A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood

Steenbergen, L.; Sellaro, R.; van Hemert, S.; Bosch, J.A.; Colzato, L.S.

DOI
10.1016/j.bbi.2015.04.003

Publication date
2015

Document Version
Final published version

Published in
Brain, behavior, and immunity

Brain, Behavior, and Immunity 48 (2015) 258–264

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood ☆

Laura Steenbergen [a,b,*], Roberta Sellaro [a,b], Saskia van Hemert [c], Jos A. Bosch [d], Lorenza S. Colzato [a,b]

[a] Leiden University, Institute for Psychological Research, Cognitive Psychology, Wassenaarseweg 52, 2333 AK Leiden, The Netherlands
[b] Leiden Institute for Brain and Cognition, P.O. Box 9600, 2300 RC Leiden, The Netherlands
[c] Winclove Probiotics, Hulstweg 11, 1032 LB Amsterdam, The Netherlands
[d] University of Amsterdam, Psychology Department, Clinical Psychology, Weesperplein 4, 1018 XA Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 12 December 2014
Received in revised form 1 April 2015
Accepted 2 April 2015
Available online 7 April 2015

Keywords:
Probiotics
Depression
Cognitive reactivity

ABSTRACT

Background: Recent insights into the role of the human microbiota in cognitive and affective functioning have led to the hypothesis that probiotic supplementation may act as an adjuvant strategy to ameliorate or prevent depression. *Objective*: Heightened cognitive reactivity to normal, transient changes in sad mood is an established marker of vulnerability to depression and is considered an important target for interventions. The present study aimed to test if a multispecies probiotic containing *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, and *Lactococcus lactis* (W19 and W58) may reduce cognitive reactivity in non-depressed individuals. *Design*: In a triple-blind, placebo-controlled, randomized, pre- and post-intervention assessment design, 20 healthy participants without current mood disorder received a 4-week probiotic food-supplement intervention with the multispecies probiotics, while 20 control participants received an inert placebo for the same period. In the pre- and post-intervention assessment, cognitive reactivity to sad mood was assessed using the revised Leiden index of depression sensitivity scale. *Results*: Compared to participants who received the placebo intervention, participants who received the 4-week multispecies probiotics intervention showed a significantly reduced overall cognitive reactivity to sad mood, which was largely accounted for by reduced rumination and aggressive thoughts. *Conclusion*: These results provide the first evidence that the intake of probiotics may help reduce negative thoughts associated with sad mood. Probiotics supplementation warrants further research as a potential preventive strategy for depression.

1. Introduction

The intestine and the brain are intimately connected via the brain-gut axis, which involves bidirectional communication via neural, endocrine and immune pathways (Grossman, 1979; Grenham et al., 2011; Mayer, 2011; Mayer et al., 2014). In recent years it has become increasingly evident that this communication also involves interactions with the intestinal microbiota, which release immune activating and other signaling molecules that may play an important role in regulating the brain and subsequent behavior (Mayer, 2011; Cryan and Dinan, 2012; Foster and McVey Neufeld, 2013). For example, the microbiota produce neuroactive substances and their precursors (e.g., tryptophan) which can reach the brain via endocrine and afferent autonomic pathways (Desbonnet et al., 2008, 2010). Also, bacterial products, such as the gram-negative endotoxins, can influence mood and cognitive functions via indirect (e.g., immune activation) and direct (e.g., Toll-like receptors on glial cells) mechanisms (Lehnardt et al., 2003; Krabbe et al., 2005; Ait-Belgnaoui et al., 2012; McCusker and Kelley, 2013).

These novel insights have fuelled the hypothesis that modification of microbial ecology, for example by supplements containing microbial species (probiotics), may be used therapeutically to modify stress responses and symptoms of anxiety and depression (Logan and Katzman, 2005; Cryan and O'Mahony, 2011;

☆ This work was supported by a research grant from The Netherlands Organization for Scientific Research (NWO) awarded to Lorenza S. Colzato (Vidi Grant: #452-12-001). The active probiotics and placebo were provided free of charge by Winclove B.V. (Amsterdam, The Netherlands), but the company was not further involved in the study design or in the collection and analysis of data.

* Corresponding author at: Leiden University, Institute for Psychological Research, Cognitive Psychology, Wassenaarseweg 52, 2333 AK Leiden, The Netherlands. Tel.: +31 71527 3875; fax: +31 71527 3619.

E-mail addresses: L.Steenbergen@fsw.leidenuniv.nl (L. Steenbergen), R.Sellaro@fsw.leidenuniv.nl (R. Sellaro), S.vanHemert@winclove.nl (S. van Hemert), J.A.Bosch@uva.nl (J.A. Bosch), Colzato@fsw.leidenuniv.nl (L.S. Colzato).

http://dx.doi.org/10.1016/j.bbi.2015.04.003
0889-1591/© 2015 The Authors. Published by Elsevier Inc.

Bruce-Keller et al., 2015). While most of this research is relatively recent, and predominantly involves animal and pre-clinical human studies, the results appear in support of this hypothesis (Logan and Katzman, 2005; Cryan and Dinan, 2012; Foster and McVey Neufeld, 2013; Tillisch, 2014; Savignac et al., 2015). For instance, Bravo et al. (2011) observed a reduction in anxious and depressive behavior after feeding healthy mice with *Lactobacillus rhamnosus* JB-1. Similarly, Desbonnet et al. (2010) observed a reduction in depressive-like behaviors in adult rats after feeding them with *Bifidobacterium infantis* 35624. This reduction was comparable to the effects of administering the antidepressant citalopram (Desbonnet et al., 2010). Probiotic studies in humans are still scarce, but the available data are promising. For example, Benton et al. (2006) found in a non-clinical sample that a 3-week intervention with probiotics-containing milk drink (i.e., *Lactobacillus casei* Shirota) improved mood scores compared to participants who received a placebo intervention. Improvement in mood was only observed for participants who showed elevated symptoms of depression at baseline. In another pre-clinical study it was demonstrated that participants who were given a mixture of probiotics containing *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 showed significantly less psychological distress than matched controls (Messaoudi et al., 2011). Furthermore, Rao et al. (2009) demonstrated that patients with chronic fatigue syndrome, which is often comorbid with anxiety disorders, reported significantly less anxiety symptoms after ingestion of a daily dose of *L. casei* Shirota for 2 months, as compared to a placebo group. On the basis of these and other results it has been suggested that probiotics may serve as adjuvant or preventive therapy for depression (for reviews see Logan and Katzman, 2005; Cryan and Dinan, 2012; Foster and McVey Neufeld, 2013; Tillisch, 2014).

These novel discoveries come at an opportune time. The increasing incidence of depression is alarming and development of preventive measures has been identified as a priority (World Health Organization, 2012). According to cognitive theories of depression, cognitive reactivity plays a central role in the development, maintenance, and recurrence of depression and therefore is a relevant target for interventions (Beck, 1967; Kovacs and Beck, 1978; Abramson et al., 1989; Haaga et al., 1991; Scher et al., 2005; Ingram et al., 2006). Cognitive reactivity refers to the activation of dysfunctional patterns of thinking that are triggered by subtle changes in mood, such as ruminative (e.g., recurrent thoughts about possible causes and consequences of one's distress), aggressive (e.g., to think about hurting others or oneself), hopelessness (e.g., loss of motivation and expectations about the future), and/or suicidal thoughts (e.g., to think that one's death is the only way to end the suffering). Such dysfunctional cognitive responses are assumed to stem from latent negative beliefs that become reactivated during low mood (Beck, 1967).

The degree to which these dysfunctional thoughts are activated seems to be critical in determining whether sad mood will be a transient state or will become protracted, increasing the risk of developing clinical depression (Beck, 1967; Kovacs and Beck, 1978; Abramson et al., 1989; Haaga et al., 1991; Scher et al., 2005; Ingram et al., 2006). Indeed, cognitive reactivity is considered one of the most predictive vulnerability markers of depression (Beck, 1967; Segal et al., 1999, 2006; Moulds et al., 2008). Among these dysfunctional thought patterns, rumination seems to be particularly relevant (Nolen-Hoeksema et al., 1993; Kuehner and Weber, 1999; Nolen-Hoeksema, 2000; Spasojevic and Alloy, 2001; Moulds et al., 2008). For instance, Moulds et al. (2008) showed that recovered and never-depressed individuals mainly differ in the degree of activation of ruminative thoughts when experiencing sad mood. Evidence strongly suggesting a causal role of cognitive reactivity in depression onset is provided by a recent study of Kruijt et al. (2013), who showed that higher cognitive reactivity precedes and predicts the episode of depression: never-depressed individuals with high scores on cognitive reactivity were more likely to develop a clinical depression during the subsequent two years, as compared to individuals with lower scores (see also van der Does, 2005, for a review). These associations were independent of a range of confounding factors including baseline mood, life events, and family history of mood disorders (Kruijt et al., 2013). Thus, interventions targeting cognitive reactivity may offer a promising approach to prevent and/or to reduce the incidence of depression-related disorders in the population.

In light of the preceding discussion, the present study aimed to complement previous findings by assessing the possible beneficial effect of probiotics on cognitive reactivity to sad mood, a vulnerability marker for depression. To this end, healthy individuals without any current mood disorder underwent a 4-week intervention period, during which they were supplied with either probiotics or an inert placebo. We tested the effect of multispecies probiotics containing different stains and species of the genera *Lactobacillus*, *Lactococcus* and *Bifidobacterium* (see methods for further details). These genera have been found to be effective in ameliorating anxious and depressive symptoms (Benton et al., 2006; Rao et al., 2009; Yamamura et al., 2009; Desbonnet et al., 2010; Bravo et al., 2011; Messaoudi et al., 2011).

Importantly, studies have shown that multispecies probiotics (i.e., combining different strains of specific genera) can have increased effectiveness through an additive effect of specific strain properties such as colonization of different niches, enhanced adhesion and induction of an optimal pH range, as compared to monospecies supplements (Timmerman et al., 2004; Chapman et al., 2011). Each bacterial strain of the multispecies probiotics used in this study has been found to improve epithelial barrier function both when tested separately and in combination (Van Hemert and Ormel, 2014). However, some probiotics may compete with each other in terms of functionality and therefore the assumption that combinations of different strains may have additive effects needs verification on a preparation by preparation basis.

Before and after the intervention, perceived cognitive reactivity to transient changes in sad mood was measured by means of the revised Leiden Index of Depression Sensitivity (LEIDS-r; van der Does and Williams, 2003), which has been shown to be predictive of depression in multiple longitudinal studies (van der Does, 2005; Kruijt et al., 2013). It was hypothesized that the probiotics intervention would lower the activation of negative thoughts that accompany sad mood, i.e., it would decrease cognitive reactivity as measured by the LEIDS-r.

2. Material and methods

2.1. Participants

Forty non-smoking young adults, with no reported cardiac, renal, or hepatic conditions, no allergies or intolerance to lactose or gluten, no prescribed medication or drug use, and who reported to consume no more than 3–5 alcohol units per week participated in the study. All participants were screened via a phone interview by the experiment leader before inclusion. During the phone interview, the Mini International Neuropsychiatric Interview (M.I.N.I.; Sheehan et al., 1998) was administered too. The M.I.N.I. is a short structured interview, taking about 15 min, which screens for several psychiatric disorders (Sheehan et al., 1998; Colzato et al., 2008, 2010). Participants with no psychiatric or neurological disorders, no personal or family history of depression or migraine were considered suitable to take part in the study. Participants were equally and randomly assigned to receive a 4-week intervention of either placebo or probiotics. Twenty participants (3 male) with a mean age of 19.7 years (SD = 1.7) and a mean body mass index (BMI) of 21.5 (SD = 2.0) were assigned to the placebo condition, and twenty participants (5 male) with a mean age of 20.2 years (SD = 2.4) and a mean BMI of 22.6 (SD = 2.2) were assigned to the probiotics condition (see Table 1). Female participants were not controlled for the menstrual cycle. No information was provided about the different types of intervention (probiotics vs. placebo) or about the hypotheses concerning the outcome of the experiment. All participants believed they were supplied with probiotic supplementation. When informed about the different conditions during the debriefing, none of the participants brought up the deception. Written informed consent was obtained from all participants and the protocol was approved by the local ethical committee (Leiden University, Institute for Psychological Research).

2.2. Design and procedure

A blind at three levels (group allocator, participants, outcome assessor), placebo-controlled, randomized, pre- and post-intervention assessment design was used to investigate the effect of multispecies probiotic intervention on cognitive reactivity to sad mood, as well as reported symptoms of depression and anxiety in healthy young students. Participants received a 4-week food supplementation intervention of either placebo or probiotics. In the probiotics intervention participants were provided with 28 sachets (one for each day of intervention), each containing 2 g freeze-dried powder of the probiotic mixture Ecologic®Barrier (Winclove probiotics, The Netherlands). Ecologic®Barrier (2.5 × 109 CFU/g) contains the following bacterial stains: *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *L. casei* W56, *Lactobacillus salivarius* W24, and *Lactococcus lactis* (W19 and W58). In the placebo intervention, participants were provided with 28 sachets, each containing 2 g freeze-dried powder of the carrier of the probiotic product: maize starch and maltodextrins. The placebo was indistinguishable from the probiotics sachets in color, taste, and smell, but contained no bacteria. The bacteria in Ecologic Barrier have been identified by using 16S rRNA sequencing and the results have been compared with the bacterial nucleotide database of the National Center for Biotechnology Information (NCBI). The viability of the probiotic bacteria was checked both by the producer and by an independent lab (Institut für Mikroökologie GmbH, Herborn, Germany, specialized in microbial analysis, ISO15189 certificated) by determining the number of colony forming units. 1 g of the product was mixed well with 9 ml of a physiological salt solution (0.9% NaCl in ddH_2O). This mixture was tenfold serial diluted in the same physiological salt solution, and 50 μl of each dilution was plated on Mann Rogosa Sharpe (MRS) + 0.5% cysteine agar plates. The plates were incubated anaerobically for 48–72 h at 37 °C. The number of colonies was counted and the total number of colony forming units was calculated based on the dilution and the number of colonies. The batch used for the present experiments contained $>2.5 \times 10^9$ CFU/g, whereas the placebo contained $<1 \times 10^4$ CFU/g. Rehydration of freeze-dried lactic acid bacteria in milk, water and physiological salt solution has been shown to result in equal survival rates (de Valdez et al., 1985). Stability studies, whereby the number of colony forming units was determined every three months, showed that the freeze-dried product is stable for at least 1.5 years when stored at 25 °C with 60% relative humidity.

At the pre- and post-intervention assessments, participants filled out a questionnaire to assess cognitive reactivity to sad mood and questionnaires that assessed symptoms of depression and anxiety. E-prime 2.0 software system (Psychology Software Tools, Inc., Pittsburgh, PA) was used to present the questionnaires and to collect participants' responses, which were to be given using the computer mouse. After having filled out the questionnaires, participants performed two social cognitive tasks tapping into reactions to fairness (ultimatum game) and interpersonal trust (trust game) unrelated to the purposes of the present study (data not reported here).[1] In each session, the complete test battery lasted about 20 min.

At the end of the pre-intervention assessment, participants were provided with the 28 sachets of powder (containing either the inert placebo or the multispecies probiotics) for the 4-week intervention. Participants were instructed, using their own supplies, to dissolve the powder in water or lukewarm milk and to drink it in the evening before going to bed. Compliance was facilitated by reminding the participants via a text message sent by the experimenter.

Table 1
Demographic characteristics for the Placebo and Probiotics groups. Standard deviations are shown within parentheses.

	Placebo	Probiotics
N (M:F)	20 (3:17)	20 (5:15)
Age (years)	19.7 (1.7)	20.2 (2.4)
Body Mass Index (BMI)	21.5 (2.0)	22.6 (2.3)

2.3. Questionnaires

The LEIDS-r (van der Does and Williams, 2003) is a self-report questionnaire with 34 items that assesses to what extent dysfunctional thoughts are activated when experiencing mild dysphoria (i.e., it measures cognitive reactivity to sad mood, also referred to as vulnerability to depression). LEIDS-r scores have been found to predict depression incidence in multiple longitudinal studies and to correlate with depression risk factors, such as depression history (Moulds et al., 2008), genetic markers of depression (Antypa and van der Does, 2010), and reaction to tryptophan depletion (Booij and van der Does, 2007). Before answering the items, participants were asked to take a few minutes to imagine how they would feel and think if they were to experience a sad mood and then to indicate, on a 5-point Likert scale ranging from 0 (i.e. 'not at all') to 4 ('very strongly'), the extent to which each statement applied to them. It was emphasized that the statements applied to the situations when "it is certainly not a good day, but you don't feel truly down or depressed". The scale consists of six subscales that measure vulnerability with respect to:

- Aggression (e.g., When I feel down, I lose my temper more easily);
- Hopelessness/Suicidality (e.g., When I feel down, I more often feel hopeless about everything; When I feel sad, I feel more that people would be better off if I were dead);
- Acceptance/Coping (e.g., When I am sad, I feel more like myself);
- Control/Perfectionism (e.g., I work harder when I feel down);
- Risk aversion (e.g., When I feel down, I take fewer risks);
- Rumination (e.g., When I feel sad, I more often think about how my life could have been different).

Hopelessness and Acceptance/Coping both consist of 5 items, with a maximum score of 20 per subscale, whereas the other scales

[1] Since these tasks were unrelated to the aim of the current study, they are not further discussed here. Given that participants performed the two social cognitive tasks after filling out the questionnaires, we can rule out that participants' scores might have been influenced by performing these tasks.

comprise 6 items with a maximum score of 24 per subscale. The LEIDS-r total score is derived by adding up the scores from each subscale, resulting in total scores ranging from 0 to 136. Internal consistency (Cronbach's alpha; α) is 0.89 for the LEIDS total score, and ranges between 0.62 (Acceptance/Coping) and 0.84 for the subscales (Hopelessness/Suicidality; Antypa and van der Does, 2010; Williams et al., 2008).

The Beck Depression Inventory II (BDI-II) (Beck et al., 1996) is a widely used 21-item multiple-choice self-report questionnaire with high internal consistency (α = .91; Beck et al., 1996), which assesses the existence and severity of current (past 2 weeks) depressive symptoms. The study used the Dutch translation validated by Van der Does (2002b). The BDI–II has been found to be a valid indicator of depression and show good diagnostic discrimination (Dozois et al., 1998). Participants were presented with items related to sumptoms of depression and asked to choose, for each item, the statement that best described how they have been feeling during the past 2 weeks (including the current day). Items are rated on a 4-point scale ranging from 0 to 3 in terms of severity. The total score is calculated by adding-up all items, hence scores range between 0 and 63 (0–13: minimal depression, 14–19: mild depression, 20–28: moderate depression and 29–63: severe depression; van der Does, 2002a).

The Beck Anxiety Inventory (BAI) (Beck et al., 1988) is a 21-item self-report questionnaire with high internal consistency (α = .90; Beck and Steer, 1993), which assesses the existence and severity of anxiety symptoms. A validated Dutch translation was used (Bouman, 1994). Participants are presented with items describing common symptoms of anxiety (such as numbness and tingling, sweating not due to heat, and fear of the worst happening) and asked to rate, on a 4-point Likert scale (0, not at all, 1, mildly, 2, moderately, 3, severely), how much they have been bothered by each symptom over the past week. Total scores are obtained by summing all items, with values ranging between 0 and 63 (as suggested by Beck and Steer (1993); 0–9: normal anxiety; 10–18: mild-moderate; 19–29: moderate-severe and 30–63: severe anxiety).

2.4. Statistical analyses

For each questionnaire, the mean scores (total and/or partial) were calculated and submitted to a repeated measures analysis of variance (ANOVA) with time (pre- vs. post-intervention) as within-subjects factor and group (placebo vs. probiotics) as between-subjects factor. All alpha levels were set at p = .05. Tukey HSD post hoc tests were performed to clarify mean differences in case of significant interactions.

In addition to standard statistical methods, we calculated Bayesian (posterior) probabilities associated with the occurrence of the null [$p(H_0|D)$] and alternative [$p(H_1|D)$] hypotheses, given the observed data. Bayesian inference allows making inferences about both significant and non-significant effects by providing the exact probability of their occurrence. The probabilities range from with 0 (i.e., no evidence) to 1 (i.e., very strong evidence; see Raftery, 1995). To calculate Bayesian probabilities we used the method proposed by Wagenmakers (2007) and Masson (2011). This method uses Bayesian information criteria (BIC), calculated using a simple transformation of sum-of-squares values generated by the standard ANOVA, to estimate Bayes factors and generate $p(H_0|D)$ and $p(H_1|D)$, assuming a "unit information prior" (for further details, see Kass and Wasserman (1995); see also Jarosz and Wiley (2014)).

Due to a technical problem, one participant, assigned to the placebo group, did not fill out the pre-intervention BAI questionnaire. No other data were missing.

3. Results

3.1. Randomization

Table 1 presents the participant characteristics by group (probiotics versus placebo). No significant group differences were observed for age [$t(38) = -0.76$, $p = 0.45$], BMI [$t(38) = -1.64$, $p = 0.11$], and gender distribution [χ^2 (1, N = 40) = 0.63, p = 0.43].

Table 2 gives a summary of pre- and post-intervention scores on the LEIDS-R, BDI and BAI in the placebo and probiotics groups.

As anticipated on basis of participant selection, ANOVA performed on the BDI–II total score revealed no main effect of time [$F(1,38) = .41$, $p = .52$, $p(H_0|D) = .84$], group [$F(1,38) = 1.1$, $p = .31$, $p(H_0|D) = .78$], nor a time by group interaction [$F(1,38) = .41$, $p = .52$, $p(H_0|D) = .84$]. Similarly, for the BAI scores no effect was observed for time [$F(1,37) = 2.30$, $p = .14$, $p(H_0|D) = .66$], group [$F(1,37) = 0.226$, $p = .64$, $p(H_0|D) = .85$], or for the interaction between the two factors [$F(1,37) = 0.064$, $p = .80$, $p_s(H_0|D) = .86$]. Thus, the two groups of participants (placebo and probiotics) were comparable in terms of depression and anxiety scores at baseline and follow-up. Importantly, participants did not show any sign of depression and anxiety in either sessions: only minimal/mild scores were observed at both time points for the BDI–II (the mean scores were 8.53, SD = 4.47, and 8.17, SD = 5.30, for the pre- and post-intervention assessment, respectively) and BAI (the mean scores were 11.77, SD = 7.32, and 10.55, SD = 7.20, the pre- and post-intervention assessment, respectively; see also Table 2).

3.2. Probiotic treatment and cognitive reactivity

ANOVAs revealed significant time by group interactions for the LEIDS-r total score [$F(1,38) = 6.05$, $p = .019$, $\eta_p^2 = 0.137$, MSE = 40.468, $p(H_1|D) = .79$], aggression [$F(1,38) = 4.94$, $p = .032$, $\eta_p^2 = .115$, MSE = 4.255, $p(H_1|D) = .65$], and rumination [$F(1,38) = 12.16$, $p = .001$, $\eta_p^2 = .242$, MSE = 3.826, $p(H_1|D) = .98$]. Tukey HSD post hoc tests performed to disentangle the interactions revealed that participants who received a 4-week placebo intervention showed comparable scores pre- versus post-intervention (total score: $p = .63$, $p(H_0|D) = .70$; aggression: $p = .95$, $p(H_0|D) = .80$; rumination: $p = 1.0$, $p(H_0|D) = .82$; see Table 2). In contrast, participants who received a 4-week probiotics intervention scored significantly lower at post-intervention compared to the pre-intervention (total score: $p < .001$, $p(H_1|D) > .99$; aggression: $p = .004$, $p(H_1|D) > .99$; rumination: $p < .001$, $p(H_1|D) > .99$; see Table 2). Thus, our results show that the intake of multispecies probiotics for a 4-week period significantly reduced overall cognitive reactivity to depression and in particular aggressive and ruminative thoughts.

4. Discussion

The aim of the current study was to investigate the effect of a multispecies probiotic intervention on cognitive reactivity in healthy individuals not currently diagnosed with a mood disorder. As mentioned in the introduction, cognitive reactivity is an important vulnerability marker of depression; the content and the type of thoughts that are activated when an individual experiences sad mood predicts whether the sad mood will be transient or will persist, and predicts the development of clinical depression (Abramson et al., 1989; Beck, 1967; Kovacs and Beck, 1978; Haaga et al., 1991; Scher et al., 2005; Ingram et al., 2006). We found that a 4-week multispecies probiotic intervention reduced self-reported cognitive reactivity to sad mood, as indexed by the LEIDS-r (van der Does and Williams, 2003; van der Does, 2005; Kruijt et al., 2013). Further analyses showed that the strongest

Table 2
Mean pre- and post-intervention scores and standard error of the means (shown in parentheses) on the LEIDS-r, BDI and BAI in the Placebo and Probiotics groups. Asterisks indicate significant treatment effect differences between pre- and post-intervention assessments.

		Pre-intervention	Post-intervention
LEIDS-r			
Aggression	Placebo	8.80 (0.94)	8.45 (0.98)
	Probiotics**	8.68 (0.94)	6.25 (0.98)
Control	Placebo	7.65 (0.80)	6.70 (0.82)
	Probiotics	7.25 (0.83)	5.80 (0.82)
Hopelessness	Placebo	5.60 (0.85)	4.70 (0.74)
	Probiotics	4.75 (0.85)	4.0 (0.74)
Risk aversion	Placebo	9.50 (0.93)	9.25 (0.87)
	Probiotics	10.00 (0.93)	7.95 (0.87)
Rumination	Placebo	11.75 (0.90)	11.85 (0.93)
	Probiotics***	11.20 (0.90)	8.25 (0.93)
Acceptance	Placebo	1.40 (0.34)	1.35 (0.37)
	Probiotics	0.90 (0.34)	1.10 (0.37)
Total	Placebo	44.70 (3.24)	42.30 (3.51)
	Probiotics***	42.75 (3.24)	33.35 (3.51)
BDI	Placebo	9.10 (1.00)	9.10 (1.19)
	Probiotics	7.90 (1.00)	7.25 (1.19)
BAI	Placebo	12.21 (1.70)	11.21 (1.69)
	Probiotics	11.35 (1.66)	9.95 (1.65)

* $p < .05$
** $p < .01$.
*** $p < .001$.

beneficial effects were observed for the aggression and rumination subscales, indicating that in the probiotics supplementation condition participants perceived themselves to be less distracted by aggressive and ruminative thoughts when in a sad mood. Notably, studies have shown that the tendency to engage in ruminative thoughts is sufficient to turn mood fluctuations into depressive episodes, and that individuals who typically respond to low mood by ruminating about possible causes and consequences of their state have more difficulties in recovering from depression (Nolen-Hoeksema et al., 1993; Kuehner and Weber, 1999; Nolen-Hoeksema, 2000; Spasojevic and Alloy, 2001; Moulds et al., 2008). Further, the activation of aggressive thoughts has been associated with suicidal ideation and attempts (Oquendo et al., 2006; Mann et al., 2008). In sum, the present results indicate, for the first time, that probiotics intervention can influence cognitive mechanisms that are known to determine vulnerability to mood disorders.

The present sample consisted of healthy individuals with minimal to mild baseline scores on both the BAI and the BDI, and it is not surprising therefore that the beneficial effect of probiotics intervention was selective for cognitive reactivity to depression and not for self-report symptoms of depression or anxiety. This observation is consistent with the findings reported by Benton et al. (2006), who found that improvements in mood after probiotics administration only occurred in participants who showed elevated symptoms of depression at the baseline. Importantly, the selection of a nonclinical sample of participants provided the opportunity to test specifically the possible beneficial effects of probiotics intervention on cognitive reactivity, i.e., not confounded by ongoing depressive symptomatology. Further longitudinal studies in high-risk or clinical groups are necessary to confirm potentially clinically relevant effects. Given that the transition from persistent changes in mood to the development of a depressive episode can be months or longer, such studies may need to extend past the current 4-week period.

While the present study did not set out to test specific biological mechanisms that could underlie possible beneficial cognitive effects, the extant literature does allow for a number of hypotheses testable in future studies. For example, it has been proposed that intestinal microbiota increase plasma tryptophan levels, and hereby potentially facilitate serotonin turnover in the brain (Desbonnet et al., 2008, 2010). Interestingly, cognitive reactivity to sad mood has been associated with serotonin concentrations, with higher scores correlating with lower serotonin levels (Booij and Van der Does, 2007; Wells et al., 2010; see also Firk and Markus, 2009). However, other pathways are plausible as well. For instance, it has been proposed that an increased intestinal permeability can induce depressive symptoms (Ait-Belgnaoui et al., 2012), possibly by endotoxin activated inflammatory pathways or via direct activation of glial and neural cells that carry Toll-like receptors and are hereby responsive to a wide range of microbial products (McCusker and Kelley, 2013). Given that certain probiotics have been found to improve the epithelial barrier function and hereby decrease permeability (Van Hemert et al., 2013), this mechanism might account for the beneficial effects of probiotics on cognitive reactivity. Follow-up probiotics studies could explore this possibility, for example by using the lactulose/mannitol ratio in urine to evaluate intestinal permeability (Teixeira et al., 2014). Animal studies have further suggested that gut-to-brain signals are transmitted via the vagus nerve (Ter Horst and Postema, 1997; Tillisch et al., 2013). For example, a study in mice has shown that the supplementation of probiotics has a beneficial effect on anxious and depressive behavior, but only with an intact vagus nerve (Bravo et al., 2011). In humans the vagus nerve reaches, via the locus coeruleus and the raphe nuclei (the principal sources of serotonin released in the brain), the anterior cingulate cortex (ACC) and the prefrontal cortex (PFC; Thayer and Lane, 2007), in particular the mPFC (Mayer et al., 2006) – i.e., one of the brain regions associated with the processing of affective and social information (Adolphs, 2001). Stimulation of the vagus nerve has already been described as a successful method to treat patients suffering from depression (Nemeroff et al., 2006). Interestingly, Tillisch and colleagues (2013) have found that 4-week intake of a fermented probiotic milk product by healthy women was associated with altered activity of brain regions (e.g., primary interoceptive and somatosensory cortices, and precuneus) that control central processing of emotion and sensation. Therefore, it would be of interest to explore whether the treatment of depressive disorders would further benefit by combining probiotic supplementation with stimulation of the vagus nerve.

The present study has a few limitations that deserve discussion. First, we did not include dietary measures and did not control for consumption of other probiotic products or fermented foods (e.g., yogurt). Hence we cannot exclude that the consumption of probiotics was accompanied by spontaneous dietary changes that may have indirectly accounted for the effect. Second, compliance was facilitated by text message reminders, but not further confirmed e.g., by stool bacterial analysis. However, prior studies which used partly the same bacterial strains have shown presence of the strains in stool samples of healthy volunteers (Koning et al., 2008). A third limitation of the present study is that it tested a predominantly female sample, and generalizability to males is uncertain therefore."

Finally, it is worth noting that our assessment only relied on self-reported cognitive reactivity that, although established as a psychometrically reliable index of cognitive reactivity and found to be predictive of the development of depressive symptoms and depressive disorder (van der Does, 2005; Kruijt et al., 2013), would be considered to provide only indirect information on actual cognitive reactivity at times of low mood. Future studies may therefore expand these observations by experimentally inducing negative mood and/or by including ambulatory measurements, e.g., using experience sampling techniques, to evaluate possible beneficial effects of probiotics.

To conclude, the present study demonstrated, for the first time, that a 4-week multispecies probiotic intervention has a positive effect on cognitive reactivity to naturally occurring changes in sad mood in healthy individuals not currently diagnosed with a depressive disorder. More specifically, the probiotic intervention reduced aggressive and ruminative thoughts in response to sad mood. These findings provide information on a cognitive mechanism that may be responsible for the positive mood effects of probiotic supplementation (Benton et al., 2006; Rao et al., 2009; Messaoudi et al., 2011; Logan and Katzman, 2005; Tillisch, 2014). Future studies should investigate the neurobiological underpinnings of these observed effects and test the applicability of the current findings to high-risk and clinical populations.

Conflict of interest

The authors have declared that no competing interests exist. This work was supported by a research grant from the Netherlands Organization for Scientific Research (NWO) awarded to Lorenza S. Colzato (Vidi grant: #452-12-001). The active probiotics and placebo were provided free of charge by Winclove B.V. (Amsterdam, The Netherlands), but the company was not further involved in the study design or in the collection and analysis of data.

References

Abramson, L., Metalsky, G., Alloy, L., 1989. Hopelessness depression: a theory-based subtype of depression. Psychol. Rev. 96 (2), 358–372.

Adolphs, R., 2001. The neurobiology of social cognition. Curr. Opin. Neurobiol. 11 (2), 231–239. http://dx.doi.org/10.1016/s0959-4388(00)00202-6.

Ait-Belgnaoui, A., Durand, H., Cartier, C., Chaumaz, G., Eutamene, H., Ferrier, L., Theodorou, V., 2012. Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrinology 37 (11), 1885–1895.

Antypa, N., Van der Does, A.J.W., 2010. Serotonin transporter gene, childhood emotional abuse and cognitive vulnerability to depression. Genes Brain Behav. 9, 615–620.

Beck, A.T., 1967. Depression: Causes and Treatment. University of Pennsylvania Press, Philadelphia.

Beck, A.T., Epstein, N., Brown, G., Steer, R.A., 1988. An inventory for measuring clinical anxiety: psychometric properties. J. Consult. Clin. Psychol. 56 (6), 893. http://dx.doi.org/10.1037//0022-006x.56.6.893.

Beck, A.T., Steer, R.A., 1993. Manual for the Beck Anxiety Inventory. The Psychological Corporation, San Antonio.

Beck, A.T., Steer, R.A., Ball, R., Ranieri, W.F., 1996. Comparison of beck depression inventories-IA and-II in psychiatric outpatients. J. Pers. Assess. 67 (3), 588–597. http://dx.doi.org/10.1207/s15327752jpa6703_13.

Benton, D., Williams, C., Brown, A., 2006. Impact of consuming a milk drink containing a probiotic on mood and cognition. Eur. J. Clin. Nutr. 61 (3), 355–361. http://dx.doi.org/10.1038/sj.ejcn.1602546.

Booij, L., van der Does, W., 2007. Cognitive and serotonergic vulnerability to depression: convergent findings. J. Abnorm. Psychol. 116 (1), 86. http://dx.doi.org/10.1037/0021-843x.116.1.86.

Bouman, T.K., 1994. De Beck Depression Inventory (BDI). Gedragstherapie 27, 69–72.

Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Cryan, J.F., 2011. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. 108 (38), 16050–16055. http://dx.doi.org/10.1073/pnas.1102999108.

Bruce-Keller, A.J., Salbaum, J.M., Luo, M., Blanchard IV, E., Taylor, C.M., Welsh, D.A., Berthoud, H.R., 2015. Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. Biol. Psychiatry 77 (7), 607–615.

Chapman, C.M.C., Gibson, G.R., Rowland, I., 2011. Health benefits of probiotics: are mixtures more effective than single strains? Eur. J. Nutr. 50 (1), 1–17. http://dx.doi.org/10.1007/s00394-010-0166-z.

Colzato, L.S., Hertsig, G., van den Wildenberg, W.P.M., Hommel, B., 2010. Estrogen modulates inhibitory control in healthy human females: evidence from the stop-signal paradigm. Neuroscience 167, 709–715. http://dx.doi.org/10.1016/j.neuroscience.2010.02.029.

Colzato, L.S., Kool, W., Hommel, B., 2008. Stress modulation of visuomotor binding. Neuropsychologia 46, 1542–1548. http://dx.doi.org/10.1016/j.neuropsychologia.2008.01.006.

Cryan, J.F., Dinan, T.G., 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat. Rev. Neurosci. 13 (10), 701–712. http://dx.doi.org/10.1038/nrn3346.

Cryan, J.F., O'Mahony, S.M., 2011. The microbiome-gut-brain axis: from bowel to behavior. Neurogastroenterol. Motil. 23 (3), 187–192. http://dx.doi.org/10.1111/j.1365-2982.2010.01664.x.

Desbonnet, L., Garrett, L., Clarke, G., Bienenstock, J., Dinan, T.G., 2008. The probiotic *Bifidobacteria infantis*: an assessment of potential antidepressant properties in the rat. J. Psychiatr. Res. 43 (2), 164–174.

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J.F., Dinan, T.G., 2010. Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. Neuroscience 170 (4), 1179–1188. http://dx.doi.org/10.1016/j.neuroscience.2010.08.005.

de Valdez, G.F., de Giori, G.S., de Ruiz Holgado, A.P., Oliver, G., 1985. Effect of the rehydration medium on the recovery of freeze-dried lactic acid bacteria. Appl. Environ. Microbiol. 50, 1339–1341.

Dozois, D.J., Dobson, K.S., Ahnberg, J.L., 1998. A psychometric evaluation of the Beck Depression Inventory–II. Psychol. Assess. 10 (2), 83. http://dx.doi.org/10.1037/1040-3590.10.2.83.

Firk, C., Markus, C.R., 2009. Mood and cortisol responses following tryptophan-rich hydrolyzed protein and acute stress in healthy subjects with high and low cognitive reactivity to depression. Clin. Nutr. 28 (3), 266–271.

Foster, J.A., McVey Neufeld, K.A., 2013. Gut–brain axis: how the microbiome influences anxiety and depression. Trends Neurosci. 36 (5), 305–312. http://dx.doi.org/10.1016/j.tins.2013.01.005.

Grenham, S., Clarke, G., Cryan, J.F., Dinan, T.G., 2011. Brain-gut-microbe communication in health and disease. Front. Physiol. 2.

Grossman, M.I., 1979. Neural and hormonal regulation of gastrointestinal function: an overview. Annu. Rev. Physiol. 41 (1), 27. http://dx.doi.org/10.1146/annurev.ph.41.030179.000331.

Haaga, D.A., Dyck, M.J., Ernst, D., 1991. Empirical status of cognitive theory of depression. Psychol. Bull. 110 (2), 215. http://dx.doi.org/10.1037/0033-2909.110.2.215.

Ingram, R. E., Miranda, J., Segal, Z., 2006. Cognitive vulnerability to depression. Cognitive Vulnerability to Emotional Disorders, 63–91.

Jarosz, A.F., Wiley, J., 2014. What are the odds? A practical guide to computing and reporting Bayes Factors. J. Probl. Solving 7 (1), 2.

Kass, R.E., Wasserman, L., 1995. A reference Bayesian test for nested hypotheses and its relationship to the Schwarz criterion. J. Am. Stat. Assoc. 90 (431), 928–934.

Kovacs, M., Beck, A.T., 1978. Maladaptive cognitive structures in depression. Am. J. Psychiatry 135 (5), 525–533.

Koning, C.J., Jonkers, D.M., Stobberingh, E.E., Mulder, L., Rombouts, F.M., Stockbrugger, R.W., 2008. The effect of a multispecies probiotic on the intestinal microbiota and bowel movements in healthy volunteers taking the antibiotic amoxycillin. Am. J. Gastroenterol. 103, 178–189.

Krabbe, K.S., Reichenberg, A., Yirmiya, R., Smed, A., Pedersen, B.K., Bruunsgaard, H., 2005. Low-dose endotoxemia and human neuropsychological functions. Brain Behav. Immun. 19 (5), 453–460.

Kruijt, A.W., Antypa, N., Booij, L., de Jong, P.J., Glashouwer, K., Penninx, B.W., Van der Does, W., 2013. Cognitive reactivity, implicit associations, and the incidence of depression: a two-year prospective study. PLoS One 8 (7), e70245.

Kuehner, C., Weber, I., 1999. Responses to depression in unipolar depressed patients: an investigation of Nolen-Hoeksema's response styles theory. Psychol. Med. 29, 1323–1333.

Lehnardt, S., Massillon, L., Follett, P., Jensen, F.E., Ratan, R., Rosenberg, P.A., Vartanian, T., 2003. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. Proc. Natl. Acad. Sci. 100 (14), 8514–8519.

Logan, A.C., Katzman, M., 2005. Major depressive disorder: probiotics may be an adjuvant therapy. Med. Hypotheses 64 (3), 533–538. http://dx.doi.org/10.1016/j.mehy.2004.08.019.

Mann, J.J., Ellis, S.P., Waternaux, C.M., Liu, X., Oquendo, M.A., Malone, K.M., Currier, D., 2008. Classification trees distinguish suicide attempters in major psychiatric disorders: a model of clinical decision making. J. Clin. Psychiatry 69 (1), 23–31.

Masson, M.E.J., 2011. A tutorial on a practical Bayesian alternative to Null Hypothesis Significance Testing. Behav. Res. Methods 43, 679–690. http://dx.doi.org/10.3758/s13428-010-0049-5.

Mayer, E.A., 2011. Gut feelings: the emerging biology of gut–brain communication. Nat. Rev. Neurosci. 12 (8), 453–466.

Mayer, E.A., Knight, R., Mazmanian, S.K., Cryan, J.F., Tillisch, K., 2014. Gut microbes and the brain: paradigm shift in neuroscience. J. Neurosci. 34 (46), 15490–15496.

Mayer, E.A., Naliboff, B.D., Craig, A.D., 2006. Neuroimaging of the brain-gut axis: from basic understanding to treatment of functional GI disorders. Gastroenterology 131 (6), 1925–1942. http://dx.doi.org/10.1053/j.gastro.2006.10.026.

McCusker, R.H., Kelley, K.W., 2013. Immune–neural connections: how the immune system's response to infectious agents influences behavior. J. Exp. Biol. 216 (1), 84–98.

Messaoudi, M., Violle, N., Bisson, J.F., Desor, D., Javelot, H., Rougeot, C., 2011. Beneficial psychological effects of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in healthy human volunteers. Gut Microbes 2 (4), 256–261. http://dx.doi.org/10.4161/gmic.2.4.16108.

Moulds, M.L., Kandris, E., Williams, A.D., Lang, T., Yap, C., Hoffmeister, K., 2008. An investigation of the relationship between cognitive reactivity and rumination. Behav. Ther. 39 (1), 65–71. http://dx.doi.org/10.1016/j.beth.2007.05.001.

Nemeroff, C.B., Mayberg, H.S., Krahl, S.E., McNamara, J., Frazer, A., Henry, T.R., Brannan, S.K., 2006. VNS therapy in treatment-resistant depression: clinical

evidence and putative neurobiological mechanisms. Neuropsychopharmacology 31 (7), 1345–1355. http://dx.doi.org/10.1038/sj.npp.1301082.
Nolen-Hoeksema, S., 2000. The role of rumination in depressive disorders and mixed anxiety/depressive symptoms. J. Abnorm. Psychol. 109 (3), 504–511.
Nolen-Hoeksema, S., Morrow, J., Fredrickson, B.L., 1993. Response styles and the duration of episodes of depressed mood. J. Abnorm. Psychol. 102 (1), 20–28.
Oquendo, M.A., Currier, D., Mann, J.J., 2006. Prospective studies of suicidal behavior in major depressive and bipolar disorders: what is the evidence for predictive risk factors? Acta Psychiatr. Scand. 114 (3), 151–158.
Raftery, A.E., 1995. Bayesian model selection in social research. In: Marsden, P.V. (Ed.), Sociological methodology. Blackwells, Oxford, UK, pp. 111–196. http://dx.doi.org/10.2307/271063.
Rao, A.V., Bested, A.C., Beaulne, T.M., Katzman, M.A., Iorio, C., Berardi, J.M., Logan, A.C., 2009. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathog. 1 (1), 1–6. http://dx.doi.org/10.1186/1757-4749-1-6.
Savignac, H.M., Tramullas, M., Kiely, B., Dinan, T.G., Cryan, J.F., 2015. Bifidobacteria modulate cognitive processes in an anxious mouse strain. Behav. Brain Res. http://dx.doi.org/10.1016/j.bbr.2015.02.044 (Epub ahead of print).
Scher, C.D., Ingram, R.E., Segal, Z.V., 2005. Cognitive reactivity and vulnerability: empirical evaluation of construct activation and cognitive diatheses in unipolar depression. Clin. Psychol. Rev. 25 (4), 487–510. http://dx.doi.org/10.1016/j.cpr.2005.01.005.
Segal, Z.V., Gemar, M., Williams, S., 1999. Differential cognitive response to a mood challenge following successful cognitive therapy or pharmacotherapy for unipolar depression. J. Abnorm. Psychol. 108 (1), 3.
Segal, Z.V., Kennedy, S., Gemar, M., Hood, K., Pedersen, R., Buis, T., 2006. Cognitive reactivity to sad mood provocation and the prediction of depressive relapse. Arch. Gen. Psychiatry 63 (7), 749–755. http://dx.doi.org/10.1001/archpsyc.63.7.749.
Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59, 22–33.
Spasojevic, J., Alloy, L.B., 2001. Rumination as a common mechanism relating depressive risk factors to depression. Emotion 1, 25–37.
Teixeira, T.F.S., Moreira, A.P.B., Souza, N.C.S., Frias, R., Peluzio, M.D.C.G., 2014. Intestinal permeability measurements: general aspects and possible pitfalls. Nutr. Hosp. 2 (29), 269–281.
ter Horst, G.J., Postema, F., 1997. Forebrain parasympathetic control of heart activity: retrograde transneuronal viral labeling in rats. Am. J. Physiol. 273, H2926–H2930.
Thayer, J.F., Lane, R.D., 2007. The role of vagal function in the risk for cardiovascular disease and mortality. Biol. Psychol. 74 (2), 224–242. http://dx.doi.org/10.1097/00149831-200605001-00124.
Tillisch, K., 2014. The effects of gut microbiota on CNS functions in humans. Gut Microbes 5 (3). http://dx.doi.org/10.4161/gmic.29232.
Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., Mayer, E.A., 2013. Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology 144 (7), 1394–1401. http://dx.doi.org/10.1053/j.gastro.2013.02.043.
Timmerman, H.M., Koning, C.J.M., Mulder, L., Rombouts, F.M., Beynen, A.C., 2004. Monostrain, multistrain and multispecies probiotics—a comparison of functionality and efficacy. Int. J. Food Microbiol. 96 (3), 219–233. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.05.012.
van der Does, W., 2002a. Cognitive reactivity to sad mood: structure and validity of a new measure. Behav. Res. Ther. 401, 105–120. http://dx.doi.org/10.1016/s0005-7967(00)00111-x.
van der Does, W., 2002b. Handleiding bij de Nederlandse versie van Beck Depression Inventory (BDI – II – NL). Pearson, Amsterdam.
van der Does, W., 2005. Thought suppression and cognitive vulnerability to depression. Br. J. Clin. Psychol. 44, 1–14. http://dx.doi.org/10.1348/014466504x19442.
van der Does, W., Williams, J.M.G., 2003. Leiden Index of Depression Sensitivity – Revised (LEIDS-R). Leiden University.
Van Hemert, S., Ormel, G., 2014. Influence of the Multispecies Probiotic Ecologic® BARRIER on parameters of intestinal barrier function. Food Nutr. Sci. 5, 1739–1745. http://dx.doi.org/10.4236/fns.2014.518187.
Van Hemert, S., Verwer, J., Schütz, B., 2013. Clinical studies evaluating effects of probiotics on parameters of intestinal barrier function. Adv. Microbiol. 3, 212–222. http://dx.doi.org/10.4236/aim.2013.32032.
Wagenmakers, E.-J., 2007. A practical solution to the pervasive problems of p values. Psychon. Bull. Rev. 14, 779–804. http://dx.doi.org/10.3758/bf03194105.
Wells, T.T., Beevers, C.G., McGeary, J.E., 2010. Serotonin transporter and BDNF genetic variants interact to predict cognitive reactivity in healthy adults. J. Affect. Disord. 126 (1), 223–229. http://dx.doi.org/10.1016/j.jad.2010.03.019.
Williams, J.M.G., Van der Does, A.J.W., Barnhofer, T., Crane, C., Segal, Z.S., 2008. Cognitive reactivity, suicidal ideation and future fluency: preliminary investigation of a differential activation theory of hopelessness/suicidality. Cogn. Ther. Res. 32 (1), 83–104.
World Health Organization, 2012. Depression (Fact sheet No. 369). Available: <http://www.who.int/mediacentre/factsheets/fs369/en/> (accessed 28 September 2014).
Yamamura, S., Morishima, H., Kumano-Go, T., Suganuma, N., Matsumoto, H., Adachi, H., Takeda, M., 2009. The effect of *Lactobacillus helveticus* fermented milk on sleep and health perception in elderly subjects. Eur. J. Clin. Nutr. 63 (1), 100–105. http://dx.doi.org/10.1038/sj.ejcn.1602898.