Serotonin, cortisol, and stress-related psychopathology

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Chapter 4

Physiological and behavioral consequences of SSRI discontinuation

Experiment 1

Long-term administration of an SSRI depletes serotonin stores in the rat brain

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ABSTRACT

The undisputable position of serotonin reuptake inhibitors (SSRIs) in antidepressant therapy duly connects with demonstrable efficacy and good tolerability in clinical studies (204;205). Lately, cracks in their armor have shown in the form of meta-analyses suggesting that their efficacy barely exceeds placebo response (206) and reports of incidental suicide and aggression in particular with young people (207;208). Such observations might connect with an unforeseen side effect of long-term SSRI treatment, namely a depletion of serotonin stores in the central nervous system akin to the drain of serotonin from the blood platelets of patients (209).

Using a suitable rat model we here demonstrate that long-term treatment with the SSRI citalopram depletes serotonin stores by 60% on average in nine brain areas, which is not yet restored within 2 days after cessation of the drug.

This indicates that central serotonin function becomes highly dependent of newly synthesized neurotransmitter during long term SSRI treatment. Factors that adversely influence this synthesis, e.g. tryptophan and vitamin B6 deficiency (210;211), tryptophan hydroxylase polymorphisms (212) or factors that otherwise derange serotonin homeostasis, e.g. severe non-compliance (213) might lead to increased impulsivity and aggression reminiscent of somatic diseases and treatment conditions associated with decreased circulating tryptophan levels (115;116).

Given the concerns expressed by the United States Food and Drug Administration (FDA) with regard to SSRI treatment especially in children and adolescents(214) we propose to assess the blood tryptophan profile (166) and vitamin B6 status in this group of patients whenever possible, and with respect to the compliance issue developing preparations with extended drug release should also be taken into consideration.

INTRODUCTION

An effective therapy with antidepressant medication lasts several months up to one year, and often even longer. The antidepressant response is usually seen within 8 weeks and continuation of the treatment thereafter is in essence prophylactic, i.e. to reduce the risk of recurrence of the depression. The time delay required for the therapeutic response has often been attributed to adaptations of the cerebral serotonin (5-HT) receptors (215).

An implicit assumption may be that during long-term exposure to selective serotonin reuptake inhibitors (SSRIs) levels of serotonin in the brain allow optimal functioning. Despite its clinical relevance, systematic studies in laboratory animals have not been reported on the issue whether 5-HT stores are affected by chronic exposure to SSRIs. It is known that long-term SSRI medication depletes blood platelet 5-HT in patients (209;216). Besides, clinical studies have also reported decreased 5-hydroxy indoleacetic acid (5-HIAA) levels in cerebrospinal fluid of depressed subjects that could be attributed to both treatment and the depressive state (217). Central 5-HT cells are likely to rely on synthesis as well as reuptake to maintain their intracellular stores. We considered therefore the possibility that intracellular 5-HT stores become depleted during long-term exposure to an SSRI if synthesis does not compensate reuptake inhibition. The functional implications of chronic treatment are conventionally assessed by pharmacological challenges with serotonergic agents following a shorter or longer period of drug discontinuation (washout period). This approach minimizes interference by residual antidepressant, but it may also provoke rapid adaptive changes (218), which would complicate the interpretation of the results considerably.

In the present study we investigated the effect of 14 days citalopram administration on 5-HT tissue content, turnover and synthesis with and without a washout period of 48 hours. As indices we used regional brain tissue contents of 5-HT and 5-HIAA. The rate of 5-HT synthesis was estimated by the accumulation of 5-hydroxy tryptophan (5-HTP) following systemic administration of the amino acid decarboxylase inhibitor NSD 1015. The ratio of 5-HIAA/5-HT was used as an index of serotonergic metabolism or turnover.

MATERIALS AND METHODS

Animal experiments

All experiments were performed according to the governmental guidelines for care and use of laboratory animals and were approved by the Committee for Animal Research of the Medical Faculty of the Groningen University. The effects of long-term citalopram administration were studied using three chronic treatment groups (I saline, II and III citalopram), each consisting of 5 animals. Osmotic mini-pumps (2ML2 Alzet, USA, 5 µl/h, 14 days) filled with either saline or 50 mg/ml citalopram hydro bromide dissolved in saline

under aseptic conditions were implanted subcutaneously under isoflurane anesthesia (2.5 %, 400 ml/min N_2O, 600 ml/min O_2). On the 15th day the osmotic minipumps were replaced under isoflurane anesthesia by new minipumps, containing either saline (group I control, group II washout) or citalopram (group III no washout). On the 17th day, animals received an intraperitoneal injection of the amino acid decarboxylase inhibitor NSD 1015 (100 mg/kg). Forty five min thereafter animals were anesthetized with isoflurane with their mini-pumps still in place, blood was taken by cardiac puncture, brains were removed and rapidly frozen on dry ice and stored at –80 °C.

Tissue destruction

Punches (0.23 mm^3) from coronal slices were taken from nine brain areas (see Fig 1). Brain tissue was homogenized in 100 µl of 0.1 M perchloric acid and centrifuged at 14,000 rpm for 10 min at 4°C. The supernatant was assayed for 5-HT, 5-HIAA and 5-HTP(219). Citalopram in plasma was analysed as previously reported (220).

Statistics

Biochemical indices of the chronic citalopram treatment groups were calculated as percentage of the control group, the chronic saline treated animals. Statistical analysis was performed using SPSS. Treatment effects were evaluated using MANOVA followed by Tukey's HSD post-hoc test.

RESULTS

Long-term citalopram administration decreased serotonin content throughout the brain (Fig 1). This effect was not influenced by the introduction of a washout period. MANOVA showed a general effect of treatment or washout on 5-HT content ($F_{(2,110)} = 7.773$; $p<0.001$). Post-hoc analyses revealed that compared to the saline treated group, long-term citalopram treatment significantly decreased 5-HT stores with ($p=0.023$) and without ($p<0.001$) a washout period.

Introducing a washout period after chronic citalopram increased serotonin turnover, while chronic treatment itself (without washout) had little effect (Fig 1). MANOVA indicated a significant effect of treatment or washout on turnover ($F_{(2,110)} = 6.034$; $p<0.05$). Post-hoc analysis revealed that serotonin turnover was significantly increased in animals with a washout period following chronic citalopram administration, compared to both saline treated animals ($p = 0.022$) and animals treated with citalopram without a washout period ($p = 0.005$).

MANOVA indicated no significant effects of treatment or washout on serotonin synthesis ($F_{(2,110)} = 0.727$; $p=0.486$). Although no significant overall effects were observed, there were trends towards increased synthesis in the washout group and decreased synthesis in the group without washout. As observed for serotonin turnover, the two experimental

conditions (washout vs. no washout) tend to have opposite effects on 5-HT synthesis (Fig 1). Plasma levels of citalopram at termination were assessed for all treatment groups. Chronic administration of citalopram without a washout period resulted in plasma levels of 361 ± 14 nM. Citalopram levels of the other treatment groups were below the limit of detection (5 nM).

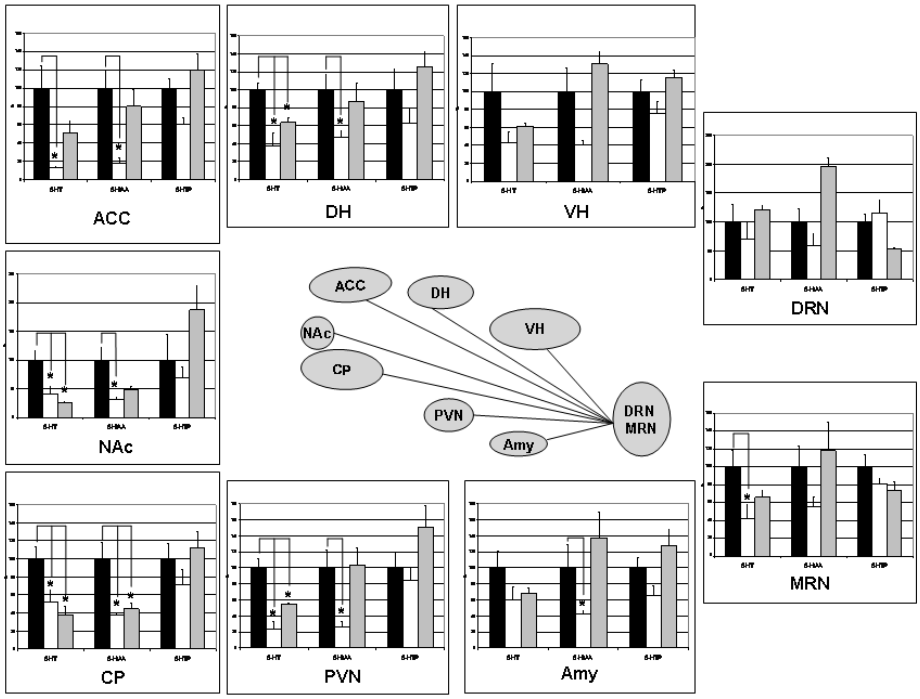

Figure 2: Schematic depiction of the rat brain: Effects of chronic citalopram administration with and without washout vs. chronic saline administration on serotonin and 5-HIAA content and on 5-HTP accumulation in nine areas of the brain. Black bars: long-term saline treatment. White bars: long-term citalopram treatment, no washout. Grey bars: long-term citalopram treatment, 48 hours washout. The saline treatment data were set to 100% and the other data related to this value. Punches of coronal slices were taken from two cell body areas, namely the dorsal (DRN) and median (MRN) raphe nuclei and seven axon terminal areas, namely the anterior cingulate cortex (ACAD), nucleus accumbens (Nac), caudate/putamen (CP), paraventricular nucleus of the hypothalamus (PVN), central nucleus of the amygdala (Amy), dorsal (dHC) and ventral hippocampus (vHC). Asterisks indicate significantly different ($p<0.05$) compared to saline control. A post-hoc test was not performed with the synthesis data, because MANOVA did not indicate statistical differences.

DISCUSSION

In this study we analyzed brain tissue of rats injected with a synthesis inhibitor shortly before termination to demonstrate that the 5-HT stores of the nerve endings are affected and not the compartment confining the newly synthesized 5-HT. We have used osmotic

minipumps to ensure that citalopram plasma levels during long-term treatment stayed within the clinically effective range (220;221). Our main observations are that the tissue stores of 5-HT become depleted during long-term citalopram treatment, while synthesis is not significantly changed. Both observations converge to the idea that the diminished reuptake of serotonin is not compensated by increased synthesis.

Considering the synthesis data of the present study, some previous studies have reported restored (222;223) or even increased levels of 5-HTP following chronic antidepressant treatment (224) , suggesting adaptation to the condition of long-term reuptake inhibition. However, in all these studies a longer washout period was included. As emphasized in the introduction, cessation of the antidepressant may provoke changes at the cellular level, thereby altering or even reversing the effects of chronic treatment (218). This is indeed confirmed by the present results, as both 5-HT turnover and the accumulation of 5-HTP tend to be decreased without and increased with a washout period. Arguably, compared with the washout strategy examining these measures in the presence of the antidepressant more accurately depicts the neurochemical consequences of the chronic exposure.

The depletion of serotonin stores suggests a diminished buffer capacity and arguably a more fragile serotonin function. It is tempting to speculate that shifts in 5-HT homeostasis become more critical in this condition and that this plays a role with some of the discontinuation symptoms associated with abrupt cessation of antidepressant treatment, but the issue of non-compliance may also not be ignored here. Because synthesis of serotonin highly depends on dietary tryptophan and vitamin B6, the nutritional state of patients could also be a critical factor with long-term SSRI treatment. Moreover, the relapse of depressive symptoms following tryptophan depletion in patients successfully treated with antidepressants also fits in this view (225). We believe that this is important information for both medical practitioners and pharmaceutical companies, but we realize also that it is difficult at this stage to judge how severe the implications of this serotonin depletion are. It is to note however that Pet-1 null mutant mice with 80-90% reductions of 5-HT and 5-HIAA content in cortex, hippocampus and caudate nucleus display heightened anxiety-like and aggressive behavior (226). Moreover, the side effect profile of the serotonin depleting anorectic drug fenfluramine also includes increased anxiety and agitation. Paradoxically, the antihypertensive drug reserpine depletes central monoamine stores including those of serotonin while inducing symptoms of depression, which observation constitutes one of the important pillars under the monoamine hypothesis of affective disorders. Further work is warranted to determine whether the depletion of intracellular serotonin stores continues, has functional consequences and occurs with other SSRIs as well. It is also worth investigating how atypical serotonergic antidepressants such as mirtazapine, tianeptine and agomelatine behave in this respect.

Experiment 2

Increased startle reflex and serotonin metabolism following citalopram discontinuation in rats

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ABSTRACT

***Background**: Abrupt termination of antidepressant treatment results in about one third of the patients in a discontinuation syndrome characterized by - among others - irritability, anxiety and impulsivity. Such symptoms have also been reported in somatic diseases that are associated with impaired serotonin function. We investigated the effect of abrupt cessation of chronically administered citalopram, a selective serotonin reuptake inhibitor, on serotonin function and responsivity to acoustic startle in rats.*

***Methods**: Rats were exposed for 14 days to citalopram via osmotic minipumps. Thereafter, these minipumps were replaced by minipumps containing either saline or citalopram. An acoustic startle paradigm and serotonin turnover indices were used to assess the behavioral and biochemical consequences in the week following drug discontinuation.*

***Results:** Startle response was higher and habituation less in the discontinuation group, concomitant with increased 5-HT turnover in the amygdala and nucleus accumbens.*

***Conclusions:** This animal study supports the idea that at least part of the selective serotonin reuptake inhibitor discontinuation symptoms observed in patients connects with alterations of serotonin function during sudden withdrawal of the SSRI. In clinical practice, the importance of slowly tapering of the antidepressant to prevent discontinuation symptoms is well recognized. Less well recognized, however, is that these symptoms may also emerge with non-compliance given the rapid and long-lasting behavioral changes observed.*

INTRODUCTION

Abrupt termination of treatment with selective serotonin reuptake inhibitors (SSRIs) results in about one third of the patients in a phenomenon referred to as the antidepressant-discontinuation syndrome. Key features of SSRI discontinuation include aggression, irritability, agitation, anxiety and low mood (114). Discontinuation symptoms are generally reported within 1-7 days after termination or dose-decrease of the treatment (114). The symptoms are typically short-lived, and in principal not life-threatening, but in some patients discontinuation symptoms cause considerable morbidity. In the clinical practice, adequate measures are taken by slowly tapering the SSRI, which reduces the frequency and severity of discontinuation symptoms considerably (111;113;227). More relevant in this respect is the high incidence of non-compliance during antidepressant treatment. Non-compliance may exceed 30% and the resulting discontinuation symptoms may not be recognized, which will negatively influence current and future treatment compliance (213;228;229).

Discontinuation symptoms are sometimes referred to as rebound depression, which could indeed play a role. However, most of the symptoms are not typical for depression, but more akin to those seen in patients suffering from somatic diseases (e.g. carcinoid syndrome) and under treatment conditions (e.g. γ-interferon). Such symptoms appear to be associated with a decreased availability of tryptophan and consequently of the formation of 5-HT in the CNS (87;115;133;137;141).Low 5-HT functioning as cause of discontinuation symptoms has also been proposed by Blier et al. (230). Previously, we have demonstrated that chronic administration of citalopram markedly decreases serotonin content in various rat brain regions, including the nucleus accumbens and amygdala. Being unable to measure serotonin in the patient brain one can only speculate, however blood platelets have been used as a peripheral model for the central 5-HT neurons, since they have similar dynamics of 5-HT uptake, receptors and monoanime oxidase activity (231). Given the fact that chronic SSRI treatment depletes blood platelet 5-HT in patients (232-234), it is imaginable that during SSRI treatment 5-HT stores are also depleted in the human central nervous system (CNS). Importantly, impaired serotonin function in rats markedly increased aggressive (70) and irritable behavior, as witnessed for instance by an increased responsiveness to acoustic stimuli (73;127;164;182) .

In the present study, we investigated the temporal effects of sudden discontinuation of long-term SSRI administration on behavioral indices in the rat. Many of the symptoms of the discontinuation syndrome have a subjective character and are therefore difficult to assess in animals. Hyperarousal on the other hand, which includes the discontinuation symptoms aggression and irritability seen with SSRIs but not with atypical antidepressants or TCAs (114), can be measured in animals. In this respect, the acoustic startle reflex (ASR) seems a good instrument because it enables measurement of reactivity, which is likely to be part of hyperarousal related symptoms. Moreover both in humans and animals, the ASR is

sensitive to changes in serotonergic activity (183;235) including the effects of acute and chronic SSRI treatment (236-238) .

To mimic steady state conditions in humans, the SSRI citalopram was administered for 14 days via osmotic minipumps. Thereafter, the minipumps were removed and replaced by minipumps containing either saline (discontinuation group) or citalopram (continuation group). An acoustic startle paradigm and brain regional 5-HT turnover measurements were used to assess behavioral and biochemical consequences of drug discontinuation.

MATERIALS & METHODS

Animals

The experiments were performed according to the governmental guidelines for care and use of laboratory animals and were approved by the Committee for Animal Research of the University of Groningen.

Male Wistar rats (Harlan, Zeist, the Netherlands), weighting 200-250 gram, were housed individually in a temperature-controlled environment (21-23 °C), with food and water ad libitum. Animals were kept on a 12h reversed light/dark cycle with lights on from 1900h to 0700h. The experiments were conducted during the active period. Animals were left undisturbed after arrival, except for daily weighing, for one week to acclimatize to their new environment.

Treatment

We considered it important to compensate for some of the pharmacokinetic differences between humans and rodents. By administering citalopram through osmotic minipumps (220), we have mimicked steady state conditions in humans and assured that citalopram blood levels were in the therapeutically effective range during the whole experiment (221). Animals were assigned to one of two treatment groups (eight rats per group): a chronic citalopram treatment group and a citalopram washout group. Citalopram was used because it currently is the most selective racemic SSRI available (109). Moreover, the compound is rapidly cleared from the brain and its metabolites are devoid of intrinsic effects on 5-HT reuptake. Osmotic minipumps (2ML2 Alzet, USA, 5µl/h, 2 weeks) were filled with 50 mg/ml citalopram hydrobromide (kindly donated by Lundbeck, Denmark, courtesy Dr. Sanchez) dissolved in saline under aseptic conditions. During isoflurane anaesthesia (2,5%, 400 ml/min N_2O, 600 ml/min), minipumps were implanted subcutaneously on the left side of the back of the rat. Rats received a single finadyne injection (2.5 mg/kg i.p) to suppress post operative pain. Fourteen days later, these minipumps were removed, the cavity was flushed with sterile saline and minipomps (1ML2 Alzet, USA, 10 µml/h 1 week) filled with either 25 mg/ml citalopram (chronic citalopram group) or saline (washout group) were implanted, under isoflurane anaesthesia (2,5%, 400 ml/min N_2O, 600 ml/min), again finadyne (2.5

mg/kg i.p) was injected post-operatively. Blood samples for the determination of plasma citalopram levels were taken by tail venous-puncture. Details of our procedures are found in Cremers et al. (220).

Acoustic startle procedures

Forty eight hours after replacement of the minipumps, we started behavioral testing for five days. At the time of the first test the citalopram plasma levels were already below the pharmacologically active range in the washout rats (220). The individual rats were tested at the same time daily. Between 0900h and 1200h, the acoustic startle response of the rats was measured, using a Startle Response System (TSE GmbH, Bad Homburg, Germany) as described before (182). After a 3 min acclimatization period, during which the rats received no stimuli except a 70 dB background noise, the test session began with 10 trials consisting of single 40 ms 120 dB pulse sound startle stimuli. The sessions then continued with 50 trials which consisted of random delivery of 20 120 dB pulse-alone trials, 10 trials during which no stimuli were delivered and 20 prepulse trials. Prepulse trials included a single 120 dB pulse preceded by 100 ms by a 20ms non-startling prepulse-stimulus of 80 dB. The last 10 trials were single 40 ms 120 dB pulse-alone startle stimuli. All the 70 trials were delivered with an interval of 10 s. During the experiment a constant background noise of 70 dB was present. Percentage of pre-pulse inhibition (PPI) was calculated as [(startle response with the pre-pulse− response to the middle twenty 120 dB pulses)/response to the middle twenty 120 dB pulses × 100]. The first and last 10 pulse-alone stimuli (block1 and block4, respectively) and the 20 pulse-alone stimuli included in the PPI block itself (block2 and block3), were used to obtain a measure of response habituation in response to repeated delivery of startling stimuli. The first 10 startle pulses of day 1 (block1day1) were used to obtain information about startle reactivity, with the least possible interference of habituation (239).

Termination and tissue dissection

Rats were decapitated 20 minutes after the 5th acoustic startle session. Trunk blood was collected for determination of plasma citalopram and corticosterone levels. The thymus and adrenal glands were removed and weighted. Brains were removed, rapidly frozen at dry ice and stored at -80 °C.

Brains were cut into 300μm slices in a cryostat and punches (0.23 mm^3) were taken from nine brain areas; anterior cingulate cortex, nucleus accumbens, caudate putamen, paraventricular nucleus of the hypothalamus, dorsal hippocampus, ventral hippocampus, amygdala, median and dorsal raphe nucleus. Brain samples were homogenized with 100 μl of 0.1 M perchloric acid and centrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant was removed and assayed for 5-HT and 5-HIAA.

Analytical procedures

Analysis of 5-HT and 5-HIAA was performed by high performance liquid chromatography (HPLC) with electrochemical detection(219). Citalopram was measured in plasma according to (240) with minor modifications (220). Assays of corticosterone were as described previously (182).

Statistical analysis

Statistical analyses were done with SPSS (version 12.0), and $P \leq 0.05$ was considered to be significant. Data are shown as mean ± S.E.M. Treatment effects were analyzed using a one-way ANOVA. Weight gain and ASR were analyzed with a repeated measures ANOVA. Correlations were determined using the Pearson correlation coefficient. Greenhouse-Geisser correlations were used when the assumption of sphericity was not met.

RESULTS

Plasma citalopram and corticosterone levels

After 14 days of citalopram administration, citalopram plasma levels were 0.27 ± 0.04 μM, with no differences between the groups. At termination, the citalopram plasma levels in the continuation group tended to be higher, amounting 0.38 ± 0.02 μM, while in the discontinuation group the levels had dropped to 0.05 ± 0.01 μM ($F = 136,153$, $P < 0.001$). Notably, elimination of citalopram after chronic administration is much slower than one would expect on the basis of the compound's halve life in rats, which is 3 hours (241). This indicates that the elimination rate after removal of the minipumps is more in line with that of patients.Plasma levels of corticosterone did not differ between the discontinuation and continuation groups at termination (797± 148 vs. 970± 137 nmol/l; $F=0.677$; $p=0.43$).

Body weight and food intake

Citalopram treatment did not affect body weights or food intake. The relative adrenal weight (discontinuation 0.013±0.001 % vs. continuation 0.014±0.001 %; $F=0.635$; $p=0.441$) or relative thymus weight (0.14±0.01 % vs. 0.16±0.01 %; $F=2.353$; $p=0.151$) did not differ between the groups.

Acoustic Startle Response (ASR)

Figure 1 shows that repeated exposure to the acoustic stimuli resulted in a decrease in motor response in all animals, both within one session (short-term habituation; main effect of block $F = 19,428$, $P < 0.001$) and across experimental days (long-term habituation; main effect of day, $F = 14,989$, $P < 0.001$). Difference in habituation between both treatment groups was seen by a block x treatment interaction effect ($F = 4,690$, $P < 0.03$) reflecting an attenuation of habituation in the citalopram wash-out rats to the acoustic stimuli.

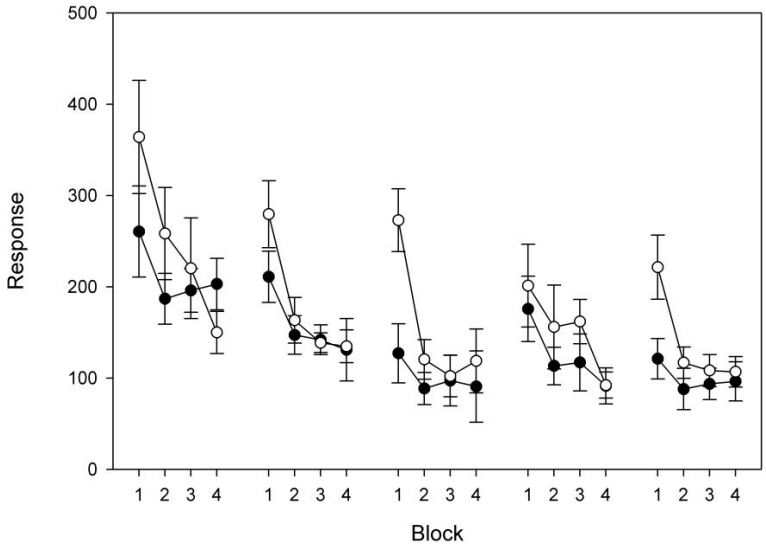

Figure 1: Acoustic startle responses. Responses are shown as blocks, consisting of 10 consecutive pulses, over 5 days. Filled circles: continuation of citalopram treatment. Open circles: discontinuation of citalopram treatment 48 hours before start of the behavioral testing. In all groups the startle response declined significantly within the daily trials and across experimental days (both P< 0.001). There was a difference between the habituation profiles of the treatment groups (P=0.03). Data of 8 rats per group. Results are expressed as average value per block ± S.E.M.

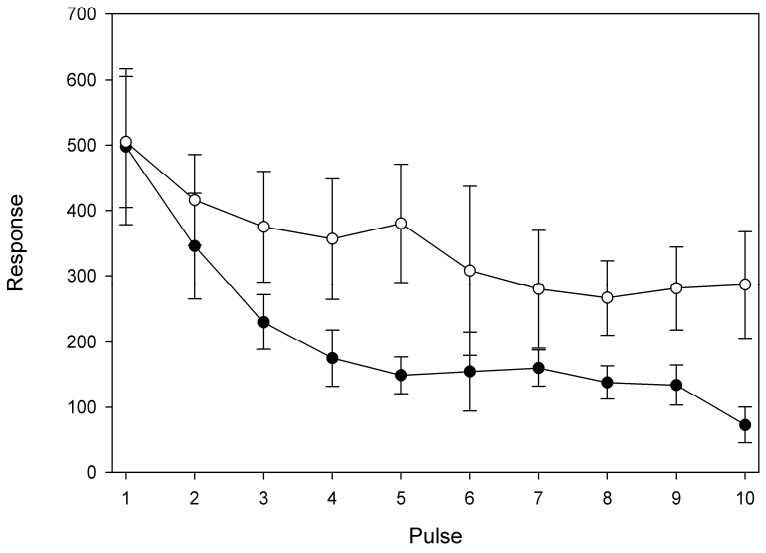

Figure 2: Response to the first 10 startle pulses (day 1, 1st block). The continuation group is depicted by filled circles, while the discontinuation group is depicted by open circles. Drug treatment affects the time courses significantly (P< 0.05). Data of 8 rats per group. Results are expressed as average value per pulse ± S.E.M

Figure 2 shows that the first 10 startle pulses at day 1 showed a significant effect of treatment on startle reactivity (F = 4,702, P = 0.05), probably the result of a diminished habituation, because a besides a significant time effect (F 32, 498, P = 0.03), also significant interaction effect of pulse*treatment (F = 18,345, P = 0.05) was observed.

Serotonin turnover

Figure 3 shows the regional brain ratio's of 5-HIAA and 5-HT. Significant differences were observed in the nucleus accumbens (Nacc, F = 6,268, P = 0.046) and the amygdala (Amy, F= 9,504, P = 0.022) with strong trends in the caudate nucleus (CP, F = 3,572, P = 0.095), the paraventricular nucleus (PVN, F = 5,968, P = 0.071), and the ventral hippocampus (vHC, F = 5,445, P = 0.067).

Pearson analysis indicated a significant correlation (r=0.714, P = 0.047) between the startle response and 5-HT turnover in the amygdala at day 5.

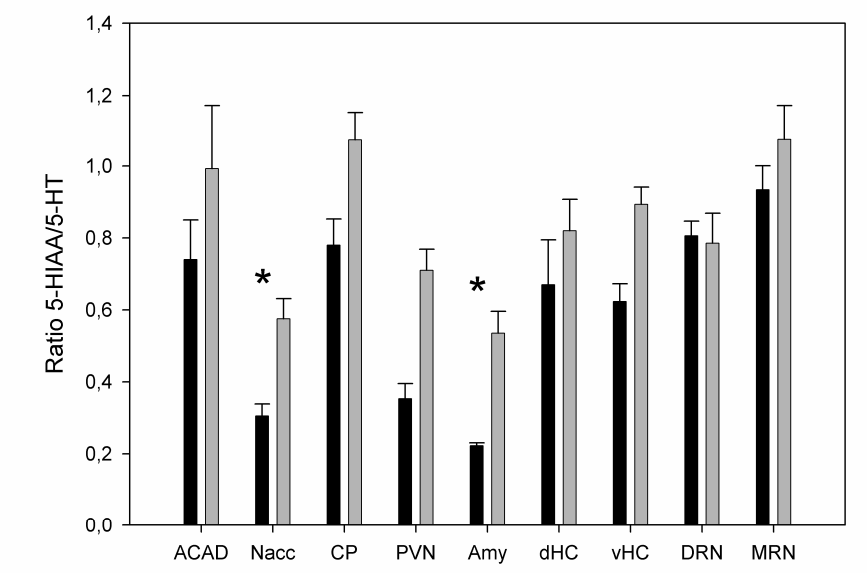

Figure 3: Ratios of 5-HIAA/5-HT in various rat brain regions. The continuation group is depicted by black bars, while the discontinuation group is depicted with gray bars. Brains were cut into 300µm slices using a cryostat, and punches were taken from nine brain areas including the anterior cingulate cortex (ACAD), nucleus accumbens (Nacc), caudate putamen (CP) praventricular nucleus of the hypothalamus (PVN), dorsal hippocampus (dHC), ventral hippocampus (vHC), amygdala (Amy), median and dorsal raphe nucleus (MRN and DRN). Groups were 8 rats. Results are expressed as means ± S.E.M. *P≤<0.05, #P≤<0.1

DISCUSSION

The present study demonstrates that abrupt discontinuation of chronic citalopram administration to rats induces long lasting increases of the acoustic startle response, while habituation is significantly decreased. At the biochemical level, serotonin turnover in both the amygdala and nucleus accumbens is significantly increased at the 5th day of behavioral assessment. Moreover, a significant correlation was found between the startle response and 5-HT turnover in the amygdala on the 5th day.

To the best of our knowledge, the present study is the first attempt to address some of the biochemical and behavioral consequences of abrupt discontinuation of a chronically administered SSRI. Measuring behavioral and biochemical indices in the same animal requires some choices to be made. Accordingly, an ex-vivo method was preferred for the biochemical analysis because it would not compromise the behavioral analysis, both in terms of brain damage and the free movement of the animals. Moreover, tissue destruction enabled the measurement of serotonin turnover in a relatively large number of brain areas. A disadvantage is that the temporal relation between behavior and serotonergic activity cannot be assessed in this way. However, using a similar design we have found 5-HT turnover to be increased in the same brain areas at day 3 of citalopram discontinuation (Jongsma thesis 2006), indicating that 5-HT turnover is increased during the whole test period.

We are not aware of studies that have investigated the effects of SSRI discontinuation on the ASR. However, one animal study reported increased locomotor activity after discontinuation of fluoxetine (242). Clearly, our study cannot provide the answer as to how citalopram discontinuation leads to an increased ASR and decreased habituation. It is noteworthy however that acute administration of citalopram also increased the ASR in rats (243). Unfortunately habituation was not measured in that study. Habituation to the ASR with a single dose of an SSRI has been studied with fluoxetine, also leading to diminished habituation (237;244). On the other hand, the effects of a single dose of citalopram in humans converged to an increased ASR, but without any clear effect on habituation (236;245). Despite the similarities in startle reaction between acute administration of an SSRI and discontinuation of chronic SSRI treatment, it is hard to imagine that the underlying biochemical mechanisms are the same. On the other hand acute administration of SSRIs to patients and discontinuation of the SSRI both lead to increased impulsive behavior (111;207). Arguably, in both cases, the SSRI related symptoms result from a transient imbalance of the serotonergic system. Recently, we found that long-term citalopram administration depletes serotonin stores in the rat brain, which could make animals more vulnerable to a rapid change of serotonin transporter function (110). It is also noteworthy that even on the 5th day of behavioral analysis the correlation between serotonin turnover in the amygdala and the ASR is still significant. Using local injection and lesion paradigms this brain area has been identified as being crucial in startle responses (129). Moreover, the amygdala is part of the

limbic system and prominently involved in stress physiology and fear. Yet, an explanation in terms of stress physiology seems less likely because corticosterone levels did not significantly differ between the treatment groups.

Finally, the rapid onset of the discontinuation effects suggests that they might play a role during periods of non-compliance in the regular treatment as well. Given the marked and relatively long lasting effects of discontinuation on both behavior and biochemistry one may even speculate that the incidental cases of suicide and aggression in young adults during SSRI treatment (246-248) are partly related to non-compliance. It is good medical practice to slowly taper when SSRI treatment is to be terminated. However, we feel that little attention has been directed to the issue of non-compliance, especially with rapidly cleared SSRIs, such as citalopram. To minimize the risk of intermittent non-compliance the development and application of formulations with extended drug release should be encouraged.

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