Clonidine augmentation in patients with schizophrenia: A double-blind, randomized placebo-controlled trial

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ABSTRACT

Introduction: Noradrenergic imbalance in the brain of schizophrenia patients may underlie both symptomatology and deficits in basic information processing. The current study investigated whether augmentation with the noradrenergic α2-agonist clonidine might alleviate these symptoms.
Methods: In a double-blind placebo-controlled randomized clinical trial, 32 chronic schizophrenia patients were randomly assigned to six-weeks augmentation with either 50 μg clonidine or placebo to their current medication. Effects on symptom severity and both sensory- and sensorimotor gating were assessed at baseline, 3- and 6-weeks. Results were compared with 21 age- and sex-matched healthy controls (HC) who received no treatment.
Results: Only patients treated with clonidine showed significantly reduced PANSS negative, general and total scores at follow-up compared to baseline. On average, also patients treated with placebo showed minor (non-significant) reductions in these scores, likely indicating a placebo effect. Sensorimotor gating of patients was significantly lower at baseline compared to controls. It *increased* in patients treated with clonidine over the treatment period, whereas it *decreased* in both the HC and patients treated with placebo. However, neither treatment nor group effects were found in sensory gating. Clonidine treatment was very well tolerated.
Conclusion: Only patients treated with clonidine showed a significant decrease on two out of the three PANSS subscales, while additionally retained their levels of sensorimotor gating. Given that there are only a few reports on effective treatment for negative symptoms in particular, our current results support augmentation of anti-psychotics with clonidine as a promising, low-cost and safe treatment strategy for schizophrenia.

1. Introduction

Schizophrenia is a heterogeneous brain disorder with unclear causes. Deviations in several neurotransmitters systems have been described (e. g. Lieberman et al., 2006; Stępnicki et al., 2018). However, current antipsychotic treatment predominantly targets dopaminergic and serotonergic neurotransmitter systems which mainly relieves positive symptomatology. Effective pharmacological treatment for negative symptomatology and basic information processing is still lacking due to insufficient knowledge about underlying biochemistry and disease mechanisms of schizophrenia. Given the theoretical association between deficient basic information processing and both cognitive dysfunctions as well as positive and negative symptomatology (Braff et al., 1999; Light and Braff, 1999) and the severe impact that these phenomena have on everyday life (Green, 1996; Kahn and Keefe, 2013; Kitchen et al., 2012), the need for new treatments in schizophrenia is high.

Noradrenaline (NA) is one of the key neurotransmitters involved in cognition and, as indicated by results from our recent pilot study, also involved in basic information processes (Kruiper et al., 2019; Oranje and Glenthøj, 2013, 2014). Furthermore, a dysregulation of noradrenaline in

the brain contributes to both positive and negative symptomatology (Fields et al., 1988; Friedman et al., 1999; Yamamoto and Hornykiewicz, 2004). From the Locus Coeruleus (LC) NA neurons project into the prefrontal cortex (PFC) where NA has distinct receptor actions. High levels of NA activate the less sensitive α1-receptors in the PFC which are associated with stress and impaired cognitive functioning, while moderate levels of NA enhance cognition through activation of the more sensitive postsynaptic α2-receptors in the PFC. In contrast, in the LC α2-receptors are located presynaptic and activation of these receptors lowers NA transmission in the PFC (Arnsten, 2004; Ramos and Arnsten, 2007). A number of studies report increased noradrenergic transmission in schizophrenia (Fields et al., 1988; Freedman et al., 1982; Friedman et al., 1999) which may be a contributing factor to dysfunctional basic information processing and increased symptomatology in these patients. Based on the above, noradrenergic α2-agonists such as clonidine will not only decrease NA transmission in the PFC by stimulating *pre*synaptic α2-receptors in the LC, but simultaneously stimulate the *post*synaptic α2-receptors in the PFC. As such, clonidine might be able to restore the disrupted α1- and α2-NA balance in the PFC of schizophrenia patients. In low doses, clonidine is considered safe, with transient sedation being the most common side-effect.

As mentioned above, patients with schizophrenia often show deficient basic information processing, such as reduced filtering of irrelevant sensory information, as reflected in reduced sensory- or sensorimotor gating (e.g. Adler et al., 1982; Braff et al., 2001; Bramon et al., 2004). P50 suppression and prepulse-inhibition of the startle reflex (PPI) are measures believed to quantify an individual's ability of, respectively, sensory- and sensorimotor gating (hereafter referred to as sensori(motor) gating). In both measures preconscious inhibitory processes reduce the response to a certain stimulus as a result of the presentation of a preceding stimulus. Numerous studies, including those from our own labs, report deficits in these measures in patients with schizophrenia (PPI: e.g. Aggernaes et al., 2010; Braff et al., 2001), (P50 suppression: e.g. Oranje et al., 2013; Patterson et al., 2008) although less consistently in P50 suppression (e.g. During et al., 2014; Light et al., 2000). It is thought that reduced capacity to filter sensory information underlies both symptomatology and cognitive impairments in schizophrenia (Braff et al., 2001; Perry et al., 1999) for which some evidence exists (e.g. Bak et al., 2017; Cullum et al., 1993; Erwin et al., 1998; Hamilton et al., 2018) although not consistent (e.g. Swerdlow et al., 2006). The common denominator in sensori(motor) gating and symptomatology is likely the PFC, given its critical role in these phenomena (Antonova et al., 2004; Bak et al., 2014; Hammer et al., 2012; Knable and Weinberger, 1997; Kumari et al., 2008; Oranje et al., 2013; Ramos and Arnsten, 2007). Previous studies from our lab have demonstrated that a single dose of clonidine was able to normalize deficient levels of PPI and P50 suppression in chronic schizophrenia patients who were stable on their antipsychotic medication (Oranje and Glenthøj, 2013, 2014).

The current study investigated the effects of a 6-week augmentation with 50 μg dd clonidine on symptomatology (primary outcome measure) as well as sensori(motor) gating (secondary outcome measures) of stably medicated patients with schizophrenia using a randomized, double-blind, placebo-controlled design. We hypothesized that augmentation with clonidine would significantly reduce clinical symptomatology as well as improve levels of PPI and P50 suppression compared to augmentation with placebo. The electrophysiological results of the patients were compared to those of sex and age matched healthy controls, who received no treatment.

2. Material and methods

2.1. Ethics approval statement

This study was registered on www.clinicaltrialsregister.eu (2014-003008-53) and approved by the ethical committee of the University

Medical Center Utrecht (UMCU; 14-145). The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All participants provided written informed consent.

2.2. Subjects and screening procedure

Schizophrenia patients were recruited through in- and outpatient clinics throughout the region of Utrecht, the Netherlands. In total, 32 patients were enrolled in the study (See flow diagram in fig. S1). All patients were aged between 18 and 55 years and were treated with antipsychotic medication (See Table 1). The screening procedure consisted of a Mini International Psychiatric Interview (M.I.N.I. 5.0.0.; Sheehan et al., 1998) to confirm a DSM-IV-TR diagnosis of schizophrenia or schizoaffective disorder. Furthermore, current symptomatology was assessed with the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987) and a physical examination was conducted to check for good physical health.

Exclusion criteria were total PANSS score below 55; presence of diseases contra-indicated with clonidine treatment; pre-existent orthostatic hypotension with a drop of systolic blood pressure (BP) of >20 mmHg or a drop of diastolic BP of >10 mmHg; supine BP of <85 mmHg and supine heart rate of <50 beats p/m; severe brady-arrhythmias and the use of beta-blockers and mirtazapine. Furthermore, pregnant and breast-feeding women were excluded. Substance use was not an exclusion criterion, but its extent and type were noted.

Patients were matched on age and sex with 22 healthy controls. Healthy controls (HC) had no current or former history of psychiatric diagnoses as confirmed with the M.I.N.I. interview. Other exclusion criteria for healthy volunteers were mental illness in a first-degree family member, current or history of substance use and/or abuse, use of prescribed medication (excluding oral contraceptives), participation in experimental drug research 30 days prior to the start of the study and a Body Mass Index below 18.

2.3. Experimental procedure

After the screening procedure, patients were randomized and allocated to either the clonidine or placebo arm of our study. The study medication was dispensed after baseline assessment. In a randomized, double-blind, placebo-controlled design, all participants were tested on three different occasions each separated by three weeks (baseline, halftime, final visit). At baseline, after screening, all participants were assessed in the pre-pulse inhibition of the startle reflex (PPI) and P50 suppression paradigms of the Copenhagen Psychophysiological Test Battery (CPTB; Oranje et al., 2008; Oranje et al., 1999). The halftime assessment consisted of these electrophysiological paradigms only. At

Table 1
Demographics Age, sex and medication use of the three experimental groups, showing no significant group differences.

	HC (n = 21)	Patients (n = 32)		F/t/ χ	p
		Clonidine (n = 16)	Placebo (n = 16)		
Mean age *(SD)*	33.95 (7.66)	36.31 (9.90)	37.63 (6.91)	1.16	0.32
Sex (m/f)	16/5	14/2	14/2	0.98	0.61
Mean years of illness duration *(SD)*	–	11.50 (67.39)	15.56 (6.65)	1.65	0.11
Antipsychotic medication (typical/atypical/ combi/missing)	–	1/14/0/1	3/10/3/0	5.67	0.13
Benzodiazepine use (Yes/ No)	–	5/11	6/10	0.14	0.71
Antidepressant (Yes/No)	–	7/9	3/13	2.33	0.13

the final visit, both the paradigms and the PANSS interview were assessed. Healthy controls underwent the same experimental procedures without treatment. Smoking was not allowed 1 h prior to EEG testing to avoid acute and/or withdrawal effects of nicotine; subjects were additionally requested to refrain from caffeinated beverages on test days.

2.4. Treatment

Clonidine (Catapresan®) and placebo were administered in identical capsules which the patients were required to take once daily in the evening for a 6-week period. The clonidine capsules consisted of 50 μg clonidine (powdered form) and were further filled with lactose monohydrate until full. The placebo capsules were filled with lactose monohydrate only. Adherence was monitored through pill counts at each visit. Treatment with antipsychotic medication and other medications as prescribed by a patient's treating physician was continued during the 6-week study period.

2.5. Electrophysiological paradigms

Both the P50 and PPI paradigm have been described before (e.g. Oranje et al., 2008). In short, auditory stimuli were binaurally presented through stereo-inserted earphones (Eartone ABR© 1996-2008 Interacoustics A/S, USA) by a computer running Presentation® (Neurobehavioral Systems Inc., USA).

2.5.1. P50

P50 suppression was assessed in three identical blocks consisting of 40, 1.4 ms white noise clicks with an interstimulus interval (ISI) of 500 ms and intertrial interval of 10 s. To avoid drowsiness, the patients were instructed to count the clicks.

2.5.2. PPI

PPI assessment started with a 5-minute acclimation period to 70 dB background white noise, directly followed by three experimental blocks superimposed on the background noise. Block one and three were identical; eight pulse alone trials (white noise bursts of 115 dB with a duration of 20 ms and a randomized ISI between 10 and 20 ms) to assess habituation and sensitization. In block two, PPI assessment consisted of four different prepulse-pulse combinations, each combination presented ten times in a pseudorandomized order. The pulse had the same intensity and duration as that of block one and three, the two different prepulse intensities were 76 dB or 85 dB (20 ms) and stimulus onset asynchrony (SOA) between prepulse and pulse was either 60 ms or 120 ms. This resulted in the following four combinations: 85dB60ms, 85dB120ms, 76dB60ms & 76dB120ms. The intertrial intervals were randomized between 10 and 20s. In combination with ten randomized pulse alone trials this added up to 50 trials in block two.

2.6. Signal recording and processing

EEG recordings were performed with BioSemi® hardware (Amsterdam, The Netherlands) using a cap with 64 Active Two electrodes. Midline electrode Cz was used to analyze the P50 ERP, given that its amplitude reached maximum value on this electrode. In addition, electromyography (EMG) was used to assess the acoustic startle reflex (right m. orbicularis oculi) for the PPI paradigm. BESA software (version 6.0, MEGIS Software GmbH, Gräfelfing, Germany) was used for processing of the data. See supplements for data processing of the PPI and P50 suppression paradigms.

2.7. Statistical analyses

Linear Mixed-Effects (LME) analyses were conducted in R (version 3.4.1) while the other analyses were performed with SPSS (version 25). Appropriate t-tests, Analysis of Variance (ANOVAs) and chi-square tests

were conducted to test for group differences in demographic characteristics. Similarly, t-tests and ANOVAs were used for screening of potential group differences in baseline PANSS scores, percentage PPI and P50 suppression (ratio T/C) between the HC and both patient groups combined, as well as between the two patient groups alone. Initially, the effect of treatment on the PANSS(-subscale) scores was analyzed with a repeated-measures ANOVA, with between factor "Treatment" (patients treated with clonidine vs treated with placebo) and within factors "SubScale (PANSS-positive, -negative, −general score) and "Time" (Baseline and 6-weeks follow-up), with baseline PANSS-Total score as covariate. Given that an effect of treatment was hypothesized in the clonidine treated patients only, a subsequent planned comparison was performed with a repeated measures ANOVA with factors SubScale and Time, split on treatment. To avoid alpha inflation, significant results from the ANOVAs were further tested and/or confirmed with non-parametric (Wilcoxon) tests.

Missing values in the PPI and P50 suppression data were replaced with LME estimates based on each treatment group separately. Subsequent analyses of these data started with ANCOVAs with the inclusion of age, sex, smoking, type of antipsychotics and illness duration as covariates. However, except for sex in the PPI analyses, all covariates were statistically non-significant; therefore, only sex was included as covariate in the PPI analyses, all others were excluded. Please note that both the PPI and P50 suppression data were not normally distributed. However, given that F-tests are quite resilient to influences of skewness in data, especially when the difference between pre- and post-treatment data is normally distributed (Vickers, 2005), which was the case with all our PPI variables but not the P50 suppression variables, we decided to test the PPI data with ANCOVAs and the P50 suppression data with non-parametric tests. Effect sizes (Cohens-d or η^2) as well as observed power were calculated for between and within-group comparisons where possible. Given that a recent report found that latency and amplitude of pulse alone trials are more robustly different and heritable than PPI (Massa et al., 2020), we additionally included analyses of these variables. We report analyses of the reduction in N100 amplitude in the P50 suppression paradigm in the supplementary for those readers interested in the effects of treatment on (neuronal) refractory processes (Budd et al., 1998; Davis et al., 1966). Last, we explored possible associations between sensori(motor) gating variables and baseline PANSS-subscale scores as well as their change between baseline to follow-up.

3. Results

3.1. Study population

Demographic and clinical characteristics of the healthy controls and patients are summarized in Tables 1 and 2 respectively. A total of 32 patients were randomized and all but two patients completed the 6-week

Table 2
Symptomatology Mean PANSS-scores (SD) at baseline and 6-weeks follow-up, showing significantly reduced PANSS negative, general, total (and trend level positive) scores at follow-up compared to baseline in patients treated with clonidine only.

	Baseline		Follow-up	
	Placebo (n = 16)	Clonidine (n = 16)	Placebo (n = 15)	Clonidine (n = 15)
Positive	21.44 (7.50)[1]	15.38 (4.56)	19.80 (6.64)	13.80 (4.4)[#]
Negative	19.63 (5.85)	17.75 (6.21)	18.73 (5.80)	15.40 (4.6)[*]
General	34.56 (6.27)	32.13 (6.54)	32.07 (7.19)	28.53 (4.6)[*]
Total	75.63 (16.59)[1]	66.25 (10.93)	70.60 (16.21)	57.73 (9.9)[*]

PANSS = Positive and Negative Symptom Scale.
[1] Significantly higher than the baseline score of clonidine patients ($p < 0.05$)
[*] Significantly reduced compared to baseline ($p < 0.05$).
[#] Trend level reduced compared to baseline ($p = 0.067$).

treatment (See fig. S1).

3.2. Tolerability

Overall, clonidine was well tolerated, and neither notable side effects were reported by the patients treated with clonidine themselves, nor were they observed by the researchers. Only three patients reported slight drowsiness in the first few days after the start of treatment which faded over the course of the study period.

3.3. Symptomatology

3.3.1. Baseline

Unexpectedly, at baseline, the placebo group had a significantly higher average PANSS positive score than the clonidine group ($F_{(1,32)}$ = 7.63, p = 0.10, η^2 = 0.203, observed power = 0.762) as well as a significantly higher average total PANSS score ($F_{(1,32)}$ = 4.37, p = 0.045, η^2 = 0.127, observed power = 0.525). The other PANSS scores showed no significant group differences ($F_{(1,32)}$ < 1.16, p > 0.291, η^2 < 0.037, observed power < 0.136). As a result, we included PANSS-Total baseline score as covariate in the between treatment analysis, to compensate for this effect.

3.3.2. Baseline to follow-up

The repeated measures ANOVA indicated a significant main effect of Subscale only ($F_{(2,26)}$ = 3.68, p = 0.039, η^2 = 0.221, observed power = 0.625), neither a main effect of Time nor a Treatment*Time interaction were found (F < 2.65, p > 0.12, η^2 < 0.089, observed power < 0.349). However, the planned comparison (repeated measures ANOVA split on treatment) showed a significant effect of Time ($F_{(1,14)}$ = 13.85, p = 0.002, η^2 = 0.50, observed power = 0.933) in the patients treated with clonidine, but not in the patients treated with placebo ($F_{(1,14)}$ = 3.66, p = 0.076, η^2 = 0.21, observed power = 0.430), indicating a significant reduction in symptoms over time in the clonidine treated patients only. Indeed, except for the positive symptoms that only showed trend level of reduction, all other symptoms reduced significantly over time in the patients treated with clonidine (PANSS-positive: z = 1.83, p = 0.067 d = 0.35; PANSS-negative: z = 2.20, p = 0.028 d = 0.43; PANSS-general: z = 2.11, p = 0.035, d = 0.71 and PANSS-total: z = 2.81, p = 0.005, d = 0.82), while none of these scores reached significance in the placebo patients: (PANSS-positive: z = 1.19, p = 0.23, d = 0.23; PANSS-negative: z = 1.11, p = 0.27, d = 0.15; PANSS-general: z = 1.68, p = 0.093, d = 0.37 and PANSS-total: z = 1.71, p = 0.088, d = 0.31). See Table 2 for baseline and PANSS scores after 6 weeks.

3.4. Electrophysiology

3.4.1. PPI paradigm

3.4.1.1. Baseline.
At baseline, the combined group of patients showed significantly less PPI to the PPI85db120ms trialtype only ($F_{(1,48)}$ = 5.60, p = 0.02, η^2 = 0.11, observed power = 0.64) compared to the HC (other trialtypes: ($F_{(1,48)}$ < 3.29, p > 0.06, η^2 < 0.08, observed power < 0.47), while PPI did not differ between the two groups of patients ($F_{(1,28)}$ < 0.74, p > 0.38, η^2 < 0.03, observed power < 0.14) (see also Table 3). This difference appeared to be caused by a non-significant increase in prepulse-pulse trials ($F_{(1,42)}$ = 0.52, p = 0.48, η^2 = 0.012, observed power = 0.11), and a non-significant decrease in pulse alone trials ($F_{(1,42)}$ = 0.102, p = 0.75, η^2 = 0.002, observed power = 0.061) within patients compared to HC. We found no significant group difference in either amplitude ($F_{(1,48)}$ = 0.05, p = 0.83, η^2 = 0.001, observed power = 0.06) or latency of pulse alone trials ($F_{(1,48)}$ = 0.04, p = 0.85, η^2 = 0.001, observed power = 0.05) (see also table S1).

3.4.1.2. Baseline to follow-up.
The overall analysis showed a Treatment*Time interaction effect ($F_{(4,455)}$ = 7.11, p < 0.001, η^2 = 0.168, observed power = 0.933), indicating *increasing* levels of PPI within patients treated with clonidine, yet *decreasing* levels within controls over time, regardless of trialtype (See Fig. 1 and Table 3). This difference was caused by the combination of significantly decreased amplitudes to pulse alone trials within both HC and patients treated with placebo ((z = 2.52, p = 0.012, d = 0.78) and (z = 2.31, p = 0.021, d = 0.22), respectively) together with stable prepulse-pulse amplitudes over time within the controls (t = 2.04, p = 0.10) and significantly decreased amplitudes to prepulse-pulse trials within the patients treated with placebo (t = 3.00, p = 0.008). Both types of amplitude remained stable over time within the clonidine patients (t < 1.62, p > 0.24). No significant treatment effects were found in latency of pulse alone trials (F < 1.17, p > 0.33, η^2 < 0.047, observed power < 0.244) (See Table S1).

3.4.2. Sensitization and habituation

3.4.2.1. Baseline.
Neither the combined group of patients nor the HC showed significant sensitization (t < 0.56, p > 0.58, d < 0.22) (see also Fig. S2). In addition, neither group nor treatment differences in habituation were found (t < 1.59, p > 0.14, d < 0.60). (See Figs. 2 & S2).

3.4.2.2. Baseline to follow-up.
No significant Group*Time interactions were found in levels of sensitization over time ($F_{(4,86)}$ = 1.31, p = 0.27, η^2 = 0.066, observed power = 0.45) (see also Fig. S1). The slope of the habituation curve significantly flattened (became less steeply descending) over time ($F_{(2,88.87)}$ = 7.20, 0 = 0.001, η^2 = 0.162, observed power = 0.96), regardless of experimental group (see Figs. 2 & S2).

3.4.3. P50 suppression paradigm

3.4.3.1. Baseline.
We found a significant group difference in amplitude to T-stimuli, indicating that patients scored higher amplitudes than controls (Kruskal-Wallis H = 5.17, p = 0.023), while no group difference in raw P50 amplitude to C-stimuli was found (Kruskal-Wallis H = 0.26, p = 0.61). These findings are indicative for reduced sensory gating in patients and are behind the nearly twice higher average baseline T/C
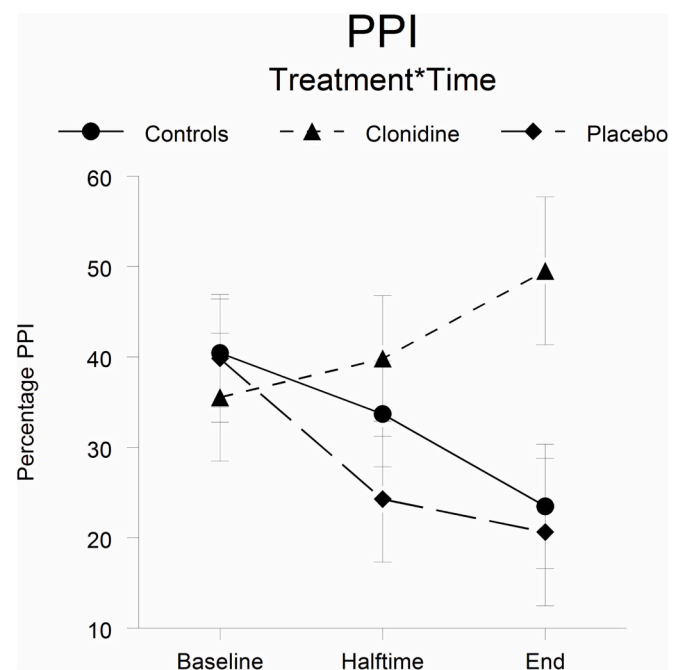

Fig. 1. PPI Percentage PPI over time showing a significant time*treatment interaction between patients treated with clonidine on the one hand, and both HC and patients treated with placebo on the other.
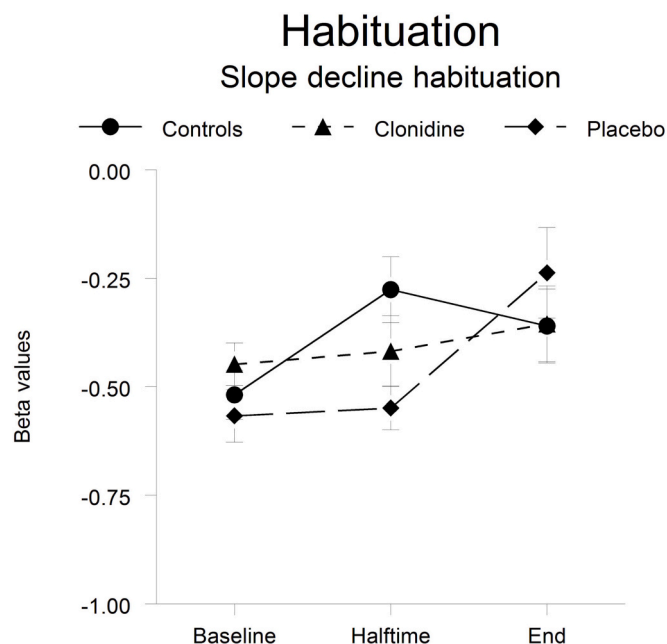
Fig. 2. Habituation B-coefficients over time for all three treatment groups, showing no group differences at baseline and 6 weeks follow-up, yet with an irregular pattern at 3 weeks follow-up.

ratio in patients compared to controls. Nevertheless, the difference in T/C ratio itself did not reach statistical significance (Kruskal-Wallis H = 2.56, $p = 0.109$). Neither did we find a significant difference between the patients treated with placebo or clonidine in any of the variables of the P50 paradigm (Kruskal-Wallis H < 0.69, $p > 0.407$). (see Table 3).

3.4.3.2. Baseline to follow-up. Neither significant effects of treatment were found *between* the 3 groups (Kruskal-Wallis H < 5.38, $p > 0.068$) (also not between the patients treated with placebo or clonidine (Kruskal-Wallis H < 197, $p > 0.160$)) nor *within* each group of subjects over time (Chi-Square < 4.217, $p > 0.121$) (See Table 3).

3.5. Correlations electrophysiology and symptomatology

Baseline measures of sensori(motor) gating did not correlate with any of the baseline measures of the PANSS subscales ($p > 0.27$). Correlations between symptomatology and the changes in sensori(motor) gating between baseline and final assessment revealed only one marginally statistically significant correlation in the placebo group; Higher baseline %PPI to trialtype 85 dB120 ms appeared to be marginally associated with a higher reduction of positive symptoms over time ($r_S = -0.548$, $p = 0.05$).

4. Discussion

Over the course of six weeks, treatment with clonidine significantly reduced PANSS-negative, −general and -total scores compared to placebo, while none of these symptomatology scores changed significantly over time in patients treated with placebo. Average percentage PPI at baseline was significantly lower in patients compared to the HC, although it reached statistical significance for one trial type only (PPI85dB120ms). Furthermore, whereas percentage PPI of HC and patients treated with placebo *decreased* over time, it *increased* in patients treated with clonidine. At baseline, patients scored significantly higher amplitude to T-stimuli than controls, indicating that patients had lower P50 suppression than controls. At follow-up no significant differences in P50 suppression were found. We found no group differences in sensitization, neither in amplitude nor latency of pulse alone trials at any point

Table 3

PPI (% with SD) and P50 suppression (amplitudes in μV, with SD), showing *increasing* levels of PPI in patients treated with clonidine over time, while these levels *decrease* in both healthy controls and patients treated with placebo as well as showing no significant group differences in any of the P50 measures at any point in time.

	HC	Clonidine	Placebo
PPI			
85 dB 120 ms			
Baseline	65.26 (18.50)	53.08 (23.17)	52.64 (26.81)
Halftime	50.70 (30.12)	64.77 (16.36)	37.46 (31.43)
Final	40.11 (30.21)	64.92 (17.39)	41.09 (24.96)
85 dB 60 ms			
Baseline	41.63 (34.91)	26.33 (48.48)	40.71 (29.19)
Halftime	27.35 (42.90)	38.31 (30.05)	21.38 (26.48)
Final	27.58 (37.28)	45.83 (35.06)	17.27 (53.08)
76 dB 120 ms			
Baseline	49.47 (24.01)	40.00 (30.67)	36.00 (30.65)
Halftime	32.80 (39.70)	43.46 (22.99)	22.15 (30.04)
Final	26.79 (36.71)	55.17 (20.15)	27.73 (22.88)
76 dB 60 ms			
Baseline	21.95 (30.48)	3.50 (55.59)	11.71 (47.74)
Halftime	10.55 (35.67)	1.31 (58.01)	−2.31 (32.37)
Final	0.50 (31.17)	38.17 (30.90)	7.00 (35.94)
P50 suppression			
T-amplitude			
Baseline	0.26 (0.10)	0.44 (0.13)	0.53 (0.15)
Halftime	0.42 (0.12)	0.49 (0.18)	0.30 (0.09)
Final	0.37 (0.09)	0.36 (0.10)	0.23 (0.07)
C-amplitude			
Baseline	1.16 (0.17)	1.24 (0.25)	1.29 (0.17)
Halftime	1.29 (0.22)	1.32 (0.3)	1.29 (0.23)
Final	1.28 (0.23)	1.03 (0.18)	1.05 (0.22)
T/C-ratio			
Baseline	0.27 (0.09)	0.37 (0.11)	0.56 (0.21)
Halftime	0.23 (0.05)	0.48 (0.14)	0.25 (0.10)
Final	0.39 (0.12)	0.47 (0.17)	0.31 (0.08)

in time. The level of habituation showed neither significant group differences at baseline nor at six weeks, while it fluctuated at the halftime assessment.

Although both patient groups on average showed fewer overall symptoms (PANSS-total score) over the study period, this only reached statistical significance in patients treated with clonidine. Please note though, that we found no significant treatment by time interaction effect, likely due to the treatment groups being relatively small, or indicating that some patients benefit more than others from treatment with clonidine. Nevertheless, we also found this pattern in the subscales, where statistically significant decreases in both PANSS-negative and –general scores were found in patients treated with clonidine only. This is an important finding given that currently no effective pharmacological treatment exists for negative symptoms, while they have a high impact on everyday life of patients with schizophrenia (Green, 1996; Kahn and Keefe, 2013; Kitchen et al., 2012). Furthermore, 50 μg clonidine is well tolerated and long-term side-effects are low.

Similar to our pilot study (Oranje and Glenthøj, 2013), baseline percentage PPI to 85 dB trialtypes was lower in patients compared to controls; however, where this reached statistical significance for both 85 dB trialtypes in our pilot study, it only reached statistical significance for one of these (PPI85dB120ms) in the current study; this is most likely caused by the modest group sizes in the current study, given that the average percentages PPI of these trialtypes in patients and HC was comparable between both studies. Also similar to our pilot study, augmentation with clonidine increased the average percentage of PPI of patients (in both studies with approximately 15 %), to levels indistinguishable from that of the HC at baseline. In contrast, PPI decreased over time in both HC and patients in the placebo arm compared to baseline in the current study, which is in line with other studies, including those from our lab (Hammer et al., 2011; Quednow et al., 2006). Similarly, the level of habituation showed a flattening of the curve over the study

period in both patients and controls, indicating long-term habituation across test-sessions.

While impaired PPI in schizophrenia is a robust finding across studies, deficits in P50 suppression are less consistently reported, even in our own lab (e.g. During et al., 2014). This may explain our current findings, where one variable indicated significantly reduced P50 suppression in the combined group of patients compared to controls at baseline (the increase of response to T-stimuli), while another did not (the T/C ratio). At follow-up no significant group or treatment differences were found. Besides that some atypical antipsychotics are able to increase levels of PPI, they also have been reported to increase levels of P50 suppression. Therefore, in contrast to PPI, the enhancing effects of atypical antipsychotics on P50 suppression may have masked some of the group and treatment effects, especially given the modest subject populations.

We did not find a relation between sensori(motor) gating measures and symptom severity. This finding supports the majority of reports in literature making it increasingly evident that there is no direct relationship between PPI, P50 suppression and symptom severity (PPI: Hass et al., 2017; Swerdlow et al., 2006) (P50: Boutros et al., 2004; Potter et al., 2006; Santos et al., 2010). An indirect relationship is therefore more likely, for instance through PFC modulation of both sensori(motor) gating and symptomatology.

As theorized above, our current results may be explained by the effects of clonidine on the noradrenergic-α2 receptors in the LC and the PFC; by activating presynaptic α2-receptors in the LC clonidine reduces noradrenergic output to the PFC thereby reducing its α1-activity. Simultaneously, clonidine will stimulate the postsynaptic α2-receptors in the PFC. These combined actions improve PFC-functioning (Ramos and Arnsten, 2007) which in turn is theorized to increase levels of PPI (Aggernaes et al., 2010; Oranje and Glenthøj, 2013) as well as decrease symptom severity.

There are strengths and limitations to this study. A strength of the study was its RCT design, with additional sex and age matched HC to disentangle the effects of clonidine from potential test-effects. Furthermore, attrition rate was low, only two out of our thirty-two included patients did not complete the study. A limitation of the current study was the modest sample size of patients in our study which resulted in reduced statistical power and was likely the cause behind the non-significant treatment by time interaction effect. Chronic schizophrenia patients are notoriously difficult to recruit in this type of funding-sponsored (read: time-limited) studies given a lack of initiative, caused by their negative symptoms. Nevertheless, we found a similar increase in percentage PPI following clonidine augmentation in the current study as in our pilot study, which makes this a robust finding. Combined, our studies strengthen the theory of involvement of noradrenaline in PPI, while more importantly, the current study shows that longer treatment with clonidine can significantly reduce symptom severity in schizophrenia as well.

5. Conclusion

We found that augmentation of antipsychotic treatment with clonidine was well tolerated and significantly reduced symptom severity in chronically ill patients with schizophrenia who were clinically stable on their medication. This leads us to believe that a low dose of clonidine added to the current medical treatment of schizophrenia patients with residual symptoms is a low-cost, safe, and clinically valuable addition to the current treatment of schizophrenia, especially to reduce the notoriously difficult to treat negative symptoms.

CRediT authorship contribution statement

CK coordinated, recruited, collected data, analyzed data and wrote the first draft of the manuscript; MK recruited and assisted with study design; IS, PB, SD assisted with study design and paper writing; BO designed, processed data, analyzed data, supervised, finalized the manuscript and submitted.

Declaration of competing interest

All authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2023.03.039.

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