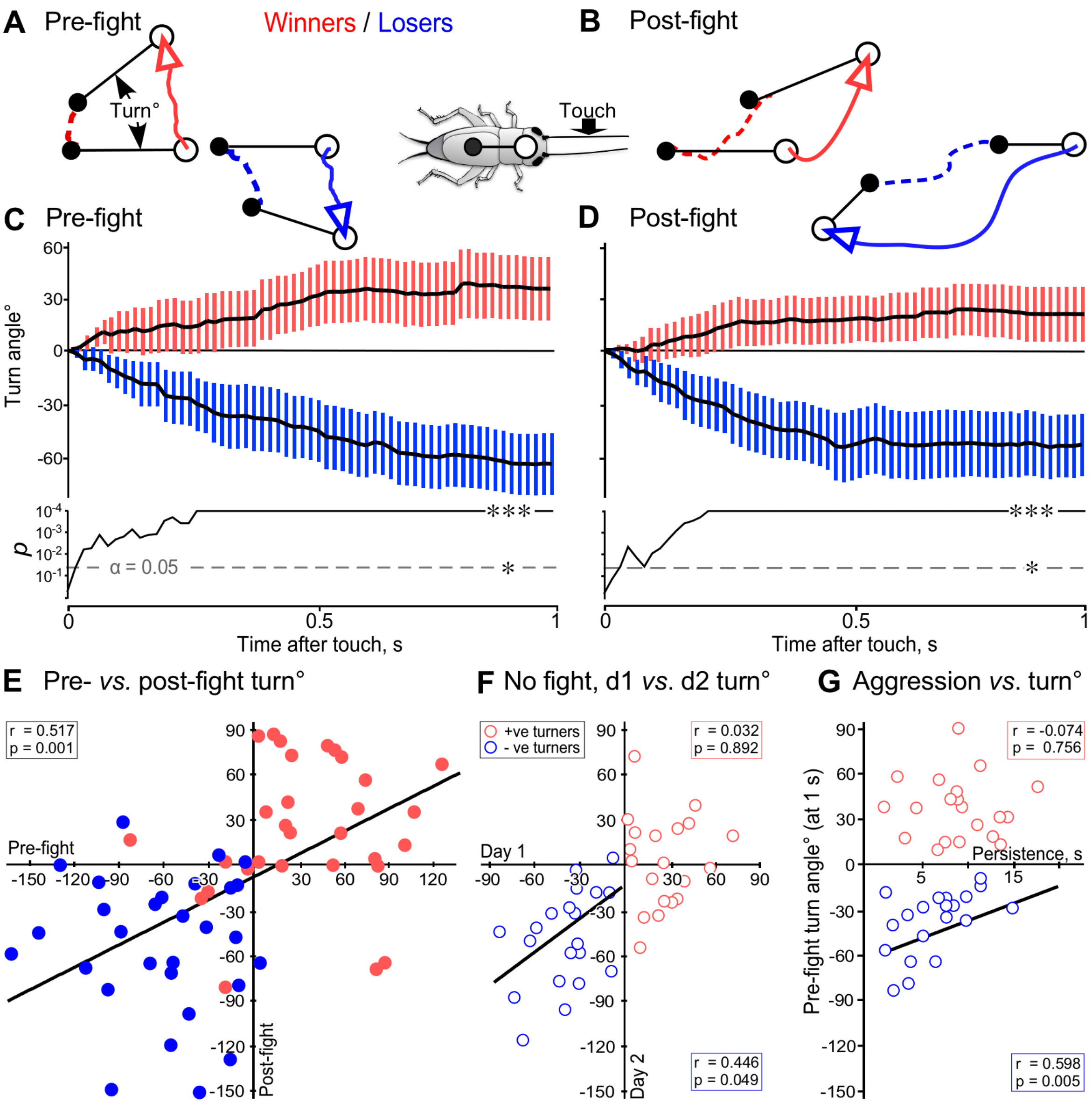


Fig. 11: Turning responses and aggression in short term isolates (STI). A Original tracks of responses to the touch stimulus in a prospective winner and loser (red, respectively blue arrows; black lines: body orientation; full circle: body; open circle: head). **B** Same animals as in A 1 h after their first fight. **C** Top graph: sequential plots of mean turn angles (black lines) for every frame with 95% CI (bars, red: prospective winners; blue: prospective losers; n = 30 each). Bottom graph: significance of difference p between prospective winners and losers, * $p < 0.05$, *** $p < 0.001$. **D** As for C 1 h after first fight. **E** Plots of pre-fight *versus* post-fight turning responses for data depicted in C and D. **F** Plots of turn angles on two consecutive days without an interposing fight. Red and blue open circles indicate individuals showing positive (+ve), respectively negative (-ve) turns at the first trial, n = 20 each. **G** Plots of fight duration against hyper-aggressive opponents *versus* pre-fight turn angle for positive and negative turners (red and blue open circles respectively, n = 20 each). (Balsam & Stevenson, 2021).

Tab. 2: Correlations between measured variables for selected test groups of individuals. ***r*** gives Pearson´s correlation coefficient and ***p*** the significance of difference. Statistically significant correlations are indicated in boldface. (Balsam & Stevenson, 2021).

X-axis	Y-axis	Test group	*r*	*p*
Turn angle pre-fight	Turn angle post-fight	STI winners & losers	**0.517**	**< 0.001**
		STI winners	0.112	0.557
		STI losers	0.087	0.647
Turn angle day 1	Turn angle day 2	STI +ve & -ve turners	**0.661**	**< 0.001**
		STI +ve turners	0.032	0.892
		STI -ve turners	**0.446**	**0.049**
Turn angle	Aggression	STI +ve & -ve turners	**0.472**	**0.002**
		STI +ve turners	-0.074	0.756
		STI -ve turners	**0.598**	**0.005**
Turn angle	Motility	STI +ve & -ve turners	**0.396**	**0.011**
		STI +ve turners	**0.570**	**0.009**
		STI -ve turners	0.106	0.658
Motility	Aggression	STI +ve & -ve turners	**0.363**	**0.021**
		STI +ve turners	0.281	0.230
		STI -ve turners	0.252	0.283
Turn angle 1 d post-6 fights	Turn angle 6 d post-6 fights	LTI$^{w/o}$ winners & losers	**0.788**	**< 0.001**
		LTI$^{w/o}$ winners	0.211	0.371
		LTI$^{w/o}$ losers	**0.762**	**< 0.001**

To check if a longer isolation period equalises the inter-individual differences in turning responses found in STI before fighting, an additional group was also taken as fully mature adults from the age- and sex-mixed breeding colony, but isolated for 7 days (Fig. 12).

No significant differences in their responses to the touch stimulus compared to the STI group isolated for 48 h were found (Fig. 12A). The prospective 7 d-isolated winners turned on average towards the stimulus and prospective losers away from it (pr- winners: mean: +24°, 95% CI: 7 to 41°; pr-losers: mean: -59°, 95% CI: -90 to -28°; significantly different to each other: $p < 0.0001$; Bonferroni correction to alpha = 0.0167; $n = 15$ each; Fig. 12B). After fighting the mean turn angles did not change significantly (winners: mean: +16°, 95%

CI: -2 to 34°; losers: mean: -43°, 95% CI: -65 to -20°; winners and losers compared to prospective winners and losers: *p* = 0.6041, respectively *p* = 0.2570) and were significantly different to each other (*p* = 0.0002; Bonferroni correction to alpha = 0.0167). Furthermore, the 7-day isolates were also not significantly different to the respective 48 h isolated groups (pr-winners: *p* = 0.4357, pr-losers: *p* = 0.7890, winners: *p* = 0.6189, losers: *p* = 0.3183).

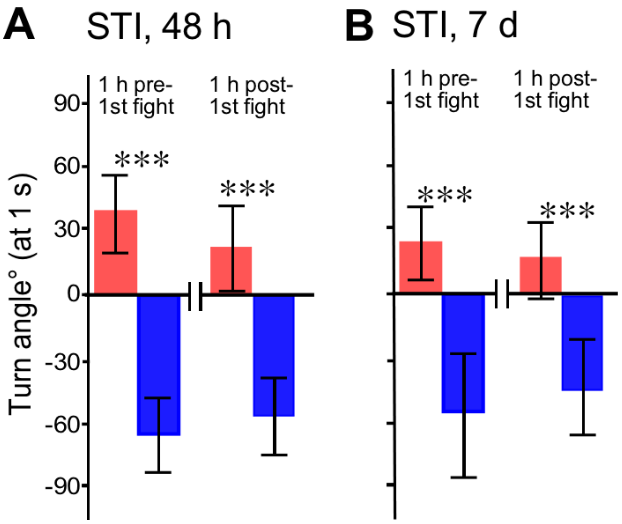


Fig. 12: Turn angles 1 s after stimulus for short term isolates of 48 h and 7 days. Adult male crickets isolated for **A** 48 h and **B** 7 d (means ± 95% CI; red bars: prospective winners and actual winners; blue bars: prospective losers and actual losers, *n* = 15 each). Asterisks indicate differences between groups (*** *p* < 0.00033; Bonferroni correction for 3 comparisons).

3.8.2 Long term isolates

Although the STI crickets were isolated for 48 h or even 7 d, they showed inter-individual differences in the response to an antennal touch before fighting. The question arose, where those differences came from and when they developed. To test, whether male nymphs also show a predisposition to approach or avoid a stimulus and also exhibit different responses after winning and losing, the same stimulus was applied to $LTI^{w/}$ and $LTI^{w/o}$ crickets after various experiences described below. The resulting turning responses are depicted as sequential plots in Fig. 13 (left side: $LTI^{w/}$, right side: $LTI^{w/o}$; *n* = 20 each), and in Figs. 14A and B as box-whisker plots 1 s after the touch stimulus.

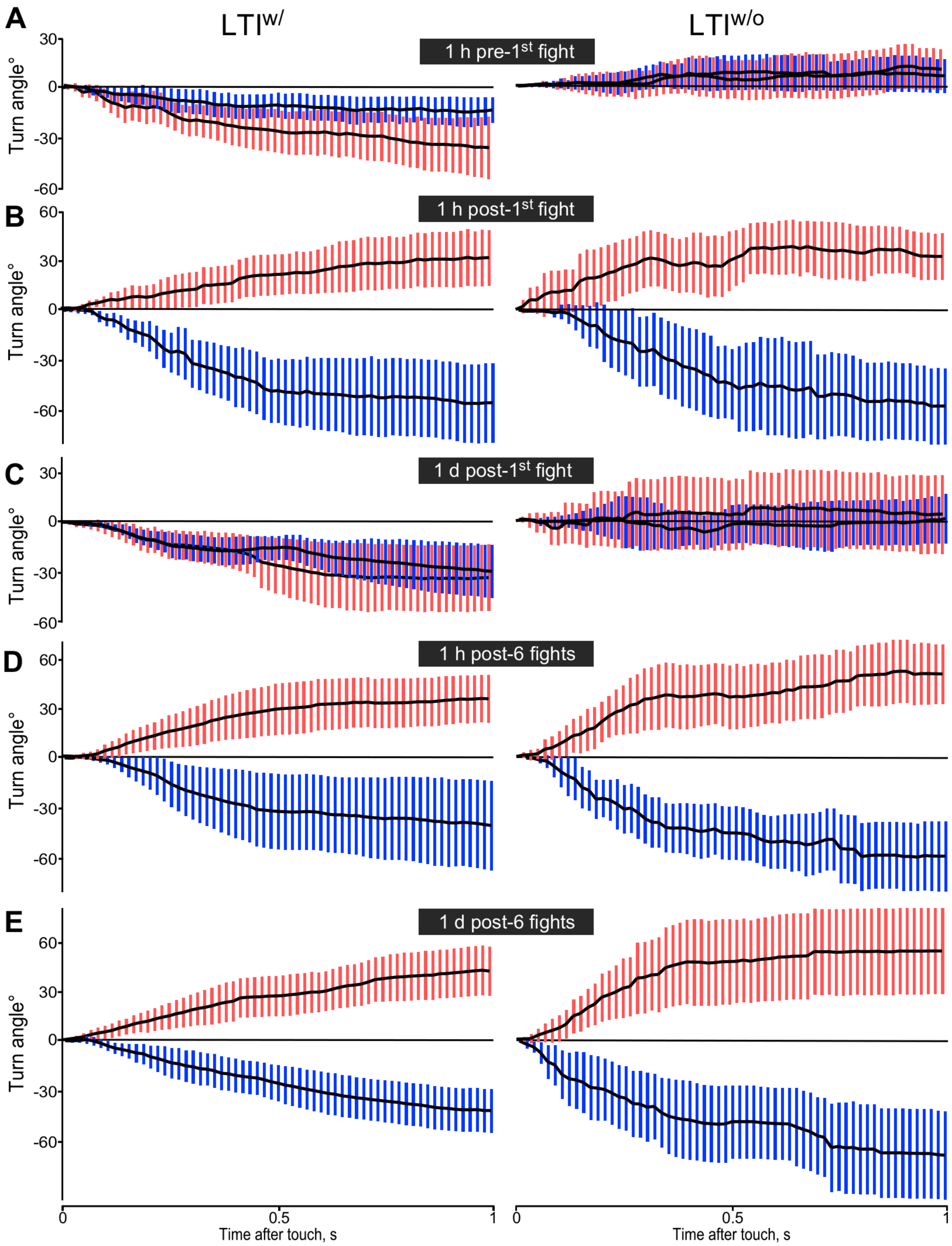


Fig. 13: Turning responses of long term isolates with and without prior contact to adult males (LTI$^{w/}$, LTI$^{w/o}$). Sequential plots of mean turn angles (black lines) for every frame with 95% CI. **A** Mature adults before fights (red and blue bars: prospective winners and losers). **B-E** Same animals as in A after various experiences: **B** 1 h after first fight, **C** 1 day later, **D** 1 h after 6 additional, consecutive fights, **E** 1 day after the 6 fights (*n* = 20 for each). (Balsam & Stevenson, 2021).

Before their first fight, nearly all LTI$^{w/}$ prospective winners and prospective losers showed negative turns in response to antennal stimulation, resulting in mean turn angles of -35° (95% CI: -53 to -17°) for the prospective winners and -19° (95% CI: -29 to -9°) for the prospective losers 1 s after the touch stimulus was applied (means significantly different to zero: both *p* = 0.0003, but not different to each other: *p* = 0.1134; Bonferroni correction to alpha = 0.0167; *n* = 20 each; Figs. 13A and 14A). Measured 1 h after the first fight, 80% of the now established winners turned towards the stimulus, whereas 90% of the losers turned away (winners: mean: 32°, 95% CI: 15 to 50°; losers: mean: -55°, 95% CI: -78 to -31°; difference significant: *p* < 0.0001; Figs. 13B and 14A). However, 24 h later, winners and losers again both avoided the stimulus (winners: mean: -33°, 95% CI: -53 to -13°; losers: mean: -28°, 95% CI: -43 to -13°; both significantly different to zero: *p* = 0.0012, *p* = 0.0005 respectively, but not to each other: *p* = 0.6655; Bonferroni correction to alpha = 0.0167; Figs. 13C and 14A). After that, the same animals were re-matched 6 times in succession in 30 min intervals to yield 6-fold winners and 6-fold losers. When analysed 1 h after these multiple winning, respectively losing experiences, winners again approached the applied stimulus and the losers avoided it (winners: mean: 36°, 95% CI: 21 to 52°; losers: mean: -39°, 95% CI: -65 to -13°; difference significant: *p* < 0.0001; Figs. 13D and 14A). In contrast to one day after a single fight, this difference between multiple winners and losers was still evident 24 h later (winners: mean: 41°, 95% CI: 27 to 56°; losers: mean: -44°, 95% CI: -58 to -30°; difference significant: *p* < 0.0001; Figs. 13E and 14A).

Contrasting to LTI$^{w/}$ adults, which had prior contact to adult males during nymphal development and initially turned away from the stimulus side, LTI$^{w/o}$ crickets showed no directional preference before their first interaction. After 1 s, prospective winners had turned on average +13° and prospective losers +8° (means; pr-winners: 95% CI: -3 to 28°; pr-losers: 95% CI: -5 to 20°; both not significantly different to zero: pr-winners *p* = 0.1039, pr-losers *p* = 0.2101; statistically different to LTI$^{w/}$ prospective winners: *p* = 0.0002; to LTI$^{w/}$ prospective losers: *p* = 0.0013; means not significantly different to each other: *p* = 0.6024; *n* = 20 each; Bonferroni correction to alpha = 0.0125; Figs. 13A right side and 14B).

LTI$^{w/o}$ made comparatively small turns, nonetheless individuals, that turned > 10° towards or away from the stimulus were checked for their predisposition to win, respectively lose a subsequent contest. This revealed that neither positive nor negative turners > 10° in magnitude showed a higher chance to win or to lose (+ve turners > 10°: *n* = 18 of which

10 won; -ve turners > 10°: *n* = 9, of which 4 won; *p* = 0.6950). After the fight, LTI$^{w/o}$ winners approached the stimulus, whereas LTI$^{w/o}$ losers avoided it (winners: mean: 33°, 95% CI: 18 to 47°; losers: mean: -60°, 95% CI: -83 to -36°; difference between groups significant: *p* < 0.0001; Figs. 13B and 14B). On the following day, this difference was no longer evident and the focal animals again showed on average no clear directional preferences (winners: mean: 4°, 95% CI: -19 to 27°; losers: mean: 0.5°, 95% CI: -16 to 17°; both not significantly different to zero: winners: *p* = 0.7180, losers: *p* = 0.9508; losers significantly different to the respective LTI$^{w/}$ cohorts at this time: losers: *p* = 0.0124, winners: *p* = 0.0146; means not significantly different to each other: *p* = 0.803; Bonferroni correction for alpha = 0.0167; Figs. 13C and 14B). Again, after 6 fights LTI$^{w/o}$ winners turned towards the stimulus and the losers away from it (winners: mean: 54°, 95% CI: 35 to 74°; losers: mean: -61°, 95% CI: -83 to -39°; difference significant: *p* < 0.0001; Figs. 13D and 14B). This time, however, the differences were still evident 6 days later (winners: mean: 33°, 95% CI: 12 to 55°; losers: mean: -57°, 95% CI: -77 to 37°; difference significant: *p* < 0.0001; Fig. 14B).

To exclude, that the presence of adult females during nymphal development had an influence on turning behaviour of adult LTI males, a separate cohort of LTI$^{w/f}$ was tested (Fig. 14C). As already demonstrated (Fig. 10), adult females neither attack nymphs in the crowded conditions nor did they have any influence on adult motility and aggression of the LTI focal animals. Furthermore, the turning responses shown by LTI$^{w/f}$ adults were similar to those exhibited by LTI$^{w/o}$ crickets. Before fighting the prospective winners and losers also exhibited no directional preference (pr-winners: mean: -1°, 95% CI: -15 to 13°; pr-losers: mean: -7°, 95% CI: -25 to 12°; both not significantly different to zero: pr-winners: *p* = 0.8492, pr-losers: *p* = 0.3840; not significantly different to the respective LTI$^{w/o}$ cohorts at this time: pr-winners: *p* = 0.1970; losers: *p* = 0.1725; not significantly different to each other: *p* = 0.6139; *n* = 15 each; Bonferroni correction to alpha = 0.0167). After 1 fight, winners showed approach and losers avoidance behaviour (winners: mean: 33°, 95% CI: 10 to 57°; losers: mean: -47°, 95% CI: -65 to -28°; difference significant: *p* < 0.0001). This difference was no longer evident on the following day (winners: mean: 5°, 95% CI: -12 to 27°; losers: mean: -2°, 95% CI: -17 to 12°; both not significantly different to zero: winners: *p* = 0.7450, losers: *p* = 0.4512 and to each other: p = 0.3845).

A plot of each individual's turn angles 1 day after 6 fights *versus* 6 days after the contests revealed a linear correlation for losers, but not for winners (winners: *r* = 0.211, *p* = 0.371;

losers: *r* = 0.762, *p* = 0.001, *n* = 20 each; Fig. 14D and Tab. 2). To further test for consistency, the turn angles after 1 s of 5 randomly selected winners and losers of the 6 consecutive fights were plotted at 4 different times after the contests. This revealed consistency over time in the angular magnitude for losers, but not for winners (Fig. 14E).

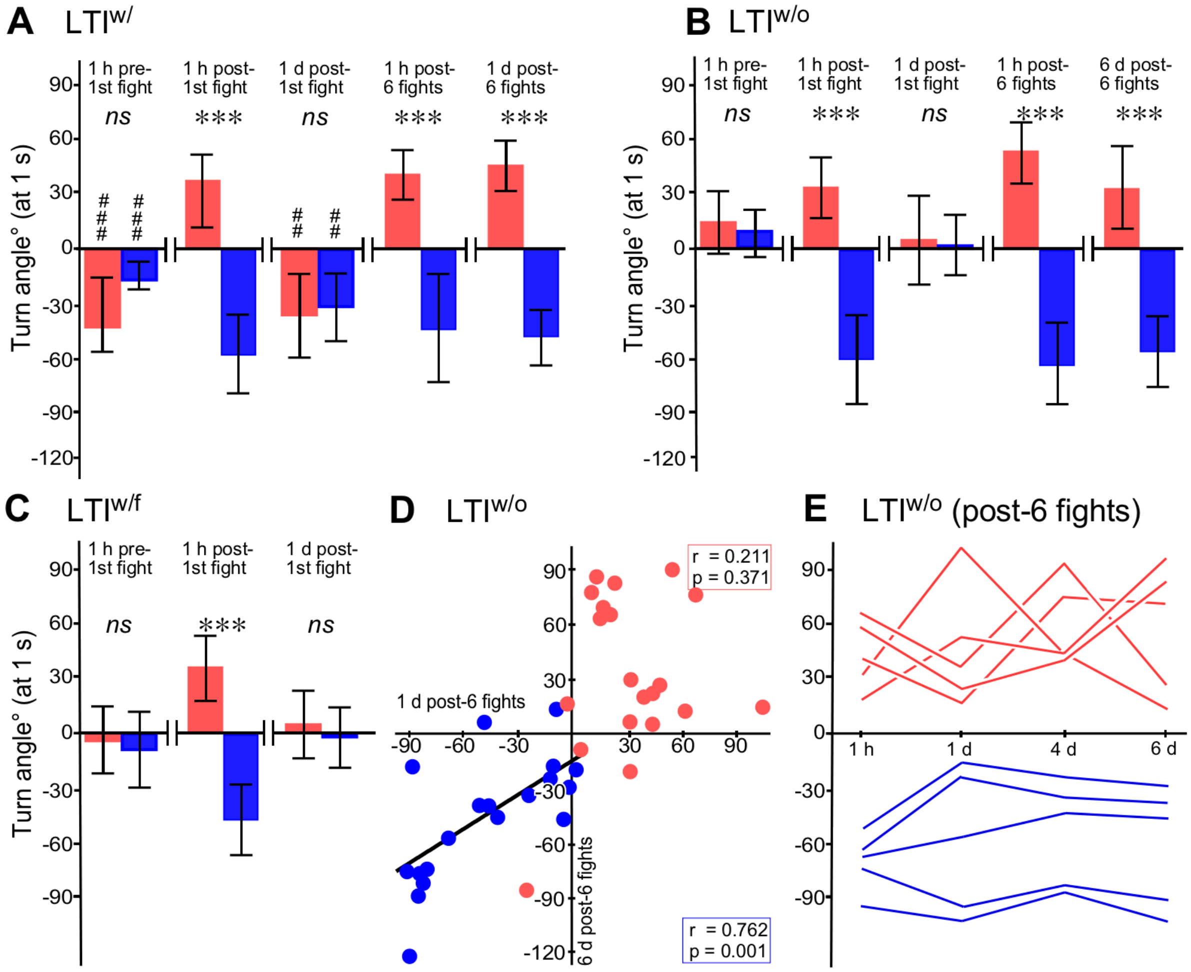


Fig. 14: Turn angles for long term isolates 1 s after stimulus. Turn angles 1 s after the touch. **A** $\mathrm{LTI}^{w/}$ and **B** $\mathrm{LTI}^{w/o}$ **C** $\mathrm{LTI}^{w/f}$ crickets (means ± 95% CI; red bars: prospective winners and actual winners; blue bars: prospective losers and actual losers). Asterisks indicate differences between groups and hashtags differences to zero (*ns* not significant, A and C: *** $p < 0.0001$; ## $p < 0.0033$ ### $p < 0.00033$; B: *** $p < 0.0001$; Bonferroni correction explicitly mentioned in results). Note: the last bar in B is for data 6 days after 6 fights, in contrast to A which depicts 1 d after the 6 fights. **D** Plots of individual turn angles on day 1 *versus* day 6 after 6 fights for $\mathrm{LTI}^{w/o}$ winners and losers (red and blue circles respectively, $n = 20$ each). **E** Sequential plots of mean turn angles at different time points over a period of 6 days following the 6 consecutive fights for randomly selected winners and losers (red and blue lines respectively, $n = 5$ each). (Adapted from Balsam & Stevenson, 2021).

3.9 Effects of neuromodulatory drugs on behavioural elements in STI and LTI crickets

3.9.1 Aggression

As shown for adult male crickets, winners of a previous interaction were more likely to persist longer in fights and escalate on higher levels than losers, which were subsequently submissive and avoided interactions with conspecifics (Fig. 7). Crickets implement the decision to persist in fighting or retreat from the interaction by modulating the behavioural threshold to flee (Rillich & Stevenson, 2015; 2017; 2018; Stevenson *et al*., 2000; 2005; Stevenson & Rillich, 2012; 2015; 2016; 2019). This threshold is raised by rewarding experiences (e. g. resource possession, winning and motor activity) and lowered by losing a previous encounter. It is known that biogenic amines (e.g. octopamine or serotonin) and nitric oxide, released in response to social experience, play a major role in the decision to approach or avoid a conspecific and whether or not to persist in fighting (Rillich & Stevenson, 2015; 2017; 2018; Stevenson *et al*., 2000; 2005; Stevenson & Rillich, 2015; 2016). Confirming these earlier findings and to check for the most effective, but not detrimental drug dosages, STI crickets were sorted in positive and negative turners after a single, antennal touch with an adult male´s antenna and subsequently given the treatments listed below (Tab. 3). After 1 h the focal animals were matched against a hyper-aggressive opponent and their persistence was measured to the nearest second (post-drug; mean ± 95% CI and the significant differences compared to the vehicle groups are listed in Tab. 3 and shown in Fig. 15). All vehicle treated groups persisted between 6 and 10 s in fights with a hyper-aggressive opponent, which is in the typical range for untreated, adult STI males. However, when injected with the OAR-antagonist epinastine, the focal animals´ aggressive persistence was lowered (statistically significant before correction). But, when treated with OAR-agonist CDM, adult males on average fought 16 to 18 s, which is significantly longer compared to the vehicle group. Similarly, when blocking the NOS with L-NAME, crickets engaged in significantly longer fights, but retreated sooner with the NO-Donor SNAP. Together these data confirm, that OA promotes aggression and aggressive motivation, whereas NO lowers the threshold to flee. Compared to this, serotonergic antagonists and agonists both had no obvious effects on subsequent aggression in a single fight against conspecifics (see also Rillich & Stevenson, 2018). No detrimental effects of the used drug dosages (see Tab. 1) or vehicle were observed.

Tab. 3: Influence of various drugs on aggressive persistence of short term isolated crickets. Mean persistence (± 95% CI) of focal animals (previously sorted in positive and negative turners) in fights against hyper-aggressive opponents after injection (20 µl) of either vehicle/control or active drug. ***p*** gives the significance of difference between treated and vehicle/control group. Statistically significant comparisons are indicated in boldface (Bonferroni correction to alpha = 0.025; *n* = 15 each).

Persistence (in seconds)							
Treatment		**positive turners**		**negative turners**		**difference to vehicle *p***	
		mean	95% CI	mean	95% CI	+ve	-ve
Octopaminergics	Vehicle	8.67	5.75 - 11.58	6.73	4.56 - 8.90		
	Epinastine	5.20	3.18 - 7.22	4.00	2.48 - 5.52	0.0451	0.0354
	CDM	18.47	14.67 - 22.26	16.80	13 - 20.6	**0.0001**	**< 0.0001**
Nitridergics	D-NAME	8.67	4.9 - 12.44	8.27	5.27 - 11.26		
	L-NAME	15.47	12.64 - 18.3	15.07	12.59 - 17.55	**0.0045**	**0.0008**
	SNAP	4.13	2.54 - 5.73	4.53	3.04 - 6.03	**0.0246**	**0.0236**
Serotonergics	Vehicle	9.33	5.81 - 12.85	6.87	3.79 - 9.94		
Antagonists	Methiothepin	9.87	5.93 - 13.8	8.2	4.84 - 11.56	0.8301	0.5354
	Ketanserin	9.2	5.81 - 12.59	8.67	5.45 - 11.88	0.9537	0.3928
	WAY	9.6	6.85 - 12.35	9.6	7.12 - 12.08	0.899	0.1492
Agonists	Vehicle	9.33	5.81 - 12.85	6.87	3.79 - 9.94		
	5HTP	8.33	4.77 - 11.9	7.8	4.52 - 11.08	0.6717	0.6598
	Fluoxetine	5.73	4.23 - 7.24	5.07	3.41 - 6.72	0.0533	0.2783
	DOI	10.2	7.25 - 13.15	8.47	5.39 - 11.54	0.6889	0.4367
	8-OH-DPAT	10.73	7.76 - 13.71	8.67	5.93 - 11.4	0.5202	0.3562

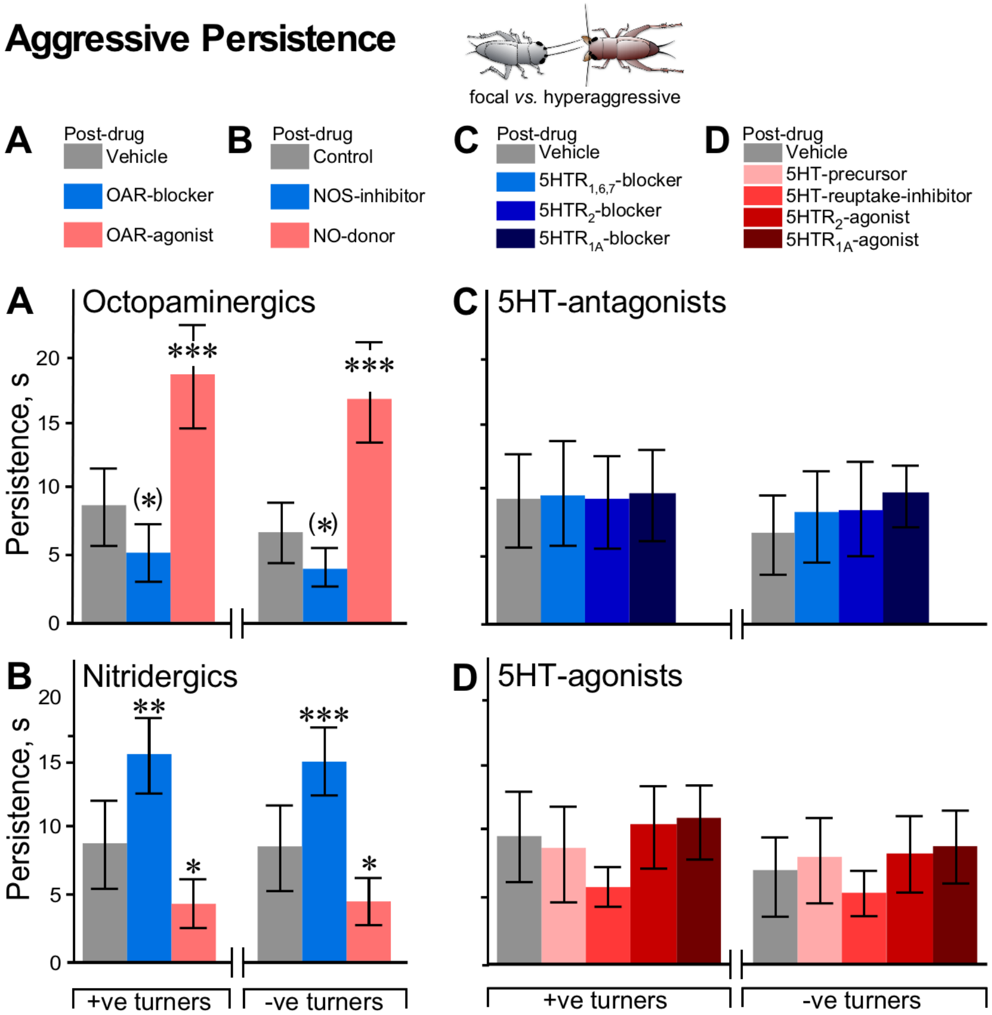


Fig. 15: Influence of various drugs on aggressive persistence of short term isolated crickets. Persistence (mean ± 95% CI; *n* = 15 each) of focal crickets in fights against a hyper-aggressive opponent 1 h after they received either vehicle (= control, grey bars) or **A** octopaminergic drugs (blue: OAR-antagonist epinastine, red: OAR-agonist chlordimeform); **B** nitridergic drugs (blue: NOS-inhibitor L-NAME, red: NO-donor SNAP); **C** 5HT-antagonists (light blue: $5HTR_{1,6,7}$-blocker methiothepin, blue: $5HTR_2$-blocker ketanserin, dark blue: $5HTR_{1A}$-blocker WAY); **D** 5HT-agonists (coral: 5HT-precursor 5HTP, light red: 5HT-reuptake-inhibitor fluoxetine, red: $5HTR_2$-agonist DOI, dark red: $5HTR_{1A}$-agonist 8-OH-DPAT). Asterisks indicate differences between treated and corresponding control group (* $p < 0.025$, ** $p < 0.005$, *** $p < 0.0005$; Bonferroni correction for 2 comparisons; (*) = significant before correction).

3.9.2 General motility

To exclude detrimental drug-effects on general motility, the total distance moved of each individual STI cricket was measured before and after treatment with either vehicle or drug (pre-drug and post-drug, respectively; mean ± 95% CI and the significant differences compared to pre-drug are listed in Tabs. 4 and 5 and shown in Fig. 16).

Video-tracking analysis of the 3 min interval revealed that the mean moving distances of all treated groups (30 to 90 cm in 3 min, see Tabs. 4 and 5) were in the range of movement of untreated STI crickets (see Fig. 6C and Rose *et al*., 2017a for reference in 5 min walking intervals) and there were no significant differences before and after treatment with octopaminergic, nitridergic and serotonergic drugs or the corresponding vehicle with one exception. Focal animals injected with the OAR-antagonist epinastine walked shorter distances during the observation period compared to the vehicle groups. This differences were just significant and equally in both positive and negative turners ($p < 0.0111$, $p < 0.0360$ respectively). However, these animals showed no signs of detrimental effects from the drug injection on their general behaviour and all of the treated animals were monitored and survived at least 24 h after the treatments.

Tab. 4: Influence of various drugs on motility of STI positive turners. Total distance moved (mean and 95% CI) of adult male crickets pre- and 1 h post-treatment with 20 µl of either vehicle/control or active drug. ***p*** gives the significance of difference between groups before and after treatment. Statistically significant comparisons are indicated in boldface (*n* = 12 each).

Motility (distance, in cm)	**positive turners**					
Treatment		**pre-drug**		**post-drug**		**difference to pre-drug *p***
		mean	95% CI	mean	95% CI	
Octopaminergics	Vehicle	59.51	33.1 - 85.92	65.9	39.21 - 92.58	0.6508
	Epinastine	67.19	47.16 - 87.22	36.7	23.35 - 50.06	**0.0111**
	CDM	69.76	47.83 - 91.69	61.77	44.51 - 79.02	0.4850
Nitridergics	D-NAME	52.41	30.84 - 73.98	58.6	36.02 - 81.18	0.7037
	L-NAME	52.94	45.59 - 60.29	64.87	48.95 - 80.79	0.1475
	SNAP	44.3	30.89 - 57.71	50.74	39.11 - 62.37	0.4287
Serotonergics	Vehicle	51.3	36.82 - 65.77	55.78	36.3 - 75.27	0.5978
Antagonists	Methiothepin	63.04	46.7 - 79.37	80.73	49.33 - 112.1	0.2751
	Ketanserin	55	34.9 - 75.11	71.69	54.03 - 89.35	0.1676
	WAY	81.22	48.27 - 114.2	97.56	77.83 - 117.3	0.2924
Agonists	Vehicle	58.96	50.68 - 67.24	53.74	34.54 - 72.94	0.5886
	5HTP	54.65	33.16 - 76.14	75.02	44.89 - 105.1	0.2463
	Fluoxetine	45.35	36.84 - 53.86	52.2	36.34 - 68.06	0.3850
	DOI	77.05	50.15 - 104	86.64	62.25 - 111	0.5843
	8-OH-DPAT	80.7	59.65 - 101.7	79.72	64.82 - 94.62	0.9123

Tab. 5: Influence of various drugs on motility of STI negative turners. Total distance moved (mean and 95% CI) of adult male crickets pre- and 1 h post-treatment with 20 µl of either vehicle/control or active drug. ***p*** gives the significance of difference between groups before and after treatment. Statistically significant comparisons are indicated in boldface (*n* = 12 each).

Motility (distance, in cm)	**negative turners**					
Treatment		**pre-drug**		**post-drug**		**difference to**
		mean	95% CI	mean	95% CI	**pre-drug *p***
Octopaminergics	Vehicle	59.57	28.27 - 90.87	59.7	40.01 - 79.4	0.9949
	Epinastine	73.46	52.72 - 94.19	45.61	26.16 - 65.06	**0.0360**
	CDM	55.94	35.61 - 76.27	65.06	44.79 - 85.33	0.3892
Nitridergics	D-NAME	31.79	21.53 - 42.05	34.8	15.9 - 53.71	0.6954
	L-NAME	49.09	25.76 - 72.41	67.44	43.93 - 90.94	0.2704
	SNAP	43.16	31.12 - 55.19	43.16	34.43 - 75.8	0.3540
Serotonergics	Vehicle	52.58	34.3 - 70.87	63.83	36.33 - 91.33	0.2649
Antagonists	Methiothepin	55.56	33.75 - 77.37	72.45	55.92 - 88.98	0.0920
	Ketanserin	39.35	22.52 - 56.18	63.89	39.09 - 88.69	0.1038
	WAY	89.23	69.26 - 109.2	89.84	48.55 - 131.1	0.9768
Agonists	Vehicle	42.01	18.88 - 65.13	40.38	27.22 - 53.55	0.9053
	5HTP	31.73	18.67 - 44.8	56.16	43.21 - 69.11	**0.0021**
	Fluoxetine	36.18	23.43 - 48.93	49.89	30.68 - 69.1	0.2562
	DOI	83.72	50.26 - 117.2	56.71	37.83 - 75.6	0.1142
	8-OH-DPAT	78.54	50.96 - 106.1	78.75	53.17 - 104.3	0.9907

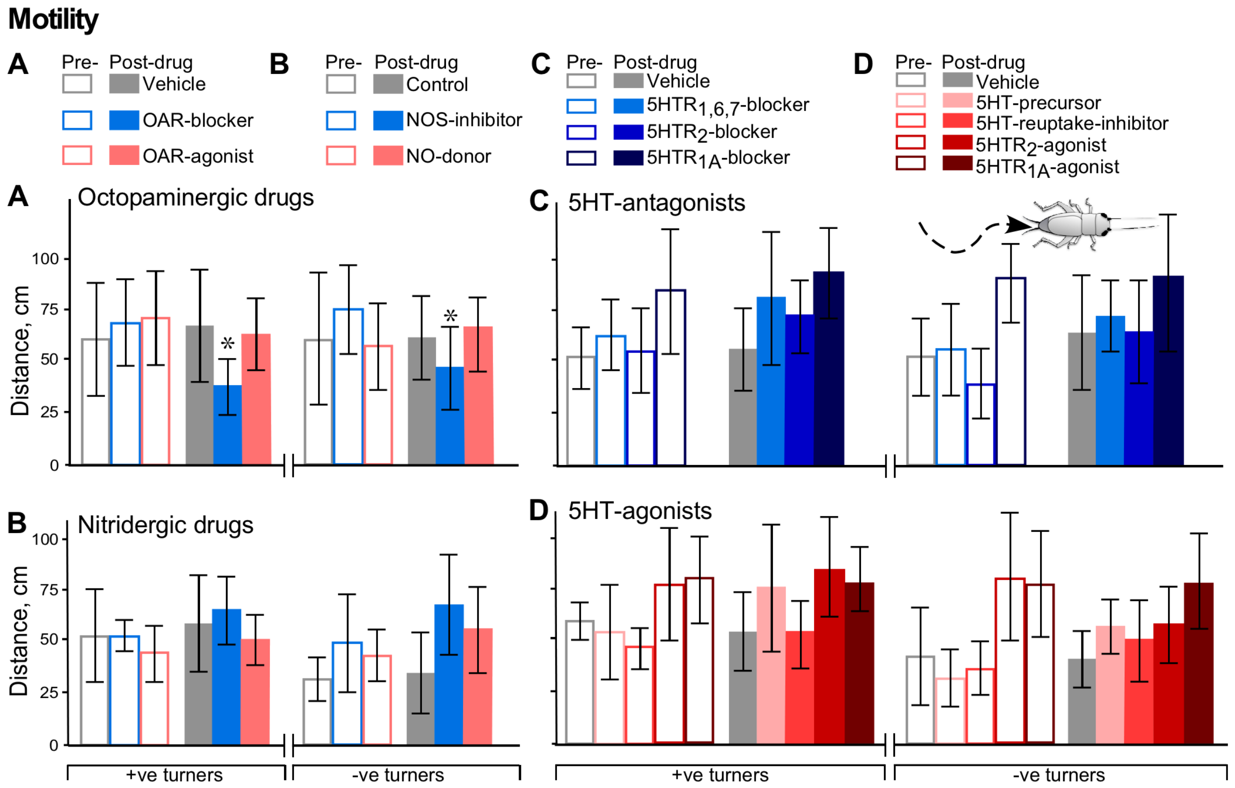


Fig. 16: Influence of various drugs on motility of short term isolated crickets. Total distance moved (mean ± 95% CI; $n = 15$ each) of adult male crickets before drug- application (outlined bars: pre-drug) and the same animals 1 h after they received either vehicle (grey bars) or **A** octopaminergic drugs (blue: OAR-antagonist epinastine, red: OAR-agonist chlordimeform); **B** nitridergic drugs (blue: NOS-inhibitor L-NAME, red: NO-donor SNAP); **C** 5HT-antagonists (light blue: $5HTR_{1,6,7}$-blocker methiothepin, blue: $5HTR_2$-blocker ketanserin, dark blue: $5HTR_{1A}$-blocker WAY); **D** 5HT-agonists (coral: 5HT-precursor 5HTP, light red: 5HT-reuptake-inhibitor fluoxetine, red: $5HTR_2$-agonist DOI, dark red: $5HTR_{1A}$-agonist 8-OH-DPAT). Asterisks indicate differences between pre-drug and corresponding treated post-drug group (* $p < 0.05$).

3.9.3 Turning responses

3.9.3.1 Influence of octopaminergic drugs

To further investigate the influences of biogenic amines and nitric oxide on the decision to approach or avoid a conspecific, turning responses, as a sensitive metric for aggressiveness, were evaluated before and after the treatment without an intervening fight. For this adult male crickets were sorted in positive and negative turners after a single, antennal touch with an adult male´s antenna and subsequently assigned randomly to a treatment group with either vehicle, various antagonists or agonists (for dosages see Tab.1). First, the influence of octopaminergic drugs on STI and $LTI^{w/o}$ crickets was evaluated (*n* = 15 each; Bonferroni correction to alpha = 0.025 for all comparisons; Fig. 17). Before treatment, in the later assigned vehicle group, positive turners had turned +28° and negative turners -39° 1 s after the touch was applied (means, 95% CI: 19 to 38° and -53 to -26° respectively; Fig. 17A). Similarly, focal animals subsequently assigned to treatment with epinastine had turned on average +34° (95% CI: 20 to 49°) for positive and -46° (95% CI: -57 to -35°) for negative turners and test animals assigned to treatment with CDM +41° for positive and -50° for negative responders (means, 95% CI: 26 to 55° and -64 to -36°). Animals that received the vehicle exhibited no change in turning direction after treatment (1 h post-drug; Fig. 17B) and were not significantly different to the corresponding pre-treatment group (+ve turners: mean: 21°, 95% CI: -5 to 48°; *p* = 0.6174; -ve turners: mean: 41°, 95% CI: -55 to -27°; *p* = 0.8527). However, adult male crickets that were predisposed to approached the stimulus and received epinastine subsequently showed avoidance (mean: -49°, 95% CI: -64 to -34°; significantly different to pre-drug: *p* < 0.0001). Negative turners treated with epinastine showed no change in responses compared to their pre-drug performance (mean: -46°, 95% CI: -57 to -35°; *p* = 0.9462). After injection of CDM positive turners still approached the stimulus (mean: 24°, 95% CI: 3 to 45°; *p* = 0.1806), but crickets that were predisposed to avoid the stimulus changed to approach after treatment (mean: 34°, 95% CI: 11 to 57°; *p* < 0.0001). Additionally, the post-drug turning responses were evaluated disregarding their direction to check whether the drugs also had an influence on turning magnitude (Fig. 17C). Although drugs altered the direction of turns in some cases, none of the treatments changed the magnitude of turns, neither for positive turners (vehicle: mean: 44°, 95% CI: 32 to 55°; *p* = 0.0589; epinastine: mean: 49°, 95% CI: 34 to 64°; *p* = 0.0927; CDM: mean: 38°, 95% CI: 25 to 51°; *p* = 0.7622) nor for negative turners (vehicle: mean: 43°, 95% CI: 32 to 54°; *p* = 0.6453; epinastine: mean: 46°, 95% CI: 35 to 57°; *p* = 0.9462; CDM: mean: 46°, 95% CI: 31 to 60°; *p* = 0.7046) compared to their respective pre-drug magnitude. Although $\text{LTI}^{w/o}$ males showed no directional preference when retrospectively sorted in prospective winners and losers (Figs. 13A right side, 14B), they could be sorted in initially positive or negative turners, but they exhibited on average smaller turn angles compared to STI males before treatment. In the later assigned vehicle group positive turners had turned +6° and negative turners -28° 1 s after the touch was applied (means, 95% CI: 0.7 to 12° and -41 to -16° respectively; Fig. 17D). The test animals subsequently assigned to epinastine treatment had turned +17° for positive and -25° for negative turners (means, 95% CI: 10 to 23° and -40 to -9° respectively) and the group assigned to CDM +10° for positive and -27° for negative responders (means, 95% CI: 3 to 17° and -43 to -10° respectively). Animals that received the vehicle neither changed the average direction (+ve turners: mean: 9°, 95% CI: -3 to 20°; *p* = 0.7238; -ve turners: mean: -20°, 95% CI: -32 to -8°; *p* = 0.2833; Fig. 17E) nor the turning magnitude after treatment compared to the corresponding pre-treatment group (+ve turners: mean: 15°, 95% CI: 6 to 24°; *p* = 0.0798; -ve turners: mean: 21°, 95% CI: 10 to 33°; *p* = 0.4498; Fig. 17F). Contrasting this, 1 h after receiving epinastine focal animals that initially approached the stimulus now avoided it (mean: -40°, 95% CI: -68 to -13°; *p* = 0.0005) and the resulting turning reactions were significantly larger in magnitude (mean: 51°, 95% CI: 31 to 72°; *p* = 0.0034). Crickets that turned away from the stimulus before treatment showed no change in direction or magnitude of turns after injection with epinastine (turn angle: mean: -36°, 95% CI: -61 to -11°; *p* = 0.4157; magnitude: mean: 36°, 95% CI: 11 to 61°; *p* = 0.4090). After receiving CDM positive turners did not change the direction, but exhibited significantly larger turns in magnitude, disregarding angular direction (turn angle: mean: 19°, 95% CI: -4 to 40°; *p* = 0.4485; magnitude: mean: 38°, 95% CI: 26 to 49°; *p* = 0.0006) and negative turners subsequently approached the stimulus, but with no change in magnitude (turn angle: mean: 14°, 95% CI: -10 to 38°; *p* = 0.0053; magnitude: mean: 32°, 95% CI: 14 to 50°; *p* = 0.6855).

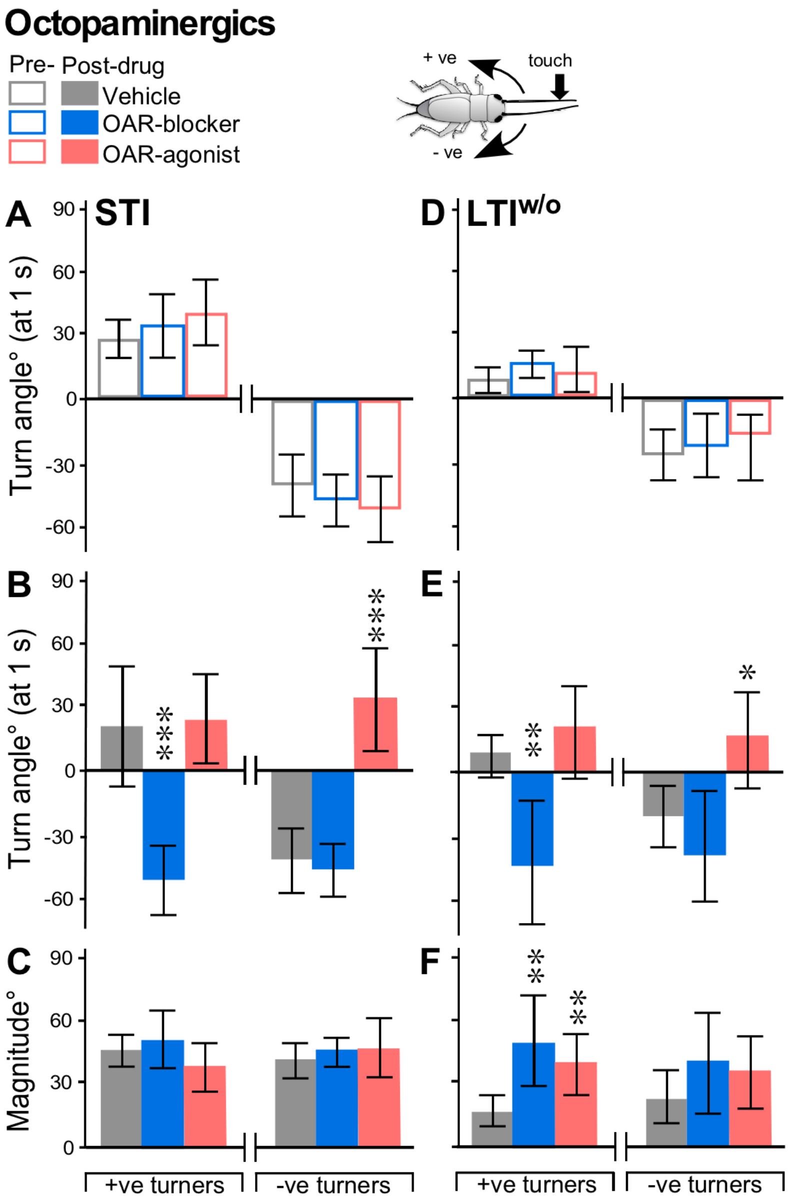


Fig. 17: Influence of octopaminergic drugs on turning responses of short and long term isolated crickets. Turn angles 1 s after the touch (mean ± 95% CI; $n = 15$ each). **A** Short term isolated positive and negative turners before treatment (STI; +ve, -ve respectively; outlined bars: pre-drug = control). **B** Same animals as in A after treatment (filled bars: 1 h post-drug; grey: vehicle, blue: OAR-antagonist epinastine, red: OAR-agonist chlordimeform). **C** Mean magnitude (± 95% CI) of turns 1 h after treatment. **D-F** As in A-C, but for long term isolates without any prior contact to adults during development ($LTI^{w/o}$). Asterisks indicate differences between post-drug groups and the respective pre-drug controls (** $p < 0.005$, *** $p < 0.0005$; Bonferroni correction for 2 comparisons).

3.9.3.2 Influence of nitridergic drugs

To examine the influence of nitridergic drugs on turning responses of STI crickets the same experimental design as for octopaminergic drugs was applied. D-NAME, an inactive isomer, was used as a control, L-NAME was applied to block NOS (antagonist) and SNAP as an NO-donor (agonist, *n* = 15 each, Bonferroni correction to alpha = 0.025 for all comparisons; Fig. 18). Average turn angles 1 s after the touch and turning magnitude were evaluated before treatment and 1 h after drug injection.

When measured before treatment, the +ve test group assigned to receive D-NAME had turned +30° and the -ve turners -36° 1 s after the touch was applied (means, 95% CI: 14 to 45° and -53 to -19° respectively; Fig. 18A). Similarly, the focal animals subsequently assigned to treatment with L-NAME had turned on average +46° for positive and -35° for negative turners (means, 95% CI: 30 to 61° and -50 to -20° respectively) and the test crickets later assigned to SNAP injection +38° and -31° (means, 95% CI: 22 to 53° and -42 to -21°).

Animals that received D-NAME showed no statistically significant change 1 h after treatment compared to the respective pre-treatment group regarding the turn angle (+ve turners: mean: 14°, 95% CI: -11 to 38°; *p* = 0.1982; -ve turners: mean: -23°, 95% CI: -39 to -7°; *p* = 0.3368; Fig. 18B) and turning magnitude (+ve turners: mean: 35°, 95% CI: 18 to 51°; *p* = 0.5323; -ve turners: mean: 31°, 95% CI: 20 to 42°; *p* = 0.6363; Fig. 18C). After treatment with L-NAME positive turners exhibited no significant differences compared to their prior performance (turn angle: mean: 23°, 95% CI: -5 to 51°; *p* = 0.1269; magnitude: mean: 40°, 95% CI: 19 to 61°; *p* = 0.6600). However, animals that were predisposed to avoid the stimulus subsequently showed approach behaviour when treated with the NOS-blocker (mean: 4° 95% CI: -9 to 17°; *p* = 0.0004), but without a significant change in magnitude of turns (mean: 19°, 95% CI: 11 to 27°; *p* = 0.0490, insignificant after correction). Contrasting this, for both positive and negative turners SNAP influenced the general response to an antennal stimulus. Positive turners had turned on average +5° and negative turners -0.8° 1 s after the touch was applied (means, 95% CI: -0.2 to 11° and -7 to 7° respectively; $p^+ = 0.0004$; $p^- = 0.0002$), but only 3 out of 15 positive turners and 4 negative turners exhibited turns > 10° in magnitude after the treatment (mean magnitude: +ve: 6°, 95% CI: 1 to 11°; *p* = 0.0005; -ve: 7°, 95% CI: 2 to 12°; *p* = 0.0008), whereas after treatment with L-NAME for both +ve and -ve turners 12 out of 15 test animals exhibited turns larger than 10° in magnitude.

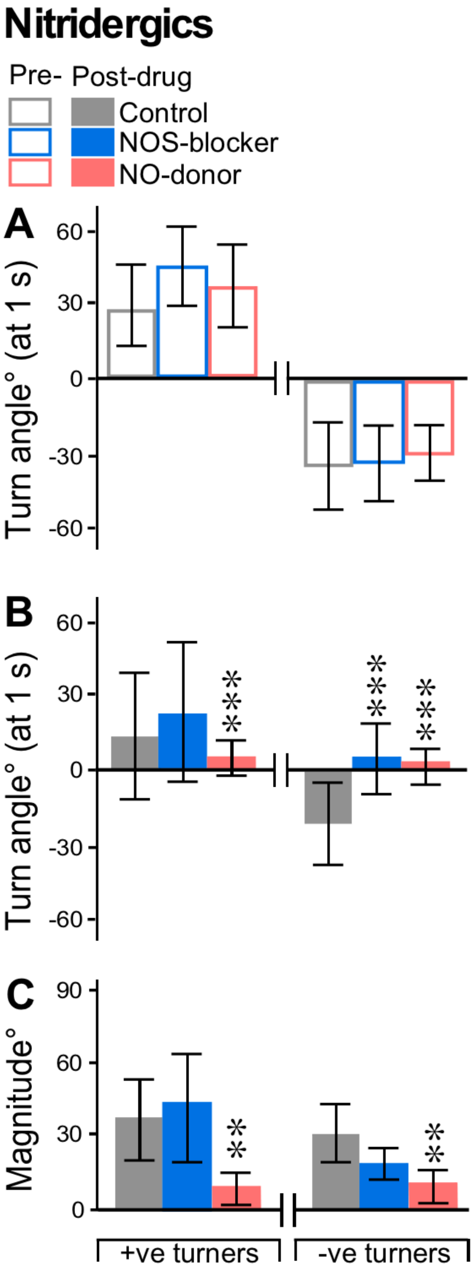


Fig. 18: Influence of nitridergic drugs on turning responses of short term isolated crickets. Turn angles 1 s after the touch (mean ± 95% CI; *n* = 15 each). **A** Short term isolated positive and negative turners before treatment (STI, +ve, -ve respectively, outlined bars: pre-drug = control). **B** Same animals as in A after treatment (filled bars: 1 h post-drug; grey: non-effective enantiomer D-NAME, blue: NOS-inhibitor L-NAME, red: NO-donor SNAP). **C** Mean magnitude (± 95% CI) of turns 1 h after treatment. Asterisks indicate differences between post-drug groups and the corresponding pre-drug groups (** $p < 0.005$, *** $p < 0.0005$; Bonferroni correction for 2 comparisons).

So far the data indicate that OA and NO are both involved in approach and avoidance behaviour of male crickets. Short term isolates that were predisposed to avoid the touch stimulus changed to approach after treatment with either the OAR-agonist CDM or after blocking NO-synthesis with L-NAME. Conversely, individuals initially predisposed to approach the stimulus showed avoidance responses after injection of the OA-blocker epinastine or no reactions after treatment with NO-donor SNAP. Differential responses to an agonistic stimulus could be elicited by modulating the OA levels in the haemolymph (Fig. 17). Furthermore, NO appeared to have also an influence on the decision to approach or avoid a stimulus, whereas SNAP lowers the perception, conduction or central integration of the stimulus signal directed at the antenna (Fig. 18).

To examine to which extend both neuromodulators contribute to the decision-making process and whether SNAP modulates the peripheral perception or central integration of the signal, combinations of drugs with opposing effects on turning responses of STI crickets were used (*n* = 15 for each group; Bonferroni correction to alpha = 0.025 for all comparisons; Fig. 19). First, the OAR-blocker epinastine was provided combined with NOS-inhibitor L-NAME, with both drugs injected alternately to avoid bias in the results in favour to the first injected drug. Focal animals later assigned to treatment with vehicle had turned on average +39° for positive and -69° for negative turners 1 s after the touch was applied (means, 95% CI: 25 to 53° and -90 to -48° respectively; Fig. 19A). Male crickets assigned to epinastine combined with L-NAME injection had turned +32° for animals that approached and -44° for animals that avoided the stimulus (means, 95% CI: 21 to 43° and -59 to -29° respectively). Vehicle treated animals exhibited no change 1 h after drug application compared to their behavioural responses pre-treatment regarding the turn angle 1 s after the touch (+ve turners: mean: 19°, 95% CI: 2 to 36°; $p = 0.0590$; -ve turners: mean: -45°, 95% CI: -67 to -24°; $p = 0.1307$; Fig. 19B) and turning magnitude (+ve turners: mean: 29°, 95% CI: 18 to 41°; $p = 0.2193$; -ve turners: mean: 52°, 95% CI: 35 to 70°; $p = 0.2532$; Fig. 19C). Crickets that were predisposed to approach the touch stimulus changed to avoidance after treatment with both drugs (mean: -42°, 95% CI: -60 to -23°; $p < 0.0001$), but without a statistically significant difference in the magnitude of turns (mean: 47°, 95% CI: 31 to 63°; $p = 0.0956$). Focal animals that initially avoided the antennal touch showed no change of direction (turn angle: mean: -43°, 95% CI: -56 to -29°; $p = 0.8649$) and magnitude (mean: 43°, 95% CI: 29 to 56°; $p = 0.9062$) compared to the corresponding pre-drug group. Second, crickets were injected with the agonists CDM and SNAP together. The vehicle group consisted of the same animals already used as a control for antagonists. STI males that initially turned towards the touch stimulus (mean: 36°, 95% CI: 28 to 44°) exhibited a decrease in mean turn angle 1 h after the treatment with both drugs which was insignificant after correction, but on average the test animals were still positive turners (mean: 16°, 95% CI: -3 to 34°; $p = 0.0317$), the average magnitude of turns was not affected (mean: 34°, 95% CI: 23 to 45°; $p = 0.7192$). Focal animals that avoided the antennal touch (mean: -48°, 95% CI: -64 to -32°) now exhibited positive turns after CDM and SNAP were injected (mean: 33°, 95% CI: 14 to 51°; $p < 0.0001$), but again without a change in magnitude (mean: 39°, 95% CI: 24 to 54°; $p = 0.4045$) compared to the respective pre-drug group. Together these were essentially the expected responses of STI positive and negative turners if epinastine or CDM were provided alone (see Fig. 17).

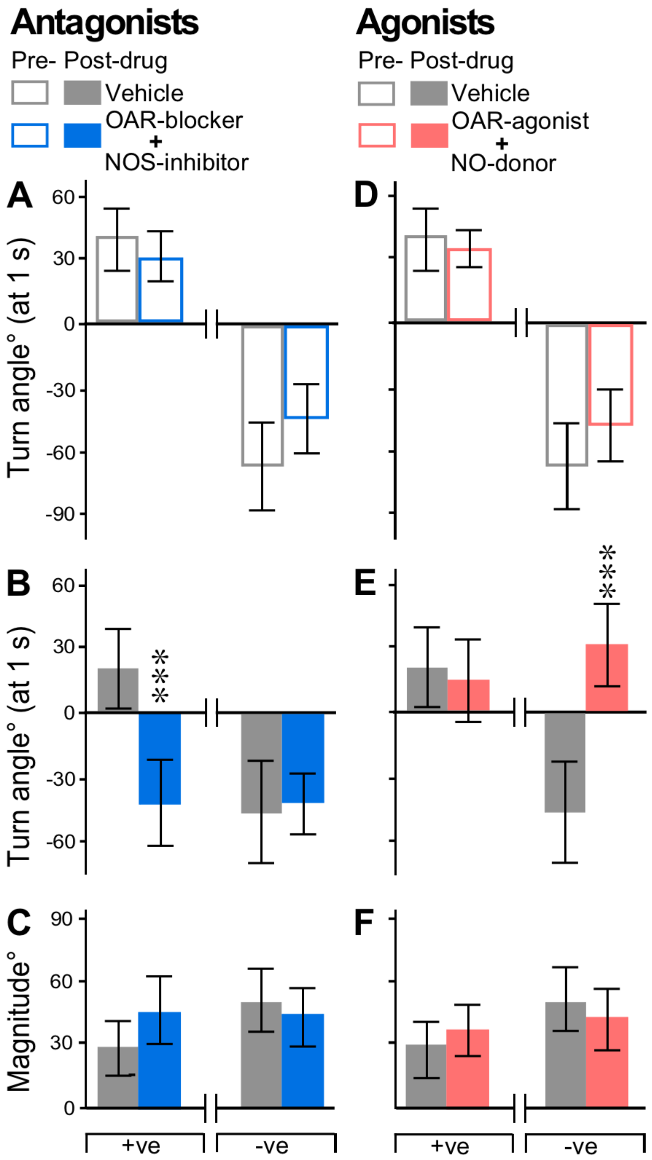


Fig. 19: Influence of octopaminergic drugs combined with nitridergic drugs on turning responses of short term isolated crickets. Turn angles 1 s after the touch (mean ± 95% CI; *n* = 20 each). **A** Short term isolated positive and negative turners before treatment (STI; +ve, -ve respectively; outlined bars: pre-drug = control). **B** Same animals as in A after treatment (filled bars: 1 h post-drug; grey: vehicle, blue: OAR-antagonist epinastine combined with NOS-inhibitor L-NAME). **C** Mean magnitude (± 95% CI) of turns 1 h after treatment. **D-F** Same as A-C, but for agonists (grey: vehicle, red: OAR-agonist CDM combined with NO-donor SNAP). Asterisks indicate differences between post-drug groups and the respective pre-drug groups (* $p < 0.025$, *** $p < 0.0005$; Bonferroni correction for 2 comparisons).

3.9.3.3 Influence of serotonergic drugs

Recovery test

For the majority of serotonergic drugs used in this experiment the most effective dosages were already established in prior studies (Rillich & Stevenson, 2017; 2018). Exceptions to this are WAY, 8-OH-DPAT and DOI, which were fed in comparable studies (3mM, Johnson *et al*., 2009) but not injected in the haemocoel. These effective dosages of DOI, WAY and 8-OH-DPAT were determined with a recovery analysis (see also Rillich & Stevenson, 2018) for 1 mM, 5 mM, 10 mM and 20 mM drug solutions.

After tests with each concentration, 10 mM was selected for further experiments (1 mM and 5 mM; no obvious effects, 10 mM: Tabs. 6 and 7, Fig. 20; 20 mM: over 20% of focal animals died within 24 h after injection in all 3 groups). In initial fights against a hyper-aggressive opponent, focal animals pre-treated with the 10 mM solution of each drug showed no statistically significant differences in the level of fights (WAY: p = 0.9288; 8-OH-DPAT: p = 0.9251; DOI: p = 0.8780; n = 15 each; Tabs 6 and 7; Fig. 20) or persistence (WAY: p = 0.3017; 8-OH-DPAT: p = 0.2156; DOI: p = 0.3223) compared to the vehicle group. Similarly, when tested 30 min and 60 min after defeat, no differences between vehicle and drug groups were observed. All test animals retreated from the hyper-aggressive opponent and fights never escalated above level 3 or lasted longer than 3 s. As shown in Tabs. 6, 7 and Fig. 20, focal animals treated with WAY and 8-OH-DPAT regained their aggressiveness 180 min after a single defeat and showed no differences in level (WAY: p = 0.7936; 8-OH-DPAT: p = 0.4495) and persistence (WAY: p = 0.7213; 8-OH-DPAT: p = 0.4511) compared to their respective vehicle control.

In marked contrast, animals treated with DOI still showed significantly depressed aggressiveness 3 h after defeat regarding to level (p = 0.0143) and persistence (p = 0.0072) of fights indicating that this $5HTR_2$-agonist acts to inhibit loser recovery and 10 mM is the effective dosage, since no effects were observed for 1 and 5 mM concentrations for all 3 drugs and after injection with 20 mM solutions, more than 20% of treated animals died within 24 h.

Tab. 6: Influence of serotonergic drugs on level of initial fights and recovery after defeat. Level (median and IQR; *n* = 15 each) of focal animals after treatment with either vehicle or active drug of initial fights against hyper-aggressive opponents and 30, 60 and 180 min after defeat. Statistically significant differences between treated and respective vehicle group are indicated in boldface.

Treatment	1st fight		30 min		60 min		180 min	
			after defeat					
	Level		Level		Level		Level	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Vehicle	4	2 - 5	1	0 - 2	2	1 - 3	4	1 - 5
WAY 10 mM	4	2 - 6	2	0 - 3	2	2 - 3	4	1 - 6
Vehicle	5	3 - 5	1	0 - 1	2	1 - 3	4	2 - 5
8-OH-DPAT 10 mM	4	2 - 6	1	0 - 1	1	1 - 3	3	2 - 4
Vehicle	4	2 - 6	0	0 - 1	2	1 - 3	**4**	**2 - 5**
DOI 10 mM	4	3 - 5	1	0 - 1	1	0 - 1	**1**	**1 - 3**

Tab. 7: Influence of serotonergic drugs on persistence in initial fights and recovery after defeat. Mean persistence (± 95% CI, *n* = 15 each) of focal animals after treatment with either vehicle or active drug in initial fights against hyper-aggressive opponents and 30, 60 and 180 min after defeat. Statistically significant differences between treated and corresponding vehicle group are indicated in boldface.

Treatment	1st fight		30 min		60 min		180 min	
			after defeat					
	Persistence		Persistence		Persistence		Persistence	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Vehicle	10.3	7.5 - 13.2	1.4	0.9 - 1.9	2.1	1.3 - 3	7.1	5.1 - 9.1
WAY 10 mM	8.3	5.2 - 11.3	1.7	0.95 - 2.4	2.3	1.9 - 2.8	7.7	4.7 - 10.6
Vehicle	9.6	7.2 - 12	1.2	0.7- 1.7	2.1	1.2 - 3	8.6	5.8 - 11.4
8-OH-DPAT 10 mM	7.6	5.2 - 10	0.9	0.5 - 1.3	1.6	0.8 - 2.4	7.1	4.1 - 10.1
Vehicle	9.9	6.4 - 13.3	1.2	0.7 - 1.7	1.8	0.9 - 2.7	**6.7**	**3.6 - 9.9**
DOI 10 mM	7.7	4.5 - 10.9	0.6	0.2 - 1	0.8	0.3 - 1.3	**2.2**	**0.96 - 3.4**

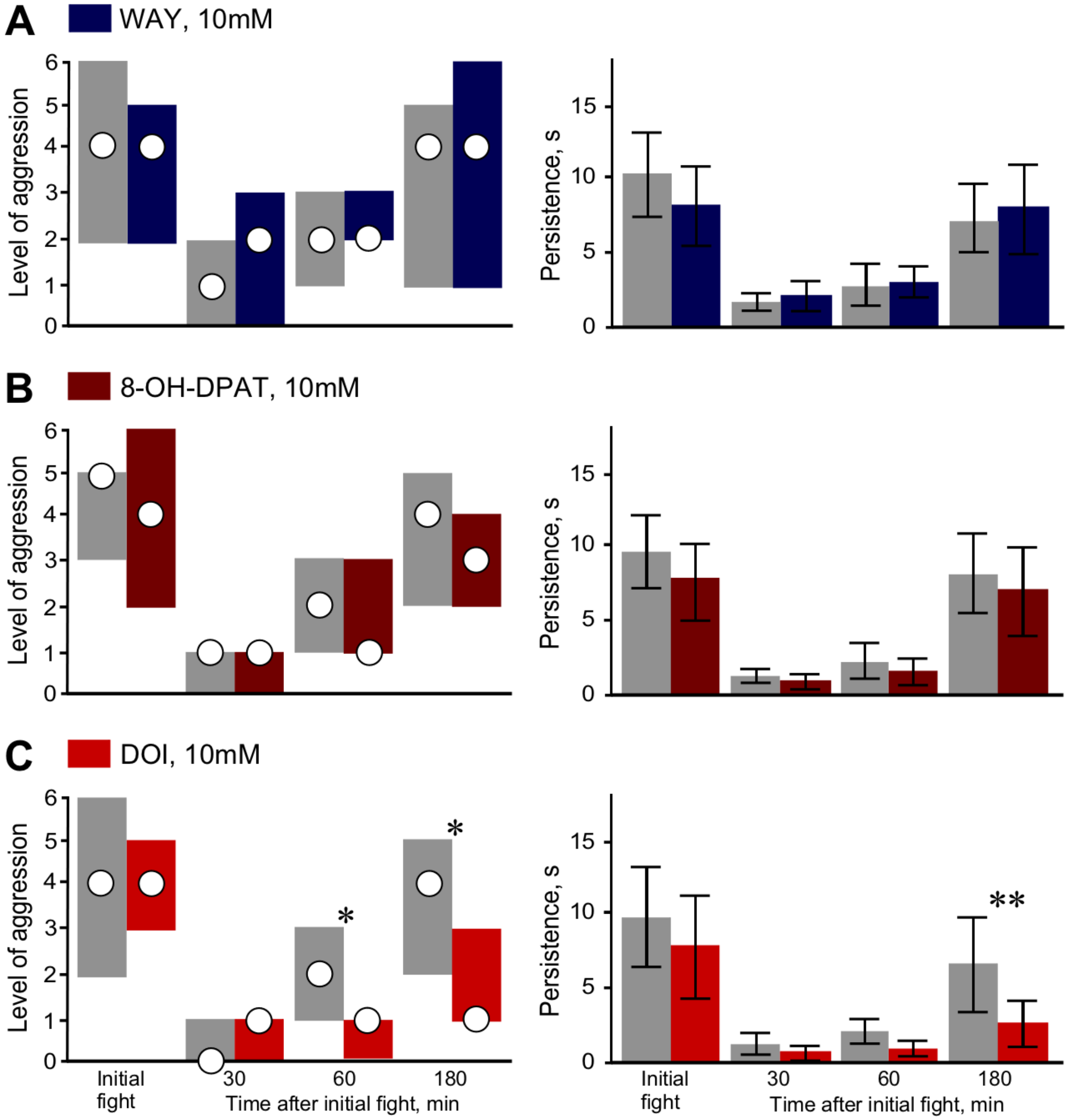


Fig. 20: Influence of serotonergic drugs on initial aggression and recovery after defeat in short term isolated crickets. Level of aggression (left; circle: median, bars: IQR) and persistence of fights (right; mean ± 95% CI) of focal crickets in fights against a hyper-aggressive opponent 1 h after they received either vehicle (= control, grey bars) or **A** $5HTR_{1A}$-blocker WAY (dark blue bars, 10mM); **B** $5HTR_{1A}$-agonist 8-OH-DPAT (dark red bars, 10mM); **C** $5HTR_2$-agonist DOI (red bars, 10mM). Focal animals were re-matched at 30, 60 and 180 min after defeat. Asterisks indicate differences between treated and corresponding vehicle groups (* $p < 0.05$, ** $p < 0.01$; $n = 15$ each).

Turning responses

The role of serotonin in aggression in invertebrates is complex and still widely discussed. So far data indicate, that serotonergic drugs have no obvious influence on initial fights (Rillich & Stevenson, 2018 and Fig. 15) and on general motility (Fig. 16) without behavioural context e.g. multiple defeats or wins. To examine the influence of serotonergic drugs on a far more sensitive metric for aggressive motivation, turning responses of STI crickets under the influence of various 5HT-antagonists (Tab. 8; Figs. 21A-C) and agonists (Tab. 9; Figs. 21D-F) were evaluated as described above for octopaminergic and nitridergic drugs (*n* = 15 for each group, Bonferroni correction to alpha = 0.025 for all comparisons; see Tabs. 8, respectively 9 for exact values).
First, all pre-treatment group (Figs. 21A and D) exhibited turn angles in the typical range of STI untreated adult male crickets for both positive and negative turners. Furthermore, 1 h after treatment with the vehicle none of the focal animals showed statistically significant differences in turn angles 1 s after the touch and magnitude of turns (Figs. 21B, C, E and F). Similarly, after treatment with the 5HTR$_{1,6,7}$-blocker methiothepin both positive and negative turners showed no change in average turn angles and magnitude of turns, disregarding direction (+ve: mean: 28°, 95% CI: 15 to 41°; p = 0.7622; -ve: mean: 37°; 95% CI: 29 to 44°; p = 0.5279). However, when treated with the 5HTR$_2$-blocker ketanserin, most animals that were predisposed to approach the stimulus now avoided it. Crickets that previously avoided the stimulus exhibited no directional changes and both groups showed no statistically significant differences in the magnitude of turns (+ve: mean: 32°, 95% CI: 21 to 43°; p = 0.8741; -ve: mean: 50°; 95% CI: 25 to 74°; p = 0.5838) compared to the respective pre-drug group. After injection of 5HTR$_{1A}$-blocker WAY focal crickets showed no clear directional preference anymore, with a mean turn angle of 0°. But this only applies to positive turners, negative turners showed no change in turn angle after the treatment and the magnitude of turns was again not affected in both groups (+ve: mean: 29°, 95% CI: 19 to 39°; p = 0.4106; -ve: mean: 42°; 95% CI: 28 to 56°; p = 0.6474).

Tab. 8: Influence of 5HT-antagonists on turning responses of short term isolated crickets. Mean turn angle (± 95% CI) of positive and negative turners before and after treatment with either vehicle or active drug. ***p*** gives the significant differences to the respective pre-drug group. Statistically significant comparisons are indicated in boldface (Bonferroni correction to alpha = 0.025; *n* = 15 each).

Turn angle ° after 1 s		**5HT-antagonists**				
Pre-drug direction	**Treatment**	pre-drug		post-drug		difference to pre-drug *p*
		mean	95% CI	mean	95% CI	
Positive turners	Vehicle	39	25 to 53	16	-3 to 34	0.0370
	Methiothepin	31	19 to 43	15	-4 to 33	0.1770
	Ketanserin	34	21 to 47	-11	-31 to 10	**0.0004**
	WAY	35	22 to 48	0	-20 to 19	**0.0010**
Negative turners	Vehicle	-45	-62 to -28	-32	-59 to -5	0.3367
	Methiothepin	-41	-56 to -27	-14	-35 to 7	0.0479
	Ketanserin	-41	-57 to -25	-27	-62 to 7	0.4841
	WAY	-38	-51 to -25	-35	-55 to -16	0.7975

After treatment with the 5HT-precursor 5HTP both positive and negative turners showed no clear directional preference in their reaction to an antennal touch, but without a change in magnitude of turns compared to their pre-drug performances (+ve: mean: 32°, 95% CI: 19 to 44°; p = 0.6389; -ve: mean: 32°; 95% CI: 22 to 42°; p = 0.0782). Similarly, injection of 5HT-reuptake-inhibitor fluoxetine disrupts group directionality of turns for both positive and negative responders. Again, the magnitude was not affected (+ve: mean: 24°, 95% CI: 16 to 33°; p = 0.8774; -ve: mean: 34°; 95% CI: 24 to 44°; p = 0.2202). Animals predisposed to approach the stimulus also showed no directional preference 1 h after treatment with 5HTR$_2$-agonist DOI. This was, however, not statistically significant after correction. Contrasting this, DOI-treated negative turners exhibited no change in turn angles and the turning magnitude was not significantly different compared to the pre-treatment groups (+ve: mean: 47°, 95% CI: 24 to 70°; p = 0.1488; -ve: mean: 47°; 95% CI: 23 to 70°; p = 0.6446). When injected with the 5HTR$_{1A}$-agonist 8-OH-DPAT, positive turners showed no statistically significant differences in turn angles and the magnitude of turns was unaffected for both groups (+ve: mean: 27°, 95% CI: 8 to 47°; p = 0.6571; -ve: mean: 24°; 95% CI: 13 to 35°; p = 0.0681) compared to the respective pre-drug group.

The response of negative turners was, however, significantly different after treatment with 8-OH-DPAT compared to the pre-drug performance, but they still avoided the stimulus on average.

Tab. 9: Influence of 5HT-agonists on turning responses of short term isolated crickets. Mean turn angle (± 95% CI) of positive and negative turners before and after treatment with either vehicle or active drug. ***p*** gives the significant differences to the corresponding pre-drug group. Statistically significant comparisons are indicated in boldface (Bonferroni correction to alpha = 0.025; *n* = 15 each).

Turn angle° after 1 s		**5HT- agonists**				
Pre-drug direction	**Treatment**	pre-drug		post-drug		difference to pre-drug *p*
		mean	95% CI	mean	95% CI	
Positive turners	Vehicle	26	16 to 35	15	5 to 25	0.1114
	5HTP	36	24 to 48	-10	-31 to 11	**0.0018**
	Fluoxetine	25	14 to 37	0	-17 to 16	**0.0034**
	DOI	47	24 to 70	-8	-52 to 36	0.0326
	8-OH-DPAT	33	17 to 48	21	-2 to 43	0.3522
Negative turners	Vehicle	-26	-35 to -17	-25	-43 to -7	0.9156
	5HTP	-58	-84 to -32	-9	-29 to 11	**0.0028**
	Fluoxetine	-42	-55 to -29	7	-14 to 29	**0.0028**
	DOI	-53	-70 to -36	-38	-66 to -10	0.3316
	8-OH-DPAT	-46	-72 to -20	-17	-31 to -2	**0.0180**

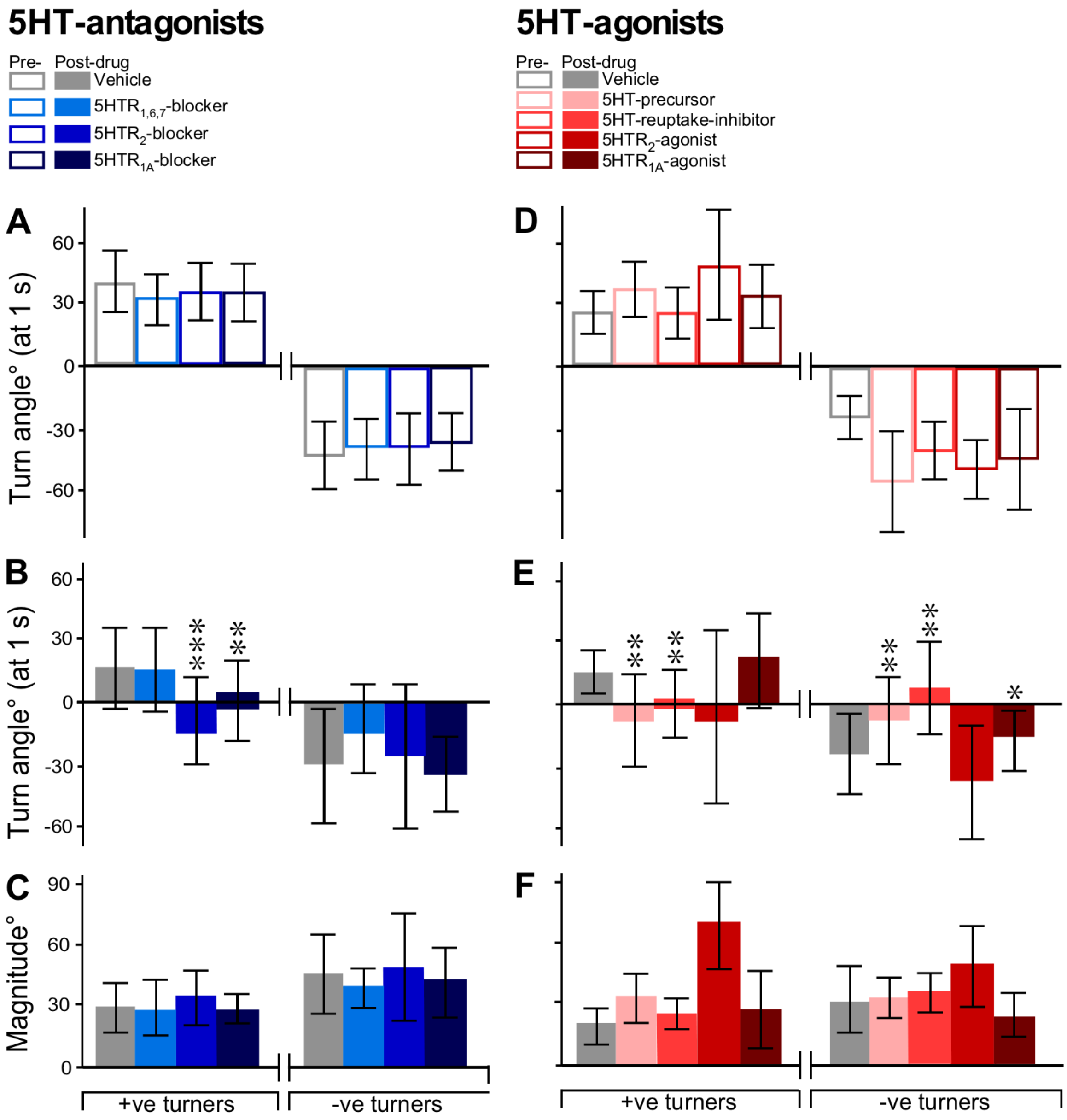


Fig. 21: Influence of serotonergic drugs on turning responses of short term isolated crickets. Turn angles 1 s after the touch (mean ± 95% CI; *n* = 15 each). **A** Short term isolated positive and negative turners before treatment (STI; +ve, -ve respectively; outlined bars: pre-drug = control). **B** Same animals as in A after treatment (filled bars: 1 h post-drug; grey: vehicle, light blue: $5HTR_{1,6,7}$-blocker methiothepin, blue: $5HTR_2$-blocker ketanserin, dark blue: $5HTR_{1A}$-blocker WAY). **C** Mean magnitude (± 95% CI) of turns 1 h after treatment. **D-F** As for A-C, but for 5HT-agonists (coral: 5HT-precursor 5HTP, light red: 5HT-reuptake-inhibitor fluoxetine, red: $5HTR_2$-agonist DOI, dark red: $5HTR_{1A}$-agonist 8-OH-DPAT). Asterisks indicate differences between post-drug groups and the corresponding pre-drug groups (* $p < 0.025$, ** $p < 0.005$, *** $p < 0.0005$; Bonferroni correction for 2 comparisons).

Influence of serotonin on the establishment of dominance and subordination

Since serotonergic drugs had none or unclear influences on turning responses in initial fights of STI male crickets (Fig. 21), and the observed effects were either the same for positive and negative turners or applied drugs disrupted directionality of the whole group, new experimental conditions were designed. To avoid effects of prior aggressive experiences with long term influences on adult behaviour in the breeding colony, LTI$^{w/o}$ crickets were used which showed no directional preferences after the antennal stimulation in previous experiments (see Fig. 13A right side and 14B). Furthermore, the focal crickets were subjected to multiple winning or losing experiences (6 times in succession at 30 min intervals). In STI male crickets, earlier studies showed that ketanserin had no effects on initial fights, but acted to prevent chronic defeat and blocked long term depression after multiple defeats (Rillich & Stevenson, 2018). DOI, as an agonist for the same receptor subtype, acted to inhibit loser recovery (Fig. 20C) when applied 1 h before the interactions. To avoid drug influences during the fights, multiple winners and losers were treated with either vehicle, $5HTR_2$-blocker ketanserin or $5HTR_2$-agonist DOI 15 min after their last fight. After 1 h, the treated animals were tested for their reaction to an antennal stimulus and compared to an additional pre-fights, non-treated group of prospective winners and prospective losers (*n* = 10 each; Bonferroni correction to alpha = 0.0167 for all comparisons; Fig. 22).

Before the fights, untreated adult male LTI$^{w/o}$ crickets exhibited similar responses to an antennal touch as shown in Fig. 14B. Prospective winners had turned +6° and prospective losers +1° (means, 95% CI: -1 to 14° and -9 to 10° respectively) with a small magnitude of resulting turns (pr-winners: mean: 9°, 95% CI: 3 to 14°; pr-losers: mean: 9°, 95% CI: 2 to 15°). Multiple winners and losers exhibited clear directional differences after fighting and treatment with the vehicle regarding to the turn angle 1 s after the stimulus (winners: mean: 29°, 95% CI: 7 to 51°; *p* = 0.0379, insignificant after correction; losers: mean: -75°, 95% CI: -111 to -39; *p* = 0.0002) and magnitude of resulting turns compared to the respective pre-fights groups (winners: mean: 35°, 95% CI: 18 to 51°; *p* = 0.0036; losers: mean: 75°, 95% CI: 39 to 110, *p* = 0.0006). After treatment with ketanserin, multiple winners were not affected from the drug-treatment and turned towards the stimulus (mean: 37°, 95% CI: 14 to 61°; *p* = 0.0099) with a significantly greater magnitude compared to prospective winners (mean: 40°, 95% CI: 20 to 61°; *p* = 0.0041). Multiple losers, however, exhibited no clear directional preference in turns and were thus not significantly different to the respective pre-fight animals (turn angle: mean: -4°, 95% CI: -12 to 5°; *p* = 0.4547; magnitude: mean: 8°, 95% CI: 2 to 15°; *p* = 0.9889). Conversely, multiple winners subsequently treated with DOI showed no statistically significant changes in turn angles compared to pre-fight prospective winners (mean: 17°, 95% CI: -21 to 53; *p* = 0.5326), but the magnitude changed significantly (mean: 47°, 95% CI: 30 to 65°; *p* = 0.0002). Multiple losers were not affected and exhibited avoidance behaviour after the stimulus was applied (turn angle: mean: -65°, 95% CI: -101 to -29°; *p* = 0.0008; magnitude: mean: 65°, 95% CI: 29 to 101; *p* = 0.0024).

Together this data indicates that ketanserin, injected after the fights, blocks the acquisition of a loser effect after chronic defeat in LTI$^{w/o}$ crickets.

Interestingly DOI appears to inhibit the establishment of a clear dominant ethotype to a certain extent after multiple wins.

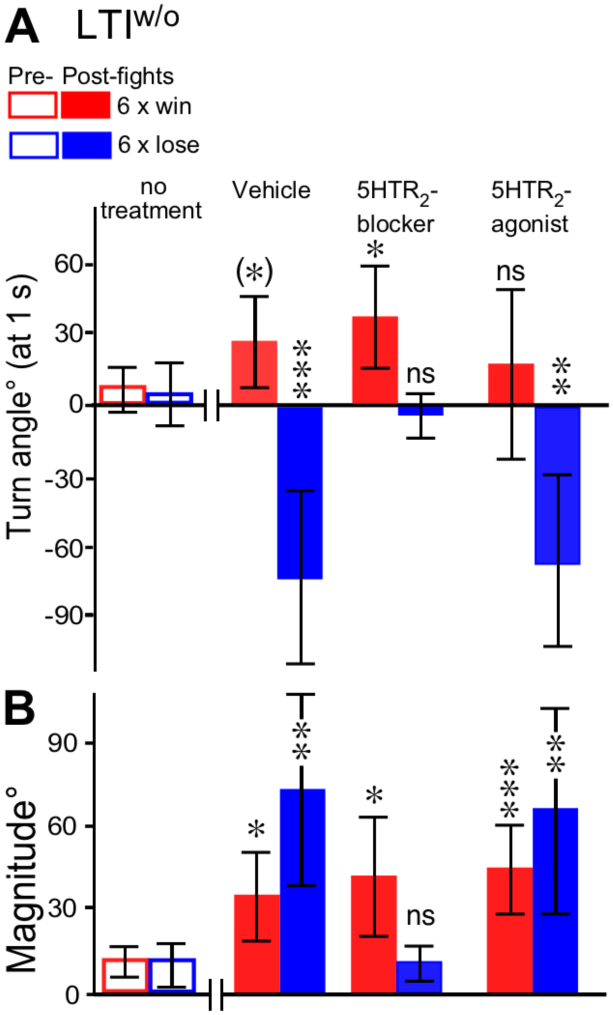


Fig. 22: Influence of 5HTR$_2$-agonist and -antagonist on turning responses after multiple wins and defeats. A Turn angles 1 s after the touch (mean ± 95% CI; *n* = 10 each) of LTI$^{w/o}$ crickets tested pre-fight (red and blue outlined bars; prospective winners and losers respectively, without treatment) and post-6 consecutive fights, 1 h after the 6 fold winners and losers (red and blue filled bars respectively) received either vehicle, 5HTR$_2$-blocker ketanserin or 5HTR$_2$-agonist DOI. **B** Same as A, but mean magnitude (± 95% CI) of turns 1 h after treatment. Asterisks indicate differences to pre-fight prospective winners and prospective losers (* $p < 0.0167$, ** $p < 0.0033$, *** $p < 0.00033$; Bonferroni correction for 3 comparisons; (*) = significant before correction).

4 Discussion

4.1 Nymphal interactions and their consequences for adult behaviour

Social interactions, particularly repeated subjugation, are considered as major factors inducing symptoms of severe depression and establishing consistent inter-individual behavioural differences between members of the same species (*animal personality*) in numerous animal groups including invertebrates (Bell *et al*., 2009; Briffa *et al*., 2015; Carter *et al*., 2013; de Boer *et al*., 2016; Gosling, 2011; 2008; Sih *et al*., 2004; 2014; Wilson *et al.*, 2019). Insects provide unique systems to investigate how early life social experiences shape adult behaviour (Westwick & Rittschof, 2021). A key question concerning the establishment of behavioural ethotypes is whether differences between individuals of the same species are inherited or acquired from social experiences - nature or nurture, and how this is mediated by neuromodulators released in response to social interactions.

This study analysed the extent to which pre-adult agonistic experiences induce consistent behavioural changes in crickets, *Gryllus bimaculatus*, which have become a model system for investigating the neurochemical control of aggression (review: Stevenson & Rillich, 2019). Therefore, the hypothesis was tested, that multiple defeats during post-embryonic development and/or multiple wins and defeats in early adult life establish inter-individual differences, which could account for different behavioural ethotypes in mature adults and the underlying role of neuromodulators therein. Previous reports imply that aggressive interactions between last instar male nymphs are essentially the same as those between adult male crickets and have long-lasting effects on adult fitness, with regards to body size and survival rate (Simmons, 1987). This study, however, which compares aggressive behaviour in male nymphs and adults, focussing primarily on the influences on future adult behaviour, calls for a different conclusion.

The results reveal that agonistic interactions between male nymphs are far less violent and that they are typified by a significantly lower level of aggressive escalation and fight duration (Fig. 7). Under normal conditions, fights escalating to a physical level were never observed. These findings are not essentially different to those of earlier investigators, who stated that nymphs escalate less (Abe *et al.*, 2018) and do not show grappling and flipping behaviour (Simmons, 1986), which corresponds to the here described level 5 and 6 fights and is typically exhibited by adults. Of more relevance to the present study is that agonistic competitions between last stage male nymphs have no lasting behavioural consequences. Their interactions do not always generate clear winners and losers, and even when this was reasonably apparent, winners were not subsequently more aggressive, nor were the losers less so (Fig. 7). In adults, those winner and loser effects influence the aggressiveness of subsequent fights and the establishment of dominant-subordinate relationships with additional consequences for the expression of other behaviours (Rose *et al.*, 2017a). Agonistic competition between last instar male nymphs were not found to have any lasting behavioural consequences.

These observations raised the question of why nymphs behave differently towards conspecifics and do not show stereotypic fighting patterns typical for adult male crickets.

On contact with conspecifics, male nymphs do exhibit some elements of aggressive behaviour as observed in adults (Fig. 5). This includes antennal fencing, mandible spreading and, far less frequently, body jerking and lunges. Even though male nymphs spread their mandibles significantly less wide compared to adults on confronting conspecifics, their mandibles are fully capable to do so, as shown on multiple occasions during feeding (Fig. 6B). Therefore, cricket nymphs seem to be just as capable as adults of displaying and initiating actual fighting behaviour, but are less inclined to do so. However, smaller mandible spread angles in nymphs are unlikely to be a cause of reduced aggressiveness since neither mandible quality (Judge & Bonanno, 2008) nor the ability to use them (Rillich *et al.*, 2007) has any influence on contest intensity or duration. Many studies of crickets and other hemimetabolous insects, e.g. locusts, indicate that the central nervous apparatus underlying specifically adult behaviours such as flight, copulation and phonotaxis are already fully functional in nymphs (Kutsch & Pener, 1997; Sakai *et al.*, 1990; Staudacher, 2009; Stevenson & Kutsch, 1988), but cannot be properly recruited by the natural occurring releasing stimuli (Stevenson & Kutsch, 1988). Therefore, the most plausible explanation for the lower aggressiveness exhibited by male nymphs is that their ability to respond to the aggression-releasing stimulus is not yet fully developed.

In adult male crickets the aggression releasing stimulus is considered to be antennal fencing, where individuals lash each other's antennae for at least 2 s at 20 Hz (Hofmann

& Schildberger, 2001). It occurs when conspecifics first meet and involves mechanical stimulation combined with the transfer of male-specific pheromonal signals that are reported to be essential for initiating normal aggression (Adamo & Hoy, 1995; Iwasaki & Katagiri, 2008; Nagamoto *et al*., 2005; Rillich & Stevenson, 2015; Sakura & Aonuma, 2013; Thomas & Simmons, 2008; Hofmann & Schildberger, 2001; Rillich & Stevenson, 2015). Conversely to adults, results reveal that male nymphs show aggressive actions such as mandible threats or lunges in the same frequency towards male and female nymph and thus do not discriminate between sexes (Fig. 5), but never towards adults of both sexes. Adult male crickets also exhibit aggressive behaviour towards male and female nymphs indiscriminately and also towards adult males, but never on contact with an adult female. Similarly, when stroked with a donor antenna, nearly all tested adult males exhibited the aggressive mandible threat display when the donor was another adult male, only about half when a male nymph´s antenna was used and hardly ever as a reaction to a female´s antenna (Fig. 6A). Contrasting this, male nymphs showed mandible threats in an equal frequency as a reaction to stroking with antennae obtained from both ages and sexes of donors (Fig. 6A).

Furthermore, adult male crickets that previously lost a fight show increasing aggression after prior antennal stimulation for 20 s with a freshly excised donor male antenna (priming; Rillich & Stevenson, 2015; Fig. 8A), but not, however, when a male nymph´s antenna is used, Nymphs, in contrast, exhibit no increase in aggression after priming with an adult male´s donor antenna with regards to level and duration of fights (Fig. 8B). Together this data imply that cricket nymphs neither produce nor respond or are fully capable to perceive adult pheromonal signatures. While differences in pheromonal compositions between males and females are documented in adult crickets (*Teleogryllus ocanicus*: Thomas & Simmons, 2008), studies of pheromones in nymphs are rare (e.g. Thibert *et al*., 2016) and there is only evidence for developmental changes in the quality and quantity during adulthood (Nagamoto *et al*., 2005).

The results further show that nymphs exhibit no actual physical fights (above level 3) when paired with conspecifics of different developmental stages and both sexes. In a further attempt to increase aggressiveness in STI male nymphs, the effect of different aggression modulators was evaluated in the present study. In marked contrast to adults, however, neither winning (Fig. 7), stimulation with an adult male’s antenna (priming; Fig. 8), nor the presence of food after starvation (Fig. 9B) lead to any significant increase in aggression exhibited by male nymphs. This difference to adult males could be caused by octopamine deficiency due to immaturity in the pheromone-linked octopamine system. Female adult cricket brains (*Acheta domesticus*: Woodring *et al*., 1988) contain 50% more octopamine compared to those of last stage nymphs and octopaminergic stimulation of the second messenger adenylate cyclase is more potent in the brain of adult locusts than in nymphs (Hiripi & Rózsa, 1984). In crickets, adult male-male contact leads to release of octopamine (Adamo & Hoy, 1995). While octopamine is not necessary for the expression (Stevenson et al., 2000) or initiation (Rillich & Stevenson, 2015) of aggression, it increases the tendency to escalate higher and persist longer in agonistic encounters by raising the threshold to flee (Stevenson & Rillich, 2019). Treatment with the OAR-agonist CDM coupled with priming with an adult male´s antenna provoked a significant increase in aggression with regard to the fight duration between nymphs (Fig. 9A) and one observed pair even interlocked the mandibles in a physical interaction otherwise seen only in adult males (level 5), supporting the hypothesis, that the central circuit regulating aggressive behaviour is fully functional in last instar nymphs, but cannot be recruited properly. Therefore, the reduced aggressive behaviour exhibited by male last instar nymphs most likely reflect some developmental immaturity in the pheromonal and octopaminergic systems which are both equally required for initiating and maintaining aggression in adult male crickets. However, observations showed that adult males aggressively intimidate nymphs in dyadic staged fights (Fig. 5) and also in the crowded colony conditions (Fig. 10). On average a nymph endures one physical attack per hour from an adult male in the age- and sex-mixed colony (Fig. 10B) and this study provides evidence that this has long-lasting influences on their future adult behaviour. Comparably, chronic social defeat in adult male crickets induce long term depression of aggression that can last for hours and even days (Rillich & Stevenson, 2018; Rose *et al*., 2017b). A comparison between nymphs raised in different social environments regarding the presence of adult males revealed that nymphs, isolated at their last instar, are significantly more active and exhibit higher levels and longer durations in fights once they are fully mature adult when raised without any contact to adult males (Figs. 10C and D). Repeated subjugation by adult males during nymphal development is thus a major determinant of long-lasting, possibly life-long inter-individual differences. However, the presence of adult females or other nymphs during development has no adverse influences on later adult behavioural profiles. Supporting this, other studies show that the population density of nymphs during rearing, and hence interactions between them, has no lasting effect on exploratory behaviour or aggression in adult crickets (Niemelä *et al*., 2012).

4.2 The decision to approach or avoid an agonistic stimulus

So far data indicate that raising adult male crickets under various social conditions evoke different behavioural profiles regarding their general motility and aggression towards conspecifics (Fig. 10). Winning and losing experiences modulate the inherent aggressive motivation, but this effects are known to last little more than 3 h and should wane after the 48 h isolation period (STI; Rillich & Stevenson, 2011; 2014; Stevenson & Rillich, 2013; 2015), but this study provides experimental evidence that social experiences gathered during nymphal development and early adult life result in consistent inter-individual differences in behavioural traits of adult male crickets.

To examine the aggressive motivation of individual crickets to engage in fighting or retreat immediately, the responses to an agonistic stimulus directed at one antenna were evaluated. Although repeated stroking with an adult male´s antenna nearly always initiates the aggressive mandible threat display in adult male crickets (Fig. 6), this was never observed after the single, brief stimulus applied in this experiments. Turning responses are, therefore, an exceptional sensitive metric for the evaluation of aggressive motivation. However, positive turners and established dominants occasionally respond with a forward lunge, body jerking or subsequently exhibited the rival song, as often shown by adult male crickets during aggressive interactions with conspecifics (Stevenson *et al*., 2000). Since initially positive turning crickets are also predisposed to become dominants in a subsequent fight (90% win chance), the directionality is also a good indicator of aggressive motivation and can be considered as an element of aggressive behaviour.

Results reveal that winners of a previously staged fight mainly turn towards a single antennal touch executed with an adult male´s donor antenna, whereas submissive individuals, which lost a preceding interaction, avoided the stimulus (Figs. 11B and D). Similar reactions were observed in earlier studies on dominant crickets that approached a stimulus directed at one cercus (Abe *et al*., 2018) and in crayfish to an unexpected touch

(Fujimoto *et al*., 2011; Song *et al*., 2006). Interestingly, the differences in behavioural responses towards an agonistic stimulus are also evident before dominants and subordinates are established in a dyadic contest and their social status is still unclear. The majority of retrospectively sorted prospective winners also approached the stimulus whereas prospective losers turned away (Figs. 11A and C). Earlier studies corroborate these results, in that winners of a preceding contest are generally more active both before and after the fight compared to losers (Rose *et al*., 2017a).

Apparently, adult male crickets taken from an age- and sex-mixed breeding colony already show distinct ethotypes: a proactive type, that is more motile, more likely to approach an agonistic stimulus and generally more aggressive with a higher probability of becoming dominant than others of the reactive type, destined to be subordinate. However, it still remains to be tested whether these seemingly stable ethotypes can be altered by multiple dominant or subjugation experiences (see on *Teleogryllus:* Rudin *et al*., 2017).

Since social isolation is generally associated with aggression, it might be argued that a longer isolation period equalises the inter-individual differences in turning responses found in STI before fighting. However, there were no difference observed in turning responses between groups of adult males isolated for 48 h and 7 days (Fig. 12). This is also corroborated by earlier studies reporting no differences in aggression between adult male crickets isolated for 1, 2 or 6 days (Briffa & Sneddon, 2007). Events during pre-adult life can have long-lasting influences on adult behaviour as already shown in a variety of vertebrates and invertebrates e.g. damselflies (Brodin, 2009), frogs (Urszán *et al*., 2015), golden hamsters (Wommack *et al*., 2003), rodents (Lukkes *et al*., 2009) and zebrafish (Groneberg *et al*., 2020). To further support the hypothesis that the two distinct ethotypes found in STI crickets result from chronic dominance or subjugation during nymphal development and early adult life, adult crickets with different social experiences were tested. Mature adults raised with adult males during development, but isolated before reaching adulthood ($LTI^{w/}$), all avoided the antennal stimulus with no difference between prospective winners and losers (Figs. 13A, left side and 14A). Interestingly, adults raised as nymphs only in the presence of other nymphs and without any contact to adult males ($LTI^{w/o}$), also exhibited no difference between prospective dominants and subordinates, but, in marked contrast to $LTI^{w/}$ adults, also no visible directional response to the touch stimulus (Figs. 13A, right side and 14B). Furthermore, adult females seemingly have no influence on later adult behaviour (Fig.10). Supporting this, nymphs raised together with adult females (LTI$^{w/f}$) showed essentially the same responses as seen in LTI$^{w/o}$ adults (Fig. 14C). Apparently, these differences to STI crickets do not result from chronic social isolation (see Fig. 12), but rather represent an effect of development in the absence of social conflicts, as recently shown in Zebrafish (Menezes *et al*., 2020). However, social isolation can have an influence on various aspects of development, leading to changes in processing environmental and social stimuli with extensive behavioural consequences in vertebrates and invertebrates alike (Cacioppo & Hawkley, 2009; Fogelman & Canli, 2019; Marsden *et al*., 2011; Scharf *et al*., 2021; Tibbetts *et al*., 2019). In cockroaches for example, which are like crickets non-eusocial insects, isolation is observed to induce exploration-avoidance, decreased foraging and reduced social interactions (Lihoreau *et al*., 2009). In adult male crickets, social isolation leads to heightened aggression and exploratory behaviour resulting from the absence of social subjugation by dominant adult males (Stevenson & Rillich, 2013). These findings also support the observation of lacking directional preference in response to an agonistic stimulus in LTI$^{w/o}$ and LTI$^{w/f}$ adults. Accordingly, and corroborating the described results of nymph-nymph interactions, nymphal contacts or the presence of adult females during development do not influence the adult behavioural profile regarding general motility and aggressive behaviour. However, nymphs are frequently attacked by adult males (about once per hour). Therefore, avoidance responses in adults that had contact to adult males, either as nymphs (LTI$^{w/}$) or as young immature adults (STI), are most likely the result of previous chronic social subjugation. After winning or losing a single fight, long term isolates (LTI$^{w/o}$, LTI$^{w/}$ and LTI$^{w/f}$) subsequently showed approach, respectively avoidance behaviour, equal to the observations in STI winners and losers. This difference, however, was no longer evident on the following day. After 6 consecutive defeats, at a frequency matching that of adult attacks endured from nymphs during development (once per hour; Fig. 10B), LTI$^{w/}$ and LTI$^{w/o}$ adult male crickets approach, respectively avoid the stimulus for at least as long as the 6 days tested in this study (Fig. 14B). In conclusion, experiencing chronic subjugation during nymphal development or repeated dominance and subordination in early adult life establishes stable, long-lasting behavioural ethotypes which could account for the predisposed approach or avoidance responses in STI crickets (Fig. 11C).

To further evaluate individual consistency in turning responses, correlation analyses were performed. Results reveal a correlation between turn angles for STI crickets before and after a single fight when social status is disregarded, but not for separate analyses of the data for prospective and actual dominants and subordinates, indicating consistent differences between the two ethotypes, but this does not, however, apply necessarily for individual crickets (Fig. 11E). The actual winner of a dyadic contest is solely determined by the opponent´s aggressive motivation and performed actions (Briffa *et al*., 2015; Rillich *et al*., 2007). Chances are, that the winner would have lost against a different, more aggressive opponent which would have persisted longer in the contest. Furthermore, the pairs were matched by weight alone, disregarding their previously exhibited turn angle. Therefore, the results might be influenced by the few winners that avoided the stimulus and the losers that approached it. Also, similar to the finding that predator stress in crickets disrupts the correlation between consecutive measures of individual *boldness* (Niemelä *et al*., 2012), the consistency of individual turning responses is most likely influenced by an intervening fight. Hence, a comparison of the turn angles of individual STI crickets on 2 consecutive days without a fight reveal a positive correlation for individuals showing avoidance, although not for those exhibiting approach behaviour (Fig. 11F). Additionally, correlation analysis was performed for LTI$^{w/o}$ crickets on day 1 vs day 6 after 6 consecutive fights. This revealed again a positive correlation for 6-fold losers, but not for 6-fold winners. Furthermore, a longitudinal analysis of randomly selected representatives of both winners and losers of 6 preceding contests reveal that inter-individual differences in turn angles were consistent in losers over a period of 6 days after the last fight, but again not for winners (Figs. 14D and E). Although winners show consistency in the positive turning directions after the contests, data reveal a lack of correlation for successive measures of individual turn angles. This is most likely a reflection of their more complex response to the applied stimulus. For example, a greater variability in turn angles for positive turners could arise because they occasionally interrupt their turns with a forward lunge of minimal angular deviation in the near vicinity of the stimulus site and generate body jerks or sing the rival song after confronted with the adult male´s antenna. But, with respect to subordinates at least, crickets do show consistent inter-individual differences in their behavioural response to an agonistic stimulus. Together these observations corroborate numerous studies conducted on crickets showing that dominant crickets tend to be more aggressive and exploratory in a new environment (Abe *et al*., 2018; Kortet & Hedrick, 2007; Rose *et al*., 2017a). The gathered data could also be interpreted as evidence for a proactive-reactive behavioural syndrome (Bell *et al*., 2009; Sih *et al*., 2004). To qualify as a behavioural syndrome, correlation should be between independent behaviours and persist over a certain time (Sih & Bell, 2008). This is, however, not fully achievable in this study, since aggression and turning responses are interdependent behaviours. Nevertheless, the results lend additional support for the hypothesis of a proactive-reactive syndrome in *G. bimaculatus*. Earlier studies show positive associations between activity, exploration and aggression for both contestants, and thus strong evidence for a proactive-reactive syndrome in *G. campestris* (Santostefano *et al*., 2016). Similarly, positive correlations between mating, exploratory and anti-predatory behaviour have been documented in *Acheta domesticus* (Wilson *et al*., 2010) No correlation was found, however, between aggression and exploratory behaviour for this species (Wilson *et al*., 2010), *G. bimaculatus* (Rose *et al*., 2017a) or *G. integer* (Niemelä *et al*., 2012). In these studies, aggression was evaluated in dyadic interactions between weight-matched individuals. As already mentioned, this could be problematic, since an individual's decisions to persist or retreat depends strongly on the opponent´s actions (Briffa *et al*., 2015; Rillich *et al*., 2007) and an intervening fight might disrupt the correlation between consecutive measurements (Niemelä *et al*., 2012). On the basis of these findings a standardised opponent as suggested by Briffa *et al*. (2015) was used to remove a source of within-individual variation. This opponent was made hyper-aggressive by flying in a windstream for 1 min which releases octopamine (Adamo *et al*., 1995; Stevenson *et al*., 2005). Since the focal animals always lost these contests, the time they persist in fighting is a direct measure of the individual aggressive motivation. This revealed a positive correlation for negative turners (Fig. 11G), individuals that exhibit large turn angles away from the stimulus also retreat earlier. Again, this correlation was not observed for positive turners. When taken all STI individuals into account, a positive correlation was also evident between activity and aggression (Tab. 2). Since the decision to approach or avoid an agonistic stimulus is taken within 34 ms (Figs. 11C and D) this excludes complex integrative processing in higher brain centres. Therefore, turning is most likely initiated by 4 fast conducting, descending interneurones, which transmit information from mechanoreceptors located in the antennae to thoracic motor centres (Schöneich *et al*.,

2011) that influence stepping and turning behaviour (Zorovi & Hedwig, 2013). With a latency of 10 ms from tactile stimulus to action potential neck-connective recordings they form a fast-conducting pathway for antennal tactile information (Schöneich *et al*., 2011, see also escape responses in cockroaches, *Periplaneta americana:* Burdohan & Comer, 1996).

4.3 The role of neuromodulators released in response to social experience

Evidence suggests that the decision-making process in crickets depends on the actions of neuromodulators released in response to aggressive social experience, mainly octopamine, serotonin and nitric oxide on which the present study focusses. The decision to persist in fighting or retreat from the interaction is implemented by modulation of the behavioural threshold to flee (review: Stevenson & Rillich, 2019).

This threshold is raised *via* the action of the biogenic amine octopamine, released in response to potentially rewarding experiences, and lowered by the gaseous neurotransmitter nitric oxide, released in response to adverse experiences and thus increases the tendency to retreat (Rillich & Stevenson, 2017; Stevenson & Rillich, 2012; 2015). To analyse how turning responses are influenced by octopaminergic drugs, CDM was used as the tissue permeable OAR-agonist and the receptors were blocked using the antagonist epinastine. This study provides strong evidence that the decision to approach or avoid an agonistic stimulus is differentially modulated *via* the action of octopamine. Furthermore, and supporting this hypothesis, activation or blockage of octopamine receptors evoke the same directional responses in LTI$^{w/o}$ crickets, without any prior agonistic experiences, as otherwise seen in winners, respectively losers of an aggressive interaction.

First, and to exclude detrimental effects of the used drug dosages, motility and aggression against a hyper-aggressive opponent were tested. This revealed no adverse effects on general behaviour of STI adult males (Figs. 15 and 16, Tabs. 3, 4 and 5). Furthermore, the applied drugs influenced both positive and negative turners equally which also confirms the general effects of OA on aggressive behaviour (Figs. 15A and 16A). It is reported that OA promotes not necessarily initiation, but escalation and persistence of fighting (Rillich & Stevenson, 2015) which data also corroborates (Fig. 15A). Those effects are rather unspecific, but with a more sensitive metric it is possible to determine the extent to which OA is involved in the rapid decision-making process to turn towards or away from an agonistic stimulus (Fig. 17). Results reveal that short term isolates, that are predisposed to avoid the touch stimulus, change to approach after treatment with CDM. Conversely, focal animals that initially approached the stimulus show avoidance responses after treatment with the OAR-blocker epinastine without an intervening fight. Furthermore, CDM has no effects on already approaching crickets and epinastine do not influence negative turners. Additionally, both drugs only change the directionality of turns in STI crickets, but not the magnitude. Supporting this, octopamine is reported to shift the behavioural response from indecision to approach (Claßen & Scholz, 2018) and increase aggression in Drosophila (Hoyer *et al*., 2008; Zhou *et al*., 2008) and crickets (Stevenson et al., 2005). As already shown, the behavioral response in reaction to contact with an adult male´s antenna is determined by social status in STI crickets (Fig. 11C). Prospective winners and thus dominant individuals are more likely to approach a stimulus directed at their antenna compared to subordinate individuals. Also the reactions of crayfish to touch depend on their social status, their current social conditions and their recent social history (Song et al., 2006). Similarly, dominant crayfish also turned to face the stimulus source, whereas subordinates avoided it (Song et al., 2006). The present study reveal that this social status-dependent, predisposed reactions can be altered by octopaminergic drugs alone. LTI$^{w/o}$ crickets initially exhibited angular responses that hardly exceeded 20° and further had no predisposition for positive turners and negative turners to win, respectively lose. However, focal animals that show turns in a positive direction again changed the directionality to avoidance after treatment with epinastine and, *vice versa*, negative turners subsequently approach the stimulus after treatment with CDM, but this time with significantly increasing magnitudes at least with respect to positive turners. Moreover, CDM-treated males were far more likely to exhibit lunges, body jerks and the rival song in response to the antennal stimulus with a male´s antenna. This behaviour was never observed in the epinastine treated crickets. All of these effects are typically exhibited by LTI$^{w/o}$ males after single or multiple fights (Figs. 13B and D, 14B), and by STI crickets with previous social experiences in the colony. However, in the present study those reactions are solely evoked by providing an OA-agonist or blocking OA-receptors selectively. Future studies have to show how long this octopaminergic drug induced effects in LTI$^{w/o}$ adult males (Figs. 17D-F) actually last and whether chronic defeat or dominance experiences could be mimicked or even reversed by treating cricket males with octopaminergic drugs over time in various intervals.

Previous studies have shown that although antennal stimulation activates octopaminergic neurons in the locust brain (Duch et al., 1999), octopaminergic drugs neither block nor promote the responsiveness to a male antenna with regards to the mandible threat frequency in crickets (Rillich & Stevenson, 2015). With the single, brief antennal touch applied in the present study a mandible threat was never triggered. Nevertheless, the choice to turn towards an adverse signal or away from it and thus the decision to approach or avoid is taken rapidly within only 34 ms. This excludes the involvement of complex integration processes necessary for initiating fighting and is, therefore, solely dependent on the actions of the accessible quantity of neuromodulators.

Furthermore, the turning responses of STI adult males are also influenced by the actions of nitric oxide (Fig. 18). NO is considered to be an important signalling molecule in various physiological processes, but the role of NO in aggression exhibited by invertebrates is still discussed. Earlier reports indicate that NO enhances aggressiveness (Dyakonova & Krushinskii, 2006). In contrast, Iwasaki et al. (2007) found that blocking NOS relieves the loser effect. Further supporting the idea of an adverse effect of NO on aggression, it has been reported that the social decision to flee can be implemented by a comparatively simple mechanism in crickets. The experience of antennal contact with a conspecific male establishes an aggressive behavioral context, and subsequent sensory aversive stimuli can act to induce production of the neuromodulator NO (Rillich & Stevenson, 2017). Rather than suppressing aggressive motivation, NO increases the susceptibility to aversive stimuli and lowers with that the threshold to flee (Stevenson & Rillich, 2015). Supporting these findings, focal animals predisposed to avoid the touch stimulus change on average to approach after inhibiting NO synthesis with L-NAME (Fig. 18). Similarly, treatment with L-NAME increased aggressiveness with regards to persistence against a hyper-aggressive opponent in both positive and negative turners (Fig. 15B). In marked contrast, NO-donor SNAP leads to an overall reduction of aggressiveness (Stevenson & Rillich, 2015) and influences the general response to an antennal stimulus. Both positive and negative turners exhibit significantly smaller turn angles after treatment with SNAP (Figs. 18B and C). Precisely, hardly any SNAP treated cricket exhibited a clear directional response exceeding 20° in magnitude during experimentation. Studies on *Manduca sexta* revealed that NO-sensitive cells are expressed in the olfactory system and NOS is expressed in high levels in the antennae and in antennal lobe neurones (Nighorn *et al*., 1998). NO signalling is, therefore, involved in communication between olfactory receptor neurones and projection neurones in the glomeruli of the antennal lobe. In crickets, antennal contact with a conspecific comprises both mechanical and olfactory information (Sakura & Aonuma, 2013; Rillich & Stevenson, 2015). Present results suggest that the NO-donor SNAP either influences the sensory perception of the applied stimulus or the conduction and integration within the antennal lobe which than blocks the approach or avoidance reaction and crickets exhibit no turns after the stimulus is applied. Similarly, *Rhodnius prolixus,* a blood sucking buck, shows reluctance to feed and focal animals were less attracted to a CO_2 source after an NO-donor was topically applied to the antennae (Sfara *et al*., 2011).

So far there is evidence that both OA and NO are involved in the rapid approach and avoidance response of male crickets. Short term isolates predisposed to avoid the touch stimulus changed to approach after OAR-agonist treatment (Fig. 17) and also after inhibiting NO-synthesis (Fig. 18). Conversely, individuals initially predisposed to approach the stimulus showed avoidance after injection of an OA-blocker. Both behavioural ethotypes subsequently exhibited no clear turning responses after treatment with a NO-donor. Combinations of drugs with opposing effects on turning behaviour revealed the extent to which both neuromodulators contribute to the decision-making process and first conclusions as to whether SNAP modulates peripheral sensory perception or rather central integration of the signal (Fig. 19). When crickets are treated with an OAR-blocker together with a NOS-inhibitor, the resulting turning reactions are equal to those exhibited after treatment with the OAR-blocker only. Adult males predisposed to approach the stimulus change to avoidance. For comparison (Fig. 18) the NOS-inhibitor should have changed the directionality of turns for negative turners. Similarly, when injected with an OAR-agonist and NO-donor, only the OAR-agonist effects the directionality of turns. Together these reactions to antennal touch after treatment with a combination of octopaminergic and nitridergic drugs are essentially the responses after epinastine or CDM were provided alone.

Interestingly, however, the NO-donor SNAP has no influence on the magnitude of turns when injected together with CDM. This provides first evidence that either the inhibitory effect of NO on sensory perception and central integration is reversible by providing OA simultaneously or the effect of NO is mainly directed at the NOS in antennal lobe neurones and also dependent on OA.

While the role of serotonin is still widely discussed, mounting evidence indicates that it plays an important role in the control of aggressive behavior in insects. However, this study hypothesised that OA and NO, but not 5HT influence general aggression, motility and the decision to approach or avoid in short term, socially isolated, adult male crickets. Activation of serotonin synthesis is reported to promote aggression (Dierick & Greenspan, 2007). Furthermore, serotonin depletion reduces the probability of winning and enhances the escape responses (Dyakonova *et al*. 1999; Stevenson *et al*. 2000). The precise role of 5HT during fighting behaviour in crickets is uncertain (Dyakonova & Kruschinsky, 2013; Stevenson & Rillich, 2017). In the present study, no specific effects on initial fights and motility (Figs. 15C and D, 16C and D) resulting from treatment with serotonin-agonists and -antagonists were found. None of the used drugs had an influence on persistence against a hyper-aggressive opponent or on general movement compared to the vehicle group. This confirms previous studies in which no clear serotonergic effect was found on socially naive crickets (Stevenson *et al*., 2000; Rillich & Stevenson, 2015; 2017). In Drosophila, a group of serotonergic neurones was described to be essential for decision-making processes during agonistic interactions (Alekseyenko *et al*., 2010). Interestingly, some of the 5HT-antagonists and -agonists used in the present study have an influence on turning responses to an antennal stimulus in STI male crickets (Fig. 21). With respect to predisposed positive turners both $5HTR_2$-blocker ketanserin and $5HTR_{1A}$-blocker WAY disrupt group directionality significantly in that an equal number of adults exhibit positive and negative turns after the treatment. This tendency is also visible in negative turners, but not statistically significant. Similarly, agonists have the same effects on both positive and negative turners. Crickets exhibit no directional preferences after treatment with serotonin agonists when the whole test group is taken into account. Importantly, however, neither antagonists nor agonist have any influence on turning magnitudes as seen in SNAP treated crickets. Apparently, the stimulus perception is unaffected, but the predisposed reaction to an agonist stimulus is disrupted and individuals are not as consistent. Therefore, the hypothesis that 5HT has no influence on the decision to approach or avoid is not fully tenable, but the effects are, however, rather unspecific in 48 h socially isolated crickets. This is no surprise since serotonin is mainly reported to be released in invertebrates after defeat to keep the threshold after the aggressive interactions low, so that losers remain submissive, it also influences loser-recovery after defeat and the establishment of long-term depression following chronic subjugation (Rillich & Stevenson, 2018).

The present study provides evidence for the additional hypothesis that 5HT only influences the decision-making process in adult male crickets when a behavioural context as chronic defeat stress or multiple winning experiences is involved. To exclude inter-individual differences, $LTI^{w/o}$ adults without any contact to adults during development and early adult life and, therefore, no predispositions to approach or avoid the touch stimulus were used. In invertebrates, several types of 5HT receptors are described, expressed in different tissues and at various expression levels (Watanabe *et al*., 2011), but $5HT_2$ has proven to be the most promising target for further investigations regarding the decision-making processes in crickets, specifically after chronic wins and defeats.

In STI male crickets, earlier studies revealed that ketanserin acts to inhibit the acquisition of submissiveness in losers, prevent chronic defeat and blocks long term depression after multiple defeats (Rillich & Stevenson, 2018). DOI, as an agonist for the same receptor subtype, decreases overall aggression in Drosophila (Johnson *et al*., 2009), acts to inhibit loser recovery and promotes long term chronic defeat (Figs. 20C and 22) when applied 1 h before the interactions. Untreated $LTI^{w/o}$ adults show little angular magnitude after the isolation period, but just after 6 wins or defeats they clearly turn towards, respectively away from the stimulus (Figs. 13D, right side and 14B). To exclude any effects of the handling during application and from the saline or DMSO, vehicle was applied 1 h before the fights. This revealed that neither the application method nor the DMSO or saline have an effect on the turning responses. Ketanserin, however, which blocks $5HTR_2$ inhibits the effect of chronic defeat in 6-fold losers completely (see also Rillich & Stevenson, 2018; Fig. 22) in that turn magnitude and direction are equal to those in untreated prospective losers. Contrasting this, the $5HTR_2$-agonist DOI partly inhibits the establishment of a dominant ethotype when the whole test group is taken into account. The average turn angle does not differ from the prospective winners, but there is a significant change in turning magnitude after 6 consecutive wins. Since DOI inhibits the recovery from chronic defeat (Fig. 20C) and alters the responses of multiple winners (Fig. 22), it may act as a modulator in the assessment of the opponent’s agonistic signals (Payne, 1998). This posits that individuals evaluate their opponent´s actions and retreat when the sum exceeds their inherent threshold to flee. During each fight, winners also receive adverse experiences performed by the opponent such as bites or pushes. The only difference to later losers is, that these experiences do not exceed the threshold to flee and the loser is the first to retreat. A possible explanation for the effect of DOI is, therefore, that this agonistic experiences are evaluated differently and that DOI, most likely together with NO, lowers the threshold to flee although the crickets previously won the fight. This would also explain the higher amount of negative turners after 6 consecutive wins after DOI treatment. However, this hypothesis needs more experimental evidence in future studies. The overall tenor of this experiments is that drugs, which pharmacologically increase, deplete or mimic the actions of naturally occurring neuromodulators, do not work independently and in isolated systems. Effects are only partly observed and the influences on the whole nervous system with behavioural consequences remain unclear. With whole-animal pharmacology, resulting effects are not always apparent, drugs target numerous receptors and receptor subtypes which all have several, partially interdependent functions. Even when the receptor chemistry and the role of a neuromodulator are well understood, behavioural context is required and important to consider. An individual’s decisions to persist or retreat depends strongly on the opponent´s actions (Briffa *et al*., 2015; Rillich *et al*., 2007). On the basis of these findings a standardised opponent was used (Briffa *et al*., 2015) to remove a source of within-individual variation. Also agonistic or rewarding experiences e.g. from an intervening fight disrupt the consecutive measurements of the turn angles so all focal animals had to be tested before an agonistic interaction just after different isolation periods. Using LTI$^{w/o}$ crickets removed inter-individual variability and thus gives an insight into the mere effect of drugs on turning behaviour. This study made also an attempt to circumvent influences and interactions by considering independent variables and behavioural traits and working with partly antagonist drugs. Environmental influences were also kept to a minimum in order to avoid any influence on the results. Turn angle, magnitude, motility and aggression are different behavioural traits, yet in part interdependent, but a possibility to narrow the precise area where treatments target and which behavioural consequences result from this. General, unspecific effects on motility and aggression were observed as well as specific, differential drug influences on turning responses, which has proven to be a highly sensitive metric.

4.4 Overall conclusion and outlook

Together this study shows that an individual´s decision to approach or avoid an agonistic stimulus is shaped by social experiences during development and early adult life. In particular, the chronic subjugation of nymphs by adult males in the breeding colony, and the absence thereof, lead to the establishment of distinct behavioural ethotypes shifting the answer to the question of whether inter-individual differences are nature or nurture in favour of nurture. Individuality in adult behaviour can thus result solely from social experiences gathered during pre-adult life. Moreover, the decision to approach or avoid a potentially agonistic stimulus is differentially modulated by the actions of the neuromodulators octopamine, serotonin and nitric oxide, which are released in response to social interactions. Interestingly, the social status dependent predisposed response to an antennal stimulus can be altered by octopaminergic drugs alone. The nitridergic and serotonergic system play a major role in the assessment of agonistic signals. Nevertheless, it is important to note that whole-animal pharmacology affects several interdependent systems and resulting effects are not always apparent. Behavioural context needs to be considered, even when the receptor chemistry and the role of a neuromodulator are reasonably clear.

Crickets can implement complex decisions by differentially modulate the threshold to persist in fighting or retreat from the interaction *via* the actions of neuromodulators. Excluding complex integration processes and consciousness with the key roles of OA and NO revealed, this opens opportunities on more complex brains in mammals since there are some notable similarities in the neurochemical control and the mechanism underlying aggression (Thomas *et al*., 2015; Stevenson & Rillich, 2017; 2019). This may spark new thoughts on how the respective amines noradrenaline, adrenaline and NO function in mammalian aggression, which is not yet fully understood. Although crickets have a comparatively simpler nervous system, practically nothing is known about the involved neurones. However, by using genetic tools neurones containing biogenic amines and thus influence aggression have been identified in Drosophila. These techniques are also attempted on crickets with promising success (Watanabe *et al*., 2011), so that crickets could develop further as a model system of neurochemical control of aggression. Interestingly, crickets are also evolving as a model insect for learning and memory. Although remarkable work is done on honey bees (Menzel, 2001; 2012) and Drosophila (Widmann *et al*., 2018), current studies on crickets employed a binary regression model for a differential olfactory learning paradigm to measure individual learning scores of adult male crickets (Borstel & Stevenson, 2021). Recent work also reveals that the experience of repeated social defeat impairs olfactory learning. The neurotransmitter systems involved are currently under investigation (Borstel & Stevenson, in preparation).

5 Summary

Aggression among conspecifics is a widely distributed and highly plastic behaviour observed in almost all species throughout the animal kingdom. Agonistic encounters serve to secure resources, as members of the same species compete for identical mating partners, food, burrows and territory. But the costs of aggression can rapidly exceed its advantages. In recent years, the two-spotted Mediterranean field cricket, *Gryllus bimaculatus*, has evolved to a model organism for studying the mechanisms underlying aggressive behaviour (reviews: Stevenson & Rillich, 2012; 2019; Simpson & Stevenson, 2015). Adult male crickets exhibit a highly stereotypic, yet easily quantifiable fighting behaviour, while their nervous system is comparatively simple containing relatively few, but individually identifiable neurones. Crickets can implement seemingly complex decisions *via* the action of well-known neuromodulators with analogues in vertebrates including humans. Experiencing chronic social subjugation and dominant-subordinate relationships are recognised as major factors inducing depression and depression-like symptoms in humans and animals alike (de Boer *et al*., 2016) and are, therefore, suspected to have a major impact on the establishment of consistent inter-individual behavioural differences in mammals, commonly referred to as *personality*. One of the most influential recent findings in the field of animal behaviour is that many invertebrate species, including insects, exhibit consistent inter-individual differences in specific suites of behaviour (Bell *et al*., 2009; Carter *et al*., 2013; Sih *et al*., 2014; Wilson *et al*., 2019), here referred to as *individuality*, a less anthropomorphic term. Moreover, behavioural traits are often found to correlate across time and different contexts forming *behavioural syndromes* (Sih *et al*., 2004a; 2014). However, the proximate mechanisms that generate individuality and distinct behavioural phenotypes are still poorly understood (Wilson *et al*., 2019).

Adult male crickets are reported to show consistent inter-individual differences in aggression, general motility and exploratory behaviour (e.g. Dochtermann & Nelson, 2014; Fisher *et al*., 2015; Niemelä *et al*., 2012;2015; Rose *et al*., 2017a; Rodríguez-Muñoz, 2010; Wilson *et al*., 2010). Interestingly, the same behavioural differences were found in the prospective winners and losers before dominance and subordinate hierarchy is established (Rose *et al*., 2017a). This supports the hypothesis that consistent inter-individual differences are shaped by events during nymphal development and early adult life.

First, the interactions of male last instar nymphs and fully mature adults with conspecifics of both ages and sexes were observed to investigate the extent to which pre-adult agonistic experiences can induce consistent behavioural changes which could account for different behavioural phenotypes in adults. This revealed that aggressive behaviour is far less fierce in nymphs, and that their interactions do not result in winner or losers effects typical for adults. Furthermore, nymphs neither produce nor recognise the typical adult pheromonal signature during antennal contact that comprises both mechanical and olfactory information and is considered to be the natural aggression releasing stimulus (Hofmann & Schildberger, 2001). Notwithstanding this, it was observed that adult males aggressively intimidate male nymphs in the age- and sex-mixed breeding colony with a frequency sufficient to induce long-term depression after chronic defeat in adults (once per hour), and this study provides indications that this has long-lasting, possibly life-long effects and thus influences future adult behavioural profiles. To further test this hypothesis, crickets were raised under different conditions with various social compositions of the colony and tested subsequently for their general motility, aggression, and the turning responses as a reaction to an agonistic stimulus directed at one antenna with the freshly excised antenna of a donor male.

STI adult males, with contact to other adult males during development and early adulthood, isolated for 48 h prior experimentation; LTI$^{w/}$, with contact to adult males during nymphal development only, but isolated at their last nymphal instar until fully mature adult; LTI$^{w/o}$, which had no contact to adult males during their whole life and LTI$^{w/f}$, which were raised together with adult females. Other nymphs of both sexes were present in each colony composition. Notably, however, contact to adult females do not result in any adverse effects in mature adult males. The experiments conducted with these test groups showed that STI adult males taken from an age- and sex-mixed breeding colony already show distinct ethotypes: a proactive type, that is more motile, more likely to approach an agonistic stimulus and generally more aggressive with a higher probability of becoming dominant than others of the reactive type, destined to be subordinate. Interestingly, crickets taken from the LTI colonies exhibited no such differences. LTI$^{w/}$ adult males all turned away from the stimulus side whereas LTI$^{w/o}$ and LTI$^{w/f}$, which never experienced repeated subjugation during development showed no directional preference and generally exhibited small turning reactions. After winning or losing just one contest, the differences in the decision to approach or avoid the stimulus were significant in that winners turned towards the stimulus side and losers away from it. This effect was, however, no longer evident 24 h later. After winning, respectively losing 6 consecutive fights the differences were consistent at least for the 6 days subsequently tested. Additionally, turn angle and aggression were also correlated and the turning magnitude was consistent over time with respect to negative turners/losers. Although these are partly interdependent metrics, the consistency across time provides evidence for a behavioural syndrome. These results indicate that an individual´s decision to approach or avoid an agonistic stimulus is shaped by social experiences gathered during development and early adult life. In particular, the chronic subjugation of nymphs by adult males in the breeding colony and the absence thereof lead to the establishment of distinct behavioural ethotypes shifting the answer to the question of whether inter-individual differences are nature or nurture in favour of nurture. Individuality in adult behaviour can thus result from social experiences during development alone. Moreover, the decision to approach or avoid a potentially agonistic stimulus is differentially modulated by the actions of the neuromodulators octopamine, serotonin and nitric oxide, which are released in response to social interactions. This study provides strong evidence that the decision to approach or avoid is differentially modulated *via* the action of octopamine. Focal animals predisposed to avoid the stimulus changed to approach behaviour after treatment with OAR-agonist CDM. Conversely, individuals initially approached the touch stimulus changed to avoidance after injection of OAR-blocker epinastine. Furthermore, and supporting this hypothesis, activation or blockage of octopamine receptors evoke strong directional responses in LTI$^{w/o}$ crickets as otherwise seen only in winners, respectively losers of an aggressive interaction, but without any aggressive experience. Furthermore, the study reveals that the nitridergic and serotonergic system play a major role in the assessment of agonistic signals. Focal animals predisposed to avoid the touch stimulus changed on average to approach after inhibiting NO synthesis with L-NAME. Injection of SNAP, however, altered the general responses to the stimulus in that crickets exhibited subsequently no turns exceeding 20°. Present results suggest that the NO-donor SNAP either influences the sensory perception of the applied stimulus or the conduction and integration within the antennal lobe which than blocks the approach or avoidance reaction and crickets exhibit no turns after the stimulus is applied. Together this indicates that both OA and NO are involved in the rapid approach and avoidance response of male crickets. Combinations of treatments with opposing effects on turning behaviour revealed the extent to which both neuromodulators contribute to the decision-making process and lead to first conclusions as to whether SNAP modulates peripheral sensory perception or rather central integration of the signal. The reactions to treatment with either CDM in combination with SNAP or epinastine combined with L-NAME were essentially the responses of CDM or epinastine provided alone. Interestingly, however, the NO-donor SNAP has no influence on the magnitude of turns when injected together with CDM. This provides first evidence that either the inhibitory effect of NO on sensory perception and central integration is reversible by providing OA simultaneously or the effect of NO is mainly directed at the NOS in antennal lobe neurones and also dependent on OA. While the role of serotonin is still widely discussed, mounting evidence indicates that it plays an important role in the control of aggressive behavior in insects. While some of the 5HT-antagonists and -agonists used in the present study have an influence on turning responses to an antennal stimulus in STI male crickets, these effects were unspecific and treatment rather disrupted group directionality than changed the individual decision to approach or avoid the stimulus. This is not surprising since serotonin is in invertebrates mainly reported to be released after defeat to keep the threshold after the aggressive interactions low, so that losers remain submissive, it also influences loser-recovery after defeat and the establishment of long-term depression following chronic subjugation (Rillich & Stevenson, 2018). The present study provides evidence for the additional hypothesis that 5HT only influences the decision-making process in adult male crickets when a behavioural context such as chronic defeat stress or multiple winning experiences is involved. In invertebrates, several types of 5HT receptors are described, expressed in different tissues and at various expression levels (Watanabe *et al.*, 2011), but $5HT_2$ has proven to be the most promising target for further investigations regarding the decision-making processes in crickets, specifically after chronic wins and defeats. Earlier studies revealed that ketanserin acts to inhibit the acquisition of submissiveness in losers, prevents chronic defeat and blocks long term depression after multiple subjugation (Rillich & Stevenson, 2018). DOI, as an agonist for the same receptor subtype (Johnson *et al*., 2009) acts to inhibit loser recovery and promotes long term chronic defeat. To exclude any effects resulting from previous interactions, $LTI^{w/o}$ males were used in this experiment. Injected 1 h before chronic defeat or winning experiences, the $5HTR_2$-blocker ketanserin inhibits the effect of chronic defeat in 6-fold losers completely (see also Rillich & Stevenson, 2018). Contrasting this, the $5HTR_2$-agonist DOI partly inhibits the establishment of a dominant ethotype when the whole test group is taken into account.

Since DOI inhibits the recovery from chronic defeat and alters the responses of multiple winners, it may act also as a modulator in the assessment of the opponent's agonistic signals. During each fight, winners receive adverse experiences performed by the opponent such as bites or pushes. The only difference to later losers is, that these experiences do not exceed the threshold to flee and the then established loser is the first to retreat. A possible explanation for the effect of DOI is, therefore, that this agonistic experiences are evaluated differently and that DOI, most likely together with NO, lowers the threshold to flee although the crickets won the fight. This would also explain the higher amount of negative turners after 6 consecutive wins after DOI treatment. Since the decision to approach or avoid an agonistic stimulus is taken rapidly within 30 to 40 ms this excludes complex integrative processing in higher brain centres. Therefore, turning is most likely initiated by 4 fast conducting, descending interneurones, which transmit information from mechanoreceptors located in the antennae to thoracic motor centres (Schöneich *et al*., 2011) and is, therefore, solely dependent on the actions of the accessible quantity of neuromodulators. Nevertheless, it is important to note that drugs which pharmacologically increase, deplete or mimic the actions of naturally occurring neuromodulators do not work independently and in isolated systems. Whole-animal pharmacology affects several, partially interdependent systems and resulting effects are not always apparent. Even when the receptor chemistry and the role of a neuromodulator are reasonably clear, consideration of the behavioural context is always required.

Parts of this study are already published:

Balsam & Stevenson, 2020: Pre-adult aggression and its long-term behavioural consequences in crickets. *PLoS One*

Balsam & Stevenson, 2021: Agonistic experience during development establishes inter-individual differences in approach-avoidance behaviour of crickets. *Scientific Reports*

6 Zusammenfassung

Aggression unter Artgenossen ist ein weit verbreitetes, hoch plastisches Verhalten, das bei fast allen Arten im gesamten Tierreich zu beobachten ist. Agonistische Begegnungen dienen der Ressourcensicherung, da Mitglieder derselben Art um Paarungspartner, Nahrung, Höhlen und Territorien konkurrieren. Doch die Kosten der Aggression können ihre Vorteile schnell übersteigen. In den letzten Jahren hat sich die Mittelmeer-Feldgrille, *Gryllus bimaculatus*, zu einem Modellorganismus für die Untersuchung der Mechanismen entwickelt, die dem aggressiven Verhalten zugrunde liegen (Reviews: Stevenson & Rillich, 2012; 2019; Simpson & Stevenson, 2015). Adulte, männliche Grillen zeigen ein hochgradig stereotypes, dabei aber leicht quantifizierbares Kampfverhalten, während ihr Nervensystem vergleichsweise einfach aufgebaut ist und relativ wenige, dafür jedoch individuell identifizierbare Neuronen enthält. Grillen können komplex erscheinende Entscheidungen durch die Ausnutzung der Wirkungsweise bereits hinreichend bekannter Neuromodulatoren treffen, die in Wirbeltieren, einschließlich Menschen, Analoga aufweisen. Die Erfahrung chronischer sozialer Unterwerfung und Dominanz-Subordination-Hierarchien gelten als wichtiger Faktor für die Entstehung von Depressionen und depressionsähnlichen Symptomen sowohl bei Menschen als auch bei Tieren (de Boer *et al*., 2016) und stehen daher im Verdacht, einen wesentlichen Einfluss auf die Entstehung konsistenter, inter-individueller Verhaltensunterschiede bei Säugetieren zu haben, die gemeinhin als Persönlichkeit bezeichnet werden. Eine der einflussreichsten und neusten Erkenntnisse auf dem Gebiet der Erforschung des Verhaltens von Tieren ist, dass viele wirbellose Tierarten, darunter auch Insekten, konsistente, inter-individuelle Unterschiede in bestimmten Verhaltensweisen ausbilden (Bell *et al*., 2009; Carter *et al*., 2013; Sih *et al*., 2014; Wilson *et al.,* 2019), die in der vorliegenden Arbeit als Individualität bezeichnet werden, ein weniger anthropomorphisierter Begriff. Darüber hinaus wird häufig berichtet, dass Verhaltensmerkmale über Zeit und verschiedene Kontexte hinweg korrelieren und Verhaltenssyndrome bilden (Sih *et al*., 2004a; 2014). Die zugrundeliegenden Mechanismen, die Individualität und unterschiedliche Verhaltensphänotypen hervorbringen, sind bisher jedoch noch wenig bekannt (Wilson *et al*., 2019). Studien zeigen weiterhin, dass erwachsene, männliche Grillen konsistente, inter-individuelle Verhaltensunterschiede in Bezug auf Aggression, allgemeine Beweglichkeit und Exploration aufweisen (siehe z. B. Dochtermann & Nelson, 2014; Fisher *et al*., 2015; Niemelä *et al*., 2012;2015; Rose *et al*., 2017a; Rodríguez-Muñoz, 2010; Wilson *et al*., 2010). Interessanterweise wurden die gleichen Verhaltensunterschiede ebenso bei den prospektiven Gewinnern und Verlierern eines Kampfes gefunden, noch bevor Dominanz und Unterordnung etabliert worden ist (Rose *et al*., 2017a). Dies unterstützt die aufgestellte Hypothese, dass konsistente, inter-individuelle Unterschiede des Verhaltens hauptsächlich durch Ereignisse während der Nymphenentwicklung und des frühen Erwachsenenlebens geprägt sind.

Zunächst wurden die Interaktionen von männlichen Nymphen des letzten Stadiums und ebenso vollständig adulten Tieren mit Artgenossen beider dieser Altersgruppen und Geschlechter beobachtet, um zu untersuchen, inwieweit agonistische Erfahrungen während der Nymphenentwicklung konsistente Verhaltensänderungen hervorrufen können, die für unterschiedliche Verhaltensphänotypen bei Adulten Grillen verantwortlich sind. Dabei zeigte sich, dass das aggressive Verhalten bei Nymphen weit weniger ausgeprägt ist und dass ihre Interaktionen nicht zu den für Adulte Männchen typischen Gewinner- oder Verlierereffekten führen. Weder produzieren, noch erkennen Nymphen die typische Pheromon-Signatur der Adulten Männchen und Weibchen während des Antennenkontakts, der sowohl mechanische als auch olfaktorische Informationen beinhaltet und als natürlich vorkommender, aggressionsauslösender Reiz gilt (Hofmann & Schildberger, 2001). Ungeachtet dessen wurde beobachtet, dass adulte Männchen männliche Nymphen in der alters- und geschlechtsgemischten Brutkolonie mit einer Häufigkeit angreifen, die ausreicht, um nach chronischen Niederlagen bei Adulten eine langfristige Depression auszulösen (ungefähr einmal pro Stunde). Weiterhin liefert diese Arbeit Anhaltspunkte dafür, dass dies langanhaltende, möglicherweise lebenslange Auswirkungen hat und somit künftige Verhaltensprofile der Adulten Grillen beeinflusst. Um diese Hypothese weiterführend zu testen, wurden Grillen unter verschiedenen Bedingungen mit unterschiedlicher sozialer Zusammensetzung der Kolonie aufgezogen und anschließend ihre allgemeine Motilität, Aggression und die Drehreaktionen als Reaktion auf einen agonistischen Reiz getestet. Dieser Stimulus wurde mit einer frisch abgetrennten Antenne eines adulten Männchens ausgeführt.

STI adulte Männchen, die während ihrer Entwicklung und im frühen Erwachsenenalter Kontakt zu anderen adulten Männchen hatten und vor den Experimenten 48 Stunden isoliert wurden; LTI$^{w/}$, welche nur während ihrer Nymphenentwicklung Kontakt zu adulten Männchen hatten, aber in ihrem letzten Nymphenstadium bis zur vollständig adulten Grille isoliert wurden; LTI$^{w/o}$, die während ihres gesamten Lebens keinen Kontakt zu adulten Männchen hatten und LTI$^{w/f}$, welche zusammen mit adulten Weibchen aufgezogen wurden. Andere Nymphen beider Geschlechter waren in jeder Koloniezusammensetzung vorhanden. Besonders hervorzuheben ist jedoch, dass der Kontakt zu erwachsenen Weibchen keine negativen Auswirkungen auf die ausgewachsenen Männchen hat. Die durchgeführten Experimente mit diesen Testgruppen zeigten, dass erwachsene STI-Männchen aus einer alters- und geschlechtsgemischten Zuchtkolonie bereits unterschiedliche Ethotypen aufweisen: einen proaktiven Typ, der beweglicher ist, sich eher einem agonistischen Reiz nähert und im Allgemeinen aggressiver ist, mit einer höheren Wahrscheinlichkeit, dominant zu werden, als andere des reaktiven Typs, die dazu bestimmt sind, untergeordnet zu sein. Interessanterweise wiesen die Grillen aus den LTI-Kolonien keine derartigen Unterschiede auf. Ausgewachsene LTI$^{w/}$-Männchen drehten sich alle von dem Reiz weg, während LTI$^{w/o}$ und LTI$^{w/f}$, die während ihrer Entwicklung nie wiederholt unterworfen wurden, keine Richtungspräferenz und im Allgemeinen nur geringe Drehreaktionen zeigten. Nach nur einem gewonnenen oder verlorenen Wettbewerb waren die Unterschiede in der Entscheidung, sich dem Reiz zu nähern oder ihn zu meiden insofern signifikant unterschiedlich, als dass die Gewinner sich dem Reiz zu- und die Verlierer sich von diesem abwandten. Dieser Effekt war jedoch 24 Stunden später nicht mehr erkennbar. Nach 6 gewonnenen bzw. verlorenen Kämpfen in Folge blieben die Unterschiede zumindest für die gemessenen folgenden 6 Tage bestehen. Darüber hinaus korrelierten auch Drehwinkel und Aggression miteinander und das Ausmaß der Drehung war, zumindest in Bezug auf negative Dreher/Verlierer, über die Zeit hinweg konsistent. Obwohl es sich hierbei teilweise um voneinander abhängige Messgrößen handelt, liefert die zeitliche Konsistenz Hinweise auf ein Verhaltenssyndrom. Diese Ergebnisse deuten darauf hin, dass die Entscheidung eines Individuums, sich einem agonistischen Reiz zu nähern oder ihn zu meiden, von sozialen Erfahrungen geprägt ist, die während der Entwicklung und im frühen Erwachsenenalter gesammelt wurden. Insbesondere die chronische Unterwerfung von Nymphen durch erwachsene

Männchen in der Brutkolonie und das Fehlen dieser Unterwerfung führen zur Etablierung unterschiedlicher Verhaltensethotypen, wodurch sich die Antwort auf die Frage, ob interindividuelle Unterschiede naturgegeben sind oder durch soziale Erfahrungen entstehen, zugunsten der Erfahrung und des Umfelds verschiebt. Individualität im Verhalten adulter Grillenmännchen kann also allein aus sozialen Erfahrungen während der Entwicklung resultieren. Darüber hinaus wird die Entscheidung, sich einem potentiell agonistischen Reiz zu nähern oder ihn zu meiden, durch die Wirkung der Neuromodulatoren Oktopamin, Serotonin und Stickstoffmonoxid, die nach und während sozialer Interaktionen freigesetzt werden, unterschiedlich moduliert.

Diese Studie liefert deutliche Hinweise darauf, dass die Entscheidung, sich einem agonistischen Reiz zu nähern oder ihn zu meiden, durch die Wirkung von Oktopamin differentiell moduliert werden kann. Zu untersuchende Tiere, die prädisponiert waren, den Reiz zu meiden, wechselten nach der Behandlung mit dem OAR-Agonisten CDM zum Annäherungsverhalten. Umgekehrt gingen Individuen, die sich dem Berührungsreiz zunächst hinwandten, nach der Injektion des OAR-Blockers Epinastin zum Meide-Verhalten über. Darüber hinaus, und diese Hypothese unterstützend, löst die Aktivierung oder Blockierung von Oktopaminrezeptoren bei LTI$^{w/o}$-Grillen ausgeprägte Richtungsreaktionen aus, wie sie sonst nur bei Gewinnern bzw. Verlierern einer aggressiven Interaktion, aber in diesem Fall ohne jegliche aggressive Erfahrung, zu beobachten sind. Darüber hinaus zeigt die Studie, dass das nitriderge und serotonerge System eine wichtige Rolle bei der Beurteilung von agonistischen Signalen spielt. Fokale Tiere, die prädisponiert waren, den Berührungsreiz zu meiden, gingen nach der Hemmung der NO-Synthese mit L-NAME im Durchschnitt dazu über, sich zuzuwenden. Die Injektion von SNAP hingegen verändert die generelle Reaktion auf den Reiz dahingehend, dass Grillen anschließend keine Drehungen von mehr als 20° zeigten. Die vorliegenden Ergebnisse deuten darauf hin, dass der NO-Donor SNAP entweder die sensorische Wahrnehmung des applizierten Reizes oder die Reizleitung und -integration innerhalb des Antennallappens beeinflusst, wodurch die Annäherungs- oder Vermeidungsreaktion blockiert wird und die Grillen nach der Applikation des Reizes keine Drehungen mehr zeigen. Zusammengenommen deutet dies darauf hin, dass sowohl OA als auch NO an der schnellen Annäherungs- und Vermeidungsreaktion der männlichen Grillen beteiligt sind. Kombinationen von Pharmaka mit gegensätzlichen Wirkungen auf das Drehverhalten zeigten das Ausmaß, in dem beide Neuromodulatoren zum Entscheidungsprozess beitragen und ließen erste Rückschlüsse darauf zu, ob SNAP die periphere sensorische Wahrnehmung oder eher die zentrale Integration des Signals moduliert. Die Reaktionen auf die Behandlung mit CDM in Kombination mit SNAP oder Epinastin kombiniert mit L-NAME entsprachen im Wesentlichen den Reaktionen nach der alleinigen Gabe von CDM oder Epinastin. Interessanterweise hat jedoch der NO-Donor SNAP keinen Einfluss auf das Ausmaß der Drehungen, wenn er zusammen mit CDM injiziert wird. Dies ist ein erster Hinweis darauf, dass entweder die hemmende Wirkung von NO auf die Sinneswahrnehmung und die zentrale Integration durch die gleichzeitige Gabe von OA umkehrbar ist oder dass die Wirkung von NO hauptsächlich auf die NOS in den Neuronen des Antennallappens gerichtet und ebenfalls von OA moduliert wird.

Während die Rolle von Serotonin noch immer diskutiert wird, gibt es immer mehr Hinweise darauf, dass es eine wichtige Rolle bei der Kontrolle von aggressivem Verhalten bei Insekten spielt. Zwar haben einige der in der vorliegenden Studie verwendeten 5HT-Antagonisten und Agonisten einen Einfluss auf die Drehantworten auf einen Antennenreiz bei männlichen STI-Grillen, doch waren diese Wirkungen unspezifisch und die Behandlung veränderte eher die Gruppenorientierung als dass sie die individuelle Entscheidung, sich dem Reiz zu nähern oder ihn zu meiden, modifizierte. Dies ist nicht überraschend, da Serotonin bei Wirbellosen hauptsächlich nach Niederlagen freigesetzt wird, um die Schwelle nach aggressiven Interaktionen niedrig zu halten, so dass Verlierer unterwürfig bleiben, es beeinflusst weiterhin die Erholung der Verlierer nach einer Niederlage und die Entstehung einer langfristigen Depression nach chronischer Subjugation (Rillich & Stevenson, 2018).

Die vorliegende Studie liefert Belege für die zusätzliche Hypothese, dass 5HT den Entscheidungsprozess bei erwachsenen männlichen Grillen nur dann beeinflusst, wenn ein Verhaltenskontext wie Stress aufgrund chronischer Niederlagen oder mehrfache Gewinnerfahrungen vorliegt. Bei Wirbellosen sind mehrere Arten von 5HT-Rezeptoren beschrieben, die in verschiedenen Geweben und auf unterschiedlichen Expressionsniveaus exprimiert werden (Watanabe *et al.*, 2011), aber $5HT_2$ hat sich als das vielversprechendste Ziel für weitere Untersuchungen zu den Entscheidungsprozessen bei Grillen erwiesen, insbesondere nach chronischen Siegen und Niederlagen. Frühere Studien haben gezeigt, dass Ketanserin die Etablierung von

Unterwürfigkeit bei Verlierern hemmt, chronische Niederlagen verhindert und langfristige Depressionen nach mehreren Niederlagen verhindert (Rillich & Stevenson, 2018). DOI, als Agonist für eben diesen Rezeptor (Johnson *et al.*, 2009), hemmt die Erholung von Verlierern und fördert langfristige chronische Niederlagen. Um jegliche Effekte auszuschließen, die aus früheren Interaktionen resultieren, wurden in diesem Experiment $LTI^{w/o}$-Männchen verwendet. Der $5HTR_2$-Blocker Ketanserin hemmt, wenn er 1 h vor chronischen Niederlagen oder Gewinnerfahrungen injiziert wird, den Effekt der Subjugation bei 6-fachen Verlierern vollständig (siehe auch Rillich & Stevenson, 2018). Im Gegensatz dazu hemmt der $5HTR_2$-Agonist DOI teilweise die Etablierung eines dominanten Ethotypen, wenn die gesamte Testgruppe betrachtet wird.

Da DOI die Erholung von chronischen Niederlagen hemmt und die Reaktionen von Mehrfachgewinnern verändert, könnte es als Modulator bei der Bewertung der agonistischen Signale des Gegners wirken. Während jedes Kampfes machen die Gewinner auch negative Erfahrungen mit dem Gegner, wie Bisse oder Stöße. Der einzige Unterschied zu späteren Verlierern besteht darin, dass diese gesammelten Erfahrungen die Schwelle zur Flucht nicht überschreiten und der dann feststehende Verlierer als erster den Rückzug antritt. Eine mögliche Erklärung für die Wirkung von DOI ist also, dass diese agonistischen Erfahrungen anders bewertet werden und dass DOI, wahrscheinlich zusammen mit NO, die Schwelle zur Flucht senkt, obwohl die Grillen den Kampf gewonnen haben. Dies würde auch den höheren Anteil an negativen Drehungen nach 6 aufeinanderfolgenden Siegen nach DOI-Behandlung erklären. Da die Entscheidung, sich einem agonistischen Reiz zu nähern oder ihn zu meiden, sehr schnell, innerhalb von 30 bis 40 ms getroffen wird, schließt dies eine komplexe integrative Verarbeitung in höheren Gehirnzentren aus. Daher wird das Drehen höchstwahrscheinlich von 4 schnell leitenden, absteigenden Interneuronen initiiert, die Informationen von Mechanorezeptoren in den Fühlern an die motorischen Zentren des Thorax weiterleiten (Schöneich *et al.*, 2011) und ist daher ausschließlich von der Wirkung der verfügbaren Menge an Neuromodulatoren abhängig.

Dennoch ist es wichtig festzustellen, dass Pharmaka, die die Wirkung natürlich vorkommender Neuromodulatoren erhöhen, vermindern oder nachahmen, nicht unabhängig und in isolierten Systemen wirken. Die Pharmakologie des ganzen Tieres betrifft mehrere, teilweise voneinander abhängige Systeme, und die daraus resultierenden Wirkungen sind nicht immer offensichtlich. Selbst wenn die Rezeptorchemie und die Rolle eines Neuromodulators hinreichend klar sind, muss immer der gesamte Verhaltenskontext berücksichtigt werden.

Teile der vorliegenden Arbeit sind bereits veröffentlicht:

Balsam & Stevenson, 2020: Pre-adult aggression and its long-term behavioural consequences in crickets. *PLoS One*

Balsam & Stevenson, 2021: Agonistic experience during development establishes inter-individual differences in approach-avoidance behaviour of crickets. *Scientific Reports*

7 References

Abe, T, Fujiyama, N, Tomimatsu, H & Nagayama T: Age-dependent and social status-dependent behavioural plasticity of the cricket *Gryllus bimaculatus*. *Animal Behaviour.* **141,** 1-7 (2018). doi.org/10.1016/j.anbehav.2018.05.003

Adamo, SA, Gomez-Juliano, A, Ledue, EE, Little, SN & Sullivan, K: Effect immune challenge on aggressive behaviour: how to fight two battles at once. *Anim. Behav.* **105**, 153–161 (2015). doi.org/10.1016/j.anbehav.2015.04.018

Adamo, SA & Hoy, RR: Agonistic behavior in male and female field crickets, *Gryllus bimaculatus*, and how behavioural context influences its expression. *Animal Behaviour.* **49(6),** 1491-1501 (1995). doi.org/10.1016/0003-3472(95)90070-5

Adamo, SA, Linn, CE & Hoy, RR: The role of neurohormonal octopamine during 'fight or flight' behaviour in the field cricket *Gryllus bimaculatus*. *J. Exp. Biol.* **198,** 1691–1700 (1995). doi.org/10.1242/jeb.198.8.1691

Alekseyenko, OV, Lee, C, Kravitz, EA: Targeted manipulation of serotonergic neurotransmission affects the escalation of aggression in adult male *Drosophila melanogaster*. *PLoS One*. **5:e10806** (2010). doi.org/10.1371/journal.pone.0010806

Alexander, RD: Aggressiveness, territoriality, and sexual behavior in Field Crickets (Orthoptera: Gryllidae). *Behaviour*. **17,** 130–223 (1961).

Balsam, JS & Stevenson, PA: Pre-adult aggression and its long-term behavioural consequences in crickets. *PLoS One*. **15,** 1-18 (2020**).** doi.org/10.1371/journal.pone.0230743

Balsam, JS & Stevenson, PA: Agonistic experience during development establishes inter-individual differences in approach-avoidance behaviour of crickets. *Sci Rep.* **11,** 16702 (2021). doi.org/10.1038/s41598-021-96201-1

Bell, AM, Hankison, SJ & Laskowski, KL: The repeatability of behaviour: a meta-analysis. *Animal Behaviour.* **77,** 771–783 (2009). doi.org/10.1016/j.anbehav.2008.12.022

Bertram, SM & Rook, V: Relationship between condition, aggression, signaling, courtship, and egg laying in the field cricket, *Gryllus assimilis*. *Ethology.* **118,** 360–372 (2012). doi.org/10.1111/j.1439-0310.2011.02019.x

Bertram, SM, Whattam, EM, Visanuvimol, L, Bennett, R & Lauzon, C: Phosphorus availability influences cricket mate attraction displays. *Anim. Behav.* **77,** 525–530 (2009). doi.org/10.1016/j.anbehav.2008.11.012

Borstel, KJ & Stevenson, PA: Individual Scores for Associative Learning in a Differential Appetitive Olfactory Paradigm Using Binary Logistic Regression Analysis. *Front. Behav. Neurosci.* **15:**741439 (2021). doi.org/10.3389/fnbeh.2021.741439

Briffa, M & Sneddon, LU: Physiological constraints on contest behaviour. *Funct. Ecol*. **21,** 627–637 (2007). doi.org/10.1111/j.1365-2435.2006.01188.x

Briffa, M & Sneddon, LU: Proximate mechanisms of animal personality among-individual behavioural variation in animals. *Behaviour.* **153,** 1509–1515 (2016). doi.org/10.1163/1568539X-00003402

Briffa, M, Sneddon, LU & Wilson, AJ: Animal personality as a cause and consequence of contest behaviour. *Biol. Lett.* **11(3)** (2015). doi.org/10.1098/rsbl.2014.1007

Brodin, T: Behavioral syndrome over the boundaries of life - Carryovers from larvae to adult damselfly. *Behav. Ecol.* **20,** 30–37 (2009). doi.org/10.1093/beheco/arn111

Bubak, AN, Renner, KJ & Swallow, JG: Heightened serotonin influences contest outcome and enhances expression of high-intensity aggressive behaviors. *Behav. Brain. Res.* **259,** 137–142 (2014). doi.org/10.1016/j.bbr.2013.10.050

Burdohan, JA & Comer, CM: Cellular Organization of an Antennal Mechanosensory Pathway in the Cockroach, *Periplaneta americana. The Journal of Neuroscience.* **16(18),** 5830-5843 (1996). doi.org/10.1523/JNEUROSCI.16-18-05830.1996

Cacioppo, JT & Hawkley, LC: Perceived social isolation and cognition. *Cell Press* 447–454 (2009). doi.org/10.1016/j.tics.2009.06.005

Carter, AJ, Feeney, WE, Marshall, HH, Cowlishaw, G & Heinsohn, R: Animal personality: what are behavioural ecologists measuring? *Biol. Rev. Camb. Philos. Soc.* **88,** 465–475 (2013). doi.org/10.1111/brv.12007

Claßen, G & Scholz, H: Octopamine shifts the behavioral response from indecision to approach or aversion in *Drosophila melanogaster*. *Front. Behav. Neurosci.* **12,** 112 (2018). doi.org/10.3389/fnbeh.2018.00131

de Boer, SF, Buwalda, B & Koolhaas, JM: Aggressive Behavior and Social Stress. In G. Fink (Ed.), *Stress: Concepts, Cognition, Emotion, and Behavior*. 293-303.(2016). doi.org/10.1016/B978-0-12-800951-2.00036-4

Dierick, HA & Greenspan, RJ: Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. Nat Genet 39(5). 678–682 (2007). doi.org/10.1038/ng2029

Dixon, KA & Cade, WH: Some factors influencing male-male aggression in the field cricket *Gryllus integer* (time of day, age, weight and sexual maturity). *Anim. Behav.* **34,** 340–346 (1986). doi.org/10.1016/S0003-3472(86)80102-6

Dochtermann, NA & Nelson, AB: Multiple facets of exploratory behavior in House Crickets (*Acheta domesticus*): Split personalities or simply different behaviors? *Ethology.* **120,** 1–8 (2014). doi.org/10.1111/eth.12284

Donoughe, S & Extavour, CG: Embryonic development of the cricket *Gryllus bimaculatus*. *Dev. Biol.* **411**, 140–156 (2016). doi.org/10.1016/j.ydbio.2015.04.009

Duch, C, Mentel, T, and Pflüger, HJ: Distribution and activation of different types of octopaminergic DUM neurons in the locust. *J. Comp. Neurol.* **403,** 119–134 (1999).

doi.org/10.1002/(SICI)1096-9861(19990105)403:1<119::AIDCNE9>3.0.CO;2-F

Dyakonova, VE & Krushinskii, AL: Effects of an NOsynthase-inhibitor on aggressive and sexual behavior in male crickets. *Neurosci.Behav.Physiol.* **36,** 565–571 (2006).

doi.org/10.1007/s11055-006-0057-1

Dyakonova, VE & Krushinsky, AL: Serotonin precursor (5-hydroxytryptophan) causes substantial changes in the fighting behavior of male crickets, *Gryllus bimaculatus. J. Comp. Physiol. A.* **199,** 601–609 (2013). doi.org/10.1007/s00359-013-0804-z

Dyakonova, VE, Sakharov, DA & Schuermann, FW: (1999) Effects of serotonergic and opioidergic drugs on escape behavior and social status of male crickets. *Naturwissenschaften* **86,** 435–437 (1999).

Fisher, DN, James, A, Rodríguez-Muñoz, R & Tregenza, T: Behaviour in captivity predicts some aspects of natural behaviour, but not others, in a wild cricket population. *Proc. R. Soc. B Biol. Sci.* **282,** (2015). doi.org/10.1098/rspb.2015.0708

Fogelman, N & Canli, T: Early life stress, physiology and genetics: A Review. *Front.Psychol.* **10,** (2019). doi.org/10.3389/fpsyg.2019.01668

Fujimoto, S, Hirata, B & Nagayama, T: Dominance hierarchy-dependent behavioural plasticity of crayfish avoidance reactions. *J. Exp. Biol.* **214,** 2718–2723 (2011). doi.org/10.1242/jeb.057752

Gosling, SD: From mice to men: What can we learn about personality from animal research? *Psychological Bulletin.* **127,** 45–86 (2001).

Gosling, SD: Personality in non-human animals. *Social and Personality Psychology Compass.* **2,** 985–1001 (2008). doi.org/10.1111/j.1751-9004.2008.00087

Groneberg, AH *et al*.: Early-life social experience shapes social avoidance reactions in larval zebrafish. *Curr. Biol*. **30,** 1–13 (2020). doi.org/10.1016/j.cub.2020.07.088

Hammels, C *et al*.: Defeat stress in rodents: From behavior to molecules. *Neurosci. Biobehav. Rev.* **59,** 111–140 (2015). doi.org/10.1016/j.neubiorev.2015.10.006

Han, CS & Dingemanse, NJ: You are what you eat: Diet shapes body composition, personality and behavioural stability. *BMC Evol. Biol.* **17,** 1–16 (2017). doi.org/10.1186/s12862-016-0852-4

Hazra, A: Using the confidence interval confidently. *J. Thorac. Dis.* **9,** 4125–4130 (2017). doi.org/10.21037/jtd.2017.09.14

Hedrick, AV & Kortet, R: Sex differences in the repeatability of boldness over metamorphosis. *Behav. Ecol. Sociobiol.* **66,** 407–412 (2012). doi.org/10.1007/s00265-011-1286-z

Hiripi, LS & Rózsa, K: Octopamine and dopamine sensitive adenylate cyclase in the brain of Locusta migratoria during its development. *Cell.Mol.Neurobiol*. **4,** 199-206 (1984).

Hofmann, HA & Schildberger, K: Assessment of strength and willingness to fight during aggressive encounters in crickets. *Anim. Behav.* **62,** 337–348 (2001). doi.org/10.1006/anbe.2001.1746

Hofmann, HA & Stevenson, PA: Flight restores fight in crickets. *Nature* **403,** 613–613 (2000). doi.org/10.1038/35001137

Hoyer, SC *et al*.: Octopamine in male aggression of Drosophila. *Curr. Biol.* **18,** 159–167 (2008). doi.org/10.1016/j.cub.2007.12.052

Hsu, Y; Earley, RL & Wolf, LL: Modulation of aggressive behaviour by fighting experience: mechanisms and contest outcomes. *Biol Rev Camb Philos Soc* **81**, 33-74 (2005). doi.org/10.1017/S146479310500686X.

Iwasaki, M; Delago, A; Nishino, H; & Aonuma, A: Effects of Previous Experience on the Agonistic Behaviour of Male Crickets, *Gryllus bimaculatus*. *Zoological science*. **23**, 863-872 (2006). doi.org/10.2108/zsj.23.863

Iwasaki, M, Nishino, H, Delago, A & Aonuma, H: Effects of NO/cGMP signaling on behavioral changes in subordinate male crickets, *Gryllus bimaculatus*. *Zool. Sci*. **24,** 860–868 (2007). doi.org/10.2108/zsj.24.860

Iwasaki, M & Katagiri, C: Cuticular lipids and odors induce sex-specific behaviors in the male cricket *Gryllus bimaculatus*. *Comparative biochemistry and physiology*. **149(3)**, 306-313 (2008). doi.org/10.1016/j.cbpa.2008.01.008

Judge, KA & Bonanno, VL: Male weaponry in a fighting cricket. *PloS one.* **3(12),** e3980 (2008). doi.org/10.1371/journal.pone.0003980

Johnson, O; Becnel, J & Nichols, CD: Serotonin 5-HT2 and 5-HT1A-like receptors differentially modulate aggressive behaviors in *Drosophila melanogaster*. *Neuroscience*. **158**, 1292–1300 (2009). doi.org/10.1016/j.neuroscience.2008.10.055

Kendler, KS & Greenspan, RJ: The nature of genetic influences on behavior: Lessons from "simpler" organisms. *Am J Psychiatry.* **163,** 1683–1694 (2006).

Kortet, R & Hedrick, AV: A behavioural syndrome in the field cricket *Gryllus integer*: intrasexual aggression is correlated with activity in a novel environment. *Biol. J. Linn. Soc.* **91,** 475–482 (2007). doi.org/10.1111/j.1095-8312.2007.00812.x

Kravitz, EA: Serotonin and aggression: insights gained from a lobster model system and speculations on the role of amine neurons in a complex behavior. *J. Comp. Physiol. A*. **186**, 221–238 (2000). doi.org/10.1007/s003590050423

Kutsch, W & Pener, MP: Muscle activity underlying sexual behaviour in male locusts (*Locusta migratoria*) after normal and hormonally disturbed development. *Physiological Entomology*. **22(1),** 47-54 (1997). doi.org/10.1111/j.1365-3032.1997.tb01139.x

Laman-Maharg, A & Trainor, BC: Mini-Review: Stress, sex and motivated behaviors. *J. Neurosci. Res*. **95,** 83–92 (2017). doi.org/10.1002/jnr.23815

Lihoreau, M, Brepson, L & Rivault, C: The weight of the clan: Even in insects, social isolation can induce a behavioural syndrome. *Behav. Processes.* **82,** 81–84 (2009). doi.org/10.1016/j.beproc.2009.03.008

Lukkes, JL, Watt, MJ, Lowry, CA & Forster, GL: Consequences of post-weaning social isolation on anxiety behavior and related neural circuits in rodents. *Front. Behav. Neurosci.* **3:18** (2009). doi.org/10.3389/neuro.08.018.2009

Marsden, CA, King, MV & Fone, KCF: Influence of social isolation in the rat on serotonergic function and memory e Relevance to models of schizophrenia and the role of 5-HT6 receptors. *Neuropharmacology*. **61**, 400e407 (2011). doi.org/10.1016/j.neuropharm.2011.03.003

Menezes, FP, de Sousa, IP & Luchiari, AC: Early mistreatment contributes to social behavior disorders in Zebrafish. *Front. Behav. Neurosci. 14,* 1–7 (2020). doi.org/10.3389/fnbeh.2020.578242

Menzel, R: Searching for the Memory Trace in a Mini-Brain, the Honeybee. *LEARNING & MEMORY*. **8,** 53–62 (2001). doi.org/10.1101/lm.38801

Menzel, R: The honeybee as a model for understanding the basis of cognition. *Nat Rev Neurosci.* **13,** 758–768 (2012). doi.org/10.1038/nrn3357

Nagamoto, J; Aonuma, H & Hisada, M: Discrimination of conspecific individuals via cuticular Pheromones by males of the cricket *Gryllus bimaculatus*. *Zoological Science*. **22(10),** 1079-1088 (2005). doi.org/10.2108/zsj.22.1079

Niemelä, PT, Lattenkamp, EZ & Dingemanse, NJ: Personality-related survival and sampling bias in wild cricket nymphs. *Behav. Ecol.* **26,** 936–946 (2015). doi.org/10.1093/beheco/arv036

Niemelä, PT, Vainikka, A, Hedrick, AV & Kortet, R: Integrating behaviour with life history: boldness of the field cricket, *Gryllus integer*, during ontogeny. *Funct. Ecol.* 450–456 (2012). doi.org/10.1111/j.1365-2435.2011.01939.x

Nighorn, A, Gibson, NJ, Rivers, DM, Hildebrand, JG & Morton. DB: The Nitric Oxide–cGMP Pathway May Mediate Communication between Sensory Afferents and Projection Neurons in the Antennal Lobe of *Manduca Sexta. Journal of Neuroscience.* **18(18),** 7244-7255 (1998). doi.org/10.1523/JNEUROSCI.18-18-07244.1998

Nosil, P: Food fights in house crickets, *Acheta domesticus*, and the effects of body size and hunger level. *Canadian Journal of Zoology Revue Canadienne de Zoologie*. **80(3),** 409-417 (2002). doi.org/10.1139/z02-018

Okada, J & Akamine, S: Behavioral response to antennal tactile stimulation in the field cricket *Gryllus bimaculatus*. *Journal of comparative physiology. A.* **198,** 557–565 (2012). doi.org/10.1007/s00359-012-0729-y

Olivier, B: Serotonin: a never-ending story. *Eur. J. Pharmacol.* **753,** 2–18 (2015). doi.org/10.1016/j.ejphar.2014.10.031

Payne, RJH: Gradually escalating fights and displays: the cumulative assessment model. *Anim. Behav.* **56(3),** 651-662. doi.org/10.1006/anbe.1998.0835

Polderman, TJC *et al*.: Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nat. Publ. Gr.* **47,** 702–709 (2015). doi.org/10.1038/ng.3285

Rillich, J; Rillich, B & Stevenson, PA: Differential modulation of courtship behavior and subsequent aggression by octopamine, dopamine and serotonin in male crickets. *Hormones & Behavior*. **114** (2019). doi.org/10.1016/j.yhbeh.2019.06.006

Rillich, J, Schildberger, K & Stevenson, PA: Assessment strategy of fighting crickets revealed by manipulating information exchange. *Animal Behaviour.* **74(4),** 823-836 (2007). doi.org/10.1016/j.anbehav.2006.11.022

Rillich, J; Schildberger, K & Stevenson, PA: Octopamine and occupancy - an aminergic mechanism for intruder-resident aggression in crickets. *Proceedings of the Royal Society London B*. **278,** 1873-1880 (2011). doi.org/10.1098/rspb.2010.2099

Rillich, J & Stevenson, PA: Winning fights induces hyperaggression via the action of the biogenic amine octopamine in crickets. *PLoS One.* **6,** (2011). doi.org/10.1371/journal.pone.0028891

Rillich, J & Stevenson, PA: A fighter's comeback: Dopamine is necessary for recovery of aggression after social defeat in crickets. *Hormones and Behavior.* **66**, 696–704 (2014). doi.org/10.1016/j.yhbeh.2014.09.012

Rillich, J & Stevenson, PA: Releasing stimuli and aggression in crickets: octopamine promotes escalation and maintenance but not initiation.
Front. Behav. Neurosci. **9,** 1–11 (2015). doi.org/10.3389/fnbeh.2015.00095

Rillich, J & Stevenson, PA: Losing without fighting - Simple aversive stimulation induces submissiveness typical for social defeat via the action of nitric oxide, but only when preceded by an aggression priming stimulus. *Front. Behav. Neurosci.* **11,** 11:50 (2017). doi.org/10.3389/fnbeh.2017.00050

Rillich, J & Stevenson, PA: Serotonin mediates depression of aggression after acute and chronic social defeat stress in a model Insect. *Front. Behav. Neurosci.* **12,** 12:233 (2018). doi.org/10.3389/fnbeh.2018.00233

Rodríguez-Muñoz, R: Natural and sexual selection in a wild insect population. *Science.* **80,** 328, (2010). doi.org/10.1126/science.1188102

Rodriguez-Munoz, R, Bretman, A & Tregenza, T: Guarding males protect females from predation in a wild Insect. *Curr. Biol.* **21,** 1716–1719 (2011). doi.org/10.1016/j.cub.2011.08.053

Roeder, T: Octopamine in invertebrates. *Progress in Neurobiology*. **59(5),** 533-561 (1999). doi.org/10.1016/S0301-0082(99)00016-7

Rose, J, Cullen, DA, Simpson, SJ & Stevenson, PA: Born to win or bred to lose: aggressive and submissive behavioural profiles in crickets. *Anim. Behav.* **123,** 441–450 (2017a). doi.org/10.1016/j.anbehav.2016.11.021

Rose, J, Rillich, J & Stevenson, PA: Chronic social defeat induces long-term behavioral depression of aggressive motivation in an invertebrate model system. *PLoS One.* **12,** 1–16 (2017b). doi.org/10.1371/journal.pone.0184121

Rudin, FS, Tomkins, JL & Simmons, LW: Changes in dominance status erode personality and behavioral syndromes. *Behav. Ecol.* **28,** 270–279 (2017). doi.org/10.1093/beheco/arw151

Sakai M, Katayama T & Taoda Y: Postembryonic development of mating behavior in the male cricket *Gryllus bimaculatus* DeGeer. *J.Comp.Physiol.[A].* **166,** 775-784 1990. doi.org/10.1007/BF00187322

Sakura, M & Aonuma, H: Aggressive behavior in the antennectomized male cricket *Gryllus bimaculatus. J Exp Biol.* **216(Pt 12),** 2221-2228 (2013). doi.org/jeb.079400[pii]10.1242/jeb.079400

Santostefano, F, Wilson, AJ, Araya-Ajoy, YG & Dingemanse, NJ: Interacting with the enemy: indirect effects of personality on conspecific aggression in crickets. *Behav. Ecol.* **27,** 1235–1246 (2016). doi.org/10.1093/beheco/arw037

Scharf, I *et al*.: Social isolation causes downregulation of immune and stress response genes and behavioural changes in a social insect. *Mol. Ecol.* **30,** 2378-2389 (2021). doi.org/10.1111/mec.15902

Schöneich, S, Schildberger, K & Stevenson, PA: Neuronal organization of a fast-mediating cephalothoracic pathway for antennal-tactile information in the cricket (*Gryllus bimaculatus* DeGeer). *J. Comp. Neurol.* **519,** 1677–1690 (2011). doi.org/10.1002/cne.22594

Sfara, V, Zerba, EN & Alzogaray, RA: Deterrence of feeding in *Rhodnius prolixus* (Hemiptera: Reduviidae) after treatment of antennae with a nitric oxide donor. *Eur. J. Entomol.* **108,** 701–704 (2011).

Sih, A & Bell, AM: Insights for behavioral ecology from behavioral syndromes. *Adv. study Behav.* **38,** 227–281 (2008). doi.org/10.1016/S0065-3454(08)00005-3

Sih, A, Bell, A & Johnson, J: Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution,* **(19),** 372–378 (2004). doi.org/10.1016/j.tree.2004.04.009

Sih, A, Bell, AM, Johnson, JC & Ziemba, RE: The quarterly review of biology, behavioral syndromes: an integrative overview. *Chicago Journals.* **79 (3),** 241–277 (2004a). doi.org/10.1086/422893

Sih, A. *et al*.: Animal personality and state – behaviour feedbacks: a review and guide for empiricists. *Cell Press* 1–11 (2014). doi.org/10.1016/j.tree.2014.11.004

Simmons, LW: Inter-male competition and mating success in the field cricket, *Gryllus bimaculatus* (de Geer). *Animal Behaviour.* **34,** 567-579 (1986). doi.org/10.1016/S0003-3472(86)80126-9

Simmons, LW: Competition between nymphs of the field cricket, *Gryllus bimaculatus* (Orthoptera: Gryllidae) and its effects on some life-history components of fitness. *Journal of Animal Ecology.* ***56*****,** 1015-1027 (1987). doi.org/10.2307/4963

Simpson, SJ & Stevenson, PA: Neuromodulation of social behaviour in insects. *The Oxford Handbook of Molecular Psychology.* (2015).
doi.org/ 10.1093/oxfordhb/9780199753888.013.014

Song, CK, Herberholz, J & Edwards, DH: The effects of social experience on the behavioral response to unexpected touch in crayfish. *J. Exp. Biol.* **209,** 1355–1363 (2006). doi.org/10.1242/jeb.02126

Staudacher, EM: The auditory system of last instars in *Gryllus bimaculatus* DeGeer.
Physiological Entomology. **34(1),** 18-29 (2009).
doi.org/10.1111/j.13653032.2008.00647.x

Staudacher, EM, Gebhardt, M & Dürr, V: Antennal Movements and Mechanoreception: Neurobiology of Active Tactile Sensors. *Advances in Insect Physiology.* **32**, 49–205 (2005). doi.org/10.1016/S0065-2806(05)32002-9

Stevenson, PA; Dyakonova, V; Rillich, J & Schildberger, K: Octopamine and experience-dependent modulation of aggression in crickets.
J. Neurosci. **25,** 1431–1441 (2005). doi.org/10.1523/JNEUROSCI.4258-04.2005

Stevenson, PA, Hofmann, HA, Schoch, K & Schildberger, K: The fight and flight responses of crickets depleted of biogenic amines. *J. Neurobiol.* **43,** 107–120 (2000). doi.org/10.1002/(SICI)1097-4695(200005)

Stevenson, PA & Kutsch, W: Demonstration of functional connectivity of the flight motor system in all stages of the locust. *Journal of Comparative Physiology A.* **162(2),** 247-259 (1988). doi.org/10.1007/BF00606089

Stevenson, PA & Rillich, J: The decision to fight or flee – insights into underlying mechanism in crickets. *PLoS One.* **15,** 1–12 (2012). doi.org/10.3389/fnins.2012.00118

Stevenson, PA & Rillich, J: Isolation associated aggression - a consequence of recovery from defeat in a territorial animal. *PLoS One.* **8,** 1–11 (2013). doi.org/10.1371/journal.pone.0074965

Stevenson, PA & Rillich, J: Adding up the odds - Nitric oxide signaling underlies the decision to flee and post-conflict depression of aggression. *Sci Adv.* 1(2), e1500060 (2015). doi.org/10.1126/sciadv.1500060

Stevenson, PA, Rillich, J: Controlling the decision to fight or flee: the roles of biogenic amines and nitric oxide in the cricket. *Current Zoology.* **62(3),** 265-275 (2016). doi.org/ 10.1093/cz/zow028

Stevenson, PA, Rillich, J: Neuromodulators and the Control of Aggression in Crickets. *H.W. Horch et al. (eds.), The Cricket as a Model Organism.* 169-195 (2017). doi.org/10.1007/978-4-431-56478-2_12

Stevenson, PA & Rillich, J: Fight or flee? Lessons from insects on aggression. *Neuroforum.* **25,** 1–9 (2019). doi.org/10.1515/nf-2017-0040

Takagi, S & Benton, RA: Animal Behavior: Neural basis of individuality. *Curr. Biol.* **30,** 710–712 (2020). doi.org/10.1016/j.cub.2020.04.052

Thibert, J, Farine, JP, Cortot, J & Ferveur, JF: Drosophila food-associated pheromones: Effect of experience, genotype and antibiotics on nymphal behavior. *PLoS One.* **11(3),** e0151451 (2016). doi.org/10.1371/journal.pone.0151451

Thomas, AL, Davis, SM & Dierick, HA: Of fighting flies, mice, and men: Are some of the molecular and neuronal mechanisms of aggression universal in the Animal Kingdom? *PLoS Genet.* **11,** e1005416 (2015). doi.org/10.1371/journal.pgen.1005416

Thomas, ML & Simmons, LW: Sexual dimorphism in cuticular hydrocarbons of the Australian field cricket *Teleogryllus oceanicus* (Orthoptera: Gryllidae). *J Insect Physiol.* **54(6),** 1081-1089 (2008). doi.org/10.1016/j.jinsphys.2008.04.012

Tibbets, EA, Desjardins, E, Kou, N & Wellman, L: Social isolation prevents the development of individual face recognition in paper wasps. *Animal Behaviour*. **152**, 71e77 (2019). doi.org/10.1016/j.anbehav.2019.04.009

Urszán, TJ *et al*.: No personality without experience? A test on *Rana dalmatina* tadpoles. *Ecol. Evol.* **5,** 5847–5856 (2015). doi.org/10.1002/ece3.1804

Vleugels, R, Verlinden, H & Broeck, JV: Serotonin, serotonin receptors and their actions in insects. *Neurotransmitter.* **2: e314 (2015).** doi.org/10.14800/nt.314

Watanabe, T, Sadamoto, H & Aonuma, H: Identification and expression analysis of the genes involved in serotonin biosynthesis and transduction in the field cricket *Gryllus bimaculatus*. *Insect Molecular Biology.* **20(5),** 619–635 (2011). doi.org/10.1111/j.1365-2583.2011.01093.x

Westwick, RR & Rittschof, CC: Insects provide unique systems to investigate how early-life experience alters the brain and behavior*. Front. Behav. Neurosci*. **15,** 660464 (2021). doi.org/10.3389/fnbeh.2021.660464

Widmann, A, Eichler, K, Selcho, M, Thum, AS & Pauls, D: Odor-taste learning in Drosophila larvae. *Journal of Insect Physiology*. **106 ,** 47–54 (2018). doi.org/10.1016/j.jinsphys.2017.08.004

Wilson, A. D. M. *et al*. Behavioral correlations across activity, mating, exploration, aggression and antipredator contexts in the European house cricket, Acheta domesticus. *Behav. Ecol. Sociobiol.* **64,** 703–715 (2010). doi.org/10.1007/s00265-009-0888-1

Wilson, V, Guenther, A, Øverli, Ø, Seltmann, MW & Altschul, D: Future directions for personality research: Contributing new insights to the understanding of animal behavior. *Animals.* **9,** 1–17 (2019). doi.org/10.3390/ani9050240

Wommack, JC, Taravosh-Lahn, K, Cavid, TJ & Delville, Y: Repeated exposure to social stress alters the development of agonistic behavior in male golden hamsters. *Hormones and Behaviour.* **43,** 229-236 (2003). doi.org/10.1016/S0018-506X(02)00029-6

Woodring JP, Meier OW & Rose R: Effect of development, photoperiod and stress on octopamine levels on the house cricket, *Acheta domesticus*. *Journal of Insect Physiology.* **34,** 759-765 (1988). doi.org/10.1016/0022-1910(88)90149-7

Zhou, C, Rao, Y & Rao, Y: A subset of octopaminergic neurons are important for Drosophila aggression. *Nature Neurosci.* **11,** 1059–1067 (2008). doi.org/10.1038/nn.2164

Zorovi, M & Hedwig, TB: Descending brain neurons in the cricket *Gryllus bimaculatus* (de Geer): auditory responses and impact on walking. *J. Comp. Physiol. A* **199,** 25–34 (2013). doi.org/10.1007/s00359-012-0765-7

Cricket illustration by Janine Wiget

8 Appendix

8.1 Figures and Tables

8.2 Publications and published abstracts

Publications

1 Balsam, JS & Stevenson, PA: Pre-adult aggression and its long-term behavioural consequences in crickets. PLoS ONE. **15(3)**: e0230743 (2020). doi.org/10.1371/journal.pone.0230743

2 Balsam, JS & Stevenson, PA: Agonistic experience during development establishes inter-individual differences in approach-avoidance behaviour of crickets. *Scientific Reports.* **11,** 16702 (2021). doi.org/10.1038/s41598-021-96201-1

Short communications (posters and talks with published abstracts)

Poster

1 Balsam, JS & Stevenson, PA: Social experience determines inter- individual behavioural differences in an insect model system. In Leipzig, 14th Research Festival for Life Sciences (2018).

2 Balsam, JS & Stevenson, PA: „Personality “in Arthropods? Early social experience in crickets produces long term changes in behaviour. In Altleiningen, ANN Spring Meeting (2018).

3 Balsam, JS & Stevenson, PA: Enhanced and depressed aggressiveness – social experience establishes behavioural differences in crickets. In Leipzig, 15th Research Festival for Life Sciences (2019).

4 Balsam, JS & Stevenson, PA: Social defeat stress induces long term behavioural changes in crickets. In Halle, Entomologentagung der Deutschen Gesellschaft für allgemeine und angewandte Entomologie e.V. (DGaaE) (2019).

5 Balsam, JS & Stevenson, PA: Aggression forges inter-individual behavioural differences in crickets. In Göttingen, 13th Meeting of the German Neuroscience Society (2019).

6 Balsam, JS & Stevenson, PA: Approach or avoid? Pre-adult social experience determines behaviour towards conspecific adults. In Leipzig, 16th Research Festival for Life Sciences (2020).

7 Balsam, JS & Stevenson, PA: Approach or avoid? Pre-adult social experience forges life-long behavioural differences in individual crickets. In Göttingen, 14th Meeting of the German Neuroscience Society (2021).

Talks

8 Balsam, JS & Stevenson, PA: Role of early social experience in inter-individual behavioural differences. In Hannover, Graduate Meeting in Animal Behavior of the DZG and the Ethologische Gesellschaft (2018).

8.3 Curriculum vitae

Persönliche Daten

Vor- und Nachname: Julia Sophie Balsam
Geburtstag:
Geburtsort:
Adresse:

Telefon:
E-Mail: julia.balsam@uni-leipzig.de

Staatsangehörigkeit: deutsch

Schulbildung

1995 – 1999	Grundschule Friedrich-Schiller
08/1999 - 07/2007	Staatliches Gymnasium Heinrich Ehrhardt *Allgemeine Hochschulreife, Abitur*

Studium

10/2007 – 06/2011	Studium der Veterinärmedizin an der Veterinärmedizinischen Fakultät Leipzig
10/2011 – 09/2014	Bachelorstudium der Biologie an der Universität Leipzig *Bachelor of Science*
10/2014 – 09/2016	Masterstudium der Biologie an der Universität Leipzig *Master of Science*

Promotion

06/2018 – 10/2021	Promotion an der Universität Leipzig Biologin mit dem akademischen Grad *Dr. rer. nat.*

Referenzen

12/2013 – 02/2014 12/2014 – 02/2015 12/2015 – 02/2016 12/2016 – 02/2017	Wissenschaftliche Hilfskraft AG Tier- und Verhaltensphysiologie Prof. Dr. Schildberger, Prof. Dr. Stevenson Institut für Biologie, Universität Leipzig
05/2014 - 07/2014 05/2015 - 07/2015	Wissenschaftliche Hilfskraft AG Mikrobiologie, Prof. Dr. Zimmermann Institut für Biochemie, Universität Leipzig
09/2015 – 09/2016	Wissenschaftliche Hilfskraft AG Zellbiologie, Institut für Biologie, Prof. Dr. Magin Institut für Biologie, Universität Leipzig
4/2017 – 3/2018	wissenschaftlicher Mitarbeiter, AG Tier- und Verhaltensphysiologie Prof. Dr. Stevenson Institut für Biologie, Universität Leipzig
06/2018 – 12/2021	Wissenschaftliche Mitarbeiterin (DFG gefördert) Promotion AG Tier- und Verhaltensphysiologie Prof. Dr. Kittel, Prof. Dr. Stevenson Institut für Biologie, Universität Leipzig

Leipzig, 20.10.2021

8.4 Acknowledgments

This doctoral thesis was financed by the German Research Foundation (DFG, Deutsche Forschungsgemeinschaft, Grant Nr. STE 714/5-1).