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Noradrenergic alpha-2 receptor agonists reverse working memory deficits induced by the anxiogenic drug, FG7142, in rats

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Abstract

Performance on working memory tasks, a measure of prefrontal cortical function, is impaired by exposure to mild stress as well as the anxiogenic drug, FG7142. Previous studies have shown that like stress, FG7142 increases catecholamine release in the prefrontal cortex (PFC) and that high levels of dopamine (DA) D_1 and norepinephrine (NE) α-1 receptor stimulation underlie the FG7142-induced cognitive impairment. Both the FG7142-induced DA turnover and working memory deficit can be blocked by pretreatment with the nonselective NE α-2/imidazoline I1 receptor agonist, clonidine. The present study examined the α-2 adrenoceptor subtype underlying this reversal in FG7142-induced working memory deficits by comparing the efficacy of clonidine with the more selective α-2A adrenoceptor agonist, guanfacine. The anxiogenic drug, FG7142 (0, 10, 20, or 30 mg/kg), dose-dependently impaired delayed alternation performance. Clonidine pretreatment (0.1 mg/kg, 30 min prior to FG7142) partially reversed the FG7142-induced impairment while guanfacine pretreatment (0.11 mg/kg) completely blocked the FG7142-induced impairment. Neither clonidine nor guanfacine had any effect on performance when administered alone. This study suggests that stimulation of the NE α-2A receptor subtype is sufficient to ameliorate the cognitive deficit induced by FG7142. Clonidine's sedative and hypotensive side effects limit its therapeutic usefulness; however, selective α-2A receptor agonists may be effective in treating prefrontal cognitive deficits in stress-related neuropsychiatric disorders with fewer side effects.

Keywords: Dopamine; Norepinephrine; Prefrontal cortex; Delay alternation; Stress; Clonidine; Guanfacine; FG7142; Working memory

1. Introduction

Cognitive functions such as working memory, behavioral inhibition, and attention regulation rely on the integrity of the prefrontal cortex (PFC). Impairments in these functions are commonly observed in aged individuals as well as in patients with neuropsychiatric disorders such as schizophrenia and Attention Deficit/Hyperactivity Disorder (ADHD). Decrements in prefrontal cortical function can also be induced experimentally by local depletion of norepinephrine (NE) and dopamine (DA), suggesting that these monoamines regulate prefrontal cortical function [16–18,46,47]. The importance of endogenous NE and DA stimulation in the PFC is supported by studies in which local infusion of either dopaminergic D_1 antagonists [43,45] or noradrenergic α-2 antagonists [34] into the PFC impairs performance on working memory tasks. Together, these data suggest that DA and NE are necessary for normal PFC functioning.

Working memory deficits are also observed following exposure to mild stressors [4,5], or the anxiogenic drug FG7142, which greatly increase catecholamine release in the PFC [13,22,24,53]. Both stress and FG7142-induced working memory deficits can be reversed by systemic pretreatment with DA D_1 receptor antagonists [5,39,40] or by local PFC infusion of an adrenergic α-1 receptor antagonist [12]. These results suggest that very high levels of DA and NE, acting via DA D_1 and NE α-1 receptors, respectively, are as detrimental to working memory as catecholamine depletion. This conclusion is supported by studies in which DA and NE agonists were directly infused into the PFC of rats. For example, infusion of either a DA D_1 agonist [57] or a NE α-1 agonist [7] impairs performance on a delayed alternation task. Thus, an optimal level of DA and NE appears to be necessary for normal PFC function, while higher

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levels of DA and NE may underlie the degradation of working memory observed during stress.

Unlike activation of NE α-1 receptors in the PFC, which impairs working memory, stimulation of NE α-2 receptors enhances PFC function. Thus, NE α-2 agonists such as clonidine improve performance in normal animals and those with catecholamine depletion, while an NE α-2 antagonist impairs working memory in these animals (reviewed in Ref. [1]). These effects are most likely mediated by post-synaptic receptors, as NE α-2 receptor agonists are most potent in animals with the greatest NE depletion [8,18]. The pharmacological profile of NE α-2 receptor agonists suggests that the α-2A receptor subtype may mediate these cognitive effects: agonists with higher α-2A receptor affinity can improve working memory with few side effects, while agents with high affinity for α-2B or α-2C receptor subtypes require higher doses and generally produce substantial side effects [8].

Clonidine can also be helpful in animals with excessive catecholamine release. For example, clonidine can restore normal performance in working memory tasks following exposure to a white noise stress [3] or the anxiogenic agent, FG7142 [41]. This behavioral improvement is accompanied by a decrease in DA turnover in the PFC [41]. However, the receptor subtype underlying these effects is not known. The current study examined the NE α-2 receptor subtype underlying clonidine's beneficial effect on FG7142-induced cognitive dysfunction. Clonidine is a nonselective NE α-2 receptor agonist with high affinity for all three receptor subtypes [54,55], as well as imidazoline I1 receptors [21,56]. In contrast, guanfacine has been shown to be 15–60 times more selective for the NE α-2A subtype than for the α-2B and α-2C subtypes [54,55], with little affinity for imidazoline I1 receptors [15,56]. There are no agonists currently available that are selective for the NE α-2B or α-2C subtypes or imidazoline I1 receptors. The contribution of the α-2A adrenergic receptor subtype in blocking stress-induced cognitive impairment was evaluated by comparing the efficacy of equimolar doses of clonidine and guanfacine in reversing the working memory deficit induced by FG7142.

2. Materials and methods

2.1. Subjects

Sixteen male Sprague–Dawley rats (Camm, Wayne, NJ) were pair-housed in a 12 light:12 dark cycle with all testings conducted during the light phase. The animals were fed Purina rat chow (15 g rat $^{-1}$ day^{-1}) immediately following behavioral testing and water was available ad libitum. Rat weights increased from an average of 125 g at the beginning of the study to 460 g by the end of the study (approximately 10 months). Food rewards during cognitive testing were highly palatable miniature chocolate chips, thus minimizing the need for dietary restriction.

2.2. Cognitive testing

The rats were handled daily during the training period and habituated to interperitonial (i.p.) injections and the testing procedure. Delayed alternation training and testing were performed in a T-maze (90 × 65 cm^2). Rats were habituated to the T-maze for several days until they were readily eating chocolate chips from the experimenter's hand. Following habituation, rats were trained on the delayed alternation task. A rat was placed in the start box of the T-maze and the gate was opened, allowing the rat to run to the choice point in the maze. On the first trial each day, animals were rewarded for entering either arm. The rat was then returned to the start box of the maze for the intertrial delay. On all subsequent trials, the rat was rewarded only if he entered the maze arm that was not chosen on the immediately preceding trial. If the correct choice was made, the rat was given a reward and returned to the start box for the intertrial delay. Following an incorrect choice, the rat was immediately returned to the start box for the intertrial delay. Each test session consisted of 11 trials, although the first trial was not scored. Rats were scored for accuracy of response and response time. Response time was measured from the time the start gate was lifted until the animal made its choice. Rats were tested once daily, five times per week.

The intertrial delay was initially "0" s (i.e., approximately 2–3 s, as true 0 delays are not feasible in this paradigm). Delays were raised by 5-s increments as needed to stabilize each rat's performance at approximately 80% correct. Training continued until a rat scored between 70% and 90% correct for 3 consecutive days, with an average score for the 3 days of 73–86%. Drug treatment was administered once stable performance was achieved, with a minimum of 1 week between drug treatments for each rat.

2.3. Drug treatment

Due to the habituation that can occur following repeated exposure to acute mild stress, we have used the anxiogenic agent, FG7142, in this study. Previous work has shown that FG7142 increases catecholamine turnover in the PFC, and induces working memory deficits similar to those observed following exposure to other acute stressors without habituation [12,40,41]. However, repeated exposure to FG7142 has been reported to induce seizures and this kindling effect is long-lasting (over 12 months [35,52]). Thus, rats in which seizure activity was observed following an FG7142 injection were not used for further testing (n=4 of the original 16 rats trained).

FG7142 (Tocris Cookson, St. Louis, MO) was suspended in a saline vehicle containing Tween 80, hydroxybetacyclodextrin and ethanol. Due to differences in potencies between different lots of this drug, all testings

in this experiment used the same lot of FG7142. Clonidine (Boehringer Ingelheim, Ridgefield, CT) and guanfacine (Wyeth-Ayerst, Princeton, NJ) were dissolved in sterile saline. In both experiments, the order of drug treatments was counterbalanced between rats.

In Experiment 1, FG7142 (10, 20, or 30 mg/kg) or an equal volume of vehicle was administered (i.p.) 30 min prior to behavioral testing in the T-maze ($n=12$). In Experiment 2, the deleterious effect of FG7142 (30 mg/kg) was challenged with equimolar doses of clonidine (0.1 mg/kg), guanfacine (0.11 mg/kg), or saline pretreatment (i.p.) 30 min prior to FG7142 or vehicle. To detect a reversal of stress-induced cognitive impairment, the rats that were most impaired (performance<70% correct) by the 30 mg/kg dose of FG7142 during Experiment 1 were used in Experiment 2 ($n=6$). In both experiments, the order of drug treatments was counterbalanced between rats.

2.4. Data analysis

Data from Experiment 1 were analyzed using a one-way analysis of variance with repeated measures (one-ANOVA-R). Planned comparisons (test of effects) were used to compare individual doses to vehicle. In Experiment 2, the effect of NE α-2 agonist pretreatment on the FG7142 response was assessed using a two-way analysis of variance with repeated measures (two-ANOVA-R) with factors of FG7142 (vehicle, FG7142) and NE α-2 agonist (saline, clonidine, guanfacine). Planned comparisons tested whether (1) saline/FG7142 impaired performance relative to saline/vehicle control; (2) whether either α-2 agonist/FG7142 treatment reversed deficits relative to saline/FG7142 performance and restored performance relative to saline/vehicle; and (3) whether treatment with either α-2 agonist/vehicle differed from saline/vehicle treatment. All statistics were done using Systat on a Macintosh LCIII.

3. Results

3.1. Experiment 1

3.1.1. Accuracy

Systemic administration of FG7142 dose-dependently impaired performance on a delayed alternation task in the T-maze (Fig. 1). The average performance for the 12 rats following vehicle treatment was 82.5% correct. The average performance following 10, 20, and 30 mg/kg FG7142 was 80.0%, 66.7%, and 64.7% correct, respectively. A one-ANOVA-R using dose as a within-subjects factor revealed a significant main effect of FG7142 dose, $F(3,33)=4.34$, $p=0.011$. Planned comparisons revealed that performance following the 20 mg/kg, $F(1,11)=5.43$, $p=0.04$, and 30 mg/kg, $F(1,11)=23.81$, $p=0.0001$, doses of FG7142 was significantly impaired as compared to vehicle administration.

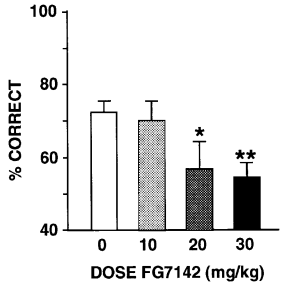

Fig. 1. The pharmacological stressor, FG7142 (10, 20, and 30 mg/kg, i.p.), administered 30 min prior to testing dose-dependently impaired delayed alternation performance in rats. Results represent mean percent correct±S.E.M. on the delayed alternation task ($n=12$). * Indicates significantly different than performance following vehicle administration, $p=0.04$, ** $p=0.0001$.

3.1.2. Response time

FG7142 also dose-dependently increased the average response time. The average response time for the 12 rats following vehicle treatment was 2.5 s. The average response time following 10, 20, and 30 mg/kg FG7142 was 9.6, 13.4, and 18.8 s, respectively. A one-ANOVA-R, using dose as a within-subjects factor, revealed a significant main effect of FG7142 dose, $F(3,33)=4.34$, $p<0.001$. Planned comparisons revealed that the response time following the 10 mg/kg, $F(1,11)=10.72$, $p=0.007$, 20 mg/kg, $F(1,11)=32.77$, $p<0.001$, and 30 mg/kg, $F(1,11)=11.12$, $p=0.007$, doses of FG7142 was significantly longer compared to vehicle administration.

3.2. Experiment 2

3.2.1. Accuracy

The results of the two-ANOVA-R for performance were as follows: main effect of FG7142, $F(1,5)=1.89$, $p=0.23$, main effect of α-2 agonist, $F(2,10)=2.23$, $p=0.16$, interaction between FG7142 and α-2 agonist, $F(2,10)=1.26$, $p=0.33$.

3.2.1.1. Saline pretreatment. Planned comparisons showed that saline/FG7142 induced impairment of performance compared to saline/vehicle as observed in Experiment 1 (average performance 53.7% correct vs. 77.5%, $F(1,5)=30.94$, $p=0.003$).

3.2.1.2. Clonidine pretreatment. Clonidine pretreatment 30 min prior to administration of FG7142 resulted in a partial restoration of performance (Fig. 2). The average performance following clonidine/FG7142 treatment was 66.7% correct, which was not significantly different from either the performance following saline/FG7142 [53.7%, $F(1,5)=0.81$, $p=0.41$] or saline/vehicle [77.5%, $F(1,5)=0.89$, $p=0.39$]. Performance following clonidine/vehicle treatment was not significantly different from

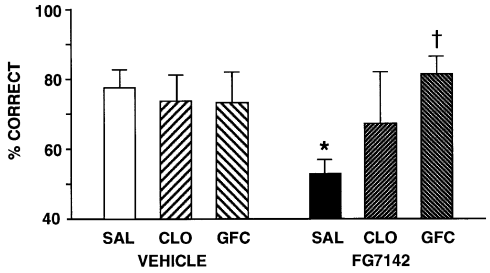

Fig. 2. The detrimental effects of FG7142 (30 mg/kg) were partially reversed by clonidine (0.1 mg/kg) and completely reversed by guanfacine (0.11 mg/kg) pretreatment. Neither clonidine or guanfacine had any effect on performance by themselves. Results represent mean percent correct±S.E.M. on the delayed alternation task (n=6). * Indicates significantly different than performance following vehicle/saline administration, p=0.003. † Indicates significantly different than performance following saline/FG7142 administration, p=0.006. SAL=saline vehicle; CLO=clonidine; GFC=guanfacine.

saline/vehicle treatment [74.2% vs. 77.5%, $F(1,5)=0.12$, $p=0.74$].

3.2.1.3. Guanfacine pretreatment. Guanfacine pretreatment 30 min prior to administration of FG7142 completely ameliorated the stress-induced cognitive deficit (Fig. 2). The average performance following the guanfacine/FG7142 treatment was 81.7% correct, which was significantly improved compared to the performance following saline/FG7142 [53.7%, $F(1,5)=20.63$, $p=0.006$], and not different from performance following saline/vehicle treatment [77.5%, $F(1,5)=0.38$, $p=0.57$]. Performance following guanfacine/vehicle treatment (73.7%) was not significantly different from saline/vehicle treatment, $F(1,5)=0.14$, $p=0.72$.

3.2.2. Response time

The two-ANOVA-R for response time revealed no main effect of FG7142, $F(1,5)=0.13$, $p=0.74$, or α-2 agonist, $F(2,10)=1.20$, $p=0.34$, but a significant interaction between FG7142 and α-2 agonist, $F(2,10)=5.89$, $p=0.02$.

3.2.2.1. Saline pretreatment. Unlike Experiment 1, the response time following saline/FG7142 administration (28 s) was not significantly slower than saline/vehicle [7 s, $F(1,5)=3.71$, $p=0.11$]. This discrepancy is likely due to the high variability in the data as well as the smaller number of animals used in Experiment 2.

3.2.2.2. Clonidine pretreatment. The response time following clonidine/vehicle (26 s) administration was significantly slower than saline/vehicle [7 s, $F(1,5)=10.45$, $p=0.02$]. The response time following clonidine/FG7142 treatment (10 s) was not significantly different from saline/vehicle, $F(1,5)=1.71$, $p=0.25$, clonidine/vehicle, $F(1,5)=1.71$, $p=0.25$, or saline/FG7142 [28 s, $F(1,5)=3.25$, $p=0.13$].

3.2.2.3. Guanfacine pretreatment. The response time following guanfacine/vehicle (10 s) administration was not significantly slower than saline/vehicle [7 s, $F(1,5)=0.58$, $p=0.48$]. The response time following guanfacine/FG7142 treatment (11 s) was not significantly different from saline/vehicle, $F(1,5)=1.48$, $p=0.28$, guanfacine/vehicle, $F(1,5)=0.07$, $p=0.78$, or saline/FG7142 [28 s, $F(1,5)=1.82$, $p=0.24$].

4. Discussion

In these studies, we have confirmed the working memory deficit in rats following administration of the anxiogenic compound, FG7142, which has previously been demonstrated [40]. Murphy et al. reported an impairment in working memory following administration of 20 mg/kg FG7142; in the current study, performance was significantly impaired by both the 20 and 30 mg/kg dose, but not the 10 mg/kg dose, of FG7142. It is important to note that while FG7142 is a benzodiazepine inverse agonist that is recognized as an anxiogenic agent, some of its effects may be different from those observed following other physical and environmental stressors (e.g., seizures). However, both FG7142 and other stressors have been shown to increase catecholamine turnover in the PFC as well as induce cognitive impairments. The DA turnover in the PFC following FG7142 was correlated with the impairment in working memory, suggesting that the cognitive deficit is due to the increased DA release, similar to that observed during stress [40]. In addition to the similar biochemical effects of FG7142 and stress, Leidenheimer and Schechter [33] have shown that rats trained in the discriminative stimulus paradigm with FG7142 will generalize to the state produced by a physiological/environmental stressor (footshock). These similarities suggest that the results from this article may generalize to other physiological and psychological stressors.

Administration of either the nonselective NE α-2 agonist, clonidine (0.1 mg/kg), or the selective NE α-2A agonist, guanfacine (0.11 mg/kg), had no effect on performance of the delayed alternation task when administered alone. However, guanfacine pretreatment 30 min prior to FG7142 (30 mg/kg) completely reversed, while clonidine pretreatment partially reversed, the cognitive deficit caused by FG7142. It has been reported previously that clonidine (0.1 mg/kg) completely reverses the FG7142-induced deficit [41]. This discrepancy is likely due to the higher dose of FG7142 employed in this study (30 vs. 20 mg/kg). In addition, clonidine's sedative side effects may have contributed to the impairment of several of the rats in the current study: clonidine, but not guanfacine, increased the reaction time consistent with its sedative actions. Further studies using a higher dose of clonidine would help determine whether the partial reversal of the cognitive effects

seen in this study was due to an insufficient dose of clonidine or to the detrimental side effects of the drug.

4.1. Receptor subtype mechanisms

Clonidine is relatively nonselective for the three NE α-2 receptor subtypes [54,55], has low affinity for imidazoline I1 receptors [15,56], and has fewer hypotensive and sedative side effects [2]. The results of this study suggest that stimulation of the NE α-2A receptor subtype is sufficient to ameliorate the stress-induced impairment of working memory. This conclusion is supported by a study in which both systemic clonidine and guanfacine blocked the stress-induced increase in DA metabolism in the PFC during a conditioned fear task [38]. In the current study, the response time of rats following clonidine administration was significantly slower (average response time following vehicle/saline administration was 7 vs. 26 s following vehicle/clonidine), while the lack of any obvious side effects (slower response time or sedation) following guanfacine further suggests that α-2A receptors mediate the beneficial cognitive effects of clonidine, while α-2B or α-2C receptor subtypes or imidazoline receptors contribute to the detrimental side effects [2,6,49,50]. Further studies using drugs selective for the α-2B and α-2C receptor subtypes or using genetically altered mice may clarify these issues [50].

4.2. Possible sites of α-2 agonist actions

In this study, all drugs were administered systemically; thus, there are many sites where clonidine and guanfacine could exert their effect. These drugs may act at the level of the ventral tegmental area, which contains the DA cell bodies projecting to the PFC, by regulating DA cell firing [25], thereby reducing DA release in the PFC as shown by Murphy et al. [40]. However, Morrow et al. [38] have shown that systemic administration of guanfacine reduced the amount of freezing during a conditioned fear task, while local administration of clonidine or guanfacine into the ventral tegmental area had no effect on freezing in this task. Thus, the beneficial effects of these systemically administered drugs during stress are unlikely to be exerted principally at the level of the ventral tegmental area. Alternatively, the NE α-2 agonists may bind to presynaptic autoreceptors on the noradrenergic cell bodies in the locus coeruleus and on dendrites in target areas, resulting in a reduction of noradrenergic tone in target areas such as the PFC and ventral tegmental area. Both NE α-2A [44] and α-2C [32] receptors have been observed on locus coeruleus neurons, and actions on these receptors likely contribute to the protective effects observed in this study. However, the current data suggest that presynaptic actions of α-2 agonists cannot account for all the protective effects of these drugs. Although guanfacine was more potent than clonidine in reversing the stress-induced impairment in the current study, guanfacine has been shown to be 10 times less potent than clonidine in decreasing the firing rate of cells in the locus coeruleus and reducing NE release from NE terminals in cortex [20], perhaps due to clonidine's actions at NE α-2C as well as α-2A receptors. Thus, stimulation of NE α-2 autoreceptors on locus coeruleus neurons may contribute to the improved behavioral performance, but it is unlikely to be the only site of action.

An additional possibility is that the NE α-2 agonists act directly on postsynaptic α-2A receptors in the PFC to protect cognitive performance during stress. NE α-2 receptors are Gi-protein-linked to the cAMP/protein kinase A (PKA) intracellular signaling pathway; thus, activation of NE α-2 receptors can decrease PKA activity. On the other hand, DA D_1 receptors are Gs-protein-linked to the PKA pathway, and activation of these receptors can increase PKA activity (Fig. 3). We have recently shown that direct activation of PKA in the PFC in rats impairs working memory performance [51]. Intra-PFC infusions of the PKA activator, Sp-cAMPS, produced delayed alternation deficits that resembled those produced by high doses of D_1 agonists or stress [51]. The delayed alternation deficits induced by Sp-cAMPS were reversed by coinfusion of the PKA inhibitor, Rp-cAMPS. These data suggest that stress-induced working memory deficits may arise, at least in part, from activation of the cAMP/PKA pathway in the PFC, and that alpha-2 agonists may help to protect cognitive performance

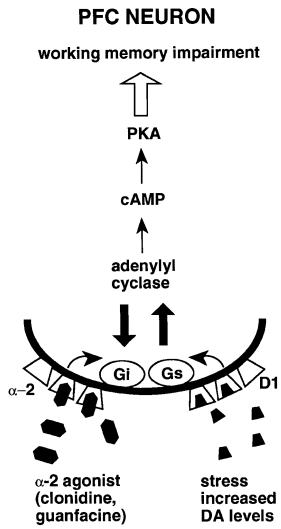

Fig. 3. A schematic representation of a possible intracellular pathway mediating the protective effects of NE α-2 receptor agonists on the stress response in the PFC. NE α-2 receptors are Gi-protein-coupled to adenylyl cyclase, inhibiting cAMP production, while DA D_1 receptors are Gs-protein-coupled to adenylyl cyclase, increasing cAMP production. Excessive release of DA during stress may increase the production of cAMP by adenylate cyclase, which in turn activates PKA and impairs working memory performance [51]. NE α-2 receptor agonists, such as clonidine and guanfacine, may counteract the effects of stress by decreasing adenylate cyclase activity and thereby reversing these intracellular pathways.

during stress by preventing activation of this intracellular pathway (as illustrated in Fig. 3).

4.3. Clinical relevance

Exposure to stress can induce prefrontal dysfunction in humans as well as in animals. For example, stress impairs performance of the Stroop Interference Task [27], which is dependent on prefrontal cortical function. Importantly, stress also contributes to the onset or exacerbation of symptoms for a number of psychiatric disorders that have been associated with prefrontal cortical deficits, e.g., schizophrenia, depression, ADHD, and post-traumatic stress disorder (PTSD; see Ref. [37] for review). It has been suggested that catecholamine dysregulation may contribute to some of the symptoms and possibly the etiology of many of these disorders [1,42,48]. For example, exposure to environmental adversity and stress greatly increases the risk for ADHD [11], a disorder with prominent PFC deficits [9,10,36]. Clonidine [31] and guanfacine [19,29,30] have been shown to improve patients with ADHD, the latter with fewer side effects. NE α-2 mechanisms may also have special relevance to PTSD. For example, administration of the α-2 antagonist, yohimbine, induces symptoms such as flashbacks and reduces metabolic activity of the PFC [14]. Conversely, clonidine [23,26] and guanfacine [28] have been used to treat PTSD. The current results in rats suggest that some of the therapeutic effects of clonidine and guanfacine in patients may arise from their ability to protect PFC function from the detrimental effects of stress.

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