

PLANT-DERIVED NANOPARTICLE TREATMENT WITH COCC 30C AMELIORATES ATTENTION AND MOTOR ABILITIES IN SLEEP-DEPRIVED RATS

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Abstract—Sleep is an essential physiological process that underlies crucial cognitive functions as well as emotional reactivity. Thus, sleep deprivation (SD) may exert various deleterious effects. In this study, we aimed to examine the adverse behavioral and hormonal effects of SD and a potential treatment with Plant-derived nanoparticle treatment – cocc 30c. The study was a 4-arm trial with randomization and double-blinding of verum and placebo treatments. SD was induced by using the Multiple Platform Method for 48 h. The effects of SD were evaluated behaviorally (pre-pulse inhibition (PPI), startle response and rotor-rod) at baseline as well as at 6, 12, 24 h, and 14 days post deprivation. cocc 30c treatment was administrated Per Os every three hours starting immediately after baseline tests and for a period of 24 h. On day 14, blood samples were taken and serum levels of corticosterone, testosterone, serotonin and leptin were tested. We found that cocc 30c improved PPI 12 and 24 h post deprivation, likewise, cocc 30c improved motor learning. On day 14 SD led to increased startle response that was ameliorated by cocc 30c. Likewise, SD led to increased levels of corticosterone and serotonin while decreasing testosterone and leptin. Interestingly, cocc 30c treatment has moderated these hormonal alterations. We conclude that the treatment with cocc 30c recovers both short-term behavioral and the long-term hormonal modulations following SD. © 2013 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: attention, nanoparticles, cocc 30c, motor learning, sleep deprivation, hormones.

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Abbreviations: 5-HT, 5-hydroxytryptamine; ANOVA, analysis of variance; D1R, dopamine receptor1; ITI, Inter-Trial-Interval; MPM, Multiple Platform Method; PPI, pre-pulse inhibition; SD, sleep deprivation.

INTRODUCTION

Sleep deprivation (SD)

In humans, sleep is an essential physiological process which, when deprived, may exert deleterious effects. In the literature, SD is divided into three categories: long-term total SD (> 45 h); short-term total SD (≤45 h); and partial SD (sleep restriction to < 7 h/24 h) (Durmer and Dinges, 2005). Considering the above, acute total SD of 24–48 h was previously shown to impair the performance in both attention (Blagrove et al., 1995; Bocca and Denise, 2006; Kendall et al., 2006) and working memory (Wimmer et al., 1992; Smith et al., 2002).

Positron Emission Tomography (PET) scans confirmed that 24 h of SD decreases glucose metabolism and synaptic activity in the prefrontal cortex, an area involved in attention processes, as well as in dorsal and ventral thalami (Thomas et al., 2000; Kato et al., 2000). Kato et al. (2000) showed that SD led to an increase in blood pressure and a decrease in muscle sympathetic nerve activity. Furthermore, Van Ieuwen et al. (2009) found that SD increases the risk of cardiovascular diseases by augmenting pro-inflammatory responses. Other studies have also showed results of severe symptoms such as irritability, fatigue, hallucinations, and delusions (Orzel-Gryglewska, 2010). Overall, SD in humans has been found to impair attention (Alhola and Polo-Kantola, 2007; McCoy and Strecker, 2011), cognitive functions, and behavioral performance (Curcio et al., 2006). While SD's deteriorating behavioral effects are suggested to be normally recovered (Schwierin et al., 1999; Faraut et al., 2012), the duration needed for this recovery was found to be dependent on the deprivation paradigm. Specifically, longer SD requires a longer normal recovery period (McCoy and Strecker, 2011).

TREATMENTS OF SD

Treatment of SD commonly involves psycho-stimulants such as caffeine, which may restore attention. However they are not effective when evaluating cognitive tasks, decision-making or motor activities (Killgore et al., 2012).

Evidence of the efficiency of homeopathic treatment with cocc 30c given to patients suffering from SD has been accumulated in our clinic (unpublished data). Patients who received cocc 30c remedy reported an improvement in their ability to sleep, reduced anxiety/

irritability as well as improvement in their cognitive capabilities. These results were based upon clinical observations and patients' reports, thus requiring systematic validation.

Homeopathy treatment, although partially disputable, is prevalent both in clinics and research (Lucertini et al., 2007; Mishra et al., 2011). Accumulating data indicate detectable effects of different types of remedies (Bell et al., 2011a), and biological effect of homeopathic treatment on physiological measures of sleep (Bell et al., 2011b). Furthermore, in 2012, the Swiss Government published a health technology assessment of homeopathy that found strong evidence supporting homeopathic treatment at least in some medical treatments (Bornhöft and Matthiessen, 2012). Specifically, already in 1927 Boericke depict that plant source material *Cocculus* has a potential for reversing the effects of sleep loss (Boericke, 1927).

Finally, new basic science data suggest that the source materials of the medicine do survive and persist in nanoparticulate form across homeopathically prepared potencies, including cocc 30c, as a function of the unique manufacturing processes (Chikramane et al., 2010, 2012). Biological effects may be mediated in part by endogenous adaptive responses (Bell and Koithan, 2012). The ability of nano-forms of plant-derived nanoparticles to cross the blood–brain-barrier was previously suggested to be mediated by nano-silica or nano-silicon vehicles and biological amplifiers that would be augmenting the *Cocculus* nanoparticles (Demangeat, 2010; Dhawan et al., 2011; Mathew et al., 2012).

ANIMAL MODELS

Previous animal studies have demonstrated the biological effects of various homeopathic treatments for SD (Ruiz-Vega et al., 2002, 2005). Nunes Junior et al. (1994) used the Multiple Platform Method (MPM) as suggested animal model to induce SD. This method was practiced in several studies (Suchecki et al., 1998; Suchecki and Tufik, 2000; Yang et al., 2010) and proved to be efficient in inducing Rapid-eye-movement (REM) deprived rats, generating less stress vis-à-vis other acceptable SD methods (Rechtschaffen and Bergmann, 2002; Machado et al., 2006). In rats, chronic SD was shown to cause death after 16–21 days from the onset of deprivation. In comparison, food deprivation was shown to cause death after 17–19 days (Orzel-Gryglewska, 2010). On the other hand, acute SD was not observed to cause destructive effects either on cells, or vital organs (Orzel-Gryglewska, 2010). However, it increases energy outlay (Martins et al., 2010), with a tendency to decrease both leptin (Rosa Neto et al., 2010) and the anabolic testosterone (Wu et al., 2011; Dattilo et al., 2012). Moreover, SD increases serum serotonin (Hipolide et al., 2005) as well as its extracellular concentrations in the hippocampus. It elevates corticosterone level (Bodosi et al., 2004; Tiba et al., 2008; Galvao Mde et al., 2009; Martins et al., 2010; Rosa Neto et al., 2010; Wu et al., 2011), while this elevation is independent of the stress response

(Galvao Mde et al., 2009; Mongrain et al., 2010) and is suggested to occur due to changes in circadian rhythm (Tartar et al., 2009) and metabolic homeostasis (Dattilo et al., 2012).

When comparing an animal model to humans, one must take into consideration physiological as well as brain growth trajectories, especially when rat and human maturation cannot be compared as linear timeline development (Erecinska et al., 2004; Quinn, 2005).

HYPOTHESIS AND AIMS

A systematic human study of the effects of SD is ethically limited. Moreover, controlling, mediating and moderating potential artifact variables are especially difficult when examining the long-term effects of SD on humans' cognitive functioning. Thus, it is customary to use an animal model in order to investigate the long-term effects of SD. Similar to humans, in rats sleep is an important physiological process regarded as a basic need for functioning and survival (Everson, 1995). Many studies have proposed different deprivation paradigms, including partial chronic (Machado et al., 2006) or acute (Suchecki et al., 1998; Schwierin et al., 1999; Suchecki and Tufik, 2000) deprivations, with similar effectiveness.

While considering treatment of SD, normal spontaneous recovery must be taken into account (Schwierin et al., 1999; Orzel-Gryglewska, 2010). Our current study aims are to explore the effects of acute SD during early adulthood, focusing on short-term behavioral effects as well as on long-term hormonal modulations. Specifically, we aimed to examine the short- (post 1-, 6-, 12- and 24-h) and long-term (post 14 days) effects of acute 48 h SD on fatigue, attention, and motor learning. Moreover, we aimed to examine the outcomes of cocc 30c treatment on both behavioral and hormonal modulations following SD.

EXPERIMENTAL PROCEDURES

Animals

Forty-four male Wistar rats (weighing between 200 and 220 gr) were purchased from Harlan (Jerusalem, Israel) and were given 7 days of acclimation in the institutional animal housing facility. Rats were housed four per cage (30_L × 30_W × 18_H cm). Room temperature was maintained at 23 ± 1 °C with 67% humidity at 12:12-day/night cycle (lights on at 0600). Food and water access were allowed *ad libitum*. This study was conducted in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering.

Procedure

Rats were randomly assigned into four groups: Naive ($n = 12$), Naive rats that were treated with cocc 30c (Naive Treated; $n = 12$), sleep deprived (SD; $n = 16$), and sleep deprived + Treatment (SD treated; $n = 16$).

Starting on Postnatal day (PND) 70 rats were subjected to SD for 48 h, using MPM (method as described below) (Suchecki and Tufik, 2000). cocc 30c or placebo was given Per Os starting immediately after baseline tests (i.e. 1 h post SD), and subsequently every 3 h during 24 h post SD. One hour after SD, prior to treatment, rats were tested in the startle box for baseline evaluation. Additional re-tests were held at 6, 12 and 24 h, as well as at 14 days post SD. Pre-pulse inhibition (PPI) was examined at 12 and 24 h. Motor learning test in the Rotor-rod was performed starting 48 h post-SD (for total of 4 days of training). Rats were handled prior to any manipulation. A description of the procedure is shown in Fig. 1.

MPM. This method is based on the muscle atonia characteristics of REM sleep. In short, this method [modified from (Suchecki and Tufik, 2000)] includes placing the animal on top of a narrow platform located inside a water tank that allows the animal to lie down. As muscle atonia occurs, the rat falls into the water and is deprived of REM sleep.

In the current study, the water tank (120 cm in diameter, 60 cm in height) contained 30 narrow platforms (6.5 cm in diameter) placed inside. Sixteen animals (in each round) were placed on top of the platforms, allowing them to move around. The tank was filled with water (6 cm) until 1 cm below the platform, thus allowing the rat to climb back in case of falling into the water. Exposure to the MPM began at 0800, and animals remained inside the tank for 48 h. In order to verify the effectiveness of MPM, rats were videotaped by an infra-red Ikegami camera. Room temperature was maintained at $23 \pm 1^\circ\text{C}$. Food and water were provided *ad libitum*. All rats accomplished the entire study (no deaths occurred).

cocc 30c treatment. cocc 30c is a homeopathic remedy made of highly diluted tincture of Anamirta cocculus seeds powder, first prepared and published by Hahnemann in 1821 (Hahnemann, 1821). The plant originates from the coast of Malabar, India, and Ceylon. The tincture is prepared by macerating the powdered seeds. The Cocculus preparation: The Cocculus was prepared in 'helios pharmacy (UK)'. It was sent in a bottle containing 99% ethanol and went through 40 succussions in 90% ethanol, by manual shaking. Sixty globules were wetted in the solution and dried for several minutes. Following this procedure, cocc 30c made from sucrose immersed in a solution of Cocculus indicus 30c (40 mg dissolved in 1 ml tap water), was administered Per Os every three hours immediately after baseline tests for a period of 24 h. Placebo treatment was 1% sucrose solution similar to the hedonic effect caused by the sweet taste of cocc 30c.

The experiment was carried out in a random double-blind design i.e. Experimenter 1 (Dr. Freed) prepared the cocc 30c or placebo treatments in two identical tubes (labeled "A" or "B") and delivered to the principal investigator (Dr. Avital) the tubes on the day of experiment. The experimenters (all the authors except

Dr. Avital and Freed) got the labeled tubes. Across total number of rats, a randomized assignment to one of four groups was made until accomplishing a total assignment of 16 rats in the treated groups and 12 in the Naive and Naive Treated rats. cocc 30c or placebo treatments were revealed after the results analysis.

Behavioral tests

PPI and startle response. The test is held in a ventilated soundproof box (Campden instruments, UK) and aims to examine Startle response as well as the function of the sensorimotor gating.

The session (a total of 90 trials) begins with three min acclimation period with a 57-dB background white noise level that is delivered continuously throughout the test session. To evaluate the startle response, each of the first ten trials consist of a single 40-ms 120-dB "pulse alone" startle stimuli (Inter-Trial-Interval (ITI) 1 min). The rest of the 80 trials (10 s ITI) consist of random delivery of: 10 "no stimuli" trials, during which no stimuli are delivered, fourteen "pre" stimuli (at 59, 61, 65, 69, 73, 78 or 85 dB), and 56 "pre-pulse" trials that include a single 120 dB pulse preceded (80 ms interval) by a 20 ms pre pulse of 2, 4, 8, 12, 16, 21 or 28 dB above background (i.e., 59, 61, 65, 69, 73, 78 or 85 dB). Finally, PPI was calculated as the percent of the habituated response as follows: $[100 - (\text{max response to "pre-pulse" trial} / \text{max response to "pulse alone" trial} \times 100)]$ (Avital et al., 2011; Ram et al., 2013).

Rotor-rod. The rotor-rod (San Diego instruments, San Diego, CA, USA) apparatus is used to assess motor functions, motor learning, coordination, and equilibrium. It is made of black lusterless Perspex, comprised of a rotating rod with four lanes separated by opaque black Perspex. The apparatus contains a sawdust cabin (45.7 cm height) for safe landing and automatic recording of latencies (in sec.) via red-beams system.

Each learning session consists of four daily trials, on four consecutive days. Rats are acclimated to the apparatus for 30 s on the first day. Each trial starts at five rpm for 15 s with constant acceleration at 0.1 rpm per second (max speed 50 rpm after 460 s).

Corticosterone, testosterone, serotonin and leptin evaluation

In order to avoid circadian variability, all samples were collected between 1100 and 1200, when plasma hormones' concentration is relatively low. Blood samples were taken immediately after decapitation and centrifuged (2000g at 4°C for 20 min), serum was collected and stored at -80°C until assayed. Serum corticosterone and testosterone levels were assessed using commercial enzyme-linked immunosorbent assay (ELISA) kits (AssayPro, St. Charles, MO, USA) according to the manufacturer's instructions.

Serum leptin was quantified using specific enzyme immunoassay (Genese®, Brazil). Serotonin concentration was determined using commercial

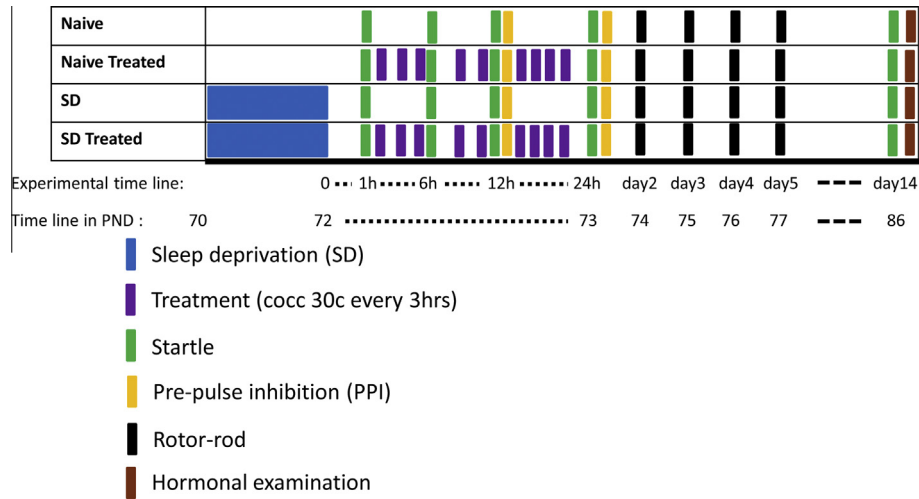


Fig. 1. A schematic description of the experimental procedure. A description of experimental time line and postnatal days, along which the exposure to sleep deprivation, treatment, behavioral and hormonal examinations took place.

enzyme assay kit (Diagnostic products Corp., Los Angeles, CA, USA) (Wu et al., 2011).

Statistical analysis

A Bonferroni multiple correction analysis was performed in order to avoid possible confounds due to the substantial amount of testing. Data were analyzed for statistical significance using an analysis of variance (ANOVA) for mixed design, with group as between-subject factor and test timing or pre-pulse intensity as within-subject factor. In order to further explore the main effects, we used a one-way ANOVA followed by a Post-Hoc Tukey test. A result was considered significant when $P < 0.05$. All tests were calculated as two-tailed using SPSS V17.0. Results are presented as means \pm standard error of the means (SEM).

RESULTS

A Bonferroni multiple correction analysis was performed in order to avoid possible confounds and the substantial amount of testing after the SD that may bound to affect the recovery sleep. The analysis yielded no effect, thus, it excludes the possible confound that may affect the sleep recovery.

Startle response

In order to examine short- and long-term progression in startle response (Fig. 2), an ANOVA for mixed design was carried-out with group as between-subject factor and test time as within-subject factor. The results indicate a significant effect for test time [$F(4, 17) = 39.96$, $P < 0.0001$] as well as between groups [$F(3, 20) = 7.2$, $P < 0.002$]. Post-hoc Tukey tests indicate a significant decrease in startle response for both SD and SD-treated groups compared with naive rats, at baseline ($P < 0.0001$), post-6 h ($P < 0.0001$) and post-12 h ($P < 0.01$). Twenty-four hours post SD all groups showed similar and low startle response that

probably reflects habituation and/or floor effect. Finally, 14 days post SD, while naive- and SD-treated rats showed a recovery (from the alleged habituation) to the naive rats' startle response, the SD rats showed a long-term increased startle response compared with naive ($P < 0.007$), naive-treated ($P < 0.0001$) and SD-treated rats ($P < 0.0001$).

PPI

PPI 12 h after SD (Fig. 3): a significant difference was found along various pre-intensities [$F(6, 47) = 102.97$, $P < 0.0001$] as well as between the groups [$F(3, 52) = 4.53$, $P < 0.007$]. Overall, a clear tendency of impaired PPI was observed following SD. Interestingly, the treatment with cocc 30c led to a beneficial effect on PPI performance. Specifically, a significant effect was found at pre-intensity 61 dB between SD-treated and SD ($P < 0.0001$), naive ($P < 0.044$) and naive-treated ($P < 0.001$) rats. Regarding pre-intensities 65 dB and 69 dB cocc 30c led to improved PPI compared with SD ($P < 0.001$; $P < 0.025$, respectively).

PPI 24 h post SD (Fig. 3): similarly to 12 h post-SD test, a significant effect was observed along different pre-intensities [$F(6, 47) = 115.97$, $P < 0.0001$] as well as between the groups [$F(3, 52) = 11.47$, $P < 0.0001$]. The treatment with cocc 30c clearly led to elevated PPI performance. In particular, SD-treated rats have exhibited a significant increase at pre-intensity 61 dB, compared with both SD ($P < 0.0001$) and naive ($P < 0.01$) rats. Following SD, the treatment cocc 30c led to improved PPI at pre-intensities 65 dB ($P < 0.0001$), 69 dB ($P < 0.0001$), 73 dB ($P < 0.001$), 78 dB ($P < 0.001$) and 85 dB ($P < 0.005$), compared with SD group.

Rotor-rod

A significant motor learning effect (Fig. 4) was found during 4 days of training [$F(3, 132) = 49.72$,

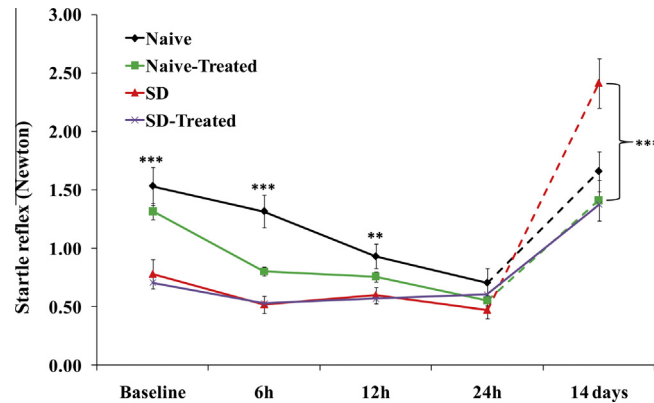


Fig. 2. Startle reflex. A significant decrease in startle response was observed in both SD and SD-treated groups compared with naive rats, at baseline, post-6 h and post-12 h. Fourteen days post SD, while naive- and SD-treated rats showed a recovery (from the alleged habituation) to the naive rats startle response, the SD rats showed a long-term increased startle response compared with naive with SD-treated rats ($***P < 0.0001$; $**P < 0.01$).

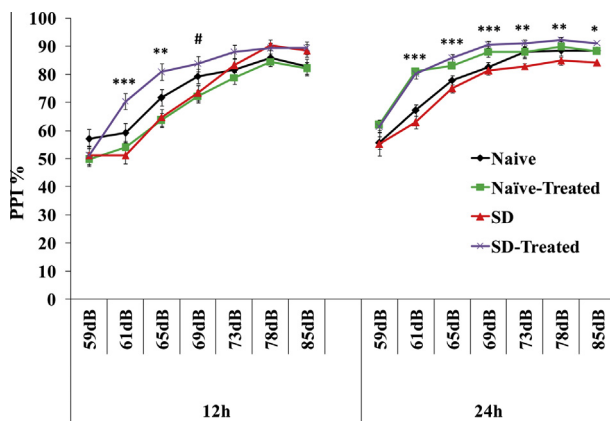


Fig. 3. Pre-pulse inhibition (PPI). The treatment with cocc 30c led to a beneficial effect on PPI performance measured both 12- and 24-h post SD ($***P < 0.0001$; $**P < 0.001$; $*P < 0.005$; $\#P < 0.025$).

$P < 0.0001$] with significant difference between the groups [$F(3,134) = 39.74$, $P < 0.0001$]. Moreover, a significant effect was found for the group \times day interaction [$F(9,392) = 2.43$, $P < 0.011$]. Post-hoc Tukey test indicated a significantly enhanced performance in the SD-treated group, compared with SD ($P < 0.0001$), naive ($P < 0.0001$) and naive-treated ($P < 0.028$), on the first day of motor learning. This enhanced performance for the treated SD was also significant ($P < 0.0001$) on the second day compared with SD, naive and naive-treated. On the third day SD-treated performed better compared with naive ($P < 0.004$) and SD ($P < 0.0001$) groups. This tendency remains on the fourth day of motor learning, as the SD-treated rats performed better than SD rats ($P < 0.0001$).

Corticosterone

A significant group effect on corticosterone serum (Fig. 5A) levels [$F(3,54) = 50.72$, $P < 0.0001$] was observed. Post-hoc Tukey test indicated a significant elevation in corticosterone following SD ($P < 0.0001$)

compared with naive and naive-treated groups. However, the treatment with cocc 30c significantly moderated this elevation toward ($P < 0.0001$; compared with SD group).

Testosterone

A significant group effect on testosterone serum (Fig. 5B) level [$F(3,51) = 13.35$, $P < 0.0001$] was observed. Post-hoc Tukey test indicated a significant decrease in testosterone level following SD ($P < 0.0001$), while cocc 30c treatment significantly restored this decrease ($P < 0.013$; compared with SD group).

Serotonin

A significant group effect on serotonin serum (Fig. 5C) level [$F(3,51) = 23.46$, $P < 0.0001$] was observed. Post-hoc Tukey test indicated a significant elevation following SD ($P < 0.0001$), while cocc 30c treatment moderated the observed elevation ($P < 0.0001$; compared with SD group).

Leptin

A significant group effect on leptin serum (Fig. 5D) level [$F(3,51) = 118.15$, $P < 0.0001$] was observed. Post-hoc Tukey test indicated a significant decrease in leptin level following SD ($P < 0.0001$), while cocc 30c treatment significantly repaired the latter decrease to a moderated level ($P < 0.0001$; compared with SD group).

DISCUSSION

Attentional and motor reactivity

Both treated and untreated SD groups showed hypo-responsiveness to the startle stimulus. Though naive rats showed habituation along the four time points that were examined (i.e. baseline, 6, 12 and 24 h post-SD), the lack of this habituation in the SD groups may be attributed to floor effect. Fourteen days post SD, all groups showed a recovery from the alleged habituation, similar to the naive group. However, the SD group

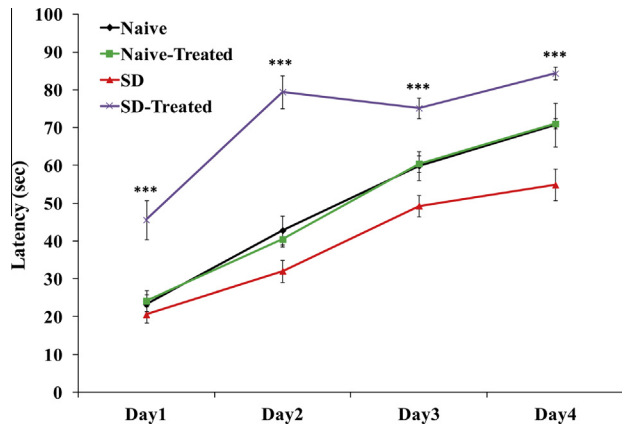


Fig. 4. Motor learning in the rotor-rod test. The treatment with cocc 30c led to a better motor learning ability (starting 48 h post SD) across all 4-days learning SD ($***P < 0.0001$).

showed a significant increased startle response. Considering the noise during the MPM procedure in which 16 rats stayed together in the same arena, one may postulate that the effects of ambient noise in the different environments can explain the hypo-responsiveness to the startle stimulus (Baldwin et al., 2006). This elevation in startle response observed in all groups, may reflect the extensive test procedure that the rats underwent, together with evidence that startle response is suggested to be age-dependent (Weiss et al., 2001).

Previously it has been shown that SD in humans impairs attention ability (Alhola and Polo-Kantola, 2007;

McCoy and Strecker, 2011). Specifically, acute SD (such as total 24–48-h deprivation) was previously shown to impair the performance in attention tasks (Blagrove et al., 1995; Kendall et al., 2006; Bocca and Denise, 2006). Thus, we aimed to test the sensorimotor gating utilizing the PPI test, which relates to attention processes (Avital et al., 2011; Ram et al., 2013). SD led to impaired PPI ability 12 h post deprivation. Surprisingly, the treated rats showed an immediate effect, with superior PPI ability compared with all other groups. Twenty-four hours post SD, though there was no significant difference in PPI performance between the control and the SD groups (presumably due to spontaneous recovery from SD), yet the SD-treated rats showed a better PPI performance compared with all other groups. The striatum is considered to be the relevant region for sensorimotor gating (Moore et al., 2006). Indeed, Lim et al. (2011) have recently found a significant decrease in dopamine receptor1 (D1R), no change in D2R, and a significant increase in D3R binding in the striatum, following SD. This pattern was not observed following stress, thus suggesting to be a specific remodeling of dopaminergic circuits after SD. Considering dopamine as the core neurotransmitter involved in attention processes in both the prefrontal cortex and striatum (Moore et al., 2006; Kumari et al., 2008; Molina et al., 2009), we postulate that the beneficial effect of cocc 30c on PPI performance, immediately following SD, may be associated to the increased binding to D3R in the striatum.

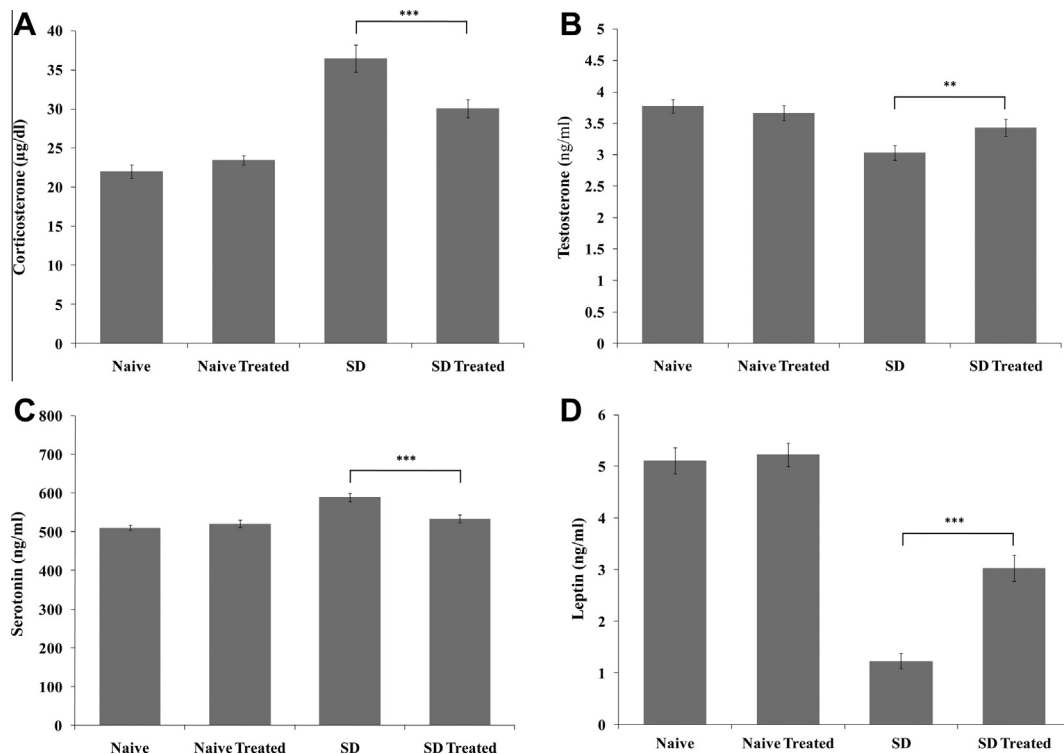


Fig. 5. Hormonal serum levels. Fourteen days after the exposure to sleep deprivation, a significant increase in both corticosterone (A) and serotonin (B) serum level was observed in the SD group. However, the treatment with cocc 30c had a long-term beneficial effect, manifested in a significant decrease of both corticosterone and serotonin. Moreover, sleep deprivation led to decreased serum levels of Testosterone (C) and Leptin (D). Interestingly, cocc 30c treatment recovered these decrements ($***P < 0.0001$; $**P < 0.013$).

Taken together, the behavioral tests have indicated significant short-term effects of SD. The treatment with cocc 30c remedy seems to improve these short-term deteriorating effects

Hormonal modulations

In order to explore whether SD has a long-term “covert” hormonal effects while the “overt” behavior effects are recovered, we examined corticosterone, testosterone, serotonin and leptin serum levels, 14 days post SD. The notion on “covert” hormonal and “overt” behavioral effects of SD is supported by Lopez-Rodriguez et al. (2003) report that extracellular concentration of serotonin remained high at the end of SD recovery day period, though displaying normal amount of sleep.

Following SD, corticosterone and serotonin serum levels were elevated and the treatment with cocc 30c recovered these elevations. Moreover, testosterone and leptin decreased following SD, and cocc 30c treatment moderated this decline. Our findings are in line with a previous study (Wu et al., 2011), which reported a reduction of serum testosterone, and elevated levels of serotonin and corticosterone, following SD. Considering the inverse secretion relation between testosterone and 5-hydroxytryptamine (5-HT) (Frungieri et al., 2002), it is plausible that the reduction in testosterone level is due to 5-HT inhibition of testosterone production, or vice versa. Similarly to our 48-h SD effect on corticosterone and leptin levels, it was previously found that 96 h of SD led to increased corticosterone as well as decreased leptin serum levels (Rosa Neto et al., 2010). Moreover, Koban and Swinson (2005) showed that leptin decreased after SD and remained low following twenty days of recovery.

CONCLUSIONS

Taken together, the treatment with cocc 30c seems to restore the deteriorating effects of 48 h of SD on attention and motor learning abilities. Examining the long-term effects of SD, cocc 30c dramatically recovered the hormonal alterations observed.

Bioavailability and biological activity of nano-forms of any material in general and specifically of cocc 30c, suggest its therapeutic potential intriguing, as CNS access across the blood–brain barrier is readily possible for the small sized nanoparticles that were already shown present in homeopathic medicines (Chikramane et al., 2010). Furthermore, recent study by Barve and Chuaughule (2013) have shown that succussions can mechanically reduce the initial particle size of plant extracts in homeopathic manufacturing processes into the very small nanoparticle range (e.g., approximately 13 nm). This observation further supports the possibility of blood–brain access.

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