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ORIGINAL ARTICLE

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Sodium nitroprusside is effective in preventing and/or reversing the development of schizophrenia-related behaviors in an animal model: The SHR strain

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Summary

Aims: The treatment of schizophrenia with antipsychotics is still unsatisfactory. Therefore, the search for new treatments and prevention is crucial, and animal models are fundamental tools for this objective. Preclinical and clinical data evidence the antipsychotic profile of sodium nitroprusside (SNP), a nitric oxide (NO) donor. We aimed to investigate SNP in treating and/or preventing the schizophrenia-related behaviors presented by the spontaneously hypertensive rats (SHR) strain.

Methods: Wistar rats (WR) and SHRs were submitted to two schemes of treatment: (i) a single injection of SNP or vehicle in adulthood; (ii) a long-term early treatment from 30 to 60 postnatal day with SNP or vehicle. The following behaviors were evaluated 24 hours after the acute treatment or 30 days after the long-term treatment: locomotion, social interaction, and contextual fear conditioning.

Results: Spontaneously hypertensive rats presented hyperlocomotion, decreased social interaction, and impaired contextual fear conditioning. Single injection of SNP decreased social interaction in both strains and induced a deficit in contextual fear conditioning in WR. Oppositely, early treatment with SNP prevented the behavioral abnormalities in adult SHRs without promoting any effects in WR.

Conclusion: Our preclinical data point to SNP as a preventive and safe strategy with a broad range of effectiveness to the positive, negative, and cognitive symptoms of schizophrenia.

KEYWORDS
animal models, prevention, schizophrenia, SHR strain, sodium nitroprusside

1 | INTRODUCTION

Schizophrenia is a debilitating disease that affects about 1% of the world population.[1] The symptomatology is characterized by positive and negative symptoms as well as cognitive deficits.[2] The onset of the disorder is marked by the first psychotic episode at late adolescence/early adulthood and is preceded by a prodromal phase characterized by attenuated negative and cognitive symptoms.[3] Although the development of antipsychotic medications that block dopaminergic type 2 receptors represented an advance in pharmacotherapy of schizophrenia, it still poses important limitations. They ameliorate mainly positive symptoms and are associated with severe motor and/or metabolic side effects and high rates of treatment nonresponse.[4,5] In this sense, the search for new treatments and, perhaps more important, for preventive strategies is crucial. Individuals with prodromal signs or recent functional decline associated with genetic risk are considered at ultra-high risk for developing schizophrenia.[6] In accordance, efforts to develop preventive and safe strategies for ultra-high-risk individuals have been made.[7]

Pharmacological, biochemical, and genetic data support the role of the nitrergic system in the pathophysiology of schizophrenia.[8-11] The blockade of glutamatergic NMDA receptors, which leads to a decrease in nitric oxide (NO) production and consequently to GMPc levels, has been extensively linked to schizophrenia. In parallel, while a diminished level of NO metabolites has been found in the serum and cerebrospinal fluid in schizophrenic patients, antipsychotics increase serum levels of NO. In addition, *postmortem* studies have shown alterations in the nitric oxide synthase activity in different brain regions, and polymorphisms of the neuronal nitric oxide synthase have been suggested as a risk factor for schizophrenia. Strikingly, recent evidence points to the antipsychotic effect of sodium nitroprusside (SNP), a nitric oxide donor. In a double-blind clinical trial, acutely relapsed schizophrenia patients present a long-lasting improvement of positive, negative, depressive, and anxiety symptoms after a single infusion of SNP.[12] In addition, cognitive deficits in these patients were also ameliorated by SNP.[13] Moreover, these antipsychotic effects of SNP were described in pharmacological animal models in which the schizophrenia-related behaviors are induced by dopaminergic agonists or glutamatergic NMDA antagonists—for review, see.[11] Nevertheless, the preventive long-term effect of an early repeated treatment with SNP has not yet been evaluated.

In contrast to the pharmacological schizophrenia animal models, the spontaneously hypertensive rats (SHR) strain presents spontaneous schizophrenia-related behavioral abnormalities: increase in locomotion (modeling positive symptoms), decreased social interaction (mimicking negative symptoms), and deficits in contextual fear conditioning (an emotional memory task). These behavioral alterations are specifically improved by antipsychotics and aggravated by proschizophrenia manipulations.[14-17] Additionally, the course of these behavioral abnormalities is in accordance with what is seen in the clinic: hyperlocomotion is presented only in adult SHRs while deficits in social interaction and in fear conditioning can be seen in 30- and 45-day-old SHRs, respectively (S.T.Niigaki, F.F.Peres, D.A.Gouvea, R.Levin, V.Almeida, N.D.Silva, M.C.Diana, M.A.Suiama, V.C.Abilio submitted). Interestingly, cardiovascular comorbidities, including hypertension, are presented in schizophrenia patients.[18] Some functional and neurochemical alterations seen in the SHR strain underlie schizophrenia physiopathology and may lead to neurovascular changes and, putatively, be related to the behavioral abnormalities presented by this strain: an increase in brain and vascular oxidative stress,[18,19] an increase in neuroinflammation,[18,20] and a hyperpermeability of the blood-brain and blood-cerebrospinal fluid barrier.[18,21,22] Considering specifically the nitrergic system, a decrease in brain and endothelial levels of NO and/or NOS activity have been reported in the SHR strain.[23,24] In addition, we have reported in the SHR strain a decrease in the gene expression of glutamate AMPA-R (*Gria1*) and NMDA-R (*Grin1*) in the nucleus accumbens as well as in *Gad2* (glutamate decarboxylase 2), *Chrnb4* (cholinergic receptor, nicotinic, beta 4), *Slc5a7* (choline transporter), and *Qrfpr* (pyroglutamylated RFamide peptide receptor) in the prefrontal cortex. We suggested that these alterations might be related to the behavioral abnormalities presented by the SHR strain.[25,26] Accordingly, this animal model has been used to investigate potential new treatments as well as early preventive strategies.[27-32] Therefore, the aim of the present work was to investigate the potential antipsychotic effect of SNP in treating and/or preventing the behavioral abnormalities presented by the SHR strain.

2 | METHODS

2.1 | Animals

Adult (4-month-old) and young (1-month-old) male Wistar rats (WRs) and SHRs from our own colony were used. SHR strain was developed by selecting WRs with hypertensive phenotype and brother-sister mating.[33] Previous experiments show that SHRs from our colony develop hypertension around 60 days of age (mean and standard error for 60-day-old WRs and SHRs, respectively, 155.58 ± 3.30 and 179.67 ± 3.10 mm Hg) remaining hypertensive in adulthood (mean and standard error for 90-day-old WRs and SHRs, respectively, 155.38 ± 3.46 and 184.08 ± 4.33 mm Hg).

Animals were maintained in groups of five in Plexiglas cages (41 × 34 × 16.5 cm) under controlled environmental conditions (22-23°C, light/dark cycle: lights on 6:30 AM-6:30 PM) with food and water available ad libitum. This study was approved by the Ethics Committee of Federal University of São Paulo (N 526654). Procedures followed the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

2.2 | Drugs and experimental design

Sodium nitroprusside (NITROP—Hypofarma, Brazil) was diluted in vehicle (0.9% NaCl saline solution). SNP and its vehicle were administered intraperitoneally in a volume of 1.0 mL/kg.

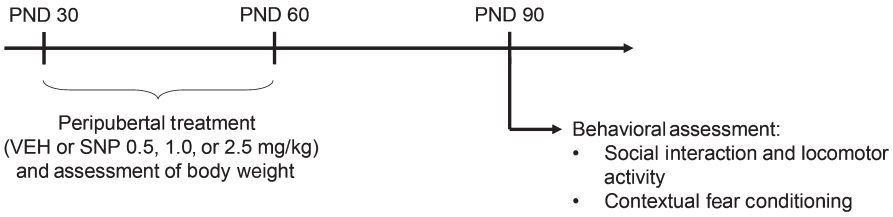

FIGURE 1 Experimental design used in experiment 2 to evaluate the preventive effects of sodium nitroprusside (SNP) on the spontaneously hypertensive rat (SHR) strain

In experiment 1, adult (4-month-old) WR and SHR (n = 8-10/group) received a single injection of VEH or SNP (0.5, 2.5 or 5.0 mg/kg). Twenty-four hours later, rats were submitted to the assessment of locomotor activity and social interaction, followed by the training session of contextual fear conditioning. On the next day, they were submitted to the testing session of contextual fear conditioning.

In experiment 2, WRs and SHRs (n = 8-10/group) were treated with VEH or SNP (0.5, 1.0 or 2.5 mg/kg) from 30 to 60 postnatal day (PND). Body weight was assessed every 4-5 days during treatment. Schizophrenia-related behaviors were assessed in adulthood (PND 90-100), with social interaction and locomotor activity being evaluated prior to the contextual fear conditioning task (Figure 1). A decrease in the doses used in this experiment was performed based on a possible effect in general activity with the highest dose used in experiment 1.

The route of administration, volume, and doses were chosen based on previous work showing beneficial effects of SNP in animal models of schizophrenia.[34-37] The interval between acute administration of SNP and the behavioral evaluations (24 hours—experiment 1) was based on previous clinical and preclinical studies evidencing the beneficial effect of a single injection of SNP 4 weeks later in patients and up to 1 week later in rats.[12,38]

2.3 | Schizophrenia-related behavioral analyses

All behavioral assessments were recorded and analyzed by trained observers—previously submitted to evaluation of concordance—that were blind to rats' experimental condition.

2.3.1 | Social interaction and locomotor activity

Social interaction and locomotor activity were evaluated simultaneously as described before.[14] Pairs of unfamiliar rats from the same experimental condition were placed in an open-field arena (97 cm in diameter and 32.5 cm high, with an open top and a floor divided into 19 similar quadrants). Social behavior and locomotor activity were quantified live during 10 minutes for each rat. Locomotor activity was assessed by the number of floor squares entered. Social interaction was calculated by the sum of time spent sniffing, following or in passive social interaction (when animals lie next to each other). In addition, the percentage of central floor squares entered was calculated to be used as an indicator of anxiety—the higher the percentage, the lower the anxiety levels.

2.3.2 | Contextual fear conditioning

The contextual fear conditioning task consisted on two consecutive days, as described previously.[15] On the first day (training session), the animals were placed in a dark chamber with a grid floor (22 × 22 × 22 cm). After 140 seconds, 0.4 mA foot shocks lasting 5 seconds were applied every 30 seconds. Twenty-four hours later each animal was placed in the same dark chamber, without receiving foot shocks. Animal's behavior was recorded by a video camera, and the assessment was conducted over a screen. Freezing (complete immobility of the animal with the absence of vibrissae movements and sniffing) duration was scored during 5 minutes.

2.4 | Statistical analysis

Levene's and Shapiro-Wilks tests were used to determine whether the data were parametric. When nonparametric (social interaction in experiment 2), data were logarithmically transformed to allow a parametric analysis.

Data were analyzed by two-way ANOVA (strain × treatment) followed by Duncan's test or by three-way ANOVA with repeated measures (strain × treatment × time) for the body weight in experiment 2. The $P < 0.05$ was used as a criterion for statistical significance. Statistical analyzes were conducted on the software SPSS 20.

3 | RESULTS

3.1 | Experiment 1: effects of single injection of SNP in adult rats

3.1.1 | Locomotor activity

Two-way ANOVA detected a significant effect of strain [$F_{(1,57)} = 6.221$; $P = 0.016$]. SHRs displayed increased locomotor activity, and treatment with SNP did not modify this behavior (Figure 2A).

3.1.2 | Social interaction

Two-way ANOVA showed a significant effect of strain [$F_{(1,57)} = 75.946$; $P < 0.001$] and treatment [$F_{(3,57)} = 3.556$; $P = 0.020$]. SHRs displayed diminished social interaction, and post hoc analysis revealed that treatment with SNP 5.0 mg/kg decreased social interaction in both strains (Figure 2B).

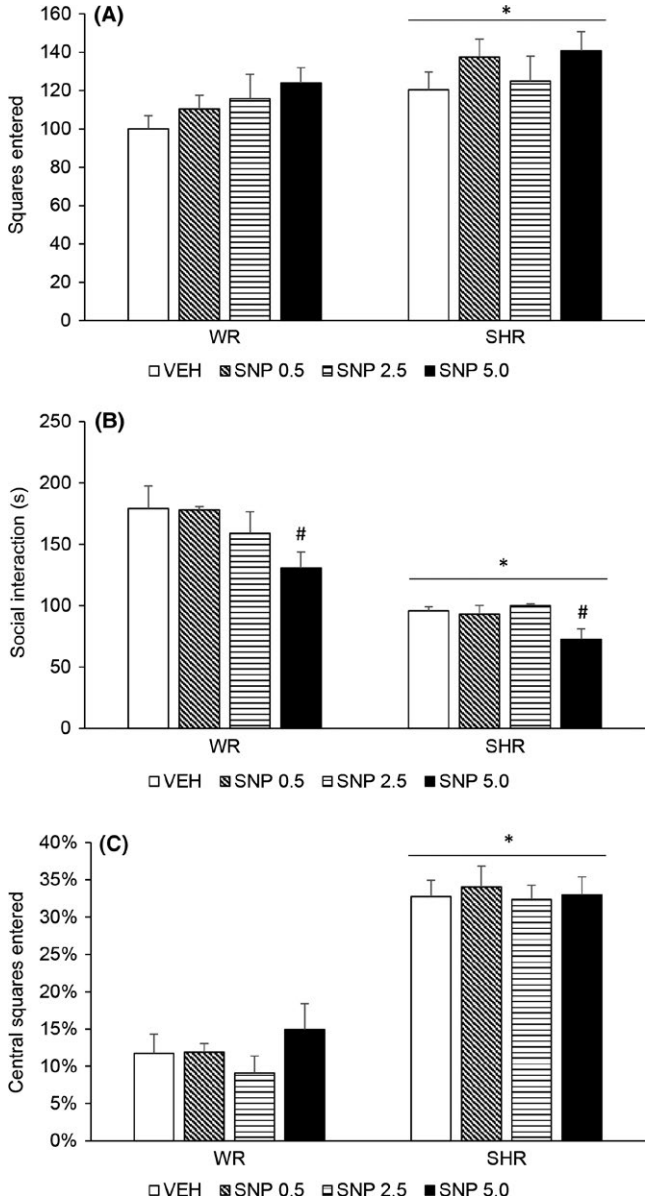

FIGURE 2 Locomotor activity (A), social interaction duration (B), and percentage of floor squares entered (C) of adult (4-month-old) WRs and SHRs (n = 8-10/group) treated with a single injection of vehicle (VEH) or sodium nitroprusside (SNP—0.5, 2.5, or 5.0 mg/kg) and submitted to behavioral assessments 24 hour later. Data reported as mean ± SE. Two-way ANOVA followed by Duncan's test. *$P < 0.05$ compared to WRs of same treatment. #$P < 0.05$ compared to VEH-treated animals of the same strain

3.1.3 | Percentage of central floor squares entered

Two-way ANOVA detected a significant effect of strain [$F_{(1,68)} = 155.856$; $P < 0.001$]. SHRs displayed increased percentage of entrance in central floor squares, which indicates lower anxiety levels. Treatment with SNP did not modify this percentage in any strain (Figure 2C).

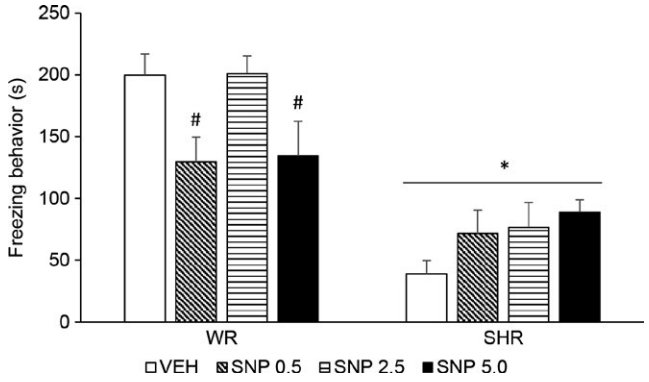

FIGURE 3 Freezing response during contextual fear conditioning test (s) of adult (4-month-old) WRs and SHRs (n = 8-10/group) treated with a single injection of vehicle (VEH) or sodium nitroprusside (SNP—0.5, 2.5, or 5.0 mg/kg) and submitted to the training and testing sessions of contextual fear conditioning 24 and 48 hour later, respectively. Data reported as mean ± SE. Two-way ANOVA followed by Duncan's test. *$P < 0.05$ compared to WRs of same treatment. #$P < 0.05$ compared to VEH-treated animals of the same strain

3.1.4 | Contextual fear conditioning

Two-way ANOVA showed a significant effect of strain [$F_{(1,72)} = 56.848$; $P < 0.001$] and an interaction between treatment and strain [$F_{(3,72)} = 4.495$; $P = 0.006$]. SHRs displayed decreased freezing behavior. Post hoc analysis revealed that treatment with SNP 0.5 and 5.0 mg/kg decreased freezing response in WRs (Figure 3).

3.2 | Experiment 2: effects of prolonged treatment with SNP during periadolescence

3.2.1 | Body weight gain

Repeated measures three-way ANOVA detected a significant effect of time [$F_{(8,576)} = 2968.104$; $P < 0.001$] and strain [$F_{(1,72)} = 177.285$; $P < 0.001$]. Body weight of both WRs and SHRs increased over time, and SHRs displayed lower body weight. An interaction between strain and time [$F_{(8,576)} = 28.021$; $P < 0.001$] suggests that the weight gain rate of SHRs is different from those of Wistar rats. Treatment with SNP did not modify animals' body weight gain (Table 1).

3.2.2 | Locomotor activity

Two-way ANOVA detected a significant effect of strain [$F_{(1,65)} = 21.251$; $P < 0.001$] and treatment [$F_{(3,65)} = 4.112$; $P = 0.010$]. SHRs displayed increased locomotor activity. Although there was no interaction effect, post hoc analysis revealed that only SHRs treated with SNP 0.5 or 2.5 mg/kg displayed decreased locomotor activity when compared to VEH-treated SHRs (without differing from WRs). Treatment with SNP 0.5 or 2.5 mg/kg attenuated the hyperlocomotion in SHRs (Figure 4A).

TABLE 1 Body weight gain evaluated every 5-7 days from the beginning of treatment of WR and SHRs treated with SNP from 30 to 60 postnatal days

Group	1	2	3	4	5	6	7	8	9
WR									
VEI	104.6 ± 4.92	131.1 ± 6.21	149.9 ± 6.58	165.0 ± 7.14	182.4 ± 7.93	192.8 ± 8.71	204.8 ± 8.29	266.3 ± 7.07	292.1 ± 8.03
SNP 0.5	106.9 ± 5.33	134.4 ± 6.70	152.6 ± 6.29	169.3 ± 6.50	186.9 ± 7.29	196.4 ± 6.34	209.0 ± 8.09	278.7 ± 8.76	304.6 ± 9.14
SNP 1	103.2 ± 3.56	129.9 ± 3.77	148.7 ± 4.04	161.2 ± 3.95	181.7 ± 4.42	191.9 ± 4.85	205.0 ± 6.48	276.5 ± 8.02	303.2 ± 9.79
SNP 2.5	100.2 ± 4.61	125.7 ± 5.67	144.9 ± 5.89	157.3 ± 5.48	175.7 ± 6.65	185.3 ± 6.94	198.0 ± 7.90	265.7 ± 7.24	294.1 ± 8.51
SHR*									
VEI	74.7 ± 2.48	87.3 ± 2.66	97.0 ± 2.39	111.8 ± 2.13	125.6 ± 2.28	138.0 ± 2.60	153.0 ± 3.04	207.0 ± 3.87	226.2 ± 4.39
SNP 0.5	75.0 ± 2.52	88.4 ± 3.25	99.3 ± 3.57	115.0 ± 3.86	130.4 ± 4.96	143.6 ± 5.31	161.9 ± 5.58	214.5 ± 6.63	236.5 ± 7.00
SNP 1	79.4 ± 3.62	90.9 ± 3.78	101.9 ± 4.61	119.1 ± 5.76	134.7 ± 7.08	149.4 ± 7.38	165.4 ± 8.04	218.8 ± 8.79	238.0 ± 8.86
SNP 2.5	75.4 ± 4.10	85.9 ± 3.27	99.0 ± 4.16	112.2 ± 3.83	126.4 ± 4.50	137.0 ± 4.88	151.4 ± 5.53	200.8 ± 6.50	222.9 ± 6.16

SHR, spontaneously hypertensive rats; SNP, sodium nitroprusside; WR, Wistar rats; *p<0.05 compared to WRs of same treatment.

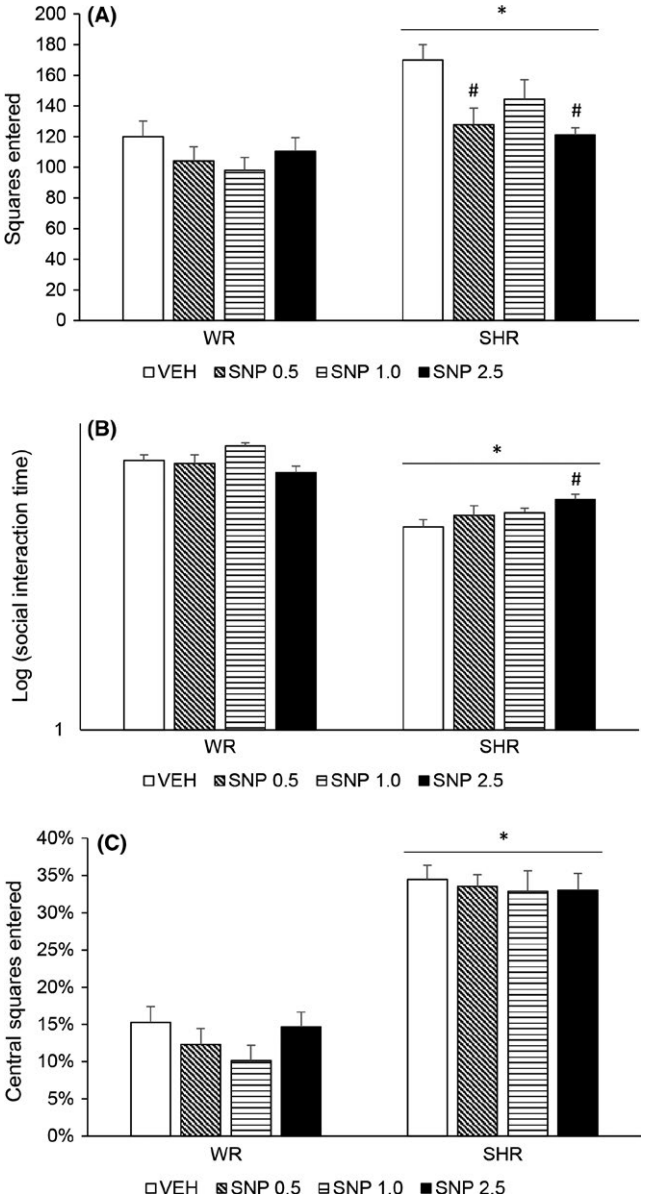

FIGURE 4 Locomotor activity (A), social interaction duration (B), and percentage of floor squares entered (C) of adult WRs and SHRs (n = 8-10/group) treated with vehicle (VEH) or sodium nitroprusside (SNP—0.5, 1.0, or 2.5 mg/kg) during periadolescence (30-60 postnatal day). Data reported as mean ± SE. Two-way ANOVA followed by Duncan's test. *P < 0.05 compared to WRs of same treatment. $^{\#}P$ < 0.05 compared to VEH-treated animals of the same strain

3.2.3 | Social interaction

Two-way ANOVA showed a significant effect of strain [$F_{(1,63)}$ = 128.823; P < 0.001] and an interaction between strain and treatment [$F_{(3,63)}$ = 3.888; P = 0.013]. Post hoc analysis revealed that SHRs displayed diminished social interaction that was attenuated by the highest dose of SNP (2.5 mg/kg) (Figure 4B).

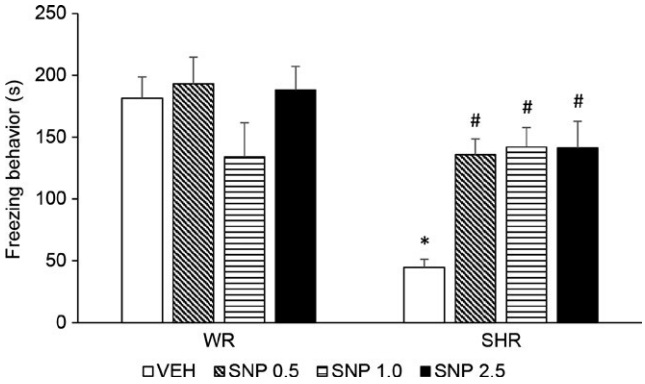

FIGURE 5 Freezing response during contextual fear conditioning test (s) of adult WRs and SHRs (n = 8-10/group) treated with vehicle (VEH) or sodium nitroprusside (SNP—0.5, 1.0, or 2.5 mg/kg) during periadolescence (30-60 postnatal day). Data reported as mean ± SE. Two-way ANOVA followed by Duncan's test. $^{*}P < 0.05$ compared to WRs of same treatment. $^{\#}P < 0.05$ compared to VEH-treated animals of the same strain

3.2.4 | Percentage of central floor squares entered

Two-way ANOVA detected a significant effect of strain [$F_{(1,66)} = 178.074$; $P < 0.001$]. SHRs displayed increased percentage of entrance in central floor squares, which indicates lower anxiety levels. Treatment with SNP did not modify this percentage in any strain (Figure 4C).

3.2.5 | Contextual fear conditioning

Two-way ANOVA showed a significant effect of strain [$F_{(1,72)} = 19.324$; $P < 0.001$], treatment [$F_{(1,72)} = 3.498$; $P = 0.020$] and an interaction between strain and treatment [$F_{(1,72)} = 5.075$; $P = 0.003$]. SHRs displayed decreased freezing behavior and treatment with SNP, in all doses, increased this behavior (Figure 5).

4 | DISCUSSION

The search for new treatments and preventive strategies for schizophrenia is paramount. In relation to advances in its treatment, SNP emerges as a potential target. SNP's antipsychotic profile was first suggested by preclinical studies—for review, see.[11] In animal models using behavioral alterations induced by NMDA antagonists, SNP was able to attenuate hyperlocomotion,[34,37,38] short- and long-term object recognition memory[34,36] and social interaction deficits.[36] In addition, the deficit in prepulse inhibition of startle induced by a dopamine agonist is also improved by SNP.[35] These promising preclinical data were followed by a randomized double-blind, placebo-controlled trial in which twenty schizophrenia inpatients in the first 5 years of the disease presented an improvement of positive, negative, anxiety, and depression symptoms within hours after a single infusion of SNP. These strikingly effects persisted for 4 weeks.[12] The cognitive deficits of these patients were also ameliorated when evaluated up to 8 hours after the infusion.[13]

In contrast to the above-mentioned data, SNP was not able to improve hyperlocomotion, deficits in social interaction and in contextual fear conditioning presented by the SHR strain when administered in the adulthood. Although schizophrenia-related behaviors acutely induced in pharmacological animal models are well-stablished, the SHR strain presents the advantage of developing schizophrenia-related behavioral abnormalities spontaneously.[14-17] Hence, the absence of effect of SNP presented herein might be related to a broader and more chronic mechanism underlying the behavioral abnormalities spontaneously displayed in this animal model when compared to the acute specific changes in the dopamine and NMDA neurotransmission induced by those pharmacological models. In this respect, an antipsychotic effect of SNP on the psychotic symptoms and on spatial working memory deficits in schizophrenia patients was not observed.[39] The authors argue that the beneficial effect of SNP previously seen[12,13] may occur in patients with a shorter history of illness and/or with a more acute exacerbation of symptoms. This same rationale could be related to the antipsychotic effect of SNP seen in the acute pharmacological-induced animal models of schizophrenia but not observed in the spontaneous, more chronic, behavioral deficits presented by the SHR strain. From another standpoint, it might be argued that the absence of effect of SNP could be due to the interval of 24 hours between its administration and the behavioral evaluations. Nevertheless, previous studies have demonstrated the effects of SNP on improving the long-term memory deficit induced by a NMDA antagonist 48 hours after its administration,[34] and a decrease in hyperlocomotion induced by a NMDA antagonist 1 week later the administration of SNP.[38]

Still considering the acute effect of SNP, it should be noticed that a decrease in social interaction was observed in both strains after treatment with the highest dose. This decrease in social interaction could be related to a decrease in general activity. Nevertheless, no changes in locomotion were observed. In parallel, an inverted U-shaped curve was observed for freezing time presented by WRs (the lowest and the highest doses decreasing it). A possible anxiolytic effect of SNP might be involved in this effect, as nitric oxide donors diminish anxiety[36,40] and anxiolytics might decrease fear conditioning.[15,41] Contrary to this assumption, an anxiety parameter observed in the open-field—percentage of central floor squares entered—was not modified by any dose of SNP. In any way, this effect was seen only in WRs, indicating that these two strains respond qualitatively different to SNP. In the same way, we have previously described that the known effect of a benzodiazepine in impairing the contextual fear conditioning is not seen in SHR rats.[15] Interestingly, we have shown that the antipsychotics haloperidol and clozapine impair contextual fear conditioning in WRs, in opposition to their beneficial effect on the contextual fear conditioning deficit presented by SHRs.[15]

In relation to a potential preventive effect, the long-term early treatment with SNP inhibited the manifestation of the schizophrenia-related behavioral abnormalities presented by adult SHRs. Hyperlocomotion was significantly prevented by the lowest and the highest dose of SNP (with a trend at the intermediate dose); social

interaction deficit (seen already in 30-day-old SHRs) was reversed by the highest dose; and contextual fear conditioning deficit (already presented at 45-day-old SHRs) was abolished by all the doses tested. Importantly, these effects were observed only in the SHR strain, indicating the specificity of SNP to schizophrenia-related behavioral abnormalities presented by this strain. As far as we know, this is the first evidence of the potential of an early treatment with SNP in preventing or reversing the development of positive-, negative-, and cognitive-related behaviors in animal model of schizophrenia.

Considering the 15% conversion rate to psychosis of ultra-high-risk patients as well as the risks of a peripubertal treatment,[7,42] it is crucial to evaluate possible harmful outcomes in healthy subjects. Noteworthy, the early treatment with SNP did not induce any behavioral changes in control animals. In addition, body weight gain of both strains was not modified by the SNP treatment. One might argue that the SHRs behavioral abnormalities in some extent could be due to the body weight difference between WRs and SHRs. For elucidating this possibility, we performed an ANCOVA analysis using weight as a covariable. The inclusion of the weight on the statistical model did not modify the results, that is, SHRs' displayed all the behavioral abnormalities and SNP prevented all of them even when controlling the results for the body weight differences.

The SHR strain has been used to evaluate pharmacological and nonpharmacological preventive strategies. Although antipsychotics (S.T.Niigaki, F.F.Peres, D.A.Gouvea, R.Levin, V.Almeida, N.D.Silva, M.C.Diana, M.A.Suiama, V.C.Abilio submitted), cannabidiol (F.F.Peres, M.C.Diana, R.Levin, M.A.Suiama, V.Almeida, A.M.Vendramini, A.W.Zuardi, J.E.C.Hallak, J.Á.Crippa, V.C.Abilio submitted), and environmental enrichment[32] have proven to present preventive effects, early treatment with SNP was the only one to prevent positive-, negative-, and cognitive-related deficits at the same time without inducing any behavioral changes in control animals. Taken as a whole, the present data point to a broad spectrum of prevention of SNP with a safe profile.

The antipsychotic mechanism of SNP has been related to a direct action on NMDA receptor function as well as to its ability to counteract the decrease in nitric oxide and in cGMP-mediated cascade due to the NMDA hypofunction associated with the pathophysiology of schizophrenia.[11,43] In accordance, a decrease in metabolites of nitric oxide has been described in the plasma and cerebrospinal fluid of schizophrenia patients.[44,45]The decreased serum level of nitric oxide is attenuated by a 6-week treatment with the antipsychotic risperidone being associated with an improvement in psychotic symptoms.[45] Also, nitric oxide and its metabolites are inversely correlated to the severity of negative symptoms.[46,47] In parallel, a modulation by nitric oxide donors of the dopaminergic system, extensively associated with the pathophysiology of schizophrenia,[48] has been postulated.[9,49] Dopamine, D_1 and D_2, receptors modulate NO production and nitric oxide synthase activity in striatal neurons.[9] In addition, it has been speculated that dopamine transporters are inhibited by NO, which could lead to an increase in dopamine levels in the prefrontal cortex and, through a modulatory control over mesolimbic areas, to a normalization of dopamine overactivity in the striatum.[49]

Considering the long-term preventive effect of SNP, it should also be taken into account that a disruption in nitric oxide signaling has been associated with long-term behavioral and neuroanatomical abnormalities that might be associated with schizophrenia—see.[50,51] The maturation of neurons and synaptogenesis are modulated by NO. Improper migration of NADPH-d, a nitric oxide synthase, neurons might lead to a disturbance in the nitrergic transmission interfering with the neurodevelopmental process culminating with the manifestation of schizophrenia later in life.[51] In this sense, neonatal nitric oxide synthase inhibition leads to an increase in dopamine agonists- and stress-induced hyperlocomotion as well as deficits in social interaction, prepulse inhibition of startle, latent inhibition, and short-term recognition memory.[50-53] In this respect, the hypertension of the SHR strain has been associated, among other factors, to a decrease of nitric oxide availability due to an increase in peripheral free radicals.[54] The same decrease in nitric oxide availability due to an increase in oxidative stress might occur in the central nervous system. Although speculative, the long-term treatment with SNP during periadolescence—which would parallel the age of ultra-high-risk individuals—might counteract a possible disruption of nitric oxide signaling in the SHR strain leading to a protective effect against the development of the schizophrenia-related behaviors seen in adulthood or during the periadolescence, corresponding to a prodromal phase. The protective mechanisms associated with the long-term beneficial effect of SNP are currently under investigation in our group. In this respect, alterations in the nitrergic system, increased brain oxidative stress, and neuroinflammation have been described in the SHR strain[19,20,23,24] and might underlie the beneficial effect of SNP presented here.

In conclusion, the present study indicates the SNP as a safe preventive strategy with a broad range of effectiveness. Translated to the clinical context, these preclinical data point to a potential of SNP in attenuating the emergence of positive, negative, and cognitive symptoms of ultra-high-risk individuals that would convert to schizophrenia. Importantly, preventive treatment of those who would not convert appears to be safe. The preventive effect and safety of SNP in other animal models of schizophrenia is currently under investigation by our group and would strengthen these findings.

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CONFLICT OF INTEREST

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