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Research article

Hypericum perforatum L. prevents the acquisition of and promotes resilience against stress-induced reinstatement of the conditioned place preference induced by cocaine

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A R T I C L E I N F O

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A B S T R A C T

Cocaine use disorder is a serious problem worldwide, and there are no approved medications for its treatment. A novel approach to the treatment of drug addiction is the use of natural products, and, in this context, preclinical evidence suggests that *Hypericum perforatum* L. (Hypericum) is effective against alcohol and other substance use disorders. We hypothesised that Hypericum could also be useful as a treatment for cocaine use disorder, and so we set out to test its effectiveness in a mice model of cocaine addiction. In the first experiment we evaluated its effects on the acquisition of cocaine-induced conditioned place preference (CPP). Adult male mice were conditioned with cocaine (25 mg/kg), cocaine with Hypericum (75, 150 or 300 mg/kg) or the plant extract alone (300 mg/kg). In the second experiment, we tested the effects of Hypericum on stress-induced reinstatement of cocaine CPP. All the mice were conditioned with cocaine (25 mg/kg) and, after extinction of CPP, the reinstating effects of social defeat (alone or with 75, 150 or 300 mg/kg of Hypericum) were evaluated. All the doses of Hypericum prevented the acquisition of cocaine-induced CPP. Furthermore, the plant extract dose-dependently reduced the reinstating effects of social defeat. Therefore, Hypericum is effective in reducing the rewarding effects of cocaine and prevents the stress-induced reinstatement of cocaine CPP in mice. The mechanisms underlying these positive effects of *Hypericum perforatum* L. need to be determined by future research. Our results endorse Hypericum as a natural treatment for cocaine dependence.

1. Introduction

Cocaine addiction has become one of the most serious public health problems affecting society. In developed countries, the incidence of cocaine consumption is high; 3.8 million adult (15–64 years old) European citizens have used cocaine in 2018 [1]. Furthermore, there are no approved medications to treat cocaine addiction, though researchers are currently exploring a variety of neurobiological targets [2].

A novel approach to the treatment of the adverse effects of drugs of abuse is the use of natural products. *Hypericum perforatum* L. (Hypericum), also known as St. John's Wort, is an herbaceous, perennial plant belonging to the Hypericaceae family and known for its important antidepressive, phytotherapeutic properties. The plant is indigenous to northern Africa, Europe and Asia, and is one of the longest used and most widely studied medicinal plants. The therapeutic form is comprised of the leaves and flowering tops. Today, the plant extract is indicated for the treatment of mild depression and is used extensively in many countries. The numerous phytochemical tests carried out on the flowering parts of this plant have highlighted the presence of over 100 components that exhibit synergistic and partially antagonistic effects,

Abbreviations: 5-HT, 5-hydroxytriptamine, serotonin; CPP, conditioned place preference; DA, dopamine; DAT, dopamine transporters; EMCDDA, European Monitoring Centre for Drugs and Drug Addiction; Hypericum, *Hypericum perforatum* L.; HPLC, high performance liquid chromatography; LC-MS, liquid chromatography mass spectrometry; NA, noradrenaline; NAT, noradrenaline transporters; Pre-C, pre-conditioning; Post-C, post-conditioning; SD, social defeat; SERT, serotonin transporters; TLC, thin-layer chromatography.

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including naphtodianthrones (hypericin and pseudohypericin, 0.05–0.30%), phloroglucinoles (hyperforin and adhyperforin); flavonoids (hyperoside, quercetin, isoquercetin and rutin, 2–4%) and catechotannins (7–15%) [3].

Preclinical studies on the central nervous system activity of Hypericum extracts have revealed a serotonergic effect that occurs through a rise in brain serotonin (5-HT) levels due to inhibition of 5-HT reuptake and an increase in 5-HT synthesis (especially in the limbic system) [4]. The lipophilic fraction of Hypericum exhibits a potent affinity for serotonin and opioid receptors [5]. In addition, St. John's wort extract has a clear inhibitory effect on the neuronal reuptake of noradrenaline (NA), dopamine (DA) and glutamate release [4,6,7]. No other antidepressant drug displays an equally broad inhibitory profile. Preclinical studies have shown that Hypericum also displays anxiolytic, sedative, nootropic, antipsychotic, anticonvulsant, and analgesic activities [6,7], and suggest it can be effective in the treatment of alcohol, nicotine, caffeine, heroin and morphine addiction in experimental animals [8].

In light of all these considerations, the objective of the present work was to test the effectiveness of the standardized dry extract (0.3% hypericin) of the flowering tops of Hypericum as a treatment for cocaine addiction in an animal model. The conditioned place preference (CPP) paradigm allows the rewarding properties of drugs to be assessed, and reinstatement of CPP after extinction is a model of the relapse that characterizes drug addiction [9]. Stress exposure is one of the most relevant environmental factors among those that induce the reinstatement of cocaine CPP [10]. Emotional stressors are primary activators of a stress response in humans, and social defeat in an agonistic encounter among rodents is considered a stressor of ecological and ethological validity that closely mimics real-life situations in the human context. Taking into account the capacity of Hypericum to inhibit the re-uptake of NA, 5-HT and DA, all of which are neurotransmitters involved in the behavioral effects of cocaine, we hypothesized that this extract can block the acquisition of cocaine-induced CPP. Furthermore, its role in glutamate transmission and its antidepressant effects suggest that Hypericum can prevent the reinstatement of cocaine CPP induced by exposure to stress.

2. Materials and methods

2.1. Plant material

A commercial extract of *Hypericum perforatum* L. obtained from the dried flowering tips of the plant and standardized to 0.3% of hypericin (Iperico, ACEF Spa, Italy) was used. To confirm the authenticity and quality of extract, we analyzed it by means of high performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and mass spectrometry (LC-MS) (see Section 3.1).

2.2. Animals

80 OF1 male mice (Charles River, France) arrived at our laboratory at 60 days of age and were housed in groups of four in plastic cages (25 × 25 × 14.5 cm) for 8 days before experiments began, under the following conditions: constant temperature, a reversed light schedule (white lights on: 19:30–07:30 h), and food and water available ad libitum, except during behavioural tests. The animals were handled on each of the 3 days prior to initiation of the place conditioning procedure in order to reduce their stress levels in response to manipulation during the experimental procedures. OF1 mice used as opponents in the agonistic encounters (n = 20) were housed in the laboratory in individual cages and were approximately the same weight as experimental mice. We selected this strain of mice because it is the most commonly used in our laboratory to study the acquisition and reinstatement of CPP induced by drugs of abuse [9] and for evaluating the effects of social defeat stress on cocaine CPP [10,11].

All procedures involving the mice and their care were conducted in compliance with national, regional and local laws and regulations, which are in accordance with the European Directive 2010/63/EU. In addition, the study was approved by the Experimental Research Ethical Committee of the University of Valencia (A1335787856485).

2.3. Drugs

Animals were injected i.p. with cocaine (Laboratorio Alcaliber SA, Madrid, Spain), alone or with Hypericum, in a volume of 0.01 ml/g. Control groups were injected with the physiological saline used to dissolve the drugs (NaCl 0.9%).

2.4. Apparatus

Twelve identical Plexiglas boxes with two equal-sized compartments (30.7 cm long × 31.5 cm wide × 34.5 cm high) separated by a grey central area (13.8 cm long × 31.5 cm wide × 34.5 cm high) were employed for place conditioning. The compartments had different coloured walls (black vs white) and distinct floor textures (fine grid in the black compartment and wide grid in the white one). Four infrared light beams in each compartment of the box and six in the central area allowed the position of the animals and their crossings from one compartment to the other to be recorded. The equipment was controlled by three IBM PC computers using MONPRE 2Z software (CIBERTEC, SA, Spain).

2.5. Experiment 1: Effects of Hypericum on acquisition of cocaine-induced CPP

2.5.1. Procedure of CPP

Acquisition of CPP consisted of three phases and took place during the dark cycle following an unbiased procedure in terms of initial spontaneous preference [11]. In brief, during preconditioning (Pre-C), mice were allowed access to both compartments of the apparatus for 15 min per day on 3 consecutive days. On day 3, the time spent by the animal in each compartment during a 15-min period was recorded. Six animals showed a strong unconditioned aversion (<33% of the time spent in both compartments) or preference (more than 67%) for one of the compartments and were eliminated from the study. In the second phase (conditioning), animals were conditioned through four pairings with the respective compartment (two pairings per day on days 4, 5, 6 and 7). Animals received an injection of physiological saline immediately before being confined to the vehicle-paired compartment for 30 min and, after an interval of 4 h, received an injection of cocaine (25 mg/kg) immediately before being confined to the drug-paired compartment for 30 min. In each group, half the animals received the drug or vehicle in one compartment and the other half in the other compartment. The central area was made inaccessible during conditioning by lowering the guillotine doors. During the third phase, or post-conditioning (Post-C), which took place on day 8, the guillotine doors separating the two compartments were removed and the time spent by the untreated mice in each compartment was recorded during an observation period of 15 min.

2.5.2. Experimental design

To study the effects of Hypericum on the acquisition of cocaine-induced CPP, 5 groups of animals (n = 8) received the following treatments during the conditioning phase: 25 mg/kg of cocaine with saline (Sal + Coc), 25 mg/kg of cocaine with 75, 150 or 300 mg/kg of Hypericum (Hp75 + Coc, Hp150 + Coc and Hp300 + Coc, respectively) and 300 mg/kg of Hypericum with saline (Hp300 + Sal). All animals received two injections in the order expressed in the name of the group – e.g. Hp75 + Coc – which were separated by an interval of 30 min (Supplementary Table 1).

2.6. Statistical analysis

The time spent by the animals in the drug-paired compartment was analysed with a mixed ANOVA, with one between-subjects variable – “Treatment”, with five levels (Sal + Coc, Hp75 + Coc, Hp150 + Coc, Hp300 + Coc, Hp300 + Sal) – and one within-subjects variable – “Days”, with two levels (Pre-C and Post-C). The Bonferroni adjustment was employed to make post hoc comparisons of all the values obtained.

2.7. Experiment 2: Effects of Hypericum on reinstatement of cocaine-induced CPP

2.7.1. Experimental design and procedure of extinction/reinstatement

In order to study the effects of Hypericum on reinstatement of cocaine-induced CPP, four groups of animals were conditioned with cocaine (25 mg/kg), as described in the previous experiment. CPP was detected in all groups during the Post-C session. Mice then underwent two extinction sessions (separated by a 15-day interval) in which they were placed in the apparatus (without the guillotine doors separating the compartments) for 15 min until the time spent by each group in the drug-paired compartment was similar to that of Pre-C and different from that of Post-C (Student’s *t*-test). Extinction of CPP was confirmed in an additional session, 5 weeks after Post-C. The reinstatement test was the same as that for Post-C (free ambulation for 15 min), except that animals were tested immediately after exposure to stress induced by social defeat (SD), as described previously [11]. The SD consisted of a 10-min agonistic encounter in a neutral cage with a defeat result for the experimental mouse. Each experimental mouse was confronted with an aggressive opponent and presented avoidance/flee and defensive/submissive behaviours after suffering aggression (threat and attack) from an opponent. To study the effects of Hypericum on SD-induced reinstatement of cocaine-induced CPP, the four groups of animals received the following treatments 30 min before the reinstatement test: physiological saline (Sal + SD, n = 8), 75 mg/kg (Hp75 + SD, n = 8), 150 mg/kg (Hp150 + SD, n = 9) or 300 mg/kg (Hp300 + SD, n = 9) of Hypericum (Supplementary Table 1).

2.8. Statistical analysis

The times spent by the animals in the drug-paired compartment were analysed with a mixed ANOVA, with one between-subjects variable - “Treatment”, with four levels (0, 75, 150 and 300) - and one within-subjects variable - “Days”, with four levels (Pre-C, Post-C, extinction and reinstatement).

3. Results

3.1. Phytochemical analysis

The phytochemical analysis showed that Hypericum contains no<0.08% of hypericins, determined and calculated by HPLC as hypericin and pseudohypericin (Supplementary Fig. 1). LC-MS highlighted the presence of 7.9% hypericin and 92.1% pseudohypericin in 10 mg of analyzed extract. The chromatogram was developed at 590 nm using a time constant of 0.64 s and a sample frequency of the 1.5625 Hz. TLC, in agreement with the current European Phamacopoeia, highlighted spots for hypericin, flavonoids, hyperoside and rutin with Rf values corresponding to the pure and active principles of the reference solution.

3.2. Experiment 1: Effects of Hypericum on acquisition of cocaine-induced CPP

The effects of Hypericum on acquisition of cocaine CPP are shown in Fig. 1a. The results of ANOVA revealed that only the interaction “Days × Treatment” was significant [$F(4,35) = 2.604$; $p < 0.05$]. Only the group that received cocaine with saline spent more time in the drug-paired compartment in Pre-C than in Post-C ($p < 0.01$). During Post-C mice treated with the low dose of Hypericum plus cocaine spent less time in the drug-paired compartment than the control group Sal + Coc

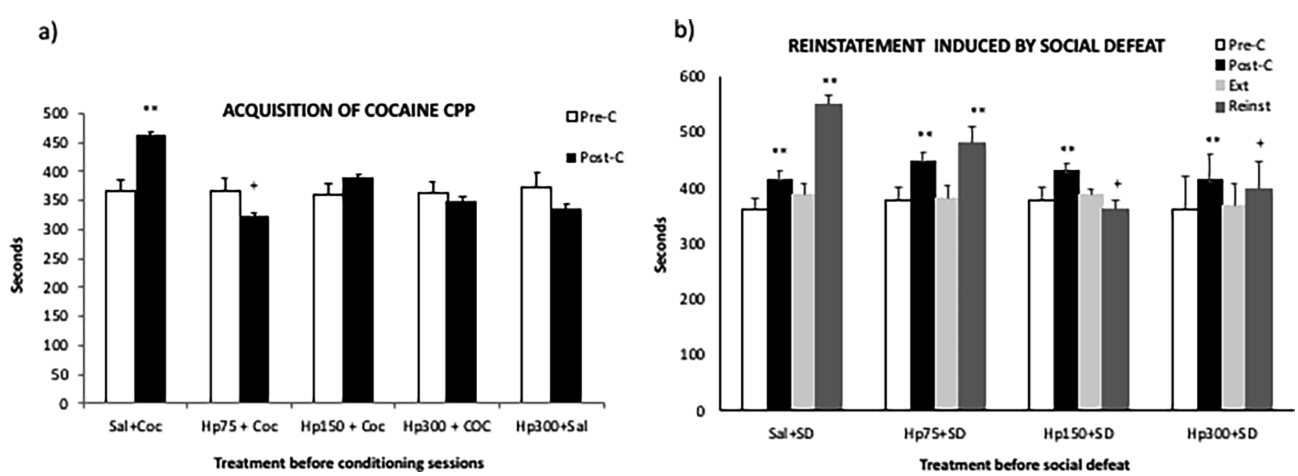

Fig. 1. Effects of Hypericum perforatum L. on the conditioned place preference (CPP) induced by cocaine. a) Effects of Hypericum on acquisition of CPP. During the conditioning phase, animals were allocated to one of the following five treatment groups (n = 8): physiological saline plus 25 mg/kg of cocaine (Sal + Coc); cocaine (25 mg/kg) with 75, 150 or 300 mg/kg of Hypericum (Hp75 + Coc, Hp150 + Coc, Hp300 + Coc, respectively); or 300 mg/kg of Hypericum with saline (Hp300 + Sal). Bars represent the time in seconds (mean ± SEM) spent in the drug-paired compartment before conditioning sessions in the pre-conditioning test (Pre-C, white bars) and after conditioning sessions in the post-conditioning test (Post-C, black bars). ** $p < 0.01$, significant difference in the time spent in the drug-paired compartment in pre-conditioning vs post-conditioning test. + $p < 0.05$, significant difference in the time spent in the drug-paired compartment with respect to control group (Coc + Sal). b) Effects of Hypericum on stress-induced reinstatement of cocaine CPP. Four groups of mice were conditioned with 25 mg/kg of cocaine and underwent extinction sessions until CPP was extinguished. Next, they received the following treatments 30 min before being exposed to social defeat (SD) stress: physiological saline (Sal + SD, n = 8), 75 mg/kg (Hp75 + SD, n = 8), 150 mg/kg (Hp150 + SD, n = 9) or 300 mg/kg (Hp300 + SD, n = 9) of Hypericum. Bars represent the time in seconds (mean ± SEM) spent in the drug-paired compartment before conditioning sessions in the Pre-C test (white bars), after conditioning sessions in the Post-C test (black bars), in the last extinction session (Ext, light grey bars), and in the reinstatement test (Reinst, dark grey bars). ** $p < 0.01$, significant difference in the time spent in the drug-paired compartment in pre-conditioning vs post-conditioning or reinstatement test. + $p < 0.05$, significant difference in the time spent in the drug-paired compartment with respect to SD + Sal on the same day.

($ps < 0.05$). These results suggest that Hypericum especially at the lower dose administered, can prevent the acquisition of cocaine-induced CPP.

3.3. *Experiment 2: Effects of Hypericum on stress-induced reinstatement of cocaine CPP*

The effects of Hypericum on reinstatement of cocaine CPP are represented in Fig. 1b. The results of ANOVA revealed that the variable "Days" [$F(3,90) = 5.922$; $p < 0.001$] and the interaction "Days × Treatment" [$F(9,90) = 3.121$; $p < 0.01$] were significant. As expected, all the groups spent more time in the drug-paired compartment in Post-C than in Pre-C ($ps < 0.01$). Regarding reinstatement, only the groups experiencing social defeat with saline or the low dose of Hypericum showed a significant increase in the time spent in the drug-paired compartment during the reinstatement test in comparison to Pre-C ($p < 0.01$). Moreover, significant differences between the treatment groups were observed only in the reinstatement test; mice treated with 150 and 300 mg/kg of Hypericum before social defeat spent less time in the drug-paired compartment than the control group (SD + Sal) exposed to social defeat ($ps < 0.05$). These results indicate that Hypericum, especially at medium and high doses, blocks the reinstatement of cocaine CPP induced by social defeat.

4. Discussion

The present study demonstrates that Hypericum is effective in blocking the conditioned rewarding effects of cocaine in the CPP paradigm and preventing stress-induced reinstatement of cocaine CPP. Our results support further evaluation by clinical trials of the potential of this plant as a treatment for cocaine abuse and addiction.

In accordance with previous studies carried out in our laboratory, administration of 25 mg/kg of cocaine induced acquisition of CPP [11,12]. However, mice treated with Hypericum (75, 150 or 300 mg/kg) before each conditioning session with cocaine did not acquire CPP. A similar effect has been observed previously with another natural compound, *Rhodiola rosea* [11]. The rewarding effects of cocaine are mainly a result of the activation of the brain dopaminergic system [13], but this drug also increases levels of other neurotransmitters, such as 5-HT [14] and NA [15]. DA receptors and DA transporters (DAT) have been implicated in the rewarding effects of cocaine [13]; however, in the absence of DAT, the role of serotonin (SERT) and NA (NAT) transporters in cocaine reward is enhanced, and DAT + SERT KO mice have been shown not to display cocaine CPP [16]. In our opinion, the blocking of the acquisition of cocaine CPP induced by Hypericum may be due to its capacity to inhibit DA, NA and serotonin re-uptake [4,6,7]. Our analyses of the hypericum extract using HPLC, LC-MS and TLC highlight the presence of hypericin and other active ingredients in the phytocomplex. Preclinical studies of the central nervous system activity of Hypericum extracts have shown that the numerous phytochemical components of this plant exhibit synergistic inhibitory effects on the neuronal re-uptake of DA, NA and 5-HT [3–5,17,18]. Such evidence suggests that the activity of Hypericum extracts is due to the synergistic interaction of numerous constituents of the phytocomplex with different pharmacological targets. In rats, acute and chronic administration of Hypericum extracts have been reported to increase in vivo DA release both in the nucleus accumbens [17,18] and the striatum [17] and to enhance serotonin levels in the nucleus accumbens [18]. Repeated application leads to rapid tolerance on DA (but not of 5-HT) neurons, while an active component of Hypericum extracts, hyperforin, also inhibits DA and 5-HT re-uptake and stimulates the release of these neurotransmitters in the nucleus accumbens [18].

Furthermore, we have previously observed that Hypericum (150 and 300 mg/kg) prevents the reinstatement of cocaine CPP provoked by exposure of mice to social defeat, an animal model of stress-induced relapse of drug-seeking after a period of abstinence [9]. Although the present study represents the first report of the effects of Hypericum on cocaine reward and cocaine addiction, our results are in line with previous research suggesting that this plant is effective in the treatment of alcohol, opioid and nicotine dependence [8,19–22]. For example, Hypericum has been found to reduce ethanol intake [23], the reinforcing properties of ethanol in the self-administration paradigm [24], and ethanol withdrawal syndrome [19] in rats. Similarly, it blocks the locomotor stimulant effects of caffeine in mice [25], attenuates the physical symptoms of withdrawal in opioid-dependent rats treated with naloxone [20,21], and reduces nicotine withdrawal signs in mice [22]. Besides the previously mentioned action of Hypericum on monoamine transporters, it has been suggested that its beneficial effects on nicotine withdrawal are related with an increase of cortical 5-HT and 5-HT1A receptors [22]. In line with this, we hypothesized that these receptors are also involved in the preventive effects of Hypericum on defeat-induced reinstatement of cocaine CPP, since several studies have demonstrated changes in 5-HT1A receptors after exposure to social defeat. In particular, a down-regulation of 5-HT1A mRNA levels in the prefrontal cortex has been observed in rats chronically exposed to social defeat [26].

Changes in glutamate and nitric oxide (NO) could also underly the effects of Hypericum on stress-induced reinstatement of cocaine CPP. Recent studies in our laboratory have demonstrated the role of glutamate receptors [27,28] and NO signalling [29] in the effects of social defeat stress in mice. Antagonism of glutamate NMDA receptors and inhibition of NO synthase (NOS) reversed the effects of social defeat on the CPP induced by cocaine [27] and MDMA [28,29]. As several components from Hypericum inhibit glutamate release [6] and NOS [25,30], the absence of stress-induced reinstatement of cocaine CPP observed in the present study may have been due, at least in part, to the effects of the plant on these neurotransmitter systems. In support of this hypothesis, a study by Uzbay et al. [25] demonstrated that the inhibitory effects of Hypericum on the hyperactivity induced by caffeine were reversed by administration of l-arginine, thus suggesting a role of NOS in said effects [25]. In addition, Hypericum has been implicated in the regulation of genes that control hypothalamic–pituitaryadrenal axis function [6] and this compound reduced stress-induced increases in plasma ACTH and corticosterone levels [31]. For these reasons, we propose that changes in the levels of stress hormones after administration of Hypericum also contribute to the prevention of social defeat-induced reinstatement. In fact, Hypericum has been demonstrated to be effective in alleviating the impairment of several spatial memory tasks induced by restrain stress exposure or prolonged corticosterone administration in rats [6]. Hypericum has anxiolytic and antidepressant effects in animal models [7,32], and controlled clinical trials have demonstrated that it possesses antidepressant properties similar to those of clinically used compounds (such as tricyclic antidepressants and selective SERT inhibitors), but with fewer and milder side effects [7]. These anxiolytic and antidepressant properties of Hypericum may contribute to the inhibition of stress-induced reinstatement of cocaine CPP, and encourage further research on the usefulness of this plant in the treatment of addictive and other stress-related disorders.

In conclusion, our study shows for the first time that Hypericum is effective in reducing the rewarding effects of cocaine and, even more importantly, the reinstatement of cocaine seeking following exposure to stress after a period of abstinence. We hypothesise that these effects are related mainly with the inhibition of monoamine re-uptake and the antidepressant properties of Hypericum, although other neurotransmitter systems and hormones could be involved. Ongoing studies in our laboratory are currently evaluating whether this compound is also effective in reducing cocaine withdrawal symptoms in mice. Although more preclinical and clinical studies are needed, our results certainly endorse the potential of Hypericum as a natural treatment for cocaine addiction.

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Author contributions

MAA and FO review the literature, design the experiments and write the paper. FF and MPGP performed the experimental work, the statistical analyses and the figures. All the authors critically contributed to the last version of the paper.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neulet.2021.136164.

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