The thyroid hormone, triiodothyronine, enhances fluoxetine-induced neurogenesis in rats: possible role in antidepressant-augmenting properties

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Abstract
The thyroid hormone triiodothyronine (T3) may accelerate and augment the action of antidepressants. Antidepressants up-regulate neurogenesis in adult rodent hippocampus. We studied the effect of T3 and T3 + fluoxetine in enhancement of hippocampal neurogenesis beyond that induced by fluoxetine alone and the correlation with antidepressant behaviour in the novelty suppressed feeding test (NSFT). Rats were administered fluoxetine (5 mg/kg.d), T3 (50 μg/kg.d), fluoxetine (5 mg/kg.d) + T3 (50 μg/kg.d) or saline, for 21 d. Neurogenesis was studied by doublecortin (DCX) immunohistochemistry in the subgranular zone (SGZ) of the hippocampus and the subventricular zone (SVZ). In the NSFT, latency to feeding in animals deprived of food was measured. Fluoxetine and fluoxetine + T3 increased the number of doublecortin-positive (DCX+) cells in the SGZ compared to saline (p = 0.00005, p = 0.008, respectively). There was a trend towards an increased number of DCX+ cells by T3 compared to saline (p = 0.06). Combined treatment with fluoxetine + T3 further increased the number of DCX+ cells compared to T3 or fluoxetine alone (p = 0.001, p = 0.014, respectively). There was no effect of any of the treatments on number of DCX+ cells in the SVZ. In the NSFT, latency to feeding in animals deprived of food was measured. Fluoxetine and fluoxetine + T3 increased the number of doublecortin-positive (DCX+) cells in the SGZ compared to saline (p = 0.00005, p = 0.008, respectively). There was a trend towards an increased number of DCX+ cells by T3 compared to saline (p = 0.06). Combined treatment with fluoxetine + T3 further increased the number of DCX+ cells compared to T3 or fluoxetine alone (p = 0.001, p = 0.014, respectively). There was no effect of any of the treatments on number of DCX+ cells in the SVZ. In the NSFT, all treatments (T3, fluoxetine + T3 and fluoxetine) reduced latency to feeding compared to saline (p = 0.0004, p = 0.00001, p = 0.00009, respectively). Fluoxetine + T3 further reduced latency to feeding compared to T3 alone (p = 0.05). The results suggest that enhancement of antidepressant action by T3 may be related to its effect of increasing hippocampal neurogenesis and that the antidepressant effect of these treatments is specific to the hippocampus and does not represent a general effect on cell proliferation.

Key words: Antidepressants, hippocampal neurogenesis, novelty suppressed feeding test, T3, thyroid hormones.

Introduction
Thyroid hormones have a profound influence on behaviour and appear to be capable of modulating the phenotypic expression of major affective illness (Bauer & Whybrow, 2001). Hypothyroidism may be associated with clinical depression in patients, which may respond to thyroid hormone replacement (Hendrick et al. 1998). The thyroid hormone, triiodothyronine (T3), is widely used to treat major depression usually as a supplement to standard antidepressants. There is evidence that T3 may accelerate and augment the actions of antidepressant drugs. In a meta-analysis (Altshuler et al. 2001) T3 was found to be effective in accelerating clinical response to tricyclic antidepressants (TCAs) in patients with non-refractory depression. A meta-analysis by Aronson et al. (1996) found that T3 augmentation was more effective than placebo for TCA-resistant major depressive disorder. In a STAR*D study, remission rate with T3 augmentation for depressed patients who experienced...
unsatisfactory results with two prior antidepressants was 24.7% (Nierenberg et al. 2006). However, as shown in a recent review (Cooper-Kazaz & Lerer, 2008) and meta-analysis (Papakostas et al. 2009) studies on the co-initiation of an SSRI and T3 in patients with major depression are inconclusive.

Adult neurogenesis, the lifelong addition of new neurons, was first documented in the rat hippocampus, and occurs in several different species, including humans. The newly generated cells mature into functional neurons (van Praag et al. 2002). Neurogenesis in the hippocampus occurs mainly in the subgranular zone (SGZ) of the dentate gyrus (DG), from which the cells migrate to the granular zone. Up-regulation of neurogenesis occurs in response to enriched environment, exercise, and learning (Farmer et al. 2004; Kempermann et al. 1997; van Praag et al. 1999). Down-regulation occurs in response to ageing and stress (psychological or environmental) (Cameron & McKay, 1999; Kuhn et al. 1996; McEwen, 1999).

Neurogenesis is regulated by a number of growth factors and by neurotrophic factors (Cameron et al. 1998; Nakagawa et al. 2002). The hippocampus is an especially plastic and vulnerable region and a target of stress hormones (gonadal, thyroid, and adrenal hormones). Chronic stress causes atrophy of dendrites in the CA3 region, and both acute and chronic stress suppress neurogenesis of rat DG granule neurons. It is well established that the volume of the hippocampus is decreased in patients suffering from depression (Bremner et al. 2000; Sheline et al. 1996). This reduction in hippocampal volume observed in depression might be related to hippocampal cell loss.

Chronic but not acute administration of several classes of antidepressant treatment up-regulates neurogenesis in adult, rodent hippocampus (Duman et al. 2001). There is evidence that antidepressant drugs increase turnover of hippocampal neurons, i.e. increase both neurogenesis and neuronal elimination (Sairanen et al. 2005). While antidepressant treatment up-regulates neurogenesis in the SGZ of the hippocampal DG of adult rats, this treatment does not up-regulate neurogenesis in the subventricular zone (SVZ), which is another brain area that shows proliferation of neural stem cells in the adult rat brain.

Reduction in hippocampal neurogenesis is hypothesized to contribute to the development of depression. Santarelli et al. (2003) demonstrated that preventing hippocampal neurogenesis by X-irradiation in a restricted region of the brain containing the hippocampus does not cause depressive-like behaviour in naive mice. However, this study group (Santarelli et al. 2003) also showed that preventing hippocampal neurogenesis by X-irradiation prevents both hippocampal neurogenesis and behavioural antidepressant effects of antidepressants. Animal models of depression such as chronic mild stress (Surget et al. 2008) or social isolation (Lapiz et al. 2003) have been shown to reduce baseline hippocampal neurogenesis in rats (Surget et al. 2008; Westenbroek et al. 2004). Stress is probably a more accurate physiological paradigm to inhibit neurogenesis and induce a depression-like response. David et al. (2009) demonstrated that in a mouse model of depression (corticosterone-treated mice) there is a reduction in neurogenesis as well as depressive-like behaviour in some of the behavioural paradigms and these immunohistochemical and behavioural changes are reversed by antidepressant treatment. This study group (David et al. 2009) further demonstrated that stress causes depressive-like behaviour that is not reversed by antidepressant treatment when neurogenesis is prevented by X-ray irradiation of the hippocampus.

Thyroid hormones have an important role in the developing nervous system as well as in survival and maturation of newly born neurons in the adult brain. Maternal and fetal thyroid hormones have an important role in fetal neurodevelopment until birth (Chan & Kilby, 2000; de Escobar et al. 2004). It has been shown that in amphibian development, there is a direct role for thyroid hormones in regulating spinal cord neurogenesis and function (Marsh-Armstrong et al. 2004; Schlosser et al. 2002). Thyroid hormones were found to be required for adult neurogenesis both in the SVZ (Lemkine et al. 2005) and the hippocampal SGZ (Ambrogini et al. 2005; Desouza et al. 2005; Madeira et al. 1991; Montero-Pedrazauela et al. 2006; Rami et al. 1986; Uchida et al. 2005). Hypothyroidism in rat models is associated with decreased hippocampal neurogenesis which is reversed by thyroid hormone replacement (Ambrogini et al. 2005; Desouza et al. 2005; Montero-Pedrazauela et al. 2006) and decreased proliferation and apoptosis of progenitor cells in the SVZ (Lemkine et al. 2005). Manipulations of thyroid hormones have been shown to influence hippocampal morphology, physiology as well as learning and memory (Gould et al. 1991). Regulation of hippocampal neurogenesis might be an antidepressant mechanism of T3.

Regulation of hippocampal neurogenesis by T3 may be a downstream consequence of T3-induced changes in the serotonergic system. One antidepressant mechanism of T3 is thought to be at the cell membrane level involving potentiation of neurotransmission via effects on 5-HT1A and 5-HT1B serotonin receptors (Bauer et al. 2002, 2008; Gur et al. 2002; Lifschytz et al. 2006b) that...
are involved in the regulation of hippocampal neurogenesis (Zhao et al. 2008). Activation of 5-HT1A serotonin receptors was found to increase hippocampal neurogenesis while blockade of 5-HT1B serotonin receptors was found to decrease hippocampal neurogenesis (Banasr et al. 2004). 5-HT1A receptors were also found to be required for fluoxetine-induced neurogenesis (Santarelli et al. 2003). Therefore, T3 might influence hippocampal neurogenesis by regulating serotonin receptors.

Another, possibly related antidepressant mechanism is at the genomic level. T3 receptors (TRs) are transcription factors that suppress and in some cases activate target gene expression in a hormone-dependent or -independent fashion (Zhang & Lazar, 2000). T3 activity is mediated by two nuclear receptors, TRα and TRβ, encoded by two genes, TRα and TRβ, respectively (Baxter et al. 2001). TRα and TRβ receptors display remarkably conserved sequences in their DNA-binding, ligand-binding and ligand-dependent transactivation domains, but their sequences differ completely in the N-terminal region. The consequences of these structural homologies are that these receptors bind the same ligand and the same motifs on DNA. Despite these similarities, each of them is likely to mediate a limited number of the physiological activities of T3 (Gauthier et al. 1999). For example, both TRα and TRβ are involved in the regulation of thyroid-stimulating hormone production, although in different ways: disruption of the TRβ gene leads to hyperthyroxinaemia (Forrest et al. 1996; Weiss et al. 1998); disruption of the TRα1 and TRΔα1 genes leads to moderate hypothyroxinaemia and mild hypothyroidism (Wikström et al. 1998); while disruption of the TRα1 and TRα2 genes leads to hypothyroxinaemia which increases upon ageing and severe hypothyroidism (Fraichard et al. 1997). T3 nuclear receptors have been localized in neurons, glial cells and ependymal cells of adult rat central nervous system, including the DG of the hippocampus (Puymirat et al. 1991). Regarding neuronal proliferation, TRα, but not TRβ, was found to be expressed in nestin-positive progenitor cells of the SVZ (Lemkine et al. 2005), but no data exists regarding the differential proliferating activity of TRα or TRβ in the DG of the hippocampus.

The aim of this work was to determine whether chronic administration of T3 to euthyroid rats influences neurogenesis and whether combined administration of T3 and the antidepressant drug, fluoxetine, results in enhancement of hippocampal neurogenesis beyond that induced by fluoxetine alone. A further aim of the study was to examine the effect of chronic administration of T3 alone and in combination with fluoxetine in a behavioural paradigm that is sensitive to the effects of antidepressant drugs, the novelty suppressed feeding test (NSFT).

Methods

Animals

Male albino rats (Sabra strain) were used in all experiments, six animals per group in the immunohistochemistry study and 12 animals per group in the behavioural study. The rats were housed four per cage in a temperature-controlled environment (22 ± 2 °C) with a regular 12-h light/dark cycle (lights on 07:00 hours). Food and water were freely available. The animals weighed 150–225 g at the beginning of the experiment. All procedures were carried out according to the guidelines of the Institutional Animal Care and Use Committee of the Hebrew University Faculty of Medicine and Hadassah Medical Organization which approved the research protocol.

Treatments

Drugs were administered once daily for 21 d: (i) 5 mg/kg fluoxetine by intraperitoneal (i.p.) injection; (ii) 50 μg/kg T3 subcutaneously (s.c.); (iii) 5 mg/kg i.p. fluoxetine plus 50 μg/kg T3 s.c.; (iv) 1 ml/kg i.p. saline. Drugs were administered at doses equivalent to those administered in our previous microdialysis and behavioural experiments (Gur et al. 2004a; Lifschytz et al. 2004, 2006a). Chronic administration of the drugs (21 d) was based on previous studied that showed that both hippocampal neurogenesis (Malberg et al. 2000) and NSFT (Dulawa et al. 2004) are responsive only to chronic but not acute administration of antidepressants. Fluoxetine was kindly provided by Teva Pharmaceutical Industries, Israel. T3 was obtained from Sigma-Aldrich-Israel (cat. no. T2752).

In the first study animals were deeply anaesthetized on day 22 and intracardially perfused with paraformaldehyde. Brains were removed and post-fixed for immunohistochemical analysis. In the second study, NSFT was performed on day 22, after which the animals were killed.

Immunohistochemistry

Tissue preparation for immunohistochemistry

Twenty-four hours after the last injection animals were deep anaesthetized using an overdose of pentobarbital, and intracardially perfused with PBS followed by cold 4% paraformaldehyde (PFA/PBS). Brains were removed and post-fixed in 4% PFA/PBS
overnight, and then transferred into 20% sucrose. Brains were deep frozen in liquid nitrogen, and stored at −70°C. Serial 8–10 μm coronal cryostat sections were performed spanning the striatum and hippocampus for immunohistochemical analysis.

**Immunohistochemistry for doublecortin (DCX)**

Sections were fixed in 4% formaldehyde for 10 min at room temperature. After three PBS washes, sections were incubated in 3% H₂O₂ in methanol for 30 min. Following two washes with double diluted water and one wash with PBS, sections were incubated in 0.1% Tween in PBS for 5 min. After one more wash with PBS, sections were incubated with the primary antibody (goat anti-DCX 1:50, Santa Cruz Biotechnology Inc., USA) for 1 h at 37°C, and at +4°C overnight. The next morning after three washes with PBS sections were incubated with the secondary antibody (biotinylated anti-goat IgG, made in horse, 1:100, Vector Laboratories, USA) for 1 h at room temperature, and visualized using an avidin-biotin-peroxidase complex system (Vectastain ABC Elite kit, Vector Laboratories). As substrate for the peroxidase reaction, diaminobenzidine (DAB; Dako, Denmark) was applied until a brown end-product was visualized. Sections were gently washed, mounted with glycerol, covered and air dried (Ben-Hur et al. 2003).

**Immunohistochemistry quantification**

All quantitative histological evaluations were performed in a uniform fashion by an observer who was blinded to the experimental group. The total number of DCX-positive (DCX⁺) cells in the SGZ of the DG of the hippocampus, or the anterior-lateral SVZ, were counted on coronal brain sections and then extrapolated for each brain (n = 6). Basically, the DCX⁺ cells were counted in bregma areas −1.3 mm to −4.3 mm, in 0.5-mm intervals. In each bregma area, 28 sections (8–10 μm) were performed and every third or fourth section was stained for DCX immunohistochemical analysis (i.e. a total of six brain sections in each bregma area). To distinguish single cells all counts were performed at 40 × under a light microscope (Leica). A cell was counted as being in the SGZ if it was within or as far as two cells away from the SGZ. Comparison of the effect of treatments on neurogenesis in the hippocampus vs. the SVZ indicated whether the effect is general or specific to the hippocampus.

**Behavioural studies**

The NSFT was based on the paradigm first described by Bodnoff et al. (1988). NSFT is responsive only to chronic administration of antidepressants (Dulawa et al. 2004) and therefore more closely represents the clinical response to treatment in humans. Following the last injection food was removed from the home cage, although access to water was free. Twenty-four hours later each animal was placed in a corner of an open-field apparatus (1 m × 1 m, 40 cm high) containing approximately 10 pellets of food in the centre. The base of the arena was divided into 10 cm × 10 cm squares. The main measurement was the time taken for the animal to reach the food and begin eating. If the animal did not reach the food within 10 min it was taken out of the arena, the test was ended and the latency to feeding was recorded and analysed as 10 min. Motor activity was defined as the average change in the location of an animal during a time unit. Thigmotaxis was defined as the percentage of motor activity performed in the centre of the open field. After being taken out of the open field the animal was placed in a cage containing a weighed amount of food. The food was weighed again after 30 min, and the difference between the two weighings constituted the ‘home cage consumption’. This measurement represents the degree of the animal’s appetite.

**Data analysis**

Data were analysed by one-way analysis of covariance (ANCOVA) with weight gain by the animals as a covariate in both the neurogenesis and NSFT analyses and motor activity as an additional covariate in the NFST analysis ANCOVA was followed by contrast analysis employed to test specific a priori hypotheses if the p value for the overall treatment effect was <0.1.
Results

Effect of fluoxetine, T3 and fluoxetine + T3 on neurogenesis in the SGZ of the hippocampus and the SVZ

Figure 1 shows immunohistochemistry for DCX+ cells in the SGZ of rats administered saline, fluoxetine, T3, and fluoxetine + T3. Since ANOVA revealed a significant effect of treatment on weight gain [68%±12%, 66±13%, 50%±14% and 50%±18% in the saline-, fluoxetine-, T3-, and fluoxetine + T3-treated animals, respectively (F=3.6, d.f. =3, 20, p=0.031)], this variable was entered as a covariate in all the following analyses. A main effect of antidepressant treatment on number of DCX+ labelled cells in the SGZ of the hippocampus was found (F=10.10, d.f. =3, 19, p=0.003). As shown in Fig. 2a, contrast analysis revealed that fluoxetine and fluoxetine + T3 increased the number of DCX+ cells compared to saline (p=0.00005, p=0.008, respectively). There was a trend towards an increased number of DCX+ cells in T3-treated rats compared to saline-treated rats, but this did not reach a statistically significant level (p=0.06). Combined treatment with fluoxetine + T3 further increased the number of DCX+ cells compared to T3 or fluoxetine alone (p=0.001, p=0.014, respectively). There was no effect of treatments on number of DCX+ cells in the SVZ, confirming that this effect is specific to the hippocampus (Fig. 2b).

Effect of fluoxetine, T3 and fluoxetine + T3 on rat behaviour in the NSFT

The main outcome measure of the NSFT is latency to feeding. Since ANOVA revealed a significant effect of treatment on weight gain and on general motor activity in the open field (Table 1), these variables were entered as covariates in all the following analyses. A main effect of antidepressant treatment on reducing latency to feeding was found (F=10.18, d.f. =3, 34, p=0.00006). As shown in Fig. 3a, contrast analysis revealed that all the antidepressant treatments (T3, fluoxetine + T3, and fluoxetine) reduced latency to feeding compared to control rats (p=0.0004, p=0.00001, p=0.00009, respectively). Combined treatment with fluoxetine + T3 further reduced latency to feeding compared to T3 alone (p=0.05) but not compared to fluoxetine alone. There was no significant effect of treatment on thigmotaxis (Fig. 3b). Reduced latency to feeding was not due to enhanced appetite since there was no effect of treatments on the amount of food consumed in the home cage following the test (Table 1).

Discussion

The results of this study suggest that the effect of T3 to enhance antidepressant action may be related to its effect on hippocampal neurogenesis. In rats administered a combination of fluoxetine + T3, neurogenesis in the SGZ of the hippocampus was increased to a significantly greater extent than in rats administered fluoxetine alone or T3 alone. In the behavioural paradigm that we studied in parallel, the NSFT, our results show that T3 induced a significant reduction in latency to feeding. In rats administered fluoxetine + T3 concurrently, latency to feeding was significantly shorter than with T3 alone. However, fluoxetine + T3 was not more effective than fluoxetine alone, possibly due to a ceiling effect of fluoxetine in the behavioural paradigm. T3 (alone and in combination with fluoxetine) was associated with a lesser degree of weight gain than in saline-treated rats and both fluoxetine (alone and in combination with T3) were associated with increased motor activity in the open field. The reason for the increase in motor activity is not clear. However, these effects were controlled for in the statistical analyses. Additional variables that might have been included in the analysis were serum hormone concentrations and fluoxetine and norfluoxetine levels
but these were not available. With regard to hormone concentrations, we identified two previous studies (including one from our own group) in which T3 and T4 levels were measured in rats administered T3. In the report of Gur et al. (2004b) 100 μg/kg/d T3 for 7 d did not alter fT3 levels but caused a dose-dependent reduction in fT4 levels. In Moreau et al. (2001) the T3 dose was 4 μg/kg/d. T3 induced a significant decrease in fT4 concentrations on both 7-d and 21-d treatments; there was no significant effect on fT3 serum concentration.

T3 might increase the total number of functional newborn neurons by enhancing mitotic activity of the neural precursors, enhancing new cell survival or reducing apoptosis rate. As shown by Ambrogini et al. (2005) and by Desouza et al. (2005), hypothyroidism in adult rats causes a decrease in newborn cell survival but not in mitotic activity of the neural precursors in the SGZ and the net effect is a lowering of the number of immature neurons added to the granule cell layer. Montero-Pedrazuela et al. (2006) showed a 30% reduction in the number of proliferating cells in the DG of hypothyroid rats, as measured by both bromodeoxyuridine (BrdU) incorporation and DCX staining. In this study, it was also shown that hypothyroid rats displayed abnormal behaviour in the forced swim test (FST), indicating a depressive-like disorder, and that both immunohistochemical and behavioural changes were normalized by restoring euthyroid status (Montero-Pedrazuela et al. 2006). Desouza et al. (2005) reported that adult onset hyperthyroidism did not influence hippocampal neurogenesis. However, in this study, hyperthyroidism was induced by a very high T3 dosage (animals received s.c. injections of 1000 μg/kg.d T3), while in the same study, restoring euthyroid status in hypothyroid rats was achieved by daily s.c. injections of T3 (10 μg/kg) and T4 (10 μg/kg) (Desouza et al. 2005). We found that fluoxetine +T3 (50 μg/kg s.c.) increased hippocampal neurogenesis and that T3 (50 μg/kg s.c.) alone also increased hippocampal neurogenesis but this did not reach statistical significance. In the SVZ, Lemkine et al. (2005) found that hypothyroidism decreases both proliferation and apoptosis of progenitor cells and decreases migration of neuroblasts out of the stem cell niche. We found no effect of T3 on subventricular neurogenesis in naive rats (i.e. without hypothyroidism), as was also found by Lemkine et al. (2005). This suggests that the antidepressant effect of fluoxetine, T3 and the combination of fluoxetine +T3 is specific to the DG and does not represent a general effect on cell proliferation. This differential effect of antidepressants on hippocampal

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**Table 1.** Effect of fluoxetine, T3 and fluoxetine +T3 on weight gain, general motor activity in the open-field and home cage consumption in the novelty-suppressed feeding test experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight before treatment (g)</th>
<th>Weight after treatment (g)</th>
<th>Weight gain (%)</th>
<th>Motor activity (lines/min)</th>
<th>Home cage consumption (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>185 ± 17</td>
<td>312 ± 28</td>
<td>69 ± 11%</td>
<td>17.6 ± 14.45</td>
<td>3.4 ± 1.4</td>
</tr>
<tr>
<td>Fluoxetine (5 mg/kg)</td>
<td>190 ± 17</td>
<td>311 ± 22</td>
<td>64 ± 10%</td>
<td>38.4 ± 7.71</td>
<td>3.5 ± 1.2</td>
</tr>
<tr>
<td>T3 (50 μg/kg)</td>
<td>185 ± 20</td>
<td>288 ± 25</td>
<td>56 ± 15%</td>
<td>34.7 ± 10.33</td>
<td>4.1 ± 1.2</td>
</tr>
<tr>
<td>Fluoxetine (5 mg/kg) + T3 (50 μg/kg)</td>
<td>189 ± 17</td>
<td>281 ± 33</td>
<td>49 ± 14%</td>
<td>33.2 ± 12.26</td>
<td>3.5 ± 1.9</td>
</tr>
</tbody>
</table>

Results are mean ± s.d.  
*p < 0.05 (for weight gain vs. saline); ^p < 0.05 (for motor activity vs. saline). Other comparisons are not significant.

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**Fig. 3.** Effect of fluoxetine, triiodothyronine (T3), and fluoxetine +T3 on (a) latency to feeding in the novelty-suppressed feeding test and (b) thigmotaxis, controlled for weight gain and motor activity. Animals per group (n = 12), *p ≤ 0.05, **p < 0.001. Bars represent standard error of the mean.
neurogenesis but not on proliferation in other brain areas is well documented (Malberg et al. 2000).

Adult neurogenesis is typically detected by incorporation of BrdU into dividing cells and co-labelling of BrdU-positive cells with markers for mature neurons. In this study we used DCX, an alternative indicator for adult neurogenesis, which is transiently expressed in proliferating progenitor cells and newly generated neuroblasts and decreases as the newly generated cells express mature neuronal markers (Brown et al. 2003; Couillard-Despres et al. 2005). The efficacy of DCX as a marker to analyse the number of newly generated neurons in the adult DG was demonstrated by Rao & Shetty (2004). They found that BrdU was expressed in 90% of DCX + neurons in the DG of these animals and that almost all DCX + cells expressed early neuronal antigens but lacked antigens specific to glia, undifferentiated cells or apoptotic cells, and therefore they concluded that DCX expression was specific to newly generated neurons (Rao & Shetty, 2004). However, further investigation, using specific markers of cell mitosis, survival and apoptosis, is needed in order to determine the specific characteristics of the increased hippocampal neurogenesis induced by T3 and their relationship to the antidepressant effect of the hormone.

Although fluoxetine alone and fluoxetine + T3 increased hippocampal neurogenesis compared to saline, T3 alone did not do so to a statistically significant level. A possible explanation might be that in our study we used naïve healthy, rats. A design that might yield stronger effects would be to employ rats exposed to a validated animal model of depression such as chronic mild stress (Surget et al. 2008), social isolation (Lapiz et al. 2003) or corticosterone-treated mice (David et al. 2009). Since all animal models have been shown to reduce baseline hippocampal neurogenesis in rats (David et al. 2009; Surget et al. 2008; Westenbroek et al. 2004), the effect of T3 on hippocampal neurogenesis might be magnified using these models.

Compared to models of depression per se, behaviourial models of antidepressant-like effect need not have high face validity but must have a high predictive value for antidepressant efficacy in the clinic. Since antidepressants have strong anti-anxiety effects, behavioural tests with a significant anxiety component are also employed. It has been consistently shown that antidepressant drugs shorten the latency period in the NSFT after chronic treatment and that this test can serve as a model for anti-anxiety and antidepressant-like activity (Dulawa et al. 2004; Dulawa & Hen, 2005). Our results suggest that the effect of all treatments (T3, fluoxetine, and fluoxetine + T3) on latency to feeding is related to an antidepressant-like effect rather than an anxiolytic-like effect, since there was no significant effect of treatments on thigmotaxis. In a previous report from our laboratory, some behavioural effects were reported in the FST paradigm in male rats with the combination of fluoxetine + T3 (100 mg/kg) but not with T3 administered alone for a short period (100 mg/kg for 7 d) or at a low dosage (20 mg/kg for 2 wk) (Lifschytz et al. 2006a). However, in recent work in our laboratory, antidepressant-like effects were found in three behavioural tests (FST, the tail suspension test and the NSFT) with administration of 50 mg/kg T3 for 21 d (Lifschytz et al. unpublished observations).

In conclusion, our results show that enhanced hippocampal neurogenesis might be the antidepressant mechanism of T3. Research on the molecular basis of adult hippocampal neurogenesis in depression models and after antidepressant treatment, as well as a focus on the specific effects of T3, may help to explain how depression develops and how antidepressant treatments including T3 reverse its development.

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Statement of Interest
None.

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