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ORIGINAL INVESTIGATION

Acute escitalopram but not contextual conditioning exerts a stronger "anxiogenic" effect in rats with high baseline "anxiety" in the acoustic startle paradigm

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Abstract
Rationale Acute administration of selective serotonin reuptake inhibitors (SSRIs) may enhance anxiety in humans, those with anxiety disorders being more susceptible than others. Fear-conditioned or unconditioned acoustic startle and freezing are common measures of fear and/or "anxiety" in rodents that may be used to study this effect of SSRIs preclinically.
Objectives Our aim was to shed further light on the effect of acute administration of an SSRI, escitalopram (10 mg/kg), on startle and freezing in the absence or presence of prior contextual conditioning. Repeated testing also enabled us to evaluate (i) if there are stable inter-animal variations with respect to these parameters in a batch of outbred Wistar rats, (ii) the possible relationship between the two and (iii) if baseline behaviour predicts the response to escitalopram.
Results Inter-animal test-retest correlations were found for both startle and freezing at baseline, and the two parameters also correlated with each other. Both escitalopram and contextual conditioning increased freezing and startle but without exerting any synergistic effect. While animals displaying high startle at baseline showed higher susceptibility to respond to escitalopram, the effect of conditioning was more pronounced in those with low baseline startle.
Conclusions The results support the usefulness of both conditioned and non-conditioned startle and freezing to capture an "anxiogenic" influence of SSRIs. Also, they suggest that baseline non-conditioned startle may predict this response in a manner reflecting the clinical situation in the sense that subjects with high baseline "anxiety" are particularly prone to respond with enhanced "anxiety" following acute SSRI administration.

Keywords Serotonin · Selective serotonin reuptake inhibitor (SSRI) · Anxiety disorder · Freezing · Startle · Fear conditioning · Contextual fear

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Introduction

Animals exposed to a sudden stimulus, such as a disturbing noise, may display a response known as startle, which is characterized by a rapid muscle contraction (Davis 2001; Davis et al. 1993), or another response known as freezing (Brandao et al. 2008; Riess 1945), which is the complete absence of body movements (except respiration). In the present study, these two parameters were assessed, both in animals that had been fear conditioned to a certain context, hence displaying exaggerated responses, and in those who had not been the subject of context conditioning, the aim being to shed further light on the anxiogenic effects displayed by acute administration of a selective serotonin reuptake inhibitor (SSRI), escitalopram.

Both startle and freezing are influenced by factors such as strain, habituation and fear conditioning, and both have been utilized as putative animal models of anxiety (Brandao et al. 2008; Davis 2001; Davis et al. 1993; Grillon 2002). Though many studies have explored both startle and freezing in the same animals (Borszcz et al. 1989; Davis and Astrachan 1978; de Oliveira et al. 2011; Kiernan and Cranney 1992; Leaton and Borszcz 1985; Luyten et al. 2011; Plappert et al. 1993; Santos et al. 2005, 2006), it remains poorly described to what extent animals within an outbred batch may be characterized as high or low responders with respect to any of the two

behaviours and, if so, to what extent an animal responding relatively strongly with respect to one of the parameters is prone to respond strongly also with respect to the other, i.e. to what extent the two behaviours correlate.

Though effective for the treatment of anxiety disorders when administered at a continuous basis (Nutt et al. 1999), at acute administration, SSRIs may exert a paradoxical increase in anxiety. This response is, however, the subject of considerable inter-individual variation: while often absent in healthy volunteers, it may be very striking in subjects with anxiety disorders such as panic disorder (Grillon et al. 2007; Rammsayer and Netter 1990; Sinclair et al. 2009). Attempts have been made to shed light on the anxiogenic influence of SSRIs by means of various animal models of anxiety, including startle and freezing, but the results have been conflicting; while some studies suggest an SSRI to enhance conditioned fear measured as startle (Santos et al. 2006) as well as freezing (Burghardt et al. 2004, 2007; Montezinho et al. 2010), others have reported the opposite effect in the freezing paradigm (Hashimoto et al. 1996, 2009; Inoue et al. 1996; Li et al. 2001; Montezinho et al. 2010; Muraki et al. 2008; Nishikawa et al. 2007; Santos et al. 2006).

In the present study, an outbred batch of male Wistar rats was exposed to two assessments of unconditioned startle and freezing followed by a third assessment in which half of them had been exposed to foot shock-based contextual conditioning; moreover, at the last assessment, half of the animals in each group (context conditioned and not context conditioned, respectively) were exposed to an acute dose of the SSRI escitalopram immediately before the test. For contextual conditioning, which may be regarded as a variant of conditioned fear, an aversive stimulus, i.e. a series of foot shocks, was delivered in a context that was deemed sufficiently neutral for this purpose, i.e. the startle/freezing chamber, in order to evoke fear of this context (Davis 2001; Fendt and Fanselow 1999; Grillon 2002; Phillips and LeDoux 1992; Santos et al. 2006).

The major aim of this study was to shed further light on the possible "anxiety"-enhancing effect of acute SSRI administration in animals that had or had not been exposed to contextual conditioning, hence shedding further light on the possible importance of fear conditioning for the "anxiogenic" effect of escitalopram to be at hand. In addition, the repeated testing of the animals before they were exposed to context conditioning or escitalopram enabled us to assess the possible inter-individual, test-retest stability with respect to the two parameters in an unconditioned situation, as well as to what extent the two responses correlate with each other, i.e. to assess if it is possible to use these parameters to characterize animals within a batch as more or less "fear prone" or "anxious". In the same vein, the experimental design enabled us to examine whether startle and freezing at baseline could predict the response to the SSRI on context-conditioned or unconditioned startle and freezing, the hypothesis being that escitalopram should exert a more pronounced "anxiogenic" effect in rats with high reactivity at baseline, just as patients with anxiety disorders or anxiety traits are more likely than others to experience enhanced anxiety upon their first exposure to serotonin reuptake inhibition (Rammsayer and Netter 1990; Ramos et al. 1993; Sinclair et al. 2009).

Methods

Animals

Eighty-eight male Wistar rats (Taconic, Denmark), 9 weeks of age on arrival, were housed with four animals per cage. Prior to experiments, each rat was habituated to human contact. The animals were 11–12 weeks old when test 1 was performed. Due to a problem with a loudspeaker of the startle equipment, seven animals had to be disqualified from the experiments. Thus, 81 animals remained qualified for analyses. The Animal Ethics Committee at the University of Gothenburg had approved all procedures involved before any of the experiments were undertaken.

Settings

All procedures were performed during the light phase of the light/dark cycle. During experiments performed on consecutive days, the protocol was so designed that the time elapsing between two procedures was the same for every rat.

Startle/freezing

The experiments were performed in a startle reflex system (Med Associates, St Albans, VT, USA) consisting of two identical ventilated and sound-attenuated plywood chambers measuring 64×60×40 cm. In each chamber, the rat was placed inside a stabilimeter consisting of a wire mesh cage (16.5×7.6×7.5 cm) that was attached to a response platform.

Electric foot shocks were delivered through the grid floor of the stabilimeter, consisting of six stainless steel bars with a diameter of 0.5 cm. The space between each bar was 1.5 cm. Sound was delivered through loudspeakers located 10 cm from the stabilimeter. A light bulb (6 W) located on top of the loudspeakers delivered a constant red light for camera detection. A constant 55-dB background noise (white noise) was delivered during all experimental sessions.

The startle amplitude was recorded from the pressure sensed by the response platform following movement of the animal using Startle Reflex Software® (Version 6.00, Med Associates). The startle-eliciting noise bursts consisted of 20-ms bursts of white noise with an intensity of 95 dB and a rise and fall time of 5 ms. The startle amplitude was recorded

using the default settings of the equipment, i.e. beginning 20 ms before and ending 280 ms after the onset of each noise burst. Following a 5-min acclimation period, 30 noise bursts with an inter-burst interval of 30 s were delivered for 15 min. The startle peak was defined as the maximal voltage peak evoked by the response platform and usually appeared within 100 ms after the burst. The magnitude of the startle response is expressed as the mean of these 30 peak amplitudes.

Gross observation of animals exposed to noise bursts, as well as of context-conditioned animals before the onset of bursts, revealed these to display various degrees of typical freezing behaviour, i.e. the complete absence of body movements (except respiration) combined with arched back, retracted ears and piloerection. For the measuring of freezing, this behaviour was defined as lack of motion for more than 1 s as assessed by means of automated scoring (Video Monitor Software, Med Associates) of video recordings obtained using a monochrome, near-infrared video camera (Med Associates), and expressed as the percentage of time the animal displayed immobility during the period before (5 min) or during (15 min) noise bursts.

Procedures

In order to obtain baseline levels of anxiety-related behaviour, each rat was first subjected to an assessment of startle and freezing (Table 1, test 1). Fourteen to 17 days after test 1, startle and freezing were once again recorded (test 2) in a session identical to test 1. The purpose of this session (test 2) was to evaluate the intra-animal consistency of startle and freezing in an unconditioned situation, as well as to enable a proper evaluation of the possible relationship between baseline behaviour and the effect of drug and context conditioning, respectively; thus, by expressing the effect of drug and conditioning as the difference between test 2 and test 3, rather than between test 1 and test 3, the regression-towards-the mean phenomenon caused by random variation at test 1 could be effectively avoided.

Before test 2, the animals had been divided into the four different treatment groups, the only purpose being to avoid any marked accidental mean differences in baseline behaviour between animals that would later be given different treatments. To this end, the animals were first ranked based on their startle response at test 1 and then on their freezing response at test 1, the summation of these rankings constituting the basis for the stratification.

On the following day, each rat was returned to the stabilimeter for 20 min (training). After 5 min of acclimation in the context, half of the rats, the context-conditioned-NaCl (CC-NaCl) and the context-conditioned-escitalopram (CC-Escit) groups, underwent contextual fear conditioning, during which they were presented to ten foot shocks each with amperage of 0.8 mA and duration of 250 ms, the intervals between the shocks being 90 s in accordance with a protocol optimized for simultaneous studies of startle and freezing (Luyten et al. 2011). The other half of the rats, the no-context-conditioned-NaCl (NCC-NaCl) and no-context-conditioned-escitalopram (NCC-Escit) groups, underwent a 20-min habituation session in the stabilimeter during which no foot shocks were delivered.

On the day after the training, every rat was again tested for startle and freezing (test 3) in a session identical to the test 1 and 2 sessions. Sixty minutes prior to test 3, all animals received a single subcutaneous injection of either escitalopram or saline (see below).

Treatment

Half of the context-conditioned (CC-Escit) and half of the non-conditioned rats (NCC-Escit) received a single injection of escitalopram (escitalopram oxalate, 10 mg/kg, from a solution of 4 mg per ml of 0.9 % saline) 1 h before test 3. The two remaining groups (CC-NaCl and NCC-NaCl) received 1 ml of 0.9 % saline. No injections were given prior to test 1, test 2 or training.

Statistical analysis

All statistical calculations were performed using the SPSS software® version 20. The main effects and interaction effects of drug and contextual conditioning on startle and freezing at test 3 were analysed using two-way ANOVA adjusted for the test 2 value of the studied parameter by using this as a covariate. This analysis was both carried out on the whole sample and after stratification based on baseline "anxiety" as defined by startle or freezing at test 1 (≥median, high;

Table 1 Experimental design

Test 1	Test 2	Training	Test 3
Context exposure+noise bursts	Context exposure+noise bursts	Habituation in context (n=20)	Saline+context exposure+noise bursts
		Contextual fear conditioning (n=21)	Saline+context exposure+noise bursts
		Habituation in context (n=21)	Escitalopram+context exposure+noise bursts
		Contextual fear conditioning (n=19)	Escitalopram+context exposure+noise bursts

<median, low). To further evaluate the possible effect of baseline behaviour on the response to contextual conditioning or escitalopram, respectively, an analysis of changes in response from test 2 to test 3 was regressed on test 1 values in the four treatment groups, after which the difference in regression slopes was tested by an interaction term between the test 1 value and treatment group. Correlations were investigated using Pearson correlation analyses.

Results

Effects of noise bursts on startle and freezing

As expected, noise bursts reliably induced startle reactions during all tests and regardless of treatment. There was no evidence for any habituation with respect to the effect of bursts on startle during the 15 min of recording in any of the tests as assessed using a linear mixed model with noise burst number used as factor (data not shown). Supporting an influence of noise bursts also on freezing, the percentage time spent on freezing was higher during exposure to noise bursts than during the preceding 5-min period at test 1 (before bursts 3.9±0.6 %, during bursts 45.5±3.1 %, paired *t* test: $p<0.0001$); a similar difference was observed also in test 2 and in the NCC animals of test 3 (data not shown). While in test 3 the context-conditioned animals displayed enhanced freezing also before the onset of bursts (NCC-NaCl before bursts 6.9±1.6 %, CC-NaCl before bursts 55.1±4.2 %, unpaired *t* test: $p<0.0001$), the percentage of time spent freezing was higher during bursts also in these animals (CC-NaCl during bursts 65.8±2.4 %, paired *t* test vs. before bursts: $p<0.05$).

Test-retest stability and inter-paradigm correlations

Values in test 1, i.e. before treatment, were similar in all treatment groups (mean±SEM for startle: NCC-NaCl 513.8 ±54.6, CC-NaCl 476.7±58.9, NCC-Escit 514.6±63.7, CC-Escit 492.2±64.0; mean±SEM for freezing (%): NCC-NaCl 47.1±6.3, CC-NaCl 45.4±6.4, NCC-Escit 47.1±5.8, CC-Escit 42.1±6.3).

For both startle and freezing, the responses at test 1 correlated strongly with the responses at test 2 (Fig. 1a, b). Significant correlations were also found between startle and freezing at both tests 1 (Fig. 1c) and 2 (not shown).

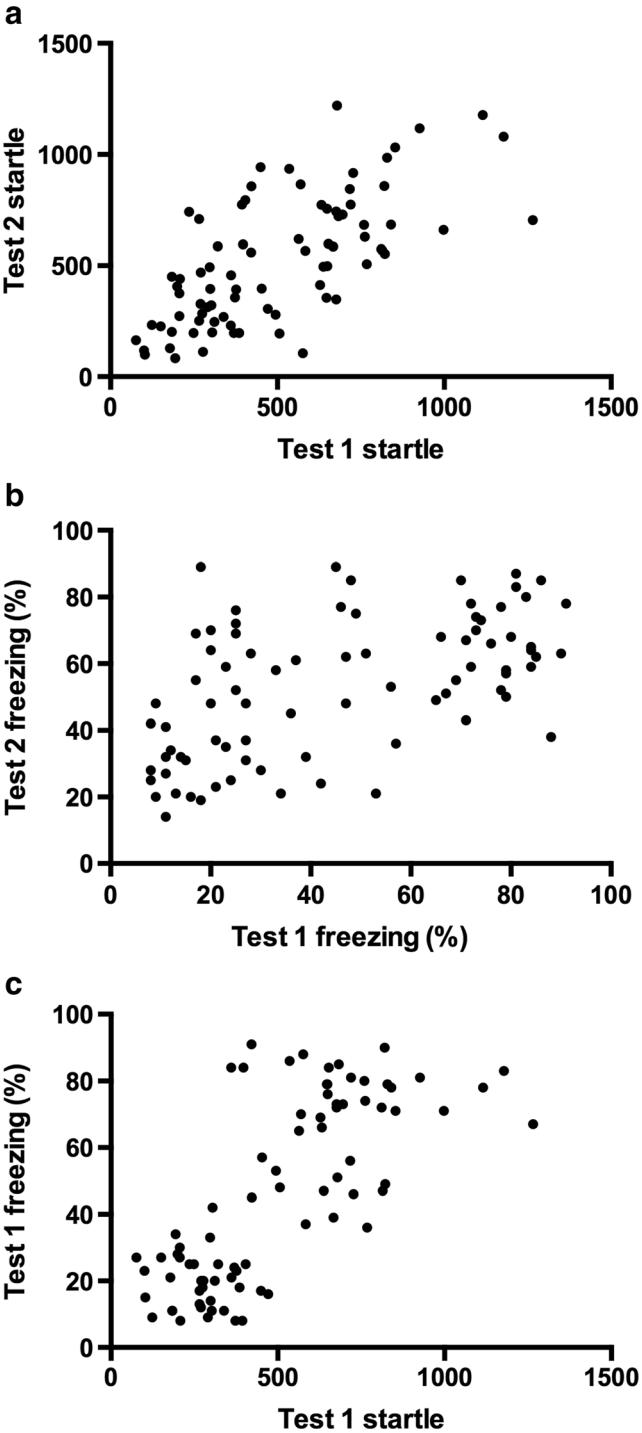

Fig. 1 The between-test relation for startle and freezing as well as the relation between the two parameters. Each *dot* represents the value of one animal ($n=81$). Significant correlations were found between **a** test 1 startle and test 2 startle ($r=0.68$; $p<0.0001$), **b** test 1 freezing and test 2 freezing ($r=0.54$; $p<0.0001$) and **c** test 1 startle and test 1 freezing ($r=0.73$; $p<0.0001$)

General effect of contextual conditioning and drug

Two-way ANOVA of startle at test 3, adjusted for measurements at test 2, showed significant main effects of drug and contextual conditioning but no interaction between the two factors (Table 2). Similar analyses of freezing also showed significant main effects of drug and contextual conditioning but no interaction.

Table 2 Startle at test 3 presented as mean±SEM displayed by each treatment group shown for all animals as well as after division based on test 1 startle (high vs. low test 1 startle). Shown also are effect sizes (ES), *F* values and levels of significance (*p* value) for the effects of contextual conditioning (CC) and escitalopram (Escit), respectively, on test 3 startle adjusted for test 2 startle

	NCC-NaCl	CC-NaCl	NCC-Escit	CC-Escit		CC	Escit
All animals	499.5±59.2 (*n*=20)	633.2±60.3 (*n*=21)	661.7±84.3 (*n*=21)	839.7±79.9 (*n*=19)	ES	146.3	166.7
					F value	7.0	9.1
					p value	0.01	0.004
High test 1 startle	631.5±89.3 (*n*=10)	677.8±85.9 (*n*=10)	848.5±124.3 (*n*=11)	986.0±118.6 (*n*=10)	ES	99.2	245.0
					F value	0.2	9.2
					p value	0.2	0.004
Low test 1 startle	367.6±54.1 (*n*=10)	592.7±86.6 (*n*=11)	456.3±72.8 (*n*=10)	677.2±80.4 (*n*=9)	ES	208.7	84.5
					F value	8.5	1.4
					p value	0.006	0.2

NCC no contextual conditioning

Influence of baseline anxiety-like behaviour on the effect of contextual fear conditioning and drug

When repeating the assessment of a possible effect of drug or contextual conditioning or the combination thereof on test 3 startle (adjusted for test 2 startle) after dividing the animals into the 41 with "high" and the remaining 40 with "low" unconditioned startle at test 1, a significant main effect of escitalopram was found only in those displaying high test 1 startle (Table 2). In contrast, a significant main effect of contextual conditioning was observed only in those displaying low test 1 startle (Table 2). A similar picture emerged when the change in startle from test 2 to test 3 was related to the baseline startle at test 1 for the four treatment groups (Fig. 2); thus, while the context-conditioned animals (CC-NaCl) displayed a negative regression slope, the drug-treated (NCC-Escit) animals showed a positive slope, the difference in slopes being significant ($p<0.05$). As should be expected from a combination of the two different trends, the slope of the combined treatment group (CC-Escit) was similar to that of the non-treated group (NCC-NaCl).

When assessing the effects of escitalopram and/or contextual conditioning on test 3 freezing (adjusted for test 2 freezing) after dividing the animals on the basis of freezing response at day 1 (high $n=41$, low $n=40$), the effects of both contextual conditioning and drug turned out to be significant only in the group that had displayed low test 1 freezing (Table 3).

Discussion

The main findings of this study are the following: (i) outbred Wistar rats display relatively stable inter-individual differences with respect to unconditioned startle and unconditioned freezing, and the two responses correlate in the sense that rats displaying high unconditioned startle responses are also more inclined to display high unconditioned freezing responses; (ii) contextual conditioning and acute escitalopram enhance both startle and freezing and exert an additive but not a synergistic effect when administered in combination; and (iii) startle in animals displaying high unconditioned startle at baseline is more influenced by escitalopram but less influenced by contextual conditioning than in those displaying low unconditioned startle at baseline. We suggest that the influence of escitalopram on context-conditioned as well as unconditioned startle in Wistar rats displaying high unconditioned startle at baseline may serve as a model for SSRI-induced anxiety in man.

The marked correlation between unconditioned startle at tests 1 and 2 suggests assessment of unconditioned startle to be useful for characterizing animals within a batch of outbred Wistar rats as more or less "anxious" using this paradigm. The inter-test correlation was similarly high for unconditioned freezing, and the two parameters also correlated with each other. To the best of our knowledge, this is the first study to investigate the inter-test correlation with respect to these measures in a batch of experimental animals. The observation that startle and freezing correlate with each other, on the other hand, is a replication of a previous finding by Leaton and co-workers (Leaton and Borszcz 1985). Furthermore, the notion that startle and freezing partly reflect similar mechanisms gains further support from studies comparing the two paradigms, e.g. with respect to response to behavioural and neurosurgical manipulations (Borszcz et al. 1989; de Oliveira et al. 2011; Leaton and Borszcz 1985; Luyten et al. 2011; Plappert et al. 1993; Santos et al. 2005, 2006).

Acute administration of SSRIs exerting anxiogenic effects in rodents is well in accordance with previous studies using different tentative models of anxiety (Brandao et al. 2008; Burghardt et al. 2004, 2007; Dekeyne et al. 2000; Martinez

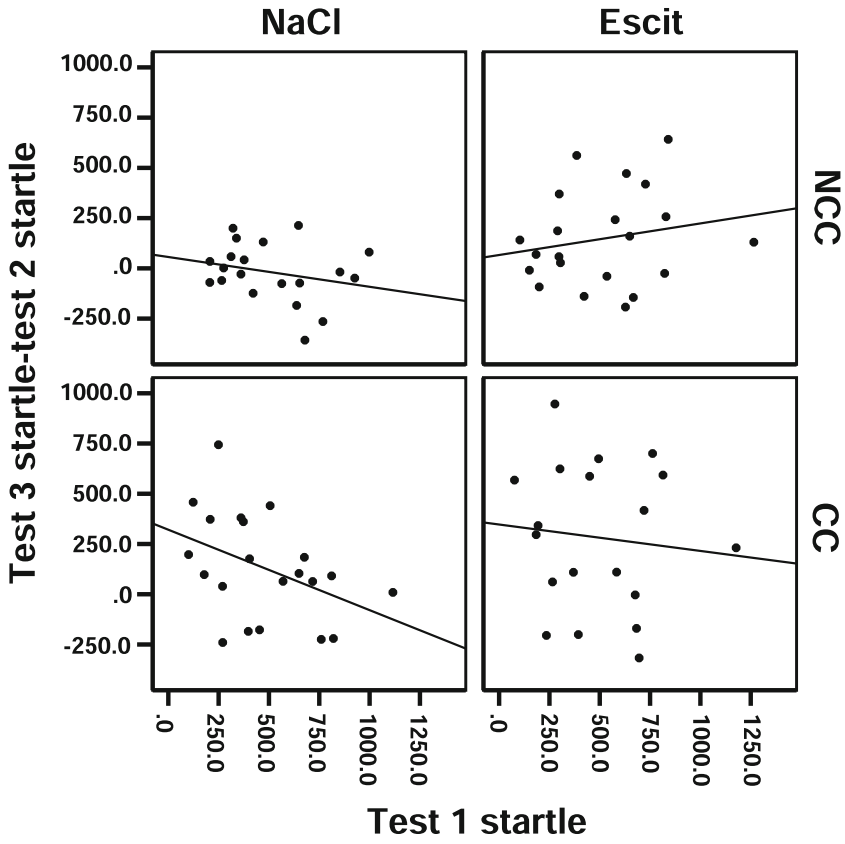

Fig. 2 Test 3 startle minus test 2 startle in relation to test 1 startle. Each *dot* represents the value of one animal, and the *regression lines* indicate the trends for different treatment groups. The difference in slopes between animals treated with either escitalopram only (Escit/NCC) or context conditioning only (NaCl/CC) was significant ($p<0.05$)

et al. 2007; Matto and Allikmets 1999; Santos et al. 2006) and also in line with reports of increased fear-potentiated startle in man following acute SSRI administration (Grillon et al. 2007); moreover, it is likely to have bearing on the clinical observation that SSRIs may exert a paradoxical increase in anxiety during the first days of treatment (Browning et al. 2007; Grillon et al. 2007; Hetem et al. 1996; Rammsayer and Netter 1990; Sinclair et al. 2009). While our observation that acute escitalopram enhances startle after contextual conditioning is in line with previous animal studies (Santos et al. 2006), to our knowledge, this is the first study showing an SSRI to enhance startle also without prior fear conditioning (apart from repeated exposure to the box in which the startle-eliciting noise has been administered).

With respect to the possible effect of acute administration of SSRIs on conditioned freezing, previous studies are not unanimous; many authors have thus found acute SSRIs to reduce rather than enhance conditioned freezing behaviour in rats (Hashimoto et al. 1996, 2009; Inoue et al. 1996; Muraki et al. 2008; Nishikawa et al. 2007; Santos et al.

Table 3 Freezing at test 3 presented as mean (%)±SEM displayed by each treatment group shown for all animals as well as after division based on test 1 freezing (high vs. low test 1 freezing). Shown also are effect sizes (ES), F values and levels of significance (p value) for the effects of contextual conditioning (CC) and escitalopram (Escit), respectively, on test 3 freezing adjusted for test 2 freezing

	NCC-NaCl	CC-NaCl	NCC-Escit	CC-Escit		CC	Escit
All animals	56.0±3.9 (n=20)	65.8±2.4 (n=21)	62.9±2.7 (n=21)	74.8±2.2 (n=19)	ES	10.5	8.1
					F value	14.0	8.3
					p value	0.0003	0.005
High test 1 freezing	64.0±5.0 (n=10)	63.3±3.4 (n=11)	64.5±3.8 (n=12)	73.8±4.5 (n=8)	ES	3.6	5.4
					F value	4.1	3.0
					p value	0.4	0.2
Low test 1 freezing	48.1±5.0 (n=10)	68.5±3.4 (n=10)	60.8±4.0 (n=9)	75.5±2.2 (n=11)	ES	17.2	9.4
					F value	10.8	6.0
					p value	<0.0001	0.01

NCC no contextual conditioning

2006). Methodological aspects, including the duration and intensity of the foot shocks during fear conditioning, as well as the dose and time of SSRI administration, can potentially account for these differences. In this context, it should be mentioned that we measured startle and freezing simultaneously and applied a protocol designed to enhance startle, hence using a higher number of foot shocks, but with shorter duration, as compared to the protocols usually applied for eliciting conditioned freezing (Hashimoto et al. 1996, 2009; Inoue et al. 1996, 2004; Muraki et al. 2008; Nishikawa et al. 2007). It should, however, be noticed that escitalopram, in our hands, enhanced freezing also in animals that had not been the subject of contextual conditioning.

While acute administration of an SSRI may exert marked increase in anxiety in patients with an anxiety disorder, such as panic disorder (Grillon et al. 2007; Ramos et al. 1993; Sinclair et al. 2009), and in subjects with anxiety-related personality traits (Rammsayer and Netter 1990), they are often entirely free from this side effect in others. A major impetus for this study hence was to assess if rats displaying enhanced non-conditioned startle or freezing at baseline are more inclined to display enhanced startle or freezing when exposed to acute escitalopram than those displaying less baseline reactivity. With respect to SSRI-enhanced startle, this was indeed found to be the case; a significant effect of escitalopram on startle was thus only observed in the half of the rats displaying relatively high startle at baseline (Table 2). Not surprisingly, given the strong correlation between baseline startle and freezing, also baseline freezing could predict the startle response to escitalopram. While the mechanisms underlying this enhanced "anxiogenic" response to an indirect serotonergic agonist in subjects displaying enhanced baseline anxiety remain unclear, it is tempting to speculate that serotonergic pathways may exert a stronger anxiety-enhancing influence in such subjects, evident both in the presence and absence of pharmacological provocation (Esler et al. 2007), and that the beneficial anxiety-reducing effect of long-term administration of SSRIs in patients with anxiety disorders may be best explained as an adaptive downregulation of such an influence.

Our observation that, in the group comprising all animals, prior exposure to foot shock in the startle/freezing chamber enhanced both startle and freezing responses supports our assumption that this chamber was sufficiently neutral a stimulus to be paired with foot shocks in order to create context-dependent fear conditioning. However, while the proneness in animals with high baseline startle to respond to a startle-enhancing stimulus was specific for the SSRI, we observed the opposite pattern with respect to the effect of contextual conditioning. Thus, while rats displaying low unconditioned baseline startle or freezing responded markedly to contextual conditioning, no such effect was seen in those with high baseline reactivity. This phenomenon was found to be proportional; the higher the baseline reactivity, the lower the response to contextual conditioning and the higher the response to escitalopram (Fig. 2). A likely explanation to this phenomenon is that animals with high startle at baseline, unlike those with low startle at baseline, may have reached a level of startle that can be further increased by acute SSRI administration but not by fear conditioning. We suggest that Wistar rats displaying high levels of unconditioned startle behaviour may serve as an animal model for the phenomenon that SSRIs enhance anxiety in some but not all subjects exposed to them, and aid to shed light on the underlying mechanisms.

SSRI-induced freezing, on the other hand, was not more pronounced in rats displaying high baseline freezing (or startle; data not shown); in contrast, both contextual conditioning and acute escitalopram enhanced freezing significantly only in animals with low baseline freezing (Table 3). A possible explanation for this observation is that, in our experimental setting, many animals may have reached a maximal effect with respect to freezing but not startle; animals with high freezing at baseline hence may have had less potential for a further increase because of a ceiling effect.

When interpreting all results regarding freezing in the paper, it should be taken into consideration that we did not actually record freezing behaviour but immobility; however, gross observation of the animals did support the notion that the immobility displayed during noise bursts, and also before noise bursts in context-conditioned rats, was accompanied by the typical features of freezing such as piloerection and arched back. The pros and cons of automated recording of freezing as applied in this study were recently discussed by Luyten and co-workers (Luyten et al. 2014).

To conclude, this study demonstrates (i) that animals within a batch of outbred Wistar rats display stable and consistent inter-individual differences with respect to proneness for unconditioned startle and freezing and also that there is a considerable correlation between unconditioned startle and unconditioned freezing; (ii) that contextual conditioning and acute exposure to an SSRI, escitalopram, both enhance startle and freezing and display an additive but not synergistic effect when combined; and (iii) that animals displaying high baseline startle are more inclined than less "anxious" animals to experience an increase in startle when exposed to escitalopram. We suggest that further studies on this issue may shed light on why acute administration of SSRIs may exert anxiogenic effects in man and why certain individuals are considerably more susceptible to this effect than others.

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Conflict of interest None of the authors declare any conflict of interest.

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