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Transthyretin is involved in depression-like behaviour and exploratory activity

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Abstract

Transthyretin (TTR), the major transporter of thyroid hormones and vitamin A in cerebrospinal fluid (CSF), binds the Alzheimer β-peptide and thus might confer protection against neurodegeneration. In addition, altered TTR levels have been described in the CSF of patients with psychiatric disorders, yet its function in the CNS is far from understood. To determine the role of TTR in behaviour we evaluated the performance of TTR-null mice in standardized tasks described to assess depression, exploratory activity and anxiety. We show that the absence of TTR is associated with increased exploratory activity and reduced signs of depressive-like behaviour. In order to investigate the mechanism underlying these alterations, we measured the levels of cathecolamines. We found that the levels of noradrenaline were significantly increased in the limbic forebrain of TTR-null mice. This report represents the first clear indication that TTR plays a role in behaviour, probably by modulation of the noradrenergic system.

Keywords: behaviour, norepinephrine, retinoids, thyroid hormone, thyroxine, transthyretin.

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Transthyretin (TTR), a major protein synthesized by the choroid plexus and the liver, is a plasma and cerebrospinal fluid (CSF) transporter of thyroxine (T4) and vitamin A [retinol (through association with retinol-binding protein) Kabat *et al*. 1942; Hagen and Elliott 1973]. Thyroid hormones (THs) and vitamin A are essential for normal brain development (Morriss-Kay and Ward 1999; Anderson 2001; Bernal 2002) and fluctuations of their circulating levels are reported to interfere with mood (Bauer *et al*. 2002). Triiodothyronine and retinoic acid, the biologically active compounds generated locally from T4 and retinol, respectively, affect the expression of a wide range of genes upon interacting with their nuclear receptors. Transcriptionally active heterodimers of these two receptors have been characterized indicating possible synergistic actions of both molecules (Anderson 2001; Bernal 2002). Among the processes regulated by THs and retinoids are differentiation of the cerebellum, axonal migration and myelination, and transcriptional regulation of enzymes, receptors and transporters of the neurotransmitter cascades (Rodrigues-Pena 1999; Smith *et al*. 2001; Balmer and Blomhoff 2002).

Given its ontogenetic and phylogenetic expression patterns, TTR was considered to be essential for delivering its ligands to target tissues, particularly to the CNS (Southwell *et al*. 1993; Schreiber *et al*. 1995). However, studies in TTR-null mice have questioned whether TTR is indeed essential for TH transfer into the brain (Palha *et al*. 2000, 2002; Palha 2002). TTR-null mice display significantly reduced levels of THs in the choroid plexus and no other protein appears to replace TTR in the transport of T4 in the CSF (Palha *et al*. 2000). Furthermore, under normal physiological conditions, distribution of THs within the brain is not impaired by the

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Abbreviations used: CSF, cerebrospinal fluid; HPLC, high-performance liquid chromatography; NE, norepinephrine; T4, thyroxine; TH, thyroid hormones, TTR, transthyretin.

absence of TTR (Palha *et al*. 2002). In addition, TTR-null mice do not present classical features of altered TH homeostasis (Episkopou *et al*. 1993; Palha *et al*. 1994, 1997).

Several reports suggest that TTR might have other biologically relevant ligands (Palha 2002). Decreased levels of TTR in the CSF have been observed in patients with psychiatric disorders such as depression (Sullivan *et al*. 1999). Decreased TTR levels in the CSF have also been found in patients with Alzheimer's disease, although no identified mutations of TTR are associated with the disease (Riisoen 1988; Palha *et al*. 1996). TTR binds the Alzheimer β-peptide (Schwarzman *et al*. 1994), possibly protecting against neurodegeneration (Stein and Johnson 2002) and mediating clearance of the brain β-peptide (Carro *et al*. 2002). TTR also binds pterins (cofactors in the biosynthesis of cathecolamines; Ernström *et al*. 1995) and oxidation products of norepinephrine (NE; Boomsma *et al*. 1991), and therefore might influence cathecolaminergic function.

These observations underline the need to investigate further the effect of TTR on behaviour, using established behavioural paradigms. In order to assess its possible role in regulating brain functions relevant to mental illness we characterize, in the present study, the behaviour phenotype of TTR-null mice. We show that TTR is involved in depression and exploratory activity, probably as a consequence of altered NE neurotransmission in the limbic forebrain.

Experimental procedures

Animals

Fifty TTR-null (25 female and 25 male) and 50 wild-type (25 female and 25 male) mice of 129Sv/EV genetic background, bred separately; and six TTR-null and six littermate wild-type were kept and used strictly in accordance with National and European Union guidelines for the care and handling of laboratory animals. Animals in groups of five were housed in 26 × 20.5 × 14 cm cages, separated by gender and genotype, weaned at 3 weeks of age and given standard rodent chow and water *ad libitum*. Animals were kept with a light/dark cycle of 12 h (light period from 08.00 to 20.00 h, 40 W), at 22.5°C ± 1 and 55% ± 5 humidity.

All behavioural tests were carried out during the light phase of the light/dark cycle. Between each test, animals were allowed to rest for the same amount of time, for a period of at least 1 week. The following experimental sequence of tests was performed in all animals: forced swim test, elevated plus maze and open field. A separate group of animals was used for neurochemical analysis. Animals were killed by careful and rapid decapitation; the brains were removed, rapidly frozen and stored at −80°C until analysis.

Eye opening and cage behaviour

Mice were qualitatively observed once a day from birth until 96 days of life and from then on once a week. The day of eye opening was registered. Details of home cage locomotion, social behaviour between males and maternal behaviour (nesting, nursing and pup retrieval) were recorded.

Forced swim test

We used the forced swim test to evaluate the failure to seek escape from an aversive stimulus. This test has been proposed to reflect 'behavioural despair', and shown to respond specifically to a wide range of antidepressant drugs (Porsolt *et al*. 1978). Mice aged 10–12 weeks were placed in a 25 cm diameter, 10 cm water depth, glass pool containing water at 25 ± 1°C. During each trial the mouse was left in the pool for 10 min. The animals were submitted to two trials, each on two consecutive days. To score activity, behaviours recorded on videotapes were rated in five categories: immobility, slow swimming, fast swimming, struggling and total turns in the pool; and analysed using the The Observer Basic 3.0 program (Noldus International Technology, Leesburg, VA, USA). The frequency and duration of behavioural categories were scored for both trials. The number of defecations was recorded.

Elevated plus maze

The elevated plus maze was used to measure anxiety-like behaviour. In this test the mouse is presented with a conflict between its innate tendency to explore novel spaces and its fear of open and/or elevated places (File *et al*. 2000). Increased time spent in the open arms of the maze is interpreted as a decrease in anxiety behaviour.

The elevated plus maze consisted of a plastic cross of two closed arms (25 × 10 × 15 cm) and two open arm, (25 × 10 × 0 cm), raised 50 cm above the floor. For each test, the subject was placed in the centre of the cross, facing an open arm and was allowed to explore the maze for 5 min. The session was videotaped. Using the The Observer Basic 3.0 the numbers of entries into open arms, closed arms and new arm; the percentage of time spent in open arms, closed arms and arms intersection; and the head-dipping over sides of open arms and the latency to enter in the first arm were measured. The number of defecations was recorded and the maze was wiped with damp cloth with a neutral detergent.

Open field

The open field test is commonly used to measure exploratory, locomotor and anxiety-like behaviour (Bolivar *et al*. 2000). The open field used was a square arena (41 × 41 × 37 cm) with infrared beams located 2 cm above the arena floor on both X and Y axes (to monitor the central and peripheral horizontal activities) and 9 cm above the floor on two sides of the chamber (to monitor vertical activity; San Diego Instruments, San Diego, CA, USA). The mouse was placed in the centre of the arena and allowed to explore the arena for 12 min. Two trials each on two consecutive days were performed. The number of defecations was recorded. Between sessions, the arena was wiped as described above.

Neurochemistry

On the day of the analysis, brains were thawed and the following regions dissected: cerebellum, brain stem, hippocampus, hypothalamus, striatum, limbic forebrain (containing nucleus accumbens, septal nuclei, prefrontal cortex, olfactory tuberculi and amygdala) and cerebral cortex. All regions were homogenized in 20–40 vol of cold 150 mmol/L potassium phosphate buffer, pH 6.8. An aliquot was used for the analysis of protein concentration.

The contents of dopamine and NE were analysed using high-performance liquid chromatography (HPLC) with electrochemical detection according to our previously published method (Romero

et al. 1995; González *et al*. 1999). For serotonin and 5-hidroxyindolacetic acid, homogenates were diluted (½) in ice-cold 0.4 N perchloric acid containing 0.4 mM sodium disulphite and 0.90 mM EDTA. *N*-methyl-serotonin was added as an internal standard. Supernatants of diluted homogenates were injected into the HPLC system which consisted of a Spectra-Physics 8810 isocratic pump and an RP-18 column (Spherisorb ODS-2; 125 mm, 4.6 mm, 5 μm particle size; Waters, MA, USA). The mobile phase consisted of 100 mM citric acid, 100 mM sodium acetate, 1 mM EDTA and 7% methanol (pH 4.2) and the flow rate was 0.8 mL/min. The effluent was monitored with a Metrohm bioanalytical system amperometric detector using a glassy carbon electrode. The potential was 0.80 V relative to an Ag/AgCl reference electrode with a sensitivity of 50 nA (approx. 2 ng/sample). The signal was recorded on a Spectra-Physics 4290 integrator. The results were given as area under the peaks and calculated by comparison with the area under the corresponding internal standard peak.

The activity of tyrosine hydroxylase was measured as previously described (Nagatsu *et al*. 1979). The number of tyrosine hydroxylase neurones was evaluated by immunohistochemistry with antityrosine hydroxylase (Affiniti, Exeter, UK), on 40-μm brain sections, counterstained with thionin using standard staining procedures.

The activity of monoamine oxidase A was measured in the cerebellum and limbic forebrain homogenates (40 μg protein per assay), using the Amplex Red Monoamine Oxidase Assay Kit (Molecular Probes, Eugene, OR, USA).

Statistical analysis

The results were analysed using the statistical package Statistica 5.5 (StatSoft, Tulsa, OK, USA) and expressed as mean ± SEM. Significance was calculated using Student's *t*-test, ANOVA or MANOVA. Post-hoc Tukey test was performed when necessary. Differences were considered significant when $p < 0.05$.

Results

Eye opening and cage behaviour

Home cage behaviour did not differ between the two groups of animals. Males did not display abnormal aggressive behaviour and females presented typical maternal behaviours. Bilateral eye opening for both groups of animals occurred, on average, at day 12.

Forced swim test

Using a three-way analysis of variance design – group (TTR-null mice, wild-type) versus sex (male, female) versus trials (trial 1, trial 2) – and as shown in Fig. 1, TTR-null mice were significantly more active than wild-type in both trials. TTR-null mice exhibited less percentage of immobility time ($F_{1,95} = 63.05$, $p < 0.0001$), increased active behaviours [slow swimming ($F_{1,95} = 55.32$, $p < 0.0001$) and struggling ($F_{1,95} = 28.02$, $p < 0.0001$)] and made more turns in the pool ($F_{1,95} = 37.28$, $p < 0.0001$) than the control mice. No statistically significant gender differences within each genotype were found.

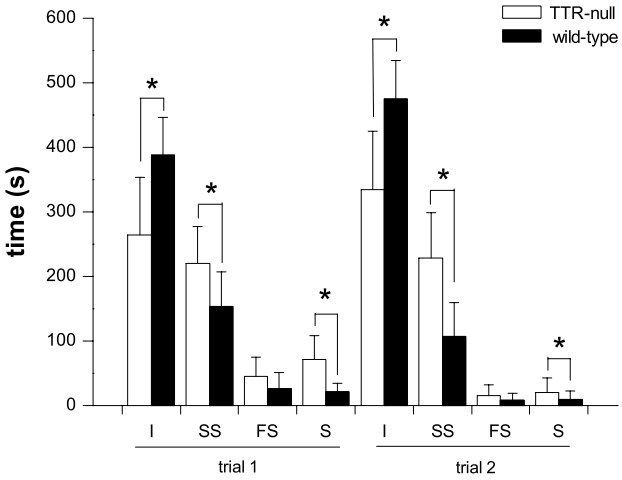

Fig. 1 Forced swim test reveals increased activity of TTR-null mice. Immobility (I), slow swimming (SS), fast swimming (FS) and struggling (S) during first (1) and second (2) trials; $*p < 0.0001$.

A slight habituation was observed for both TTR-null and wild-type mice from the first to the second trial, as revealed by the increase in immobility time ($F_{1,95} = 78.26$, $p < 0.0001$) and the total number of turns ($F_{1,95} = 26.73$, $p < 0.001$), and it was observed an interaction group versus trials with a decrease in struggling time ($F_{1,95} = 46.83$, $p < 0.0001$). Only wild-type mice showed habituation for slow swimming ($F_{1,95} = 10.65$, $p < 0.001$).

When only the last 6 min of the first trial were analysed using non-paired Student's *t*-test, the data were consistent with the previous analysis.

Identical results were obtained when littermate TTR-null and wild-type mice were used.

Elevated plus maze

Using a three-way analysis of variance design – group (TTR-null mice, wild-type) versus sex (male, female) versus trials (trial 1, trial 2, trial 3) – the results revealed that both the TTR-null and the wild-type mice show a preference for the closed versus the open arms of the maze (Fig. 2). For all behavioural categories scored (time spent in the arms intersection, closed arms, open arms; the number of entries into closed arms and the number of head-dips), there were no statistical significant differences between the groups. No statistically significant gender differences within each genotype were found.

Open field activity

Using a three-way analysis of variance design – group (TTR-null mice, wild-type) versus sex (male, female) versus trials (trial 1, trial 2) – and as seen in Fig. 3, both male and female TTR-null mice had statistically significant increased locomotor activities when compared to wild-type mice.

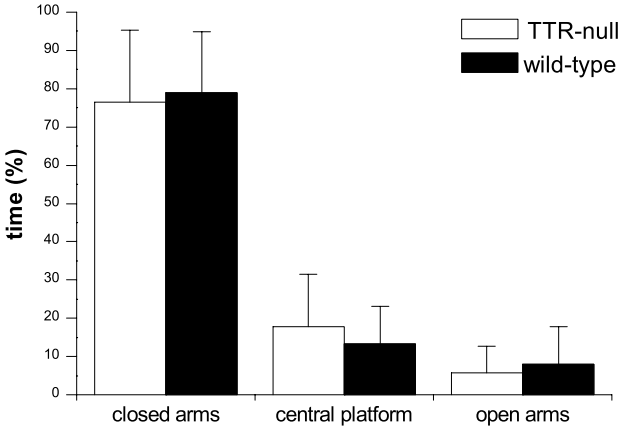

Fig. 2 The elevated plus maze shows that both TTR-null and wild-type mice were very anxious, spending most of the time in the closed arms; $p > 0.05$.

When horizontal activity was analysed, the TTR-null mice were significantly more active in central ($F_{1,96} = 30.49$, $p < 0.0001$) (Fig. 3b) and in peripheral activity ($F_{1,96} = 26.73$, $p < 0.0001$) (Fig. 3c). There was a significant interaction between genotype and sex. Tukey test analyses indicated that, even though males were generally more active than females in both groups, only for the wild-type a main effect was observed ($p < 0.006$). From the first to the second trial, both groups displayed habituation, which was reflected by a decrease in the central ($F_{1,96} = 32.74$, $p < 0.0001$) and peripheral ($F_{1,96} = 186.87$, $p < 0.0001$) horizontal activities in the second trial. Specifically, TTR-null mice perform increased rearing relative to wild-type mice ($F_{1,96} = 79.05$, $p < 0.0001$) (Fig. 3a). An interaction between group versus sex versus trials was observed ($F_{1,96} = 3.97$, $p < 0.049$), but only the TTR-null males showed a significant decrease between first and second trials ($p < 0.002$).

TTR-null mice showed increased defecation compared to wild-type mice ($F_{1,96} = 26.50$, $p < 0.0001$). For both the TTR-null and wild-type mice, an increased number of defecations was observed from the first to the second trials, but no significant interaction between genotype and trial was found.

Identical results were obtained when littermate TTR-null and wild-type mice were used.

Neurochemistry

TTR-null mice showed increased levels of NE in the limbic forebrain while no differences were found in the other brain regions examined (Fig. 4a). The data of the three different groups of analyses were combined for presentation.

In addition, there were no statistically significant differences between the two groups of animals for dopamine, even though there is an evident trend of increased levels in the TTR-null hippocampus (Fig. 4b) or for the activity of tyrosine hydroxylase (data not shown).

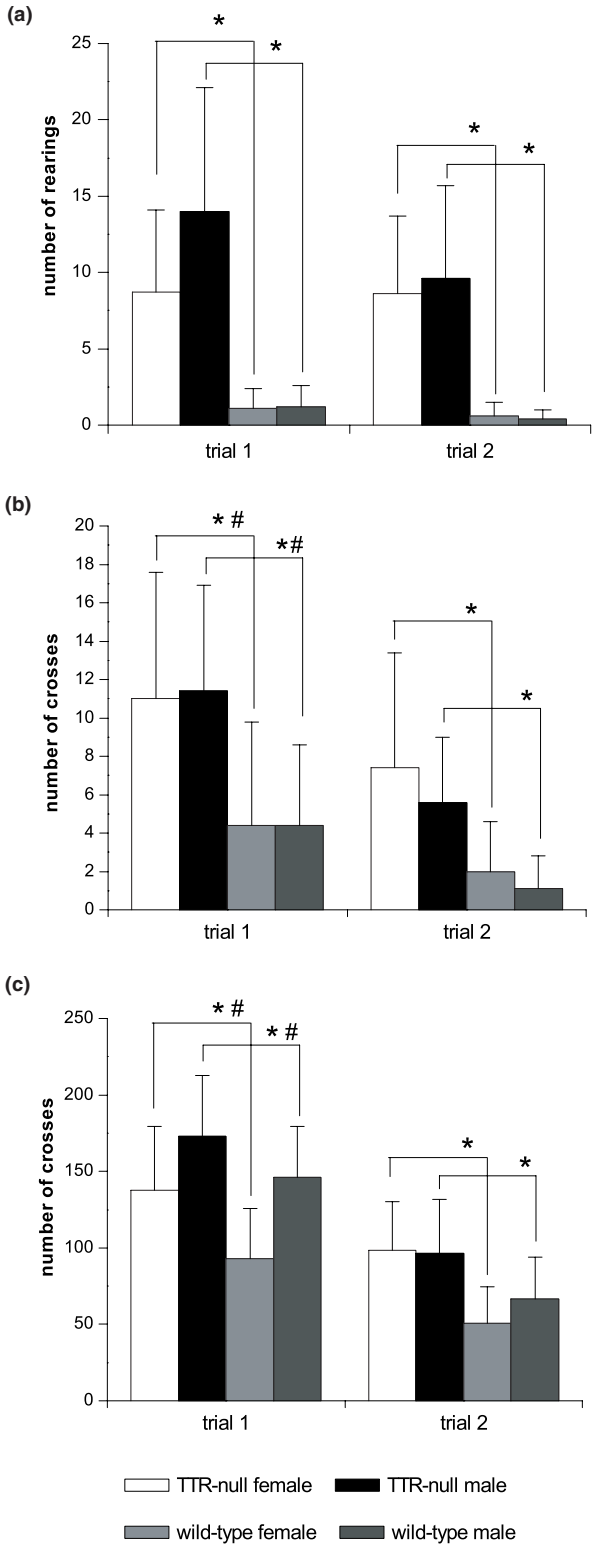

Fig. 3 TTR-null mice are more active than the wild-type, as assessed by the open field vertical (a), horizontal central (b) and horizontal peripheral activities (c); *$p < 0.0001$. Both genotypes revealed habituation from the first to the second trials in the central ($F_{1,96} = 32.74$, $p < 0.0001$) and peripheral ($F_{1,96} = 186.87$, $p < 0.0001$) activities (#).

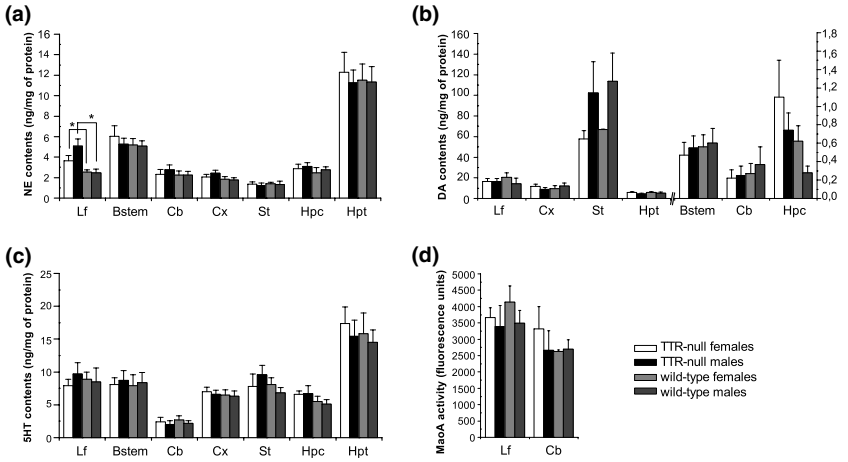

Fig. 4 HPLC measurements in brain regions revealed that TTR-null mice have increased levels of norepinephrine (NE) in the limbic forebrain (a) while no differences are observed for dopamine (DA; b) and serotonin (5-HT; c). The enzymatic activity of monoamine oxidase A (MaoA) is normal in the TTR-null mice (d). Five to 11 animals were measured for each parameter. Lf, limbic forebrain; Bstem, brain stem; Cb, cerebellum; Cx, cortex; St, striatum; Hpc, hippocampus; Hpt, hypothalamus; $^*p < 0.005$.

The TTR-null mouse brain presented the same regional distribution and number of tyrosine hydroxylase immunopositive neurones as the control mice, as determined by immunohistochemistry (data not shown).

The level of serotonin (Fig. 4c) and of its major catabolic metabolite, 5-hidroxyindolacetic acid (data not shown), were not altered in the TTR-null mouse brain.

The mRNA level of monoamine oxidase A, determined by RT–PCR (data not shown) was similar in both groups of animals, as was the enzymatic activity of monoamine oxidase A (Fig. 4d) as measured in tissue homogenates.

Discussion

Altered levels of TTR have been linked to a number of neuronal dysfunctions. Reduced CSF TTR have been associated with Alzheimer's disease and depression in both human and animal models (Riisoen 1988; Sullivan *et al*. 1999; Carro *et al*. 2002; Stein and Johnson 2002). Here we show that TTR-null mice displayed decreased immobility in the forced swimming test and increased exploratory activity in the open field test. Both alterations suggest an increased behavioural response to stress, reflecting either decreased behavioural inhibition or, alternatively, increased reactivity in brain circuits mediating motor responses to stressful environments. Yet, to the best of our knowledge, this is the first report demonstrating that TTR is implicated in behaviour.

It is well known that animals forced to swim in a restricted space will rapidly cease apparent attempts to escape and adopt a characteristic posture, termed 'immobility'. Previous experiments have consistently showed that immobility is reduced by a variety of antidepressant agents and this test has been extensively used as a screening model for antidepressants (Porsolt *et al*. 1977). Thus, the present demonstration that TTR-null mice are less prone to immobility in forced-swimming might be viewed as a potential resistance to depression. In support of this assumption, is the recent report of decreased expression and synthesis of TTR in the choroid plexus (Pulford *et al*. 2002) following chronic administration of lithium chloride, a mood-stabilizing agent used in the treatment of bipolar disorders. These consistent observations apparently contradict the decreased levels of TTR found in the CSF of a reduced sample of depressed individuals (Sullivan *et al*. 1999). However, these might in fact be in agreement if confirmed, as discussed by Sullivan *et al.* (2002) that the reduced levels of TTR could result from the long-lasting effects of the psychotropic medication in course prior to the analysis.

Performance in the open field revealed that mice lacking TTR showed increased motor activity and/or decreased anxiety-like behaviours. It is more likely that the absence of TTR influences locomotor activity since the use of a more specific test for anxiety, the elevated plus maze, failed to reveal increased signs of anxiety in wild-type mice.

The mechanism underlying the behaviour phenotype of the TTR-null mice is probably related to the increased levels of NE present in the limbic forebrain, as the concentrations of serotonin and dopamine remain unaffected. In fact, drugs increasing central dopaminergic and α-adrenergic activities (Porsolt *et al*. 1979) reduce immobility in the forced swimming test. In addition, the apparent discrepancy observed between the open field and elevated plus maze tests (increased locomotor activity without associated hypo-anxiety traits) probably reflects the specific activation of distinct limbic regions. Indeed, while NE administration in the nucleus accumbens or hippocampus is known to induce increased locomotion in the open field (with concomitant reduced immobility in the forced-swimming test), NE administration into the amygdala either reduces exploratory activity or produces no effects in the elevated plus maze (respectively if the release is in the basomedial or central divisions; Plaznik *et al*. 1985a, 1985b).

In order to determine the mechanism by which the TTR-null mice exhibit increased levels of NE in the limbic

forebrain, we have explored if such a difference resulted from changes in NE metabolism. Neither tyrosine hydroxylase activity, the rate-limiting enzyme in the cathecolamine biosynthetic pathway, nor monoamine oxidase A, the major NE catabolic enzyme, are affected in the TTR-null. Thus, the increased levels of NE found in the limbic forebrain appear to be the result of changes in NE not dependent on metabolic activity. It is also possible that the TTR-null mice have an altered limbic synaptic network; preliminary data showing a reorganization of dendritic length and spines in the amygdala of TTR-null mice strongly supportsthis hypothesis (Pêgo *et al*. 2003). Further studies will address whether manipulation of the noradrenergic system with agonists and antagonists influence the observed phenotype.

The mechanism by which the absence of TTR relates to the increased limbic levels of NE is still enigmatic. Although TTR is the major carrier of thyroid hormones in the CSF, TTR-null mice are euthyroid and therefore we suggest that the role of TTR in behaviour is unlikely to result from impaired hormonal homeostasis (Palha 2002; Palha *et al*. 1994, 1997, 2000). However, the possibilities of impaired occupancy of TH receptors or TH imbalance earlier in development cannot be ruled out at this point. Furthermore, we cannot exclude that the stressful exposure to behaviour testing alters TH availability. In addition, it must be remembered that TTR binds vitamin A, the precursor of active 9-*cis*- and all-*trans* retinoid acid, which interact with nuclear receptors (RXR and RAR) and influence the expression of genes such as dopamine D2 receptor, dopamine β-hydroxylases and synapsins (Kim *et al*. 2001; Balmer and Blomhoff 2002); yet, whether the absence of TTR affects brain retinoid metabolism remains to be elucidated.

Irrespective of the underlying mechanism, data presented herein clearly reveal a role for TTR in behaviour. Such involvement was shown to be associated with variations of NE levels in the limbic system. The importance of this finding deserves further investigation regarding both the molecular mechanisms underlying this effect, as well as the specific regions implicated in the process.

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References

Anderson G. W. (2001) Thyroid hormones and the brain. *Front Neuroendocrinol.* **22**, 1–17.

Balmer J. E. and Blomhoff R. (2002) Gene expression regulation by retinoic acid. *J. Lipid Res.* **43**, 1773–1808.

Bauer M., Heinz A. and Whybrow P. C. (2002) Thyroid hormones, serotonin and mood: of synergy and significance in the adult brain. *Mol. Psychiatry* **7**, 140–156.

Bernal J. (2002) Action of thyroid hormone in brain. *J. Endocrinol. Invest.* **25**, 268–288.

Bolivar V., Cook M. and Flaherty L. (2000) List of transgenic and knockout mice: behavioral profiles. *Mamm. Genome* **11**, 260–274.

Boomsma F., Man in 'T. Veld A. J. and Schalekamp M. A. (1991) Not norepinephrine but its oxidation products bind specifically to plasma proteins. *J. Pharm. Exp. Therap.* **259**, 551–557.

Carro E., Trejo J. L., Gomez-Isla T., LeRoith D. and Torres-Aleman I. (2002) Serum insulin-like growth factor I regulates brain amyloid-β levels. *Nat. Med.* **8**, 1390–1397.

Episkopou V., Maeda S., Nishiguchi S., Shimada K., Gaitanaris G. A., Gottesman M. E. and Robertson E. J. (1993) Disruption of the transthyretin gene results in mice with depressed levels of plasma retinol and thyroid hormone. *Proc. Natl Acad. Sci. USA* **90**, 2375–2379.

Ernström U., Petterson T. and Jörnvall H. (1995) A yellow component associated with human transthyretin has properties like a pterin derivate, 7, 8-dihydropterin-6-carboxaldehyde. *FEBS Lett.* **360**, 177–182.

File S. E., Lippa A. S., Beer B. and Lippa M. T. (2000) Animal tests of anxiety. In: *Current Protocols in Neuroscience* (Taylor G. P., ed.), pp. 8.3.1–8.3.19. John Wiley & Sons, Inc, New York.

González S., Romero J., de Miguel R., Lastres-Becker I., Villanua M. A., Makriyannis A., Ramos J. A. and Fernandez-Ruiz J. J. (1999) Extrapyramidal and neuroendocrine effects of AM404, an inhibitor of the carrier-mediated transport of anandamide. *Life Sci.* **65**, 327–336.

Hagen G. A. and Elliott W. J. (1973) Transport of thyroid hormones in serum and cerebrospinal fluid. *J. Clin. Endocrinol.* **37**, 415–422.

Kabat E. A., Moore D. H. and Landow H. (1942) An electrophoretic study of the protein components in cerebrospinal fluid and their relationship to the serum proteins. *J. Clin. Invest.* **21**, 571–577.

Kim H. S., Hong S. J., LeDoux M. S. and Kim K. S. (2001) Regulation of the tyrosine hydroxylase and dopamine β-hydroxylase genes by the transcription factor AP-2. *J. Neurochem.* **76**, 280–294.

Morriss-Kay G. M. and Ward S. J. (1999) Retinoids and mammalian development. *Int. Rev. Cytol.* **188**, 73–130.

Nagatsu T., Oka K. and Kato T. (1979) Highly sensitive assay for tyrosine hydroxylase activity by high-performance liquid chromatography. *J. Chromat.* **163**, 247–252.

Palha J. A. (2002) Transthyretin as a thyroid hormone carrier: a function revisited. *Clin. Chem. Lab. Med.* **40**, 1292–1300.

Palha J. A., Episkopou V., Maeda S., Shimada K., Gottesman M. E. and Saraiva M. J. (1994) Thyroid hormone metabolism in a transthyretin-null mouse strain. *J. Biol. Chem.* **269**, 33135–33139.

Palha J. A., Moreira P., Wisniewski T., Frangione B. and Saraiva M. J. (1996) Transthyretin gene in Alzheimer's disease patients. *Neurosc. Lett.* **204**, 212–214.

Palha J. A., Hays M. T., Morreale de Escobar G., Episkopou V., Gottesman M. E. and Saraiva M. J. (1997) Transthyretin is not essential for thyroxine to reach the brain and other tissues in a transthyretin null mouse strain. *Am. J. Physiol.* **272**, E485–E493.

Palha J. A., Fernandes R., Morreale de Escobar G., Episkopou V., Gottesman M. and Saraiva M. J. (2000) Transthyretin regulates thyroid hormone levels in the choroid plexus but not in the brain parenchyma: study in a transthyretin-null mouse model. *Endocrinology* **141**, 3267–3272.

Palha J. A., Nissanov J., Fernandes R. *et al.*. (2002) Thyroid hormone distribution in the mouse brain: the role of transthyretin. *Neuroscience* **113**, 837–842.

Pêgo J. M., Cerqueira J. J., Palha J. and Sousa N. (2003) Morphological changes in the basolateral division of the amygdala of TTR-null mice. In: *Sixt IBRO World Congress of Neuroscience*. Abstract book, pp 207, number 2265, Prague.

Plaznik A., Danysz W. and Kostowski W. (1985a) A stimulatory effect of intraaccumbens injections of noradrenaline on the behavior of rats in the forced swim test. *Psychopharmacology* **87**, 119–123.

Plaznik A., Danysz W. and Kostowski W. (1985b) Some behavioral effects of microinjections of noradrenaline and serotonin into the amygdaloid body of the rat brain. *Physiol. Behav.* **34**, 481–487.

Porsolt R. D., Bertin A. and Jalfre M. (1977) Behavioral despair in mice: a primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Ther.* **229**, 327–336.

Porsolt R. D., Anton G., Blavet N. and Jalfre M. (1978) Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur. J. Pharmacol.* **47**, 379–391.

Porsolt R. D., Bertin A., Blavet N., Deniel M. and Jalfre M. (1979) Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. *Eur. J. Pharmacol.* **57**, 201–210.

Pulford D. J., Henry B., Adams F., Harries D. N., Mallinson D. J., Reid I. C. and Stewart C. A. (2002) Chronic administration of lithium chloride down-regulates transthyretin mRNA expression in rat brain. In: *Society for Neuroscience 32nd Annual Meeting*. Abstract Viewer/Itinerary Planner Program No. 308.2. Society for Neuroscience, Washington, DC. Online.

Riisoen H. (1988) Reduced pre-albumin (transthyretin) in CSF of severely demented patients with Alzheimer's disease. *Acta Neurol. Scand.* **78**, 455–459.

Rodrigues-Pena A. (1999) Oligodendrocyte development and thyroid hormone. *J. Neurobiol.* **40**, 497–512.

Romero J., de Miguel R., García-Palomero E., Fernández-Ruiz J. J. and Ramos J. A. (1995) Time-course of the effects of anandamide, the putative endogenous cannabinoid receptor ligand, on extrapyramidal function. *Brain Res.* **694**, 223–232.

Schreiber G., Southwell B. R. and Richardson S. J. (1995) Hormone delivery systems to the brain transthyretin. *Exp. Clin. Endocrinol.* **103**, 75–80.

Schwarzman A. L., Gregori L., Vitek M. P. *et al.* (1994) Transthyretin sequesters amyloid β-protein and prevents amyloid formation. *Proc. Natl Acad. Sci. USA* **91**, 8368–8372.

Smith D., Wagner E., Koul O., McCaffery P. and Drager U. C. (2001) Retinoic acid synthesis for the developing telencephalon. *Cereb. Cortex* **11**, 894–905.

Southwell B. R., Duan W., Alcorn D., Brack C., Richardson S. J., Kohrle J. and Schreiber G. (1993) Thyroxine transport to the brain: role of protein synthesis by the choroid plexus. *Endocrinology* **133**, 2116–2226.

Stein T. D. and Johnson J. A. (2002) Lack of neurodegeneration in transgenic mice overexpressing mutant amyloid precursor protein is associated with increased levels of transthyretin and the activation of cell survival pathways. *J. Neurosci.* **22**, 7380–7388.

Sullivan G. M., Hatterer J. A., Herbert J. *et al.* (1999) Low levels of transthyretin in the CSF of depressed patients. *Am. J. Psychiatry* **156**, 710–715.