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St John's wort *(Hypericum perforatum)* counteracts deleterious effects of the chronic restraint stress on recall in rats

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Abstract. This study aimed at verifying a hypothesis that St. John's wort *(Hypericum perforatum)* alleviates stress-induced memory impairments. Administration of *Hypericum perforatum* (350 mg kg^{-1} daily for 21 days) significantly enhanced recall of passive avoidance behavior (PAB), but had no effect on the acquisition of conditioned avoidance responses (CARs). Rats stressed chronically (2 h daily for 21 days) displayed diminished recall of the PAB and this effect was abolished by St John's wort. Chronic administration of the "equivalent" to the stress dose of exogenous corticosterone (5 mg kg^{-1} daily for 21 days) also impaired recall of PAB, and this effect was also reversed by *Hypericum perforatum*. None of our treatments produced significant motor coordination impairments as tested in a 'chimney' test. It appears that *H. perforatum* prevents stress-induced deterioration of memory in rats.

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Key words: *Hypericum perforatum*, stress, corticosterone, passive avoidance, conditioned behavior, learning and memory, rats

INTRODUCTION

There is extensive evidence that chronic stress impairs cognition in a number of aspects, such as acquisition of memory, consolidation, and recall (Arnsten and Goldman-Rakic 1998, Baker and Kim 2002, Bowman et al. 2003, Lupien and Lepage 2001, Mizoguchi et al. 2000, Park et al. 2001, Sarnyai et al. 2000). Exposure to mild uncontrollable stress leads to an array of biochemical, physiological and behavioral changes. Prolonged stress causes exhaustion of the adaptive mechanisms and deregulation of the hypothalamo-pituitary-adrenal (HPA) axis resulting in an overproduction of glucocorticosteroids. In human stress, excessive cortisolaemia, and administration of the exogenous glucocorticosteroids can have severe adverse repercussions including a number of neurochemical and neuroanatomical changes in the brain, similar to these demonstrated in patients with Cushing's syndrome, depression or patients with post-traumatic stress disorder (PTSD) (Arnsten and Goldman-Rakic 1998, Belanoff et al. 2001, Bremner 1999, Diamond et al. 1992, Lupien and McEwen 1997, McEwen 2000, 2001, Moghaddam 2002, Roozendaal et al. 2003, Sapolsky 2000). The neurogenesis is disturbed (Brezun and Daszuta 2000, Duman et al. 2001, Lupien and Lepage 2001, McEwen 2000, 2001) and the neuronal atrophy (Lupien and Lepage 2001, Magarinos et al. 1996, McEwen 2001, Sapolsky 2000, Sousa et al. 2000), lowering total number of neurons (McEwen 2001) and their ramifications (Galea et al. 1997), occurs in the hippocampal areas CA1 and CA3. Multiple hormone and neurotransmitter systems are involved in producing stress-induced malfunction including cerebral aminergic systems, especially 5-HT1A and dopaminergic receptors in prefrontal cortex, striatum and hippocampus (Arnsten and Goldman-Rakic 1998, Beck and Luine 1999, Butterweck et al. 2001, Flugge 1995, Grundemann et al. 1998, Imperato et al. 1989, Lindley et al. 1999, Lupien and Lepage 2001, McEwen 2001, Mizoguchi et al. 2000, Piazza et al. 1996). Considerable harm results from the disadvantageous alterations in the excitatory, particularly glutamate (NMDA receptors) as well as from inhibitory ($GABA_A$ receptors) amino acid systems (Kim et al. 1996, McEwen 2000, 2001, Sarnyai et al. 2000, Singh et al. 1999). Also, an adverse influence of stress was observed on the levels of nerve growth factor (NGF) and the brain-derived neurotrophic factor (BDNF) (Belanoff et al. 2001, Duman et al. 2001, Schaaf et al. 2000).

There are several reports concerning the search for efficient pharmacological agents that neutralize memory deficits occurring in affective disorders (Butterweck et al. 2001, Di Carlo et al. 2001, Duman et al. 2001, Greeson et al. 2001, Lupien and Lepage 2001, McEwen 2000, 2001). The effectiveness of antidepressant drugs including tricyclic antidepressants and some antidepressive agents of a new generation (Duman et al. 2001, Lupien and Lepage 2001, McEwen 2001) is well known. However, administration of traditional antidepressants entails numerous adverse effects and their prophylactic use would be unwise. Obviously, a drug of an efficacy similar to antidepressants' efficacy, but with less undesirable effects, would be most wanted for that purpose.

Hypericum perforatum extracts used for the treatment of affective disorders have comparable efficacy to that of drugs routinely used in mild to moderate depression (Butterweck et al. 1998, 2001, Chatterjee et al. 1998, Gambarana et al. 2001, Greeson et al. 2001, Muller et al. 1997). *H. perforatum* effectively restores normal cortisolaemia (Butterweck et al. 2001, Gambarana et al. 2001, Holsboer-Trachsler et al. 2002, Neary and Bu 1999). Also, it was found that one from the active components of the extract (pseudohypericin) selectively antagonizes release of corticotropin-releasing factor (CRF) (Simmen et al. 2003), that regulates HPA axis trough the CRF1 receptors. Another agent, hyperforine, being one of the major active lipophilic constituents of the extract, causes effects comparable to reserpine in catecholaminegic and serotonergic terminals triggering neurotransmitter release (Butterweck et al. 2002, Chatterjee et al. 1998, Roz and Rehavi 2003). By elevating intracellular Na^+, it potently inhibits serotonin uptake and normalizes number and sensitivity of the 5-HT1A (Fornal et al. 2001, Neary and Bu 1999, Yu 2000) and 5-HT2 (Muller et al. 1997, Teufel-Mayer and Gleitz 1997) receptors. It also reverses stress-induced downregulation of cortical β-adrenoceptors (Flausino et al. 2002) as well as the dopamine neurotransmission in prefrontal cortex, striatum and hippocampus (Kehr et al. 2002, Raffa 1998). *H. perforatum* also abolishes deleterious effect of stress on glutamate (Belanoff et al. 2001, McEwen 2000, 2001, Vandenbogaerde et al. 2000) and gabaergic (Neary and Bu 1999, Nathan 2001, Vandenbogaerde et al. 2000) neurotransmission. Moreover, the Na^+-dependent uptake of choline (the precursor of acetylcholine) was found to be changed in the brain by the plant, causing possibly increased acetylcholine release (Kiewert et al. 2004). *H.*

perforatum extracts disinhibits neurogenesis as well as restores BDNF levels in CNS (Duman et al. 2001, Greeson et al. 2001, Muller et al. 1997). Also, the plant has been shown to inhibit intrleukin-6 (IL-6) expression (Vandenbogaerde et al. 2000), a cytokine that may stimulate release of cortisol (Thiele et al. 1994). Therefore, the use of St John's wort in protecting against the negative effects of stress on learning and memory, proposed in our earlier study (Trofimiuk et al. 2005), seems to be an attractive possibility. Importantly, treatment with *H. perforatum* extracts (in recommended daily doses) is not connected with any serious adverse effects in humans and in animals (Butterweck et al. 1998, Holsboer-Trachsler et al. 1999, Trautmann-Sponsel and Dienel 2004). Some problems could be due to drug interactions. As with synthetic antidepressants, pharmacokinetic interactions may occur infrequently as a result of activity changes of drug-metabolising and drug-transporting proteins, especially CYP 3A4 and P-gp. Hence, *Hypericum* extracts preparations should not be taken at the same time with other antidepressants, with coumarin-type anticoagulants, the immunosuppressants cyclosporine and tacrolimus, protease and reverse transcriptase inhibitors used in anti-HIV treatment or with certain antineoplastic agents (Schulz 2006). However, such cases are extremely rare and, with medical supervision, easily avoided.

To confirm and extend our previous findings presently we used the rat model of chronic stress caused by their daily immobilization for two hours (Avital et al. 2001). Recall of passive avoidance behavior (PAB) (Ader et al. 1972) and learning of conditioned avoidance responses (CARs) (Braszko et al. 1987) were used to assess cognitive aspects of behaviour. A 'chimney' test was applied to exclude adverse effects of our treatments on the rats' motor performance (Boissier et al. 1960).

METHODS

Animals

One hundred and twenty four male, two-month old Wistar rats, weighing 140–150 g at the beginning of the study, were used. The rats were maintained in a temperature (23°C) and humidity (50–60%) controlled vivarium in groups of five under a constant 12/12 h light/dark schedule (lights on at 7:00 A.M.) with free access to the standard lab chow and tap water.

Drugs

Dried crude herb of *H. perforatum* (Labofarm, Poland) in the form of a brown powder, standardized to 0.3% hypericins was used in all experiments. Its constituents were naphthodiantrones (hypericin and its derivatives), phloroglucinols (up to 6% hyperforin), flavonoids 2–4 % (rutine, quercetine, quercitrine, isoquercitrin, biapigenin, hyperoside) and procyanidins 8–12 % (procyanidin, catechin, epicatechin polymers). It was suspended in a 2% caboxymethyl-cellulose (2% CMC) and administered at the dose of 350 mg kg^{-1} daily for three weeks by gastrointestinal gavage (p.o.) in the volume of 1.5 ml kg^{-1}. This dose corresponds to the human recommended daily dose of 0.9 mg total hypericins (Widy-Tyszkiewicz et al. 2002). Corticosterone (5 mg kg^{-1}, Sigma, Germany) was dissolved in absolute ethanol and subsequently diluted in water, to the final ethanol concentration 10% (Sandi et al. 1997). It was administered subcutaneously (s.c.) at the volume of 1ml kg^{-1} daily for 21 days. This dose of corticosterone induces plasma levels of the steroid comparable to these evoked by substantial stress (Sandi et al. 1997).

The injections did not produce any noticeable side effects in our rats resulting from the presence of ethanol.

Animals were divided into six groups treated as follows (Table I): (1) Twenty-one rats received 2% CMC p.o. and 30 min later 10% ethanol s.c. (Control); (2) twenty-one rats received 2% CMC and 30 min later

Table I

Treatment		
Group	p.o.	s.c.
Control	2% CMC [a)]	10% ethanol [b)]
Stress	2% CMC	10% ethanol
Cort.	2% CMC	5 mg kg^{-1}
Hyper	350 mg kg^{-1}	10% ethanol
Hyper + Stress	350 mg kg^{-1}	10% ethanol
Hyper + Cort.	350 mg kg^{-1}	5 mg kg^{-1}

(a) 2% solution of carboxymethyl-cellulose (CMC) in water was used as a vehicle for the oral administration of *Hypericum perforatum*; (b) 10% ethanol solution was used as vehicle for the subcutaneous administration of corticosterone

10% ethanol s.c. followed by stress procedure described below (Stress); (3) twenty-one rats received a 2% CMC p.o. and 30 min later corticosterone (5 mg kg^{-1}) s.c. in 10% ethanol (Cort); (4) twenty-one rats received 350 mg/kg *H. perforatum* (about 13 μg kg^{-1} hypericins) p.o. in 2% CMC and 30 min later 10% ethanol s.c. (Hyper); (5) twenty rats received 350 mg kg-1 *H. perforatum* p.o. in 2% CMC and 30 min later 10% ethanol s.c. followed by stress procedure described below (Hyper+Stress); (6) twenty rats received 350 mg kg-1 H. perforatum in 2% CMC p.o. and 30 min later corticosterone 5 mg kg-1 in 10% ethanol (Hyper+Cort).

The experimental procedures were carried out according to the European Council Directive of 24 November 1986 (6/609/EEC) and were approved by the Local Ethics Commission for Animal Experimentation.

Stress procedure

Two groups of animals (41 rats) were subject to chronic restraint stress (Avital et al. 2001), 2 h daily for 21 days. The restraint was performed during the light cycle from 9:00 A.M. to 11:00 A.M. The restrainer was made of transparent perforated plastic tube, 20 cm long, and 7 cm in diameter. The rats were eased into the restrainer, head first, and once in, the tubes were closed with plexiglass lids. The animals fit tightly into the restrainers and it was not possible for them to move or turn around. Non-stressed control rats were at the same time briefly handled and returned to their home cages.

Behavioral tests

'CHIMNEY' TEST

Locomotor coordination was estimated using a 'chimney' test characterized originally for mice (Boissier et al. 1960). Three days after stress and medication procedures, rats (64 animals) were allowed to enter a glass laboratory cylinder 452 mm long and 57 mm in diameter laid on its side. As soon as the animal reached the far end of the cylinder its orientation was quickly changed from horizontal to vertical position and a timer was started. The animal immediately began to withdraw from the upper end. The timer was stopped after the rat left the cylinder and assumed sitting posture on the top of the vessel. The time of exit from the cylinder was approved as a measure of motor coordination and, possibly, anxiety. This test was used on the same rats (64 animals), which were tested in the passive avoidance task and performed after that.

PASSIVE AVOIDANCE

Passive avoidance behavior was studied in a one trial learning, step-trough situation (Ader et al. 1972), which utilizes the natural preference of rats for dark environments. The apparatus consists of the platform (250 × 80 mm) connected with a dark compartment – a metal box (400 × 400 × 400 mm) with an opening (60 × 100 mm) in the middle of the frontal wall length. After a 2-min habituation to the dark compartment, the rat was placed on the illuminated platform and allowed to enter the dark compartment. Two more approach trials were given on the following day with a 2-min interval. At the end of the second trial unavoidable scrambled electric footshock (0.25 mA, AC, 2 s) was delivered through the grid floor of the dark compartment (learning trial). Twenty-four hours later retention of the passive avoidance response was tested after an additional 15-min by placing the animal on the platform and measuring the latency to re-enter the dark compartment to a maximum of 300 s. The four rats were excluded from test probe after training sessions because they did not effectively learn the rules of that test. As a result, their data for the PAB are not available.

CONDITIONED AVOIDANCE RESPONSES

Conditioned avoidance responses (CARs) were studied in a shuttle box (48 × 27 × 23 cm) and divided in two equal parts by a wall with a 7 cm wide and 8 cm high opening in the middle of its length (Columbus Instruments, Columbus, USA) (Braszko et al. 1987). A buzzer (sound intensity set at 5, 5000 Hz, conditioned stimulus, CS) was sounded for 5 s. If the rat did not make a positive (+)CAR, i.e. move to the other compartment within 10 s, a 0.3 mA scrambled electric shock (unconditioned stimulus, US) was delivered through the box floor which was made of stainless steel rods 4 mm in diameter and spaced at 18 mm intervals. The US was terminated when the animal escaped to the other compartment of the box. CARs acquisition training consisted of five daily 20-trial sessions. The number of (+) CARs (avoidance responses) was recorded every day and expressed as the percentage of the total number of trials. The intertrial interval was 10 s. The gird floor

was kept clean throughout the training sessions. Seven rats were excluded from the test after training sessions because they did not effectively learn the rules of that test. As a result, their data for the CARs are not available.

Statistical analysis

Data were presented as means ± standard error of mean (SEM). One-way analysis of variance (ANOVA), followed by Bonferroni test for chosen group comparisons, was applied for the 'chimney' and passive avoidance tests. A two way analysis of variance (ANOVA II) (treatment × days) with repeated measures on factor 1, followed by the post hoc Newman-Keuls test for multiple comparisons, was used for results obtained in the conditioned avoidance responses (CARs). *F* values, degrees of freedom, and *P* values are quoted only for significant differences. The probability levels less than 0.05 were accepted as significant. The daily percentages of (+)CARs for each rat were summed up first and overall group means were then calculated.

RESULTS

Effects of stress, exogenous corticosterone, and *H. perforatum* on locomotor coordination of rats in the 'chimney' test

ANOVA of times of the animals' exit from the glass cylinder obtained in the 'chimney' test yielded $F_{5,63}$=0.561, P>0.5 no statistically significant differences in the Figure 1, indicating that our treatments and procedures did not affect the motor coordination and anxiety aspects of the rats' performance.

Effects of stress, exogenous corticosterone, and *H. perforatum* on passive avoidance behaviour

ANOVA of the results obtained in the passive avoidance test yielded $F_{5,59}$=40.17 (P<0.001) statistically significant differences between the groups in the re-entry latencies in the passive avoidance situation (Fig. 2). *Post-hoc* comparisons in preselected pairs with Bonferroni test revealed that stressed rats re-entered the dark part of apparatus significantly earlier then both control (P<0.01), and treated with *H. perforatum* stressed (P<0.001) or not stressed (P<0.001) rats. In the animals treated with corticosterone, re-entry latencies

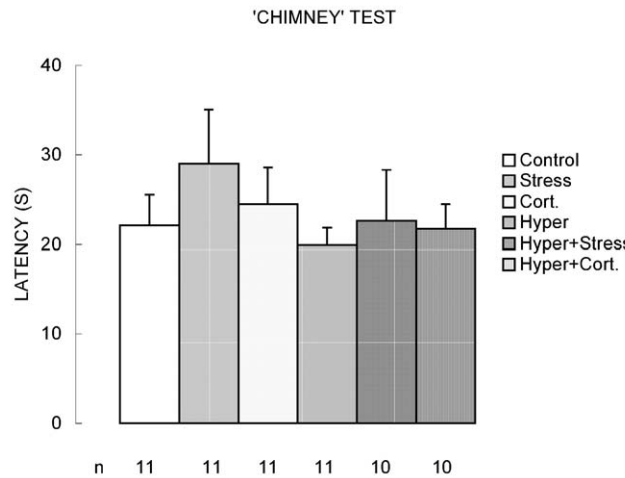

Fig. 1. Effects of chronic stress, chronic corticosterone Hypericum perforatum on time of rats' exit from 'chimney'. Columns represent means ± SEM of the values obtained from n rats indicated at the bottom of the figure.

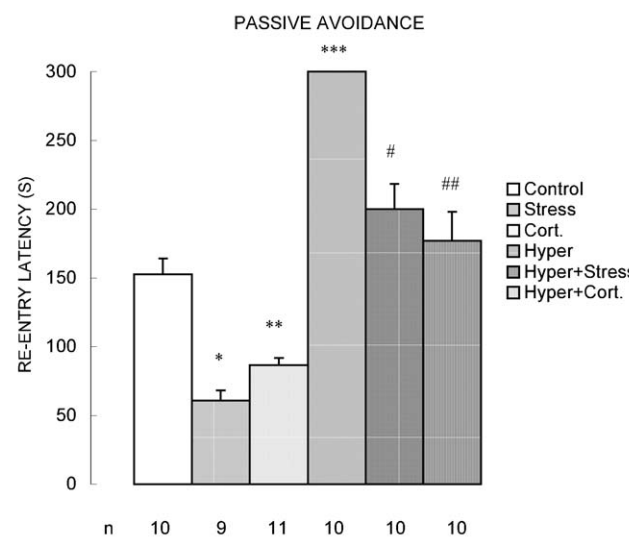

Fig. 2. Effects of chronic stress, chronic corticosterone and Hypericum perforatum on the re-entry latencies in the passive avoidance situation. Columns represent means ± SEM of the values obtained from n rats indicated at the bottom of the figure. The three groups demonstrated significantly different results in re-entry latencies in comparison with Control: (*) P<0.01 vs. Stress; (**) P<0.05 vs. Cort; (***) P<0.001 vs. Hyper. The statistically significant differences were also in Hyper+Stress [(#) P<0.001] vs. Stress; and Hyper+Cort [(##) P<0.01] vs. Cort.

were significantly (P<0.05) earlier than controls, as well as rats pretreated with *H. perforatum*, subsequently treated (P<0.01) with corticosterone were significantly different (P<0.001) than corticosterone treated.

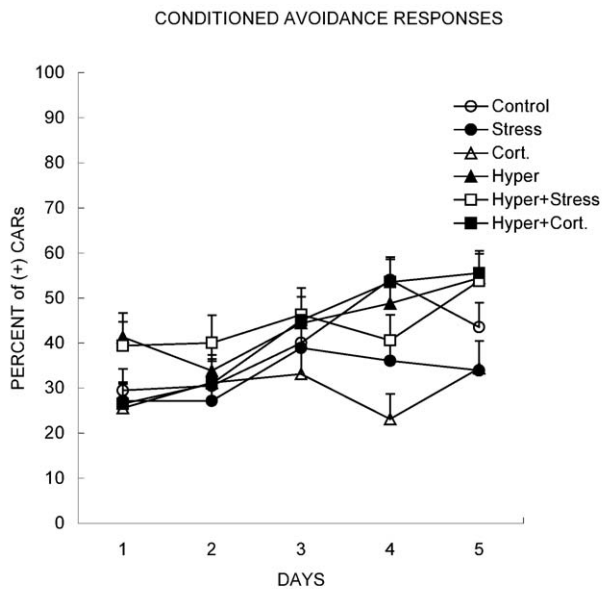

Fig. 3. Effects of chronic restraint stress, chronic corticosterone and Hypericum perforatum on acquisition of CARs over 5 days. Columns represent means ± SEM of the values obtained from 8 to 10 subjects.

This pattern showed that substantial adverse effects of stress as well as exogenous corticosterone administration on learning and avoidance behavior were abolished by the simultaneous *H. perforatum* administration. Moreover, in naive rats the herb improved memory.

Effects of stress, exogenous corticosterone, and H. perforatum on conditioned avoidance response (CARs)

ANOVA II of the cumulative (over 5 days), rates of (+)CARs revealed no significant treatment effect ($F_{5,47}$=2.07; P>0.05) but a significant day effect ($F_{4,20}$=15.645; P<0.001) and significant treatments × days interaction ($F_{20,188}$=2.23; P<0.01) (Fig. 3). It means that all rats effectively learned the task but no specific treatment effect could be detected. Alternatively, the treatment effect could be masked especially in the Cort group on the fourth day.

DISCUSSION

The present results clearly show a remarkable improvement of memory for the passive behaviour in rats treated with the crude preparation of St John's wort. The plant also alleviated the negative influence of chronic stress and multiple glucocorticosteroid injections. Moderate (nonsignificant) decrease of the rate of conditioned avoidance responses (CARs) acquisition (learning) in the corticosterone-treated, as well as the stressed group was also reversed by our doses of *Hypericum perforatum*.

Noteworthy, the stimulating effect of *H. perforatum* on CARs acquisition was disappointingly small, almost none. It was somewhat surprising given the considerable memory-enhancing action of the plant seen in the water maze previously (Trofimiuk at al. 2005). It might be caused by the pain-relieving activity of *Hypericum* (Apaydin et al. 1999, Sanchez-Mateo et al. 2006) decreasing strength of the foot shock perceived by the animals diminishing thus expression of any cognitive activity of the drug. Also, it is well known that electric stimuli themselves activate antinociceptive including opioid-mediated mechanisms (Gamaro et al. 1998, Raffa 1998) that may interact with these of the herb through its influence on the NA^+-dependent noradrenaline and serotonin uptake (Roz and Rehavi 2003).

As motor coordination and fear may affect results of memory tests, we checked for these aspects of rats' behaviour in a 'chimney' test. The lack of any greater influence of our treatments on these results means that there were no impairments of motor performance/coordination and possibly fear in the extent that might influence the effects of the cognitive tests conducted in our animals.

The cognitive effects of *H. perforatum* in animals and humans (Johnson et al. 1994, Khalifa 2001, Widy-Tyszkiewicz et al. 2002) have been reported as qualitatively comparable with these of piracetam (Kumar et al. 2000). These data are consistent with our previous (Trofimiuk et al. 2005) and present results showing significant improvement of rat cognitive functions by *H. perforatum* in rats. However, in this investigation we focused on the issue of the *H. perforatum* potential to protect the central nervous system against memory impairing effects of chronic restraint stress. The results appear quite promising. Administration of *H. perforatum* clearly prevented the deficits of cognitive functions caused by both, chronic stress and application of exogenous corticosterone. It has been noted that the severity of dysfunction caused by stress was much higher than that caused by corticosterone. This observation points to the importance of several other than prolonged cortisolaemia factors that must contribute to the stress-induced impairment of cognition. Multiple

hormone and neurotransmitter systems are involved in producing stress-induced brain malfunction, not only glucocorticosteroid overproduction with ensuing hypercortisolaemia.

CONCLUSIONS

This study seems to justify the conclusion that St John's wort in crude form has considerable stress relieving and memory enhancing activity. Our data demonstrate that *H. perforatum* is able to protect against several changes evoked by chronic stress at different levels of neuronal function in various structures involved in the memory processes and even reverse such changes. *H. perforatum*, in addition to its well-known antidepressive actions, has positive effects on other cognitive as well as non-cognitive aspects of behaviour and perhaps should be more widely used as a foolproof and inexpensive remedy in prevention of, not only stress memory disorders, but also these associated with advancing age.

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REFERENCES

Ader R, Weijnen JAWM, Moleman P (1972) Retention of passive avoidance response as function of the intensity and duration of electric shock. Psychon Sci 26: 125–129.

Apaydin S, Zeybek U, Ince I, Elgin G, Karamenderes C, Oztruk B, Tuglular I (1999) Hypericum triquetrifolium Turra extract exhibits antinociceptive activity in the mouse. J Ethnopharmacol 67: 307–312.

Arnsten AFT, Goldman-Rakic PS (1998) Noise stress impairs prefrontal cognitive function in monkeys. Arch Gen Psychiatry 55: 362–368.

Avital A, Richter-Levin G, Leschiner S, Spanier I, Veenman L, Weizman A, Gavish M (2001) Acute and repeated swim stress effects on peripheral benzodiazepine receptors in the rats hippocampus, adrenal, and kidney. Neuropsychopharmacology 25: 669–678.

Baker KB, Kim JJ (2002) Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. Learn Mem 9: 58–65.

Beck KD, Luine VN (1999) Food deprivation modulates chronic stress effects on object recognition in male rats: role monoamines and amino acids. Brain Res 830: 56–71.

Belanoff JK, Gross K, Yager A, Schatzberg AF (2001) Corticosteroids and cognition. J Psych Res 35: 127–145.

Boissier PJR, Tardy J, Diverres JC (1960) Une nouvelle methode simple pour explorer l`action´tranquilisante: le test de la cheminee. Med Ezp 3: 81–84.

Bowman RE, Beck KD, Luine VN (2003) Chronic stress effects on memory: sex differences in performance and monoaminergic activity. Horm Behav 43: 48–59.

Braszko JJ, Wiśniewski K, Kupryszewski G, Witczuk B (1987) Psychotropic affects of angiotensin II and III in rats: Locomotor and exploratory vs. cognitive behaviour. Behav Brain Res 25: 195–203.

Bremner JD (1999) Alterations in brain structure and function associated with post-traumatic stress disorder (PTSD). Semin Clin Neuropsychiatry 4: 249–255.

Brezun JM, Daszuta A (2000) Serotonin may stimulate granule cell proliferation in the adult hippocampus, as observed in rats grafed with foetal raphe neurons. Eur J Neurosci 12: 391–396.

Butterweck V, Böckers T, Korte B, Wittkowski W, Winterhoff W (2002) Long-term effects of St. John's wort and hypericin on monoamine levels in rat hypothalamus and hippocampus. Brain Res 930: 21–29.

Butterweck V, Petereit F, Winterhoff H, Nahrstedt A (1998) Solubilized hypericin and pseudohypericin from Hypericum perforatum exert antidepressant activity in the forced swimming test. Planta Med 64: 291–294.

Butterweck V, Winterhoff H, Herkenham M (2001) St John's wort, hypericin, and imipramine: a comparative analysis of mRNA levels in brain areas involved in HPA axis control following short-term and long-term administration in normal and stressed rats. Mol Psychiatry 6: 547–564.

Chatterjee SS, Noldner E, Koch E, Erdelmeier C (1998) Antidepressant activity of Hypericum perforatum L. and hyperforin: The neglected possibility. Pharmacopsychiatry 31: 7–15.

Diamond DM, Bennet MC, Fleshner M, Rose GM (1992) Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal prime burst potentiation. Hippocampus 2: 421–430.

Di Carlo G, Borrelli F, Ernst E, Izzo AA (2001) St John's wort: Prozac from the plant kingdom. Trends Pharmacol Sci 22: 292–297.

Duman RS, Nakagawa S, Malberg J (2001) Regulation of adult neurogenesis by antidepressant treatment. Neuropsychopharmacology 25: 836–844.

Flausino OA Jr, Zangrossi H Jr, Salgado JV, Viana MB (2002) Effects of acute and chronic treatment with Hypericum perforatum L. (LI 160) on different anxiety-related responses in rats. Pharmacol Biochem Behav 71: 251–257.

Flugge G (1995) Dynamics of central nervous 5-HT1A-receptors under psychosocial stress. Neuroscience 15: 7132–7140.

Fornal CA, Metzler CW, Mirescu C, Stein SK, Jacobs BL (2001) Effects of standardized extracts of St. John's wort on the single-unit activity of serotonergic dorsal raphe neurons in awake cats: Comparisons with fluoxetine and sertraline. Neuropsychopharmacology 25: 858–870.

Galea LA, McEwen BS, Tanapat P, Deak T, Spence RL, Dhabhar FS (1997) Sex differences in denditic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. Neuroscience 81: 689–697.

Gamaro GD, Xavier MH, Denardin JD, Pilger JA, Ely DR, Ferreira MBC, Dalmaz C (1998) The effects of acute and repeated restraint stress on the nociceptive response in rats. Physiol Behav 63: 693–697.

Gambarana C, Tolu PL, Masi F, Rinaldi M, Giachetti D, Morazzoni P, De Montis MG (2001) A study of the anti-depressant activity of Hypericum perforatum on animal models. Pharmacopsychiatry 34: S42–44.

Greeson JM, Sanford B, Monti DA (2001) St Jonh's wort (Hypericum perforatum): A review of the current pharmacological, toxicological and clinical literature. Psychopharmacology 153: 402–414.

Grundemann D, Schechinger B, Rappold GA, Schoming E (1998) Molecular identification of the corticosterone-sensitive extraneuronal catecholamine transporter. Nat Neurosci 1: 349–351.

Holsboer-Trachsler E, Hatzinger M, Hemmeter U (2002) The influence of hypericum extract on the hypotalamic-pituitary adrenocortical (HPA) system regulation in depressive patients. Eur Neuropsychopharmacol 12: 241.

Holsboer-Trachsler E, Henauer S, Vanoni C (1999) Efficacy and tolerability of the hypericum special extract LI 160 in young and elderly outpatients with depressive disorders: A drug monitoring study. Eur Neuropsychopharmacol 9: 226–227.

Imperato A, Puglisi-Allegra S, Casolini P, Zocchi A, Angelluci L (1989) Stress-induced enhancement of dopamine and acetylcholine release in limbic structure: Role of corticosterone. Eur J Pharmacol 165: 337–339.

Johnson D, Ksciuk H, Woelk H, Sauerwein-Giese E, Frauendorf AJ (1994) Effects of hypericum extract LI 160 compared with maprotiline on resting EEG and evoked potentials in 24 volunteers. J Geriatr Psychiatry Neurol 7: S44–S46.

Kehr J, Yoshitake T, Yoshitake S, Ögren SO (2002) Differential effects of acute and repeated administration of hypericum perforatum (St. John's wort) on extracellular levels of serotonin, noradrenaline and dopamine in prefrontal cortex, striatum and hippocampus of the rat. Eur Neuropsychopharmacol 12: 203.

Khalifa AE (2001) Hypericum perforatum as a nootropic drug: Enhancement of retrieval memory of a passive avoidance conditioning paradigm in mice. J Etnopharmacol 76: 49–57.

Kiewert C, Buchholzer ML, Hartmann J, Chatterjee SS, Klein J (2004) Stimulation of hippocampal acetylcholine release by hyperforin, a constituent of St. John's Wort. Neurosci Lett 364: 195–198.

Kim JJ, Foy MR, Thompson RF (1996) Behavioral stress modifies hippocampal plasticity trough N-methyl-D-aspartate receptor activation. Proc Natl Acad Sci U S A 93: 4750–4753.

Kumar V, Singh PN, Muruganandam AV, Bhattachrya SK (2000) Effect of Indian Hypericum perforatum Linn on animal models of cognitive dysfunction. J Ethnopharmacol 72: 119–128.

Lindley SE, Bengoechea TG, Schatzberg AF, Wong DL (1999) Glucocorticoids effects on mesotelencephalic dopamine neurotransmission. Neuropsychopharmacology 21: 399–407.

Lupien SJ, Lepage M (2001) Stress, memory, and the hippocampus: Can't live with it, can't live without it. Behav Brain Res 127: 137–158.

Lupien SJ, McEwen BS (1997) The acute effects of corticosteroids on cognition: Integration of animal and human model studies. Brain Res Rev 24: 1–27.

Magarinos AM, McEwen BS, Flugge G, Fuchs E (1996) Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. J Neurosci 16: 3534–3540.

McEwen BS (2000) The neurobiology of stress: from serendipity to clinical relevance. Brain Res 886: 172–189.

McEwen BS (2001) Stress, sex, hippocampal plasticity: Relevance to psychiatric disorders. Clin Neurosci Res 1: 19–34.

Mennini T, Miari T (1991) Modulation of 3H glutamate binding by serotonin in rat hippocampus: an autoradiographic study. Life Sci 49: 283–292.

Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui D-H, Tabira T (2000) Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. J Neurosci 15: 1568–1574.

Moghaddam B (2002) Stress activation of glutamate neurotransmission in the prefrontal cortex: implications for dopamine-associated psychiatric disorders. Biol Psychiat 51: 775–787.

Muller WE, Rolli M, Schäffer C, Hafner U (1997) Effects of Hypericum extract (LI 160) in biochemical models of antidepressant activity. Pharmacopsychiatry 30: 102–107.

Murphy BL, Arnsten ATF, Goldman-Rakic PS, Roth RH (1996) Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. Proc Natl Acad Sci U S A 96: 1325–1329.

Nathan PJ (2001) Hypericum perforatum (St John's Wort): A non-selective reuptake inhibitor? A review of the recent advances in its pharmacology. J Psychopharmacol 15: 47–54.

Neary JT, Bu Y (1999) Hypericum LI 160 inhibits uptake of serotonin and norepinephrine in astrocytes. Brain Res 816: 358–363.

Park CR, Campbell AM, Diamond DM (2001) Chronic psychosocial stress impairs learning and memory and increases sensitivity to yohimbine in adult rats. Biol Psychiatry 50: 994–1004.

Pavlides C, Watanabe Y, Magarinos AM, McEwen BS (1995) Opposing roles of Type I and Type II adrenal steroid receptors in hippocampal long-term potentiation. Neuroscience 68: 387–394.

Piazza PV, Rouge-Pont F, Deroche V, Maccari S, Simmon H, Le Moal M (1996) Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission. Proc Natl Acad Sci U S A 93: 8716–8720.

Raffa RB (1998) Screen of receptor and uptake site activity of hypericin component of St. John's wort reveals sigma receptor binding. Life Sci 62: PL265–PL270.

Roozendaal B, Griffith QK, Buranday J, De Quervain DJF, McGaugh JL (2003) The hippocampus mediates glucocorticoid-induced impairment of spatial memory retrieval: Dependence on the basolateral amygdala. Proc Natl Acad Sci U S A 100: 1328–1333.

Roz N, Rehavi M (2003) Hyperforin inhibits vesicular uptake of monoamines by dissipating pH gradient across synaptic vesicle membrane. Life Sci 73: 461–470.

Sanchez-Mateo CC, Bonkanka CX, Hernandez-Perez M, Rabanal RM (2006) Evaluation of the analgesic and topical anti-inflammatory effects of Hypericum reflexum L. fil. J Ethnopharmacol Mar 18, [Epub ahead of print].

Sandi C, Loscertales M, Guaza C (1997) Experience-dependent facilitating effect of corticosterone on spatial memory formation in the water maze. Eur J Neurosci 9: 637–642.

Sapolsky RM (2000) Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiat 27: 925–935.

Sarnyai Z, Sibille EL, Pavlides C, Fenster RJ, McEwen BS, Toth M (2000) Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking 5-HT1A receptors. Proc Natl Acad Sci U S A 97: 14731–14736.

Schaaf MJ, De Kloet ER, Vreugdenhil E (2000) Corticosterone effects on BDNF expression in the hippocampus. Implications for memory formation. Stress 3: 201–208.

Schulz V (2006) Safety of St. John's Wort extract compared to synthetic antidepressants. Phytomedicine 13:199–204.

Simmen U, Bobirnac I, Ullmer C, Lubbert H, Buter KB, Schaffner W, Schoeffter P (2003) Antagonist effect of pseudohypericin at CRF1 receptors. Eur J Pharmacol 458: 251–256.

Singh LK, Pang X, Alexacos N, Letourneau R, Theoharides TC (1999) Acute immobilization stress triggers skin mast cell degranulation via corticotropin releasing hormone, neurotensin, and substance P: A link to neurogenic skin disorders. Brain Behav Immunity 13: 225–239.

Sousa N, Lukoyanov NV, Madeira MD, Almeida OF, Paula-Barbosa MM (2000) Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. Neuroscience 97: 253–266.

Teufel-Mayer R, Gleitz J (1997) Effects of long-term administrtion of hypericum extracts on the affinity and density of the central serotoninergic 5HT1A and 5HT2A receptors. Pharmacopsychiatry 30: 113–116.

Thiele B, Brink I, Ploch MJ (1994) Modulation of cytokine expression by hypericum extract. Geriatr Psychiatry Neurol 7: S60–S62.

Trautmann-Sponsel RD, Dienel A (2004) Safety of Hypericum extract in mildly to moderately depressed outpatients. A review based on data from three randomized, placebo-controlled trials. J Affect Disord 82: 303–307.

Trofimiuk E, Walesiuk A, Braszko JJ (2005) St John's wort (Hypericum perforatum) diminishes cognitive impairment caused by the chronic restraint stress in rats. Pharmacol Res 51: 239–246.

Vandenbogaerde A, Zanoli P, Puia G, Truzzi C, Kamuhabwa A, De Witte P, Merlevede W, Baraldi M (2000) Evidence that total extract of Hypericum perforatum affects exploratory behavior and exerts anxiolytic effects in rats, Pharm Biochem Behav 65: 627–633.

Widy-Tyszkiewicz E, Piechal A, Joniec I, Blecharz-Klin K (2002) Long term administration of Hypericum perforatum improves spatial learning and memory in the Water Maze. Biol Pharm Bull 25: 1289–1294.

Yu PH (2000) Effect of the Hypericum perforatum extract on serotonin turnover in the mouse brain. Pharmacopsychiatry 33: 60–65.

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