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Research report

Deuterium content of water increases depression susceptibility: The potential role of a serotonin-related mechanism

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HIGHLIGHTS

- Geographical distribution of deuterium correlates with depression rate.
- Deuterium depleted water reduces stress-induced depressive-like signs in mice.
- Hippocampal proliferation after stress is rescued by deuterium depleted water.
- Deuterium depleted water induces SSRI-like changes in EEG parameters of sleep.
- Above-indicated effects may be due to normalization of hippocampal 5-HTT level.

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ABSTRACT

Environmental factors can significantly affect disease prevalence, including neuropsychiatric disorders such as depression. The ratio of deuterium to protium in water shows substantial geographical variation, which could affect disease susceptibility. Thus the link between deuterium content of water and depression was investigated, both epidemiologically, and in a mouse model of chronic mild stress. We performed a correlation analysis between deuterium content of tap water and rates of depression in regions of the USA. Next, we used a 10-day chronic stress paradigm to test whether 2-week deuterium-depleted water treatment (91 ppm) affects depressive-like behavior and hippocampal SERT. The effect of deuterium-depletion on sleep electrophysiology was also evaluated in naïve mice. There was a geographic correlation between a content of deuterium and the prevalence of depression across the USA. In the chronic stress model, depressive-like features were reduced in mice fed with deuterium-depleted water, and SERT expression was decreased in mice treated with deuterium-treated water compared with regular water. Five days of predator stress also suppressed proliferation in the dentate gyrus; this effect was attenuated in mice fed with deuterium-depleted water. Finally, in naïve mice, deuterium-depleted water treatment increased EEG indices of wakefulness, and decreased duration of REM sleep, phenomena that have been shown to result from the administration of selective serotonin reuptake inhibitors (SSRI). Our data suggest that the deuterium content of water may influence the incidence of affective disorder-related pathophysiology and major depression, which might be mediated by the serotoninergic mechanisms.

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1. Introduction

Major depressive disorder, a common and recurrent disorder, has been projected to become the second leading cause of disability worldwide by 2020. It is associated with considerable morbidity and increased mortality and is challenging to treat effectively. Indeed, environmental factors, such as insolation and amount of day light [1,2], annual fluctuations of air temperature [3], a content of certain minerals in soil and water [4,5] were shown to be important interacting factors for neuropsychiatric disorders including depression. Isotope content of natural waters is one of the factors that greatly varies across different geographical areas [6,7] and can profoundly affect basic physiological processes [8]. However, it is unclear whether isotope content has an impact in the prevalence of affective disorders, or whether it might alter the phenotype in animal models of depression.

Natural water is a mixture of nine water isotopologues formed by stable isotopes of hydrogen [^{1}H, protium (H) and ^{2}H, deuterium (D)] and oxygen (^{16}O, ^{17}O, ^{18}O). The term 'isotopologue' refers to a molecular entity that differs only in isotopic composition [9]. The abundance of nine water isotopologues in environmental water, expressed as the deviation (δ) relative to the international standard 'Vienna Standard Mean Ocean Water 2′ (VSMOW2) standard, varies systemically by location and climatic conditions due to isotopic fractionation accompanying evaporation-condensation process, when the air mass move inland over topographic features. The content of heavy isotopologues decreases proportionally with the distance from the ocean and altitude. It is also influenced by other factors, such as latitude, humidity, and a seasonal temperature [7,10–12]. The spatial isotopic distribution in tap waters reflects the regional variation of isotopes in the local environment [13], which shows considerable variation across the United States (US). However, the lowest levels of heavy isotopologues are detected in the plateau of Antarctica whose water is used as another international standard, Standard Light Antarctic Precipitation 2 (SLAP2) (International Atomic Energy Agency, 2009). This water is characterized by 43% reduction in deuterium content (D/H ratio 89.1 vs. 155.8 ppm) and by 5% reduction in oxygen-18 content (^{18}O/^{16}O ratio 1894.9 ppm vs. 2005.2 ppm), as compared to VSMOW2.

2. Objectives

Here, we performed an epidemiological analysis of the relationship between deuterium content of drinking water in the US, the most abundant isotope after protium and isotopes of oxygen, and prevalence of depression. We then assessed the antidepressant-like properties of deuterium depleted water (D91 = 91.7 ppm) against the pharmacological reference citalopram using three well established paradigms of depression-like state and stress in mice: a chronic stress depression model [14], stress-induced suppression of hippocampal neural proliferation [15] and EEG analysis of sleep [16].

3. Methods

3.1. Epidemiological analysis

Depression rates were obtained for each state in the US from the morbidity and mortality weekly report (MMWR) on depression in adults in the US in the period 2006–2008 [17]. Multiple samples of tap water were collected over the period 2004–2013, and the proportion of deuterium was averaged to give a value for each state in the continental USA. We then performed a correlation analysis (Pearson's r) between content of deuterium in tap water and prevalence of depression across the USA.

3.2. Animals

Studies were performed using 3.5-month-old male C57BL/6J mice. 3.5-month-old male CD1 mice were used as resident intruders for social stress and 2–5-month-old Wistar rats were used for predator stress. Animals were kept in standard laboratory conditions as previously described [14]. Details on the animals' housing can be found in Supplementary methods. Experiments were carried out in accordance with the European Communities Council Directive for the care and use of laboratory animals, and were approved by respective local governmental bodies.

3.3. Water isotopologues and drug therapy

Experimental solutions replaced normal drinking water. Control water (D140 = 140.3 ppm deuterium) and deuterium-depleted water (D91 = 91.7 ppm deuterium) were obtained from Almaz Kotovsk Tambov Reg/Timantti AB, Stockholm, Sweden (for details, see Supplementary tables). Citalopram was dissolved in tap water; the solutions were changed every 3 days. The citalopram dose (15 mg/kg/day), in D140 drinking water, was based on the previously validated dosing method [18]. We measured water intake between D91 and D140 groups; no significant difference between these groups was found ($p > 0.05$) (Supplementary Fig. 1).

3.4. Chronic stress study

Mice were either assigned to control ($n = 30$) or stress ($n = 63$) groups. The group means were matched for mouse social behavior, body weight and sucrose preference, after Strekalova et al. [19]. 25 of the stressed mice were treated with D140, 19 with D91, and 19 were treated with the antidepressant, citalopram (15 mg/kg/day, via drinking water). Control mice were treated either with D91, D140 or citalopram ($N = 10$ in each group). D91, D140 and citalopram were administrated starting 7 days prior the onset of stress and lasted for the entire duration of the stress procedure (*see below*).

We employed a 10-day stress protocol [14] comprising a dark cycle rat exposure stress (predator stress) and light cycle semi-random application of two of three stressors: social defeat stress, restraint stress and tail suspension stress. Briefly, between the hours of 09:00 and 18:00 two stressors per day were employed in the following sequence: social defeat for 30 min, restraint stress for 2 h and tail suspension for 40 min with an inter-session interval of at least 4 h. All mice were weighed on the 7th and 10th day of stress, and scored for a coat state 15 h after the termination of the last stressor. 12 h after the last stressor, after being weighed and scored for coat disintegration, all mice were tested in 8-h sucrose test, to assess hedonic traits. Immediately thereafter their forced swimming was assessed to evaluate changes in affective behavior. Chronically stressed mice were killed ~30 h after the last stressor, and their hippocampi were isolated, quantitative RT-PCR of SERT expression was performed as described elsewhere [14]. For details on the behavioral and molecular methods, see Supplementary methods.

3.5. Hippocampal neural cell proliferation after a 5-day predation stress

Mice were randomized to two groups; one received D91 for 2 weeks and the other D140. Both groups underwent predator (rat exposure) stress for 5 consecutive nights; treatment with D91 or D140 continued throughout the stress exposure (see Supplementary methods). Before the first stress session, mice received four intraperitoneal injections of Bromodeoxyuridine

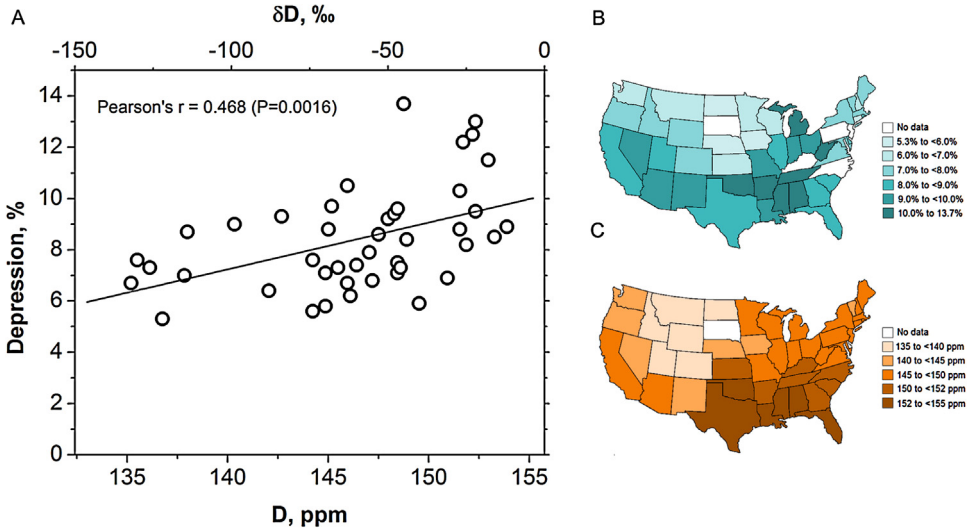

Fig. 1. Depression rates and deuterium content of tap water. (A) Correlation between reported rates of major depressive disorder in US states and content of deuterium in tap water in the USA. (B) The reported prevalence of individuals with depression in among adults aged ≥18 years in each US state [Centers for Disease Control and Prevention. Mental illness surveillance among adults in the United States. MMWR 2011;60 (Suppl):1–30]. (C) Geographical distribution of average deuterium content in the tap water in the continental USA from samples collected from each state for 2004–2013.

(Sigma–Aldrich, St. Louis, MO, USA; 200 mg/kg/kg) dissolved in 0.9% NaCl and 0.007 M NaOH, spaced one from the other by 2 h. They were killed 24 h after the last stress session, perfused with 4%-paraformaldehyde, and brains were removed and treated as described elsewhere [20]; see Supplementary methods for more detail. Immunofluorescence, confocal microscopy and quantification for detection of BrdU and Ki67 were performed as described in Supplementary methods.

3.6. EEG sleep study

12 naïve C57BL6J mice were implanted with electrodes for polygraphic recordings as described elsewhere [16]. The citalopram-treated group was not included in the EEG experiment with sleep analysis, since the effects of SSRIs on sleep were intensely investigated and are well described in a literature of the last decades (for a review, see Ursin [21]). 2 weeks after surgery and recovery period, animals were connected to recording cables and allowed to habituate to the recording chambers for 1 week. Lighting schedule in the chambers was kept reversed (12 h:12 h, light-off at 9:00), food and water were available at libitum. Thereafter, D91 or D140 delivery was started simultaneously with the polygraphic recordings that lasted 14 days. Data were recorded and analyzed as described elsewhere [22].

3.7. Statistics

Data were analyzed with GraphPad Prism version 5.0 for Windows (San Diego, CA). One- and two-way ANOVA was used followed by a Tukey's post-hoc comparisons (or more strictly, Tukey–Kramer, owing to unequal group sizes). *t*-tests were applied for two-group, two-tailed comparisons. A type I error rate of $p < 0.05$ was adopted, and data are shown as mean ± SEM.

4. Results

4.1. Depression prevalence and deuterium content in tap water

We performed correlation analysis using Pearson's *r*, for the relationship between incidence of depression and deuterium content of tap water (Fig. 1). There was a significant correlation ($r = 0.468$; $p = 0.0016$; $F = 11.49$) between deuterium content of tap water and rates of depression. From the linear equation we estimate that prevalence of depression is increased by 1.8% (95% confidence intervals 0.7–2.9%; $R^2 = 0.219$; $p = 0.0016$; $F = 11.49$) for a 10 ppm increase in deuterium level in tap water (see Supplementary Table 3 state-by-state tap water deuterium content.) In order to allow more stringent control of experimental variables, we pursued further work in animal models.

4.2. Assessment of anhedonia induction

Two-way ANOVA revealed a significant effect of stress on sucrose preference ($F_{1,120} = 37.83$, $p < 0.001$), and an interaction between stress and treatment ($F_{2,120} = 4.84$, $p = 0.009$). *Post-hoc* Tukey-corrected *t*-tests showed a significant reduction in sucrose preference from baseline for the non-treated stressed group ($p < 0.05$), but when treated with citalopram and D91 there was no difference from baseline in terms of sucrose preference. This suggests that D91, like citalopram, prevents a reduction in sucrose preference for the stressed cohort (Fig. 2A).

4.3. Effects of treatment on floating behavior

'Behavioral despair' measured in the modified Porsolt forced swim test (FST) by occasion of floating behavior is another sign of depressive-like state in rodents [23]. There was a significant main effect of stress for latency to floating in the FST as revealed by two-way ANOVA ($F_{1,87} = 14.19$, $p < 0.001$), but there was no significant main effect of treatment ($F_{2,87} = 0.82$, $p = 0.44$) and no significant interaction ($F_{2,87} = 0.92$, $p = 0.40$). Post-hoc tests showed a significant reduction in latency to floating for stressed compared with control mice treated with D140 ($p < 0.01$), but there was no significant difference between stressed and control mice that received citalopram or D91 ($p > 0.05$; Fig. 2B). There was also a significant main effect of stress for time spent floating ($F_{1,81} = 9.11$, $p = 0.0033$), with stressed mice floating for longer than controls, and a significant main effect of treatment ($F_{2,87} = 3.14$ $p < 0.05$), but no significant interaction between treatment and stress ($F_{2,87} = 1.28$, $p = 0.284$) (Fig. 2C). Post-hoc tests showed significant differences

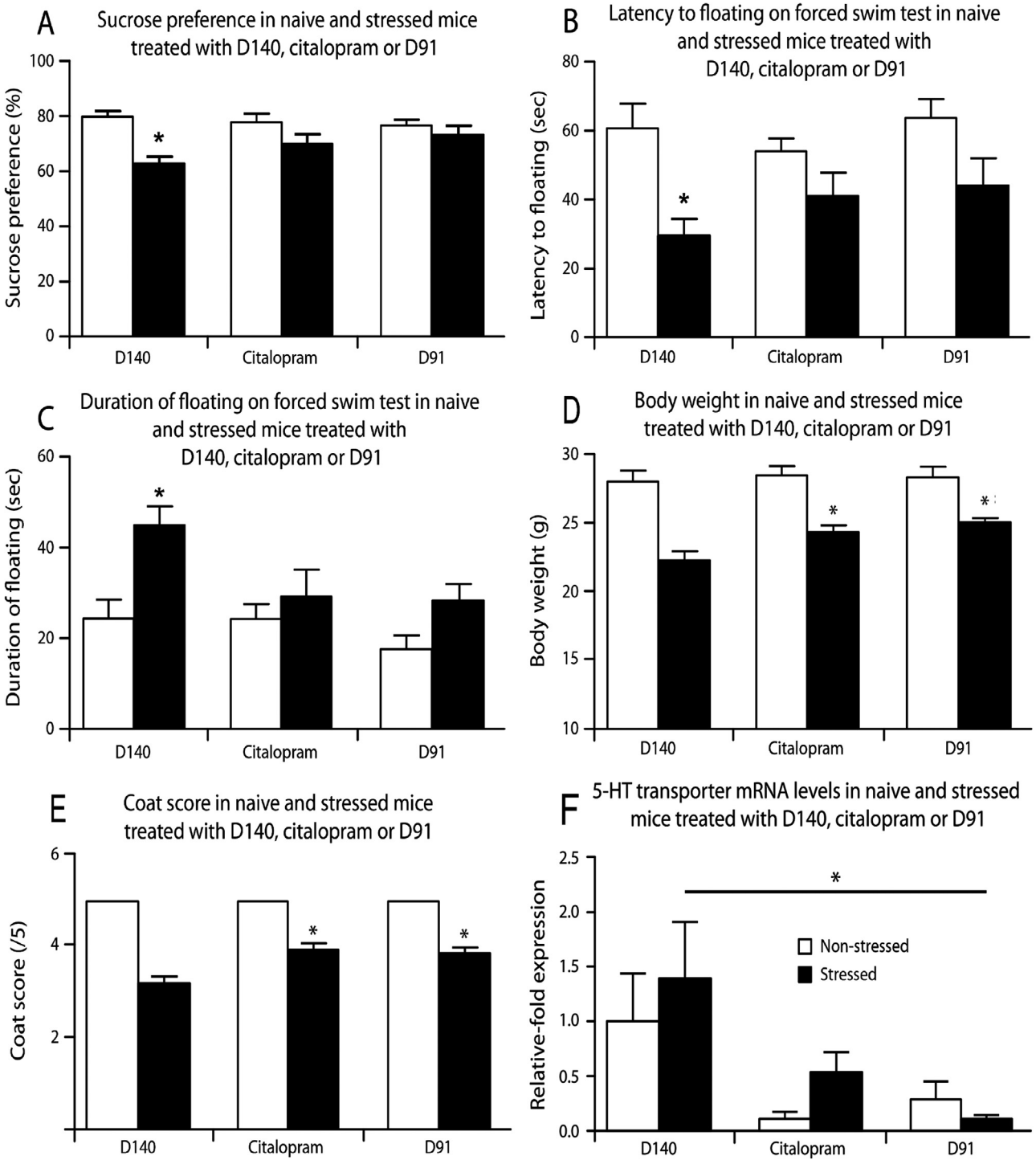

Fig. 2. Citalopram or D91 ameliorates stress-induced depressive-like changes in mice. (A) D140 treated stress mice showed a significant reduction in sucrose preference at day 10 ($p < 0.01$, Tukey's post hoc test). However, citalopram-treated and D91-treated stressed mice displayed no alteration in sucrose preference ($p > 0.05$, Tukey's post hoc test). (B) In the forced swim test, the latency to float was significantly increased by stress in the D140-treated mice ($p < 0.01$), but this was not the case in the citalopram or D91-treated groups ($p > 0.05$; Tukey's post hoc test). (C) The duration of floating was significantly elevated in stressed mice that received D140 ($p < 0.001$), but it was unaltered in D91 and citalopram treated animals ($p > 0.05$; Tukey's post hoc test). (D) All stressed groups showed a significant decrease in body weight compared with controls ($p < 0.001$ for D140 and citalopram treated animals, $p < 0.01$ for D91 treated mice). Post-hoc testing revealed that body weight was significantly higher in stressed groups treated with citalopram or D91 compared to D140 ($p < 0.01$ and $p < 0.0001$ respectively). (E) All stressed groups had significantly lower coat scores than controls while stressed citalopram or D91-treated animals had significantly higher scores than the D140-treated group ($p < 0.001$, Tukey's test). (F) The relative-fold mRNA expression level of the 5-HT transporter (SERT) was significantly lower in D91-treated stressed mice. Data are presented as relative-fold compared to control animals with all expression being normalized to the housekeeping gene GAPDH. Data are mean ± SEM (*<0.05, Tukey's post hoc test). Data are mean ± SEM.

between control and stressed D140-treated groups ($p < 0.01$) but not between other groups ($p > 0.05$).

4.4. Changes in body weight

In terms of body weight, at the end of the chronic stress paradigm there was a significant main effect of stress ($F_{1,87} = 64.09$, $p < 0.0001$), a near-significant main effect of treatment ($F_{2,87} = 2.94$, $p = 0.058$), but no significant interaction between stress and treatment ($F_{2,87} = 1.71$, $p = 0.0.187$). However, stressed mice treated with citalopram or D91 were heavier than those treated with D140 ($p < 0.01$ and $p < 0.0001$, respectively, Fig. 2D).

4.5. Evaluation of coat scores

Before the onset of the chronic stress procedure, all mice had good coat quality, with no significant difference between the groups (*data not shown*). After completion of the chronic stress procedure, a two-way ANOVA comparison showed significant main effects of both stress ($F_{1,87} = 3.59$, $p < 0.0001$); and treatment ($p = 0.0317$; Fig. 2E), and there was also a significant interaction between stress and treatment ($F_{2,87} = 3.59$, $p < 0.05$). All stressed mice showed significantly lower scores of coat state than control treated animals ($p < 0.001$). However, citalopram and D91 administration groups showed higher scores of coat state compared with the D140-treated group ($p < 0.001$).

4.6. Hippocampal gene expression of serotonin transporter (SERT)

Given the marked behavioral differences between the groups, and previous findings showing upregulation of the serotonin transporter (SERT) during anhedonia [14], we sought to elucidate whether treatment with citalopram or D91 led to changes in SERT mRNA expression. A 2-way ANOVA revealed a significant difference between treatment groups in hippocampal expression of SERT mRNA ($F_{2,47} = 4.11$, $p = 0.022$ Fig. 2F). There was no significant main effect of stress ($p = 0.542$) and no significant interaction ($p = 0.705$). There was a trend to increased SERT expression for stressed compared with non-stressed mice in the D140 and citalopram-treated group, but these were not significant on post-hoc analysis ($p > 0.05$). However, both citalopram and D91 decreased the expression of SERT, which was significantly lower in stressed D91-treated compared with stressed D140-treated mice ($p < 0.05$), but the comparison of citalopram-treated versus D140 did not survive correction for multiple comparisons ($p = 0.26$). Therefore, in addition to D91 treatment having a significant effect on behavior, it also had a significant effect on the expression level of SERT mRNA in the hippocampus.

4.7. Neural cell proliferation after a five-day stress

The number of BrdU-positive cells per mm^3 of in the dentate gyrus was significantly reduced in the stressed D140- and D91-treated groups (mean: 613.6 ± 42.59 and 838.8 ± 99.18), as compared to the non-stressed control group (mean: 1578 ± 147.1; $p = 0.0003$, $F = 18.06$, $R^2 = 0.766$, $q = 5.58$ and $q = 4.30$, respectively, one-way ANOVA). There was a significant difference in BrdU-positive cells normalized to control between stressed D91- and D140-treated mice ($p = 0.041$, $t = 2.086$, Fig. 3A). The number of Ki67-positive cells per mm^3 in the dentate gyrus was lower in the stressed D140-treated group, compared to the non-stressed control group (mean: 1405 ± 104.05, $p = 0.0008$, $t = 5.201$), but the difference between stressed D140-treated group (mean: 648.3 ± 80.1) and D91-treated mice (mean: 1371.0 ± 558.5) did not reach statistical significance in this measure normalized to control ($p = 0.102$, $t = 1.28$, Fig. 3B).

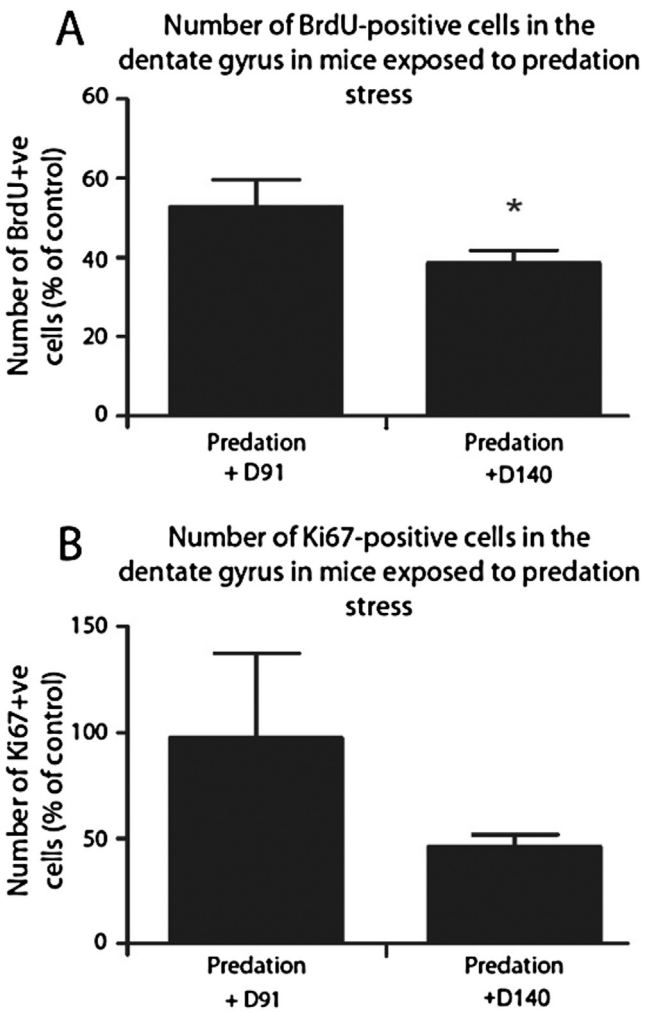

Fig. 3. Effects of D91 on hippocampal SERT and neurogenesis. In stressed animals, normalized to control, (A) the number of BrdU-positive cells per mm^3 in the dentate gyrus was significantly higher in the D91-treated than in D140-treated mice ($^*p < 0.05$, unpaired two-tailed *t*-test). (B) The number of Ki67-positive cells per mm^3 in the dentate gyrus was not significantly different between D91- treated and D140-treated mice.

4.8. EEG parameters of sleep

EEG analysis was performed two weeks after surgery and recovery/adaptation period (Fig. 4A). On day 3 following D91 administration, we noted significant changes in the duration of wakefulness (W) (5–7%, $p = 0.044$, $t = 2.30$, unpaired two-tailed *t*-test, Fig. 4B) and slow wave sleep (SWS) (5-7%, $p = 0.040$, $t = 2.35$, unpaired two-tailed *t*-test, Fig. 4C) compared to D140-treated animals, and this phenomenon remained constant throughout the recording period till day 14 ($p = 0.031$, $t = 2.50$, unpaired two-tailed *t*-test, Fig. 4A and B). D91 administration significantly suppressed REM sleep after day 4 ($p = 0.046$, $t = 2.27$, unpaired two-tailed *t*-test, Fig. 4D); this was more marked than the effect on the other parameters, and was maximal on day 11 of the recording period (40%, $p = 0.0086$, $t = 3.26$, unpaired two-tailed *t*-test). No differences between the groups were observed in the power spectra at any time period of the light cycle, or on any day ($p > 0.05$, unpaired two-tailed *t*-test), including the day 11, when maximal changes in sleep parameters were detected (Supplementary Fig. 2). The spectral

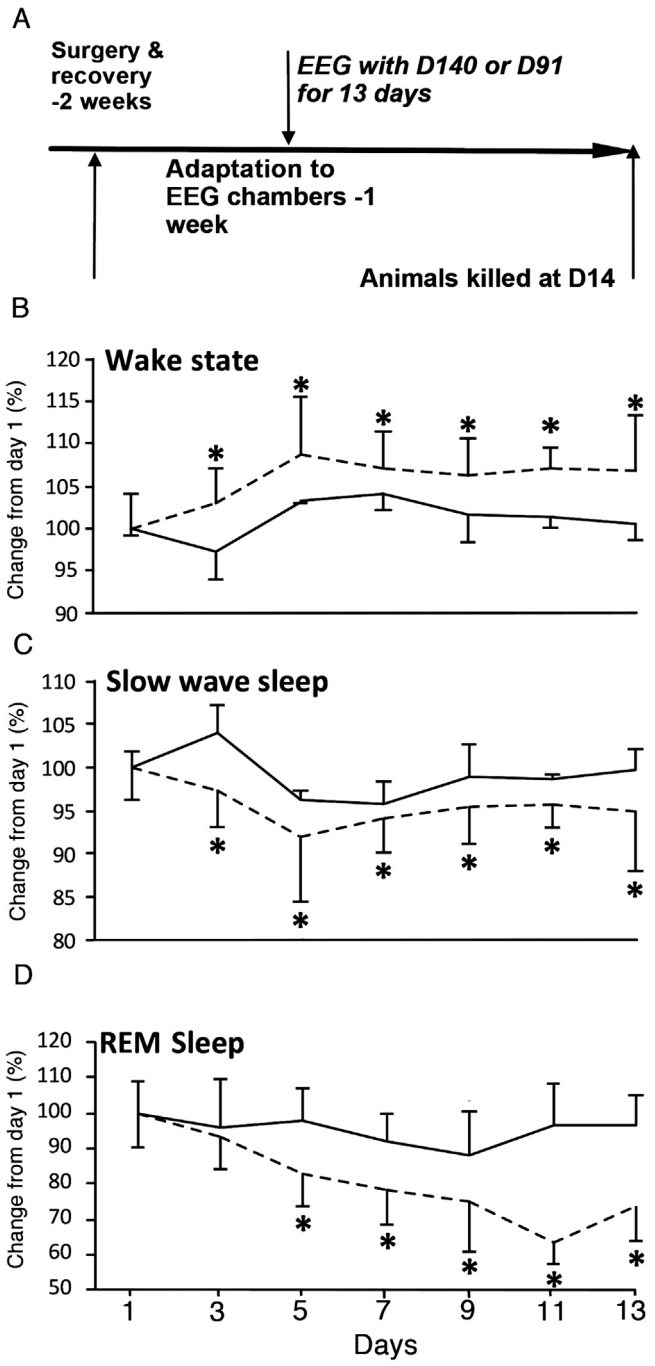

Fig. 4. Effects of D140 and D91 on EEG parameters of sleep of naïve mice. (A) Schematic timeline of the EEG experiment. After electrode implantation, habituation to the recording chambers and baseline EEG registration on the day 1, animals were continuously treated with D140 or D91 during 14-day EEG recordings. (B) Effects of D140 and D91 on waking state. There was a significant increase of the duration of the waking state in D91-treated mice in comparison to D140-treated animals throughout the treatment period. (C) Effects of D140 and D91 on the slow wave sleep. In comparison to D140-treated animals, D91-treated mice had significantly shorter duration of the slow wave sleep on days 3–13. (D) Effects of D140 and D91 on the REM sleep. A significant decrease of the duration of the REM sleep in D91-treated mice in comparison to D140-treated animals was detected throughout the treatment period. $^{*}p < 0.05$; unpaired two-tailed *t*-test. Data are mean ± SEM, expressed as percentage from the means obtained on the day 1.

bands of the EEG considered were: delta, 0.5–4 Hz; theta, 4–8 Hz, alpha, 8–11.5 Hz, sigma, 11.5–14.5 Hz; beta-1, 14.5–18.6 Hz, and beta-2, 18.6–30 Hz.

5. Discussion

We show a positive correlation between rates of affective disorder-related behavior and pathophysiology defined by the MMWR [17] and deuterium content of water, on a state-by-state basis in the USA. Given that the average daily water intake in adults ranges between 2.7 and 3.7 L per individual per day [24], the absolute consumption of the isotopologue hydrogen–oxygen–deuterium (HOD) in deuterium-containing water is around 0.7–1.2 mL for water close to the VSMOW2 standard. As such, even relatively small changes in the deuterium content of consumed water could result in substantial variations of intake. However, it should be pointed out that social, economic and other demographic factors show state-to-state variation, which could be a confounding factor, and it is not possible to highlight a causal relationship from correlational statistics. However, the strength of the association prompted further investigations in mice, which revealed that reduced deuterium intake has the potential to reverse the negative affect, and alter sleep patterns and gene expression associated with serotonergic neurotransmission.

We used a paradigm of stress-induced anhedonia in mice [14,19], using the established sucrose-preference test [25,26] which is a core feature of depression [27]. Citalopram is well documented to counteract the stress-induced decrease in sucrose preference in similar behavioral paradigms [28,29]. Chronic stress reduced sucrose preference in D140-treated mice in agreement with other reports [30,31]; however, after treatment with citalopram and D91 there was no longer a significant difference between stress groups. Importantly, neither D91 nor citalopram had an effect on sucrose test parameters in control animals ruling out a more general effect on sucrose preference. Our data using the Porsolt forced swim test showed that whilst chronic stress decreased the latency to floating, and increased the total time spent floating in mice receiving D140, both citalopram and D91 prevented pathological changes in these parameters, there was no significant difference between stressed and control mice that received citalopram or D91 ($p > 0.05$; Fig. 2B and C). Previous publications from our group showed a similar effect following chronic administration of imipramine and citalopram [18,32,33].

All stressed animals showed a decrease in body weight (Fig. 2D), but this effect was diminished in D91- and citalopram-treated animals. All treated mice showed a significant increase in body weight compared with controls and stressed mice treated with citalopram or D91 were heavier than those treated with D140, while no significant interaction between stress and treatment was revealed. A near-significant main effect of treatment was found. This was consistent with previous reports of restoration of body weight after antidepressant treatment [31,34,35]. Stressed mice also displayed a significant deterioration of coat state, which was ameliorated by both D91 and citalopram treatment (Fig. 2E). Coat disintegration is an important feature of a depressive-like (anhedonic) state in preclinical models [36,37], and improvements in coat state have been associated with antidepressant treatment [37,38].

We next wished to ascertain whether D91 treatment resulted in a corresponding change in SERT expression in the hippocampus. Whilst there was a trend to increased SERT expression in stressed compared with non-stressed D140-treated mice, in line with our previous findings [14], this comparison was not statistically significant here (Fig. 2F). Compared with stressed D140-treated mice, expression of SERT was significantly decreased in stressed mice treated with D91. Interestingly this seemed to have a greater effect size than the antidepressant citalopram, which whilst lower, did

not show a significant decrease in SERT expression. Both stressed and non-stressed animals showed a trend to decreased SERT expression with citalopram/D91 treatment compared with D140, so it is not clear that this effect is limited to stressed animals in this case. Change in SERT expression within the CNS in chronic stress and depression is supported by a number of studies [39–41], and have been associated with alterations in neuroinflammatory-inked pathways [14]. Thus, molecular changes related to the reduction of SERT expression in the CNS may underlie antidepressant-like action of both citalopram and D91 presented here.

We also found that the number of BrdU-positive cells in the dentate gyrus was reduced by stress, and this was ameliorated by treatment with D91 compared with D140 (Fig. 3A), indicating a stimulatory effect of deuterium-depleted water on hippocampal cell proliferation. The effects of stress and antidepressant/anxiolytic therapy on hippocampal neurogenesis are well established in the literature [42,43], and suggests that deuterium depletion could fulfil a similar role. Recent findings have shown that antidepressants, including SSRIs, rescue ongoing neurogenesis during stress [44] and suggested a link between suppression of SERT and the activation of the hippocampal neurogenesis [45] with a key role of BDNF-mediated processes in this effect [46].

The rapid onset in the increase of wakefulness, accompanied by a rapid decrease in slow wave sleep (SWS) remained constant over the treatment period in D91-treated animals (Fig. 4). Remarkably, the changes in REM sleep appeared progressively throughout the treatment period and reached a maximum on the 11th day when this state of sleep was decreased by about 40% in comparison to a baseline value. Analogous effects on SWS and REM sleep are reported for antidepressants inhibiting serotonin and noradrenaline reuptake [16,47]. Both acute [48,49] and 1–3 week long [49,50] dosing with citalopram or other SSRIs reduced the duration of REM and SWS and increased wakefulness. In the clinic, the REM sleep reduction observed with SSRI treatment is used as a biomarker of their therapeutic efficacy [51].

In clinical depression there is decreased latency to the first REM episode, together with an enhancement of REM during the first part of the night. Such an effect is counterbalanced by a decline in REM sleep during the second part of the night, with a decrease in total REM-sleep in a night. Conversely, SWS is decreased in the first part of the night and intermittent awakenings increased. It is possible that the changes in SERT expression may play a role in the changes to sleep EEG parameters. In contrast to the most of standard antidepressants [52,53] the power spectra of D91-treated animals was not altered throughout the treatment period regardless the light cycle. This was surprising given the reduction in REM sleep that might have been expected to alter the proportion of alpha waves.

Previous research has shown a kinetic isotope effect of water as a solvent, with 100% D_2O slowing the rate of ubiquinol derivative oxidation around 400-fold [54]. However, whilst deuterium within natural water range (89–155 ppm) is unlikely to have a significant effect on most chemical reactions, respiration in mitochondria may be a special case, as it involves a connected sequence of proton-coupled electron transfers. Very low levels of deuterium have been shown to have an impact on this process [55] and may account for some of the biological effects we have observed in our mouse paradigms.

A mechanism, by which water with reduced deuterium content exhibits antidepressant-like properties, remains to be elucidated. Based on currently available literature and own preliminary results, various speculations concerning this matter can be proposed. First, replacement of normal water with water of lower viscosity could exert physicochemical effects, leading to increased fluidity of the cell membranes and less rigid organization of phospholipid bilayers [56–58] which can, in turn alter the dispersion of neurotransmitter receptors and increase receptor affinity [59,60], affect passive blood brain barrier permeability [61] and metabolism of arachidonic acid and calcium-dependent receptor binding [62]. Currently unpublished work from our group suggests that epigenetic and post-translational regulation mechanisms may underlie the effect of D91 treatment, based on limited gene expression changes, and there may also be an involvement of factors of synaptic plasticity and BDNF/TrkB signaling.

6. Conclusions

Taken together, our study demonstrates that rates of depression correlate with geographical distribution of deuterium in the natural water in the US population. Substitution of normal drinking water with deuterium-depleted water in mice counteracts the behavioral, transcriptional and proliferative changes typical of the depressive-like state, which was comparable to the effects of the SSRI citalopram. In naïve mice, consumption of deuterium depleted water results in changes of EEG parameters of sleep that are reminiscent of the effects of noradrenaline and serotonin reuptake inhibitors. Thus, deuterium-depleted drinking water could present a novel prophylactic strategy for depression.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2014.07.039.

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