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Research report

Cotinine reduces depressive-like behavior, working memory deficits, and synaptic loss associated with chronic stress in mice

J. Alex Grizzell[a,b], Alexandre Iarkov[a], Rosalee Holmes[a], Takahashi Mori[c], Valentina Echeverria[a,d,e,f,*]

[a] Research & Development Service, Bay Pines VA Healthcare System, Bay Pines, FL 33744, USA
[b] Department of Psychiatry and Behavioral Neurosciences, Morsani College of Medicine, University of South Florida, Tampa, FL 33611, USA
[c] Departments of Biomedical Sciences and Pathology, Saitama Medical Center and Saitama, Medical University, Kawagoe, Saitama 350-8550, Japan
[d] Universidad Autónoma de Chile, Carlos Antúnez 1920, Providencia, Santiago, Chile
[e] Research Service, James A Haley Veterans' Hospital, Tampa, FL 33612, USA
[f] Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL 33647, USA

HIGHLIGHTS

- Cotinine reduces depressive-like behavior and memory loss in the restrained mice.
- Cotinine increases synaptic density in the stressed mouse brains.
- Cotinine inhibits glycogen synthase kinase 3 β in the restrained mouse brains.

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ABSTRACT

Chronic stress underlies and/or exacerbates many psychiatric conditions and often results in memory impairment as well as depressive symptoms. Such afflicted individuals use tobacco more than the general population and this has been suggested as a form of self-medication. Cotinine, the predominant metabolite of nicotine, may underlie such behavior as it has been shown to ameliorate anxiety and memory loss in animal models. In this study, we sought to investigate the effects of cotinine on working memory and depressive-like behavior in mice subjected to prolonged restraint. Cotinine-treated mice displayed better performance than vehicle-treated cohorts on the working memory task, the radial arm water maze test. In addition, with or without chronic stress exposure, cotinine-treated mice engaged in fewer depressive-like behaviors as assessed using the tail suspension and Porsolt's forced swim tests. These antidepressant and nootropic effects of cotinine were associated with an increase in the synaptophysin expression, a commonly used marker of synaptic density, in the hippocampus as well as the prefrontal and entorhinal cortices of restrained mice. The beneficial effects of cotinine in preventing various consequences of chronic stress were underscored by the inhibition of the glycogen synthase kinase 3 β in the hippocampus and prefrontal cortex. Taken together, our results show for the first time that cotinine reduces the negative effects of stress on mood, memory, and the synapse.

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1. Introduction

Memory and emotion are governed by homeostatic plasticity processes, which are susceptible to psychological stress. Chronic stress can induce deficits ranging from transient mnemonic and/or emotional disturbances to more pervasive memory and/or mood dysfunctions, resulting in various depressive and anxiety disorders such as post-traumatic stress disorder (PTSD) and major depression, among others [1,2]. Stress disorders such as PTSD are often accompanied with volumetric reductions of hippocampus [3,4] as well as associated limbic cortices such as prefrontal cortex (PFC)

Abbreviations: ANOVA, analysis of variance; BDNF, brain derived neurotrophic factor; CA, cornu ammonis region; DG, dentate gyrus; EC, entorhinal cortex; GSK3β, glycogen synthase kinase 3 β; HPA, hypothalamus–pituitary–adrenal; PF, open field; nAChR, nicotinic acetylcholine receptor; PBS, phosphate-buffered saline; PT, Porsolt's test; PTSD, post-traumatic stress disorder; PFC, prefrontal cortex; RS, restraint stress; PAWM, radial arm water maze; RT, room temperature; 5-HT, serotonin; TBS, tris-buffered saline; TBST, TBS with 0.1% Tween 20; SSRIs, selective serotonin reuptake inhibitors; TST, tail suspension test.

* Corresponding author at: 10,000 Bay Pines Blvd., Bay Pines, FL 33744, USA. Tel.: +1 727 398 6661x4425; fax: +1 727 319 1161.
E-mail address: echeverria.valentina@gmail.com (V. Echeverria).

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[5]. In addition, alterations in limbic circuitry [6,7] as well as insults to hypothalamic–pituitary–adrenal (HPA) axis function have been reported [8–10].

While incapable of fully encapsulating the wide-ranging sequelae of chronic stress in humans, animal models have been useful in elucidating many of the underlying links between psychological stress, memory, and depressive-like behavior [11,12]. In rodents, it has been shown that many of the stress-induced pathological behaviors are accompanied by morphological and neurochemical changes in the limbic system [12,13]. For instance, chronic stress induces reversible dendritic atrophy in CA3 pyramidal cells [12,14]. Chronic-stress models have also been shown to diminish or alter synapse morphology [15,16] and complexity [17], as well as dendrite morphology and length [18,19] in CA1 pyramidal neurons. Chronic stress-induced atrophy of the granule cells in dentate gyrus (DG) has also been reported [19,20]. Other limbic regions, for instance the PFC, are also implicated. Chronic stress induces pyramidal dendritic retractions within the medial PFC [21] as well as disruptions of afferent pathways from PFC to EC [22] and efferent projections from limbic regions to PFC [23]. Because these limbic regions and PFC are heavily implicated in emotional regulation and memory [24,25], the normalization of their function is a fundamental goal for new therapies to restore working memory and improve mood in individuals subjected to chronic stress.

Restraint stress (RS), is a model of chronic stress in rodents that consistently leads to neuronal remodeling of limbic regions such as PFC [21] and hippocampal CA1 [15,16] and CA3 [12,14] neuronal cells. RS also induces working memory impairment [26,27], anxiety, and depressive-like behavior [28]. These abnormalities mimic those observed in humans exposed to chronic stress or acute stress [29,30].

Cotinine, a tobacco-derived nootropic compound, is a weak agonist of the nicotinic acetylcholine receptors (nAChRs) [31]. However, it has recently been suggested that cotinine may act as a positive allosteric modulator (PAM) of the α7nAChR [32,33]. Also, cotinine acts as agonists of both α4β2 and α3α6β2 nAChR subtypes in the caudate region leading to dopamine release [34]. In addition to the nAChRs, it has been suggested that cotinine may target other potential receptors [35,36] via activation of the serotonergic (5-HT) and dopaminergic systems [37] and/or other yet-to-be characterized receptors.

Despite growing preclinical evidence, few clinical studies have investigated the effect of cotinine on memory or emotion. One study showed that cotinine-treated abstinent smokers self-reported a reduction of anxiety, though whether this effect was due to an interaction with nicotine dependence is unclear as this report did not include non-smokers [38]. In an animal model, systemic long-term cotinine administration at doses as much as ten times higher than those achieved by smoking tobacco reduced anxiety and facilitated the extinction of a contextual fear memory [39] and this latter finding was replicated following hippocampal cannulae injection of cotinine [40]. Furthermore, cotinine positively influences learning, memory [33,41–43], attention, executive function, and impulsive behavior in various animal models [41,44]. Moreover, cotinine delays the progression of memory loss in a mouse model of Alzheimer's disease (AD) [45]. Because of its ideal pharmacodynamics and pharmacokinetic properties [44,46] as well as positive safety profile in humans [46,47], cotinine has been regarded as a viable therapeutic agent to treat the symptoms or delay the progression of many psychiatric and neurological conditions [33,48,49].

In this study, we utilized RS to investigate cotinine's effect on the cognitive and emotional deficits associated with chronic-stress exposure, i.e. spatial and working memory impairment and depressive-like behavior. In addition, we sought to determine cotinine's effects on synaptic density in various limbic regions by immunolabeling the synaptic marker, synaptophysin. Finally, we investigated the effect of cotinine on the expression of glycogen synthase kinase 3 β (GSK3β) in brains of mice exposed or not to chronic restraint stress.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice at ~3 months of age (The Jackson Laboratories, Bar Harbor, ME), weighing 25–35 g were maintained on a 12-h light/dark cycle with ad libitum access to food and water and at a regulated room temperature (25 ± 1 °C). Mice were group housed and habituated to environmental conditions for 7 days. All protocols were approved by the Institutional Animal Care and Use Committee of the Bay Pines Veterans Affairs Healthcare System and followed the National Institutes of Health standards.

2.2. Drugs

Cotinine ((5*S*)-1-methyl-5-(3-pyridyl) pyrrolidin-2-one; Sigma–Aldrich, St. Louis, MO) was prepared by dissolving the powdered compound in sterile phosphate-buffered saline (PBS, pH 7.4). Based on treatment level assignment, mice were orally administered vehicle (PBS, pH 7.4) or cotinine per group assignment (below) via gavage each day at approximately 16:00 EST. The first gavage treatment occurred at least 7 days prior to any experimentation and mice in each group were continuously gavaged daily until the end of each timeline described below. Cotinine was administered via gavage because it has been established that about 80% of cotinine is absorbed with peak plasma concentration averaging 45 min when given orally [50,51]. Cotinine readily crosses the blood–brain barrier [52] at a rate of about 43–61 μg/g/day based on the analysis of unidirectional influx in rat [53]. The dosage of cotinine chosen for this study (5 mg/kg) was based on dose–response curves included in previous studies in our laboratory showing that this was the smallest yet most efficacious dose tested improving anxiety, fear extinction [39] and depressive-like behavior after RS (1 h/day for 14 days; unpublished data).

2.3. Experimental design

Following 7 days of acclimation to the vivarium and housing conditions, mice were divided into 5 cohorts (described below) and randomly assigned to treatment groups (vehicle or cotinine 5 mg/kg). For all mice, drug administration occurred daily until euthanasia, including a 7-day pretreatment period wherein no other experimental procedures were conducted.

The first cohort (C-1) was used to investigate the effects of cotinine in RS-exposed mice across an array of behavioral and neurochemical assessments (described in greater detail below). This cohort consisted of 3 experimental groups: (1) vehicle-treated, non-RS mice (NS + Veh, $n = 5$), (2) vehicle-treated RS mice (RS + Veh, $n = 8$), and (3) cotinine-treated RS mice (RS + Cot 5, $n = 8$). Following the cessation of the RS period (day 35), all mice were tested in open field (OF; day 37), radial arm water maze (RAWM; days 38–47), tail suspension test (TST; day 49) and Porsolt's forced swim test (PT; day 50). Daily treatments continued from 7 days prior to RS until euthanasia (day 51). A timeline depicting the experimental protocol for C-1 can be seen in Fig. 1A.

An additional cohort of mice (C-2) was used to elucidate the effects of cotinine in the RAWM in non-RS (NS) treatment groups (NS + Veh, $n = 8$; NS + Cot 5, $n = 8$). Mice in C-2 were exposed to identical conditions as NS mice in C-1 but were not exposed to OF and were sacrificed following RAWM testing (day 47) and