

Decreased anxiety in mice lacking the organic cation transporter 3

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Abstract The organic cation transporter 3 (OCT3; synonymous: extraneuronal monoamine transporter, EMT, *Slc22a3*) encodes an isoform of the organic cation transporters and is expressed widely across the whole brain. OCTs are a family of high-capacity, bidirectional, multi-specific transporters of organic cations. These also include serotonin, dopamine and norepinephrine making OCTs attractive candidates for a variety of neuropsychiatric disorders including anxiety disorders. OCT3 has been implicated in termination of monoaminergic signalling in the central nervous system. Interestingly, OCT3 mRNA is however also significantly up-regulated in the hippocampus of serotonin transporter knockout mice where it might serve as an alternative reuptake mechanism for serotonin. The examination of the behavioural phenotype of OCT3 knockout mice thus is paramount to assess the role of

OCT3. We have therefore subjected mice lacking the OCT3 gene to a comprehensive behavioural test battery. While cognitive functioning in the Morris water maze test and aggression levels measured with the resident–intruder paradigm were in the same range as the respective control animals, OCT3 knockout animals showed a tendency of increased activity and were significantly less anxious in the elevated plus-maze test and the open field test as compared to their respective wild-type controls arguing for a role of OCT3 in the regulation of fear and anxiety, probably by modulating the serotonergic tone in limbic circuitries.

Keywords Organic cation transporter · Anxiety · Activity · Aggression

Introduction

Drugs that interfere with the reuptake and/or metabolism of biogenic amines have been used for the treatment of depression and anxiety for more than four decades. The inhibition of the reuptake of serotonin, noradrenaline and dopamine increases the extracellular level of these neurotransmitters, thereby enhancing their availability. Two uptake systems that remove extracellular monoamines have been described. The neuronal Uptake-1 system is present at presynaptic nerve endings and mediates the reuptake of released monoamines from the synaptic cleft. Uptake-1 is a high-affinity and Na⁺ and Cl⁻ dependent system comprising the noradrenaline (NET), dopamine (DAT), and serotonin (5HTT) transporter proteins (Amara and Kuhar 1993; Chen and Skolnick 2007; Murphy and Lesch 2008). Inactivation experiments in mice demonstrated that the Uptake-1 transporter proteins are the targets for antidepressant and psychostimulatory treatment and are pivotal

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effectors in the control of synaptic monoamine concentrations with respective behavioural sequelae (Bengel et al. 1998; Giros et al. 1996; Xu et al. 2000).

Uptake-2 is a sodium- and chloride-independent, low-affinity, high-capacity transport system initially described in heart, smooth muscle, and glandular cells (Iversen 1965; Martel and Azevedo 2003; Zwart et al. 2001), thus constituting a catecholamine removal system found in peripheral tissues with sympathetic innervation. The functional characterization of this Uptake-2 system was pioneered with perfused organs and isolated tissues by the groups of Iversen and Trendelenburg (Grohmann and Trendelenburg 1984; Iversen 1970; Trendelenburg et al. 1988). Contrary to sodium-driven, high-affinity neuronal Uptake-1, Uptake-2 can be inhibited by corticosteroids and *O*-methylated catecholamines as well as cyanin-related compounds (Friedgen et al. 1996; Wu et al. 1998). The main organic cation transporters (OCT) subtypes (OCT1, OCT2, and OCT3), which have been isolated in humans and rodents, display pharmacological profiles that match these general characteristics (Grundemann et al. 1997; Grundemann et al. 1998a, b; Hayer-Zillgen et al. 2002; Wu et al. 1998) and have thus been proposed to accomplish Uptake-2. OCTs are carrier-type permeates that participate in the elimination of a variety of cationic substances, physiological compounds as well as xenobiotics, in tissues such as kidney, liver, and placenta (Koepsell 1998). Schömig and colleagues showed that OCT3 differs immensely from the high-affinity neuronal Uptake-1 transporters e.g. for dopamine (DAT) and noradrenaline (NET) (Povlock and Amara 1998) with respect to transport mechanism, drug sensitivity, substrate affinity, and substrate selectivity (Schömig et al. 2006). It was also proposed that OCT3 is the extraneuronal monoamine transport system (EMT). EMT expression was found and its function was analysed in cultured glial cells of different origin (e.g. human glioma) (Russ et al. 1996).

However, as shown by *in situ* hybridization (ISH) (Schmitt et al. 2003), OCT3 is expressed exclusively in neuronal cells of the brain. These findings are in accordance with respective studies in rats using ISH (Wu et al. 1998) and in mice using immunohistochemistry (IHC) (Vialou et al. 2004). Although these ISH and IHC experiments do not rule out an additional glial localization of OCT3 the known neuronal localization is a sufficient evidence to put into question the notion that OCT3 corresponds mainly to extraneuronal transporter system. In this respect, OCT3 localized in neurons might serve as an alternative transporter removing monoamine transmitters that might escape neuronal high-affinity reuptake and therefore is discussed to influence neuronal signalling by modulating neurotransmitter concentrations in the synaptic cleft. Additionally, there are some references for the

participation of OCT3 in monoamine-clearance pathways. Haag et al. (2004) revealed a partly overlapping expression pattern of EMT/OCT3 and the monoamine-metabolizing enzyme monoamine oxidase B (MAOB) in rat area postrema and Verhaagh et al. (2001) detected a colocalization of OCT3 with the MAOA in the placenta. Because of these characteristics and its distribution in CNS, OCT3 is regarded a promising drug target in antidepressant therapy (Schildkraut and Mooney 2004). Owing to its physiological profile and expression pattern, OCT3 also qualifies as a candidate gene for various neuropsychiatric disorders of inherent monoaminergic dysfunction. Accordingly, genetic variation in OCT3 has been implicated in obsessive-compulsive disorder (OCD) (Lazar et al. 2008). The latter described the first non-synonymous substitution in *OCT3* in a family affected by a severe form of OCD, which might constitute a potential causative or modulating role in OCD, at least in this family. From the present data, OCT3 might well be implicated in other types of anxiety disorders as well as ADHD and depression (Dell'Osso et al. 2006).

Knockout animals are valuable tools to identify the behavioural impact of a given gene. Using careful behavioural analysis, the present study thus aimed to characterize basal behavioural pattern in OCT3 wild-type (+/+), heterozygous (+/+) and knockout (-/-) mice in two traditional novelty-based models: the open field and elevated plus-maze (EPM) tests (Carobrez and Bertoglio 2005; Crawley 1999; Izidio et al. 2005). Because cognitive functions play a key role in animal exploration and activity, with serotonin and OCT3 both possibly implicated in the regulation of cognitive processes in animals and humans (Leussis and Bolivar 2006; Roiser et al. 2006, 2007), we hypothesized that cognitive functions related to exploration (such as spatial memory), may also be affected in OCT3 -/- mice along with impulsivity and aggression, as they are thought to correlate with central serotonin levels.

Methods

Construction of the OCT3 (EMTm, Slc22a3) targeting vector

Plasmid pOP6 contains the p15A ori (origin of replication) from plasmid pACYC 177 for low-copy propagation in *E. coli*, a Neo cassette, and a TK cassette. The Neo cassette (1,806 bp) contains the mouse phosphoglycerate kinase 1 promoter followed by a neomycin phosphotransferase open reading frame (orf) and the mouse phosphoglycerate kinase 1 polyadenylation site; the sequence of the Neo cassette conforms to a segment from plasmid pHM2 (accession number X76683). The TK cassette (1,929 bp) contains a

murine polyomavirus enhancer (a tandem of replication origin beta element) followed by herpes simplex virus thymidine kinase promoter, orf, and polyadenylation site.

Homology regions (long arm, 4,978 bp; short arm, 1,657 bp) were generated by long distance PCR based on cosmid MPMGc121E06731Q2 (129/ola mouse genomic DNA, library #121, Resource Center of the German Human Genome Project, Max-Planck-Institute for Molecular Genetics, Berlin, Germany) and inserted into pOP6 to yield the targeting vector pOP6/EMTm.

In pOP6/EMTm, the sequence of elements is: short arm, Neo cassette; long arm, TK cassette. Neo and TK orfs are in opposite orientation to the EMTm gene. The precise crucial junctions are junction 1, GCGGCCGC-TCAGAG GCTGTGGGAG (*Not* I site of vector pOP6—end of short arm); junction 2, ACTGTCAACTAAATGT-GGCGCG CC-GAATTCTACCGG (beginning of short arm—*Asc* I site—beginning of Neo cassette); junction 3, AGAAGCT GGTCGACCTCGAGCTGTCGAA - GCGATCGC - GTG ATCTAGATAAGAA (end of Neo cassette—*Sgf* I site—end of long arm), and junction 4, GAGTTTCAGGTGA ACA-CCGCGG-TCTAGA (beginning of long arm—*Sac* II site—beginning of TK cassette).

The targeting vector replaces exon 7 and most of exon 6 of the OCT3 gene, i.e. the code for amino acids 322–424 and thus transmembrane segments 7, 8, and 9 are fully eliminated (Gründemann and Schömig 2000). Moreover, since the first 5 bases (ATCAC) of exon 6 are retained, normal splicing will yield an mRNA that runs into a stop codon after 32 artificial amino acids.

Generation of transgenic mice

E14 mouse embryonic stem cells (129/ola) were electroporated with *Not* I-linearized pOP6/EMTm and selected with G418 and ganciclovir. Cell clones that had undergone homologous recombination were identified by PCR as described (Gründemann and Censarek 2003) and verified by Southern blotting. Cells from positive clones were microinjected into blastocysts from C57BL/6 female mice, which were then implanted into pseudo-pregnant mice. The five resultant chimeric male mice were crossed with C57BL/6 females (8 generations) to generate agouti mice with germline transmission. In the offspring of one of these mice (chimera #7), germline transmission was evident by the detection of 8 (out of 21) heterozygous animals. Following mating of these heterozygous mice, homozygotes were identified and distinguished from heterozygous and wild-type mice by PCR and real-time PCR.

For quantitative real-time PCR analysis, total RNA was extracted from different organs by acid guanidinium thiocyanate–phenol–chloroform extraction (Chomczynski and Sacchi 1987), performed according to the manufacturer's

instructions (Promega, Madison, WI). RNA was reversed transcribed into cDNA by using random hexamers and Superscript II Plus RNase H⁻ Reverse Transcriptase (Invitrogen, Carlsbad, CA). The expression level was quantified by real-time PCR using the LightCycler. Amplification was performed in a total volume of 10 µl for 40 cycles and product was detected using LightCycler FastStart DNA Master^{PLUS} SYBR Green I (Roche, Mannheim, Germany). The relative expression levels of exon 4/5 (forward: 5'-CATCTCTCTGCCAGCTTTC-3', reverse 5'-GGCTTTCTCTCCTTGCTTCC-3') and exon 7/8 (forward: 5'-CCCGGAGCTCTCTTAATCCT-3', reverse 5'-TTTCG AAGCCATGGTTAT-3') were determined by normalizing expression of each target to β -actin (forward: 5'-AG CCATGTACGTAGCCATCCA-3', reverse 5'-TCTCCGG AGTCCATCACA ATG-3') and quantified as described (Pfaffl 2001). Genotyping of the investigated animals was confirmed by four different PCR reactions of tail clip gDNA: 1. Forward 5'-TTGTGGATCTGACGTACTGAA AAT-3' binds intronic the OCT3 gene in front of exon 6 and reverse 5'-GCCGTTTTCTGGATTCA-3' specific for the Neomycin gene, yielding a 837 bp band in KO. 2. Forward 5'-GCTGGCCCTTAATTTGGTT-3' specific for the Neo Cassette and reverse 5'-GCCGTTTTCTGGAT TCA-3' specific for the Neomycin gene, yields a 429 bp band in KO. 3. Forward 5'-TTGTGGATCTGACGTACT GAAAAT-3' binds intronic the OCT3 gene in front of exon 6 and reverse 5'-TCCCAGGCGCATGACAAGTCC-3' binds within exon 7 of the EMTm gene, yielding a 389 bp band in WT. 4. Forward 5'-TTGTGGATCTGACGTACT GAAAAT-3' and reverse 5'-TCTTTGGGAACTGTCAAC TAAATGT-3' both recognize the specific intronic region flanking exons 6 and 7 of EMTm, yielding a 601 bp band in WT and 1,990 bp in KO, both bands in heterozygous mice. All PCR reactions yielded consistent results.

Investigation of experimental animals

This study was carried out with male mice, weighing 27–35 g, which were individually housed under controlled temperature (22°C) and a 12 h light/dark cycle (lights on at 6:00 h, lights off at 18:00 h). Behavioural phenotyping of knockout and wild-type littermates: experimental design and evaluation of general health. They had free access to regular mouse chow and water ad libitum. All animal protocols have been reviewed and approved by the review board of the Government of Lower Franconia and the University of Würzburg and conducted according to the Directive of the European Communities Council of 24 November 1986 (86/609/EEC).

The experiments were designed in such a way that the number of animals used and their suffering was minimized. Specifically, the experiments were performed with OCT3

–/–, OCT3 +/- mice and their wild-type OCT3 +/+ littermates.

Experimental protocols

In this study, mice were subjected to a sequence of four behavioural tests that were separated for at least 1 week. The series of behavioural tests was started with the elevated plus-maze test (EPMT), continued with the open field test (OFT), followed by the Morris water maze test (MWM) and completed with the resident–intruder test (RIT) in increasing stress-inducing order.

Behavioural tests

Prior to all behavioural tests, mice were allowed to adapt to the test room ($22 \pm 1^\circ\text{C}$, $50 \pm 5\%$ relative air humidity, lights on at 6:00 h, lights off at 18:00 h, maximal light intensity 100 lux) for 2 weeks.

Elevated plus-maze test

Animals were placed in the centre of a maze with 4 arms arranged in the shape of a plus (Pellow and File 1986; Belzung and Griebel 2001). Specifically, the maze consisted of a central quadrangle (5×5 cm), two opposing open arms (30 cm long, 5 cm wide) and two opposing closed arms of the same size but equipped with 15-cm high walls at their sides and the far end. The device was made of opaque grey plastic and elevated 50 cm above the floor. The light intensity at the centre quadrangle was 70 lux, on the open arms 80 lux and in the closed arms 40 lux. At the beginning of each trial, animals were placed on the central quadrangle facing the upper open arm. Movements of the animals during a 5-min test period were tracked by a video camera positioned above the centre of the maze and recorded with the software VideoMot2 (TSE Systems, Bad Homburg, Germany). Post-test this software was used to evaluate the animal tracks and to determine the number of their entries into the open and closed arms, the time spent on the open and closed arms and the total distance travelled in the open and closed arms during the test session. Entry into an arm was defined as the instance when the mouse placed its four paws on that arm. Additionally, the number of rearings and groomings were recorded by hand.

Open field test

The open field consisted of a box ($82 \times 82 \times 25$ cm) that was made of opaque grey plastic and illuminated by 100 lux at floor level. The ground area of the box was divided into a 70×70 cm central area and the surrounding border zone. Mice were individually placed in a corner of

the open field, and their behaviour during a 5-min test period was tracked by a video camera positioned above the centre of the open field and recorded with the software VideoMot2 (TSE Systems, Bad Homburg, Germany). Post-test this software was used to evaluate the time spent in the central area, the number of entries into the central area and the total distance travelled in the central area as well as in the whole open field. Additionally, the number of rearings and groomings were recorded by hand.

Morris water maze

The MWM consists of an open circular pool of dark grey-coloured plastic material (120 cm diameter; 60 cm depth), filled with water ($26 \pm 1^\circ\text{C}$ temperature) and a hidden platform (8 cm diameter; 29 cm height) submerged 1 cm below the water surface. The platform was camouflaged by placing opacifying materials in the water (white, non-toxic tempera paint), thereby making it indistinct given the low visual aspect ratio to the water as seen by the mice when swimming. Two principal axes of the maze were designated, each line bisecting the maze perpendicular to one another to create an imaginary '+'. The end of each line demarcated four cardinal points: North (N), South (S), East (E) and West (W) (Rubio et al. 2007). These were not true magnetic compass directions but referred to S being the experimenter's position, N being at the opposite point, E being to the experimenter's right and W being to the experimenter's left (Vorhees and Williams 2006). Dividing the maze this way created four equal quadrants. The platform was positioned half-way (30 cm) between the centre and the wall of the maze, therefore in the middle of one of the quadrants (the target quadrant). Distal visual cues for navigation were provided by the environment of the laboratory; proximal visual cues consisted of four different coloured and figured posters placed on the cardinal points on the inside wall of the tank (Rossato et al. 2006). The animals were transferred from their cages to the pool in an opaque cup and were released into the water at water-level, immediately facing the tank wall, at one of eight symmetrically placed start locations. The set of start locations was predetermined to be quasi-random and non-sequential (Table 1), therefore the start positions are not equidistant from the platform, creating short and long paths to the goal. Mice were allowed to swim a maximum time of 3 min to locate the platform. Finding the platform was defined as staying on it for at least 2-s; subjects that crossed the platform without stopping were left to swim. Those who failed to find the platform within the allotted time were picked up and placed on respectively guided to the platform to rest there for ~ 20 s, and assigned a latency of 180 s. Afterwards the subject was gently picked up using a colander and allowed to warm up and dry off under a 125-W heat lamp

Table 1 Morris water maze spatial (hidden platform) start positions

Trial	Acquisition			Reversal	
	Day 1	Day 2	Day 3	Day 4	Day 5
1	W	NE	SE	E	SE
2	N	SW	NW	S	SW
3	S	E	E	NW	N
4	E	W	S	NE	S
5	NW	S	N	W	NW
6	SE	N	W	N	NE

before it was returned to its home cage. After each mouse in the testing squad had completed one trial, the next trial was begun (Morris 1984). The entire procedure took 5 consecutive days, each animal having six trials per day, with a 40–60 min inter-trial interval. The platform was fixed in the south–east quadrant during the first 3 days (18 trials; spatial acquisition) and relocated to the opposite (north–west) quadrant for the remaining 2 days (12 trials; spatial reversal) (D’Hooge and De Deyn 2001). Trials 19 and 20 were defined as probe trials to analyse the precision of the spatial learning (Vorhees and Williams 2006).

Resident–intruder test

Following 1 week of single housing, mice were tested for social dominant and aggressive behaviour in the resident–intruder paradigm. An intruder mouse (unfamiliar age-, weight-, and sex-matched C57BL6; six of them were used during testing) was placed in the resident’s home cage. Each test session (10 min duration) was recorded on videotape using a camera placed 150 cm above the home cage. Order of testing was counterbalanced across the entire session such that wild-type, heterozygous and knockout mice were exposed equally to each of the intruder mice, thereby minimizing the possibility that phenotypic changes in behaviour might be related to intruder-related factors. After testing,

behaviours were coded and quantified from videotapes using homebrew behavioural analysis software by an observer blind to genotype. The social investigative and aggressive behaviours scored were: number of episodes and total time mice spent engaged in anogenital sniffing, grooming, following or standing next to each other. The aggressive behaviours scored were: biting, pinning, aggressive following, tail-rattling.

Statistics

Statistical evaluation of the results was performed on SPSS 15 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to dissect statistical differences for the factor genotype. In case of sphericity violations the Greenhouse–Geisser correction was applied. The homogeneity of variance was analysed with the Levene test. Post-hoc analysis of group differences was performed with the Tukey HSD (honestly significant difference) test, when the variances were homogeneous, and with the Games–Howell test, when the variances were unequal. Probability values of $P < 0.05$ were regarded as statistically significant. All data are presented as mean values \pm SEM, n referring to the number of mice in each group.

Results

Elevated plus-maze test

The anxiety-related behaviour of OCT3 +/+, +/- and -/- mice was assessed with the EPM (Fig. 1), in which the time spent on the open arms and the number of entries into the open arms were taken as indices of anxiety. These parameters were expressed as a percentage of the total time spent on any arm and of the total number of entries into any arm during the 5-min test session. ANOVA revealed a

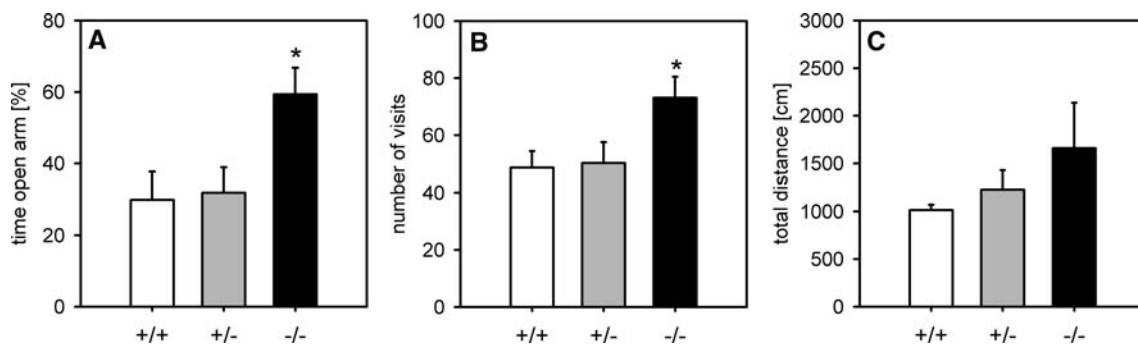


Fig. 1 Behaviour of OCT3 +/- and OCT3 -/- and control mice in the elevated plus-maze. The graphs show the time spent on the open arms (a), the number of entries into any arm (b) and the total distance travelled in the open and closed arms (c) during the 5-min test

session. The time spent on the open arms is expressed as a percentage of the total time spent on any arm. The values represent mean values \pm SEM, $n = 9–11$ per group. $*P < 0.05$ versus control mice

genotype-related difference in the time spent on the open arms ($F_{2,27} = 4.127$, $P = 0.029$). Post-hoc analysis revealed that OCT3 $-/-$ mice spent significantly more time on the open arms than heterozygous and wild-type control mice (Fig. 1a). This result was confirmed by the number of open arm entries which exhibited a genotype-related difference ($F_{2,27} = 3.877$, $P = 0.042$), as OCT3 $-/-$ mice entered the open arms more often than heterozygous and wild-type control animals (Fig. 1b). In order to assess locomotor activity on the elevated plus-maze, the total distance travelled in the open and closed arms and the total number of entries into any arm during the 5-min test session was analysed. Both the total travelling distance ($F_{2,27} = 1.577$, $P = 0.227$; Fig. 1c) and the number of total arm entries ($F_{2,27} = 1.428$, $P = 0.334$; not shown) however did not differ between genotypes.

Open field test

The OFT was used to examine the locomotor/exploratory and anxiety-related behaviour (Fig. 2). The time spent in the

central area and the number of entries into the central area was considered indices of anxiety, with longer stays in the centre indicating less anxiety. ANOVA revealed a genotype-related difference in the time spent in the central area ($F_{2,27} = 3.602$, $P = 0.049$) and post-hoc analysis showed that both OCT3 $-/-$ and OCT3 $+/-$ mice spent significantly more time in the central area than both OCT3 $-/-$ and control mice (Fig. 2a). The number of central area entries exhibited no genotype-related difference ($F_{2,27} = 2.506$, $P = 0.1$, Fig. 2b). Further analysis revealed that knockout of the OCT3 gene did not cause any changes in the locomotor/exploratory activity in the open field (Fig. 2c). The total travelling distance in the open field ($F_{2,27} = 1.503$, $P = 0.24$) during the test session did not differ between the genotypes.

Morris water maze

Figure 3 presents the data of memory performance during the Morris water maze. All groups readily acquired the task. OCT3 $+/+$ mice found the platform in an average of

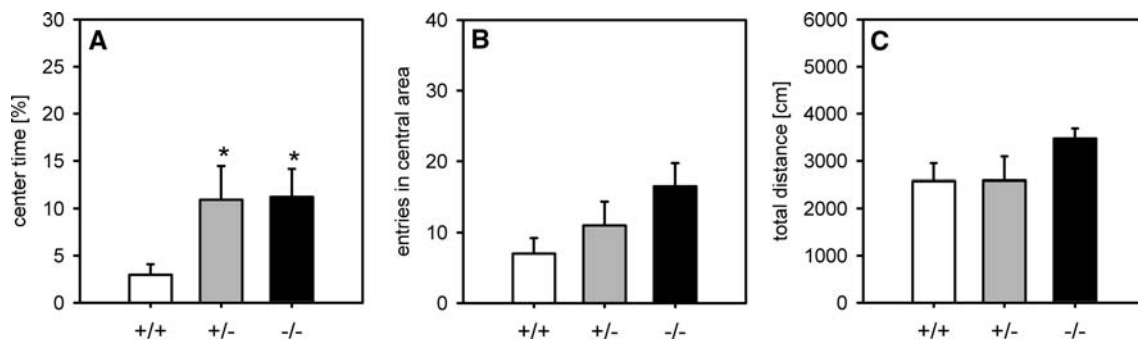
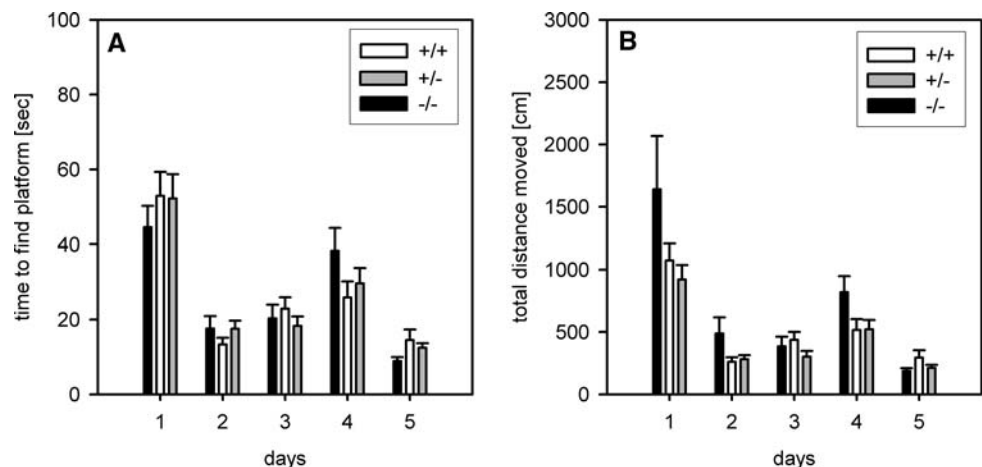


Fig. 2 Behaviour of OCT3 $+/-$ and OCT3 $-/-$ and control mice in the open field test. The graphs show the time spent in the central area (a), the number of entries into the central area (b), and the total distance travelled in the open field (c) during the 5-min test session. The time spent in the central area is expressed as a percentage of the

total test duration, and the number of entries into the central area is given as a percentage of the total number of entries into any zone during the whole test session. The values represent mean values \pm SEM, $n = 9$ –11 per group. * $P < 0.05$ versus control mice

Fig. 3 Behaviour of OCT3 $+/-$ and OCT3 $-/-$ and control mice in the Morris water maze. The graphs show the latency (a) and the total swim distance (b) to find the hidden platform positioned in south–west direction (days 1–3). On day 4, the platform switched from south–west to north–east direction. The values represent mean values \pm SEM, $n = 9$ –11 per group



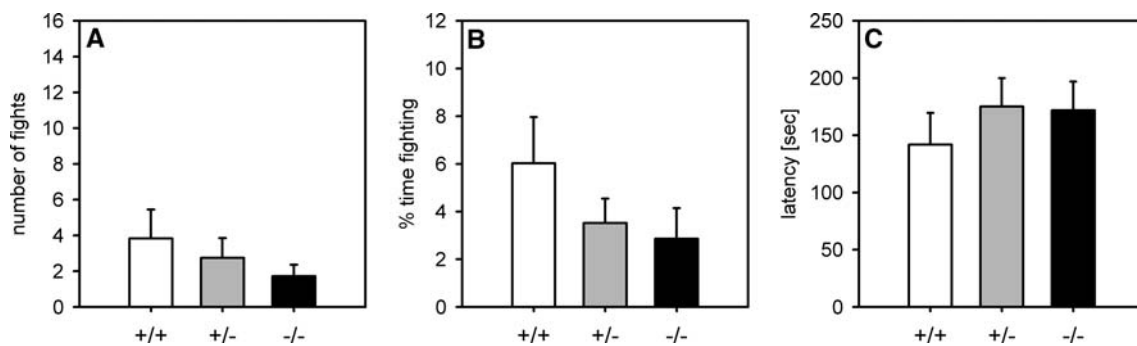


Fig. 4 Behaviour of OCT3 +/+ and OCT3 -/- and control mice in the resident-intruder test. The graphs show the overall number of fights initialized by the resident (a), the time spent fighting (b), and

latency until the first fight (c) during the 10-min test session. The time spent fighting is expressed as a percentage of the total test duration. The values represent mean values \pm SEM, $n = 9$ –11 per group

54.0 ± 6.4 , OCT3 +/- mice in 52.2 ± 6.5 s and OCT3 -/- mice in 45.4 ± 5.8 s during the first day. The latency decreased steadily during the following 3 days, reaching an average latency of 22.9 ± 3.0 , 18.3 ± 2.5 and 20.3 ± 3.7 s for wild types, heterozygous and knockouts, respectively, during the 18th and final session before switching the platform to the opposite quadrant. There were no significant differences between the three groups during cued training with respect to latency ($F_{2,27} = 1.929$, $P = 0.32$) or swim distance ($F_{2,27} = 1.721$, $P = 0.41$). Again, there were no significant differences between genotypes during retrieval period, consisting of an additional 12 trials with respect to latency ($F_{2,27} = 0.080$, $P = 0.92$) or swim distance ($F_{2,27} = 0.91$, $P = 0.42$). During the probe trial, tested immediately following the last acquisition session, no significant between groups difference were detected by ANOVA ($F_{2,23} = 0.485$, $P = 0.622$).

Resident-intruder test

Aggressive behaviour was assessed using the resident-intruder paradigm (Fig. 4). ANOVA revealed no differences between genotypes in either the number of fights ($F_{2,27} = 1.094$, $P = 0.36$) or in the overall time spent fighting ($F_{2,27} = 1.563$, $P = 0.24$).

Discussion

Organic cation transporters (OCTs) were initially described as eliminators of cationic endobiotics and xenobiotics in tissues such as kidney and liver. They function both as sodium-independent and as sodium-dependent transport systems (Koepsell 1998). Three subtypes of potential-sensitive organic cation transporters were cloned and characterized, with OCT3 being the sole isoform which is found to be expressed in the rodent brain (Schmitt et al.

2003; Wu et al. 1998), whereas in human brain also (beneath OCT3 expression) OCT2 expression at mRNA and protein level could be detected (Busch et al. 1998).

Our data suggest a robust anxiolytic phenotype of OCT3 -/- mice, as knockout of the OCT3 gene resulted in a 100% increase in the time spent on the open arms of the EPM. Likewise, OCT3 -/- mice entered the open arms of the elevated plus-maze more frequently, whereas the number of entries into the central area of the open field did not differ from the other two groups. OCT3 knockout did not impact significantly on the overall locomotor activity as reflected by the total distance travelled in the open and closed arms during the EPMT and the OFT, arguing against any influence of motor activity on the anxiolytic phenotype.

These findings thus appear to be the converse of the behavioural profile of serotonin transporter (5HTT) knockout mice, an extensively used genetic model which consistently displays anxiety-like behaviour (Holmes et al. 2003a, b; Kalueff et al. 2007a, b) indicating a less pronounced role of OCT3 knockout in the development of the 5HT homeostasis and its effects on the behavioural phenotype, in contrast to 5HTT knockout (Holmes et al. 2003a). Interestingly, we have previously detected a significant and region specific increase of OCT3 mRNA in the hippocampus, but not in other brain regions, including cortex, striatum, cerebellum, and brainstem of 5HTT-deficient (Schmitt et al. 2003), arguing for an involvement of OCT3 in region specific hippocampal 5HT reuptake, a finding which yet has to be confirmed by neurochemical studies presently conducted by our group.

In addition to cationic neurotoxins, such as tetraethylammonium and MPP+, monoamine neurotransmitters including noradrenaline and 5HT are taken up by OCT3 (with low affinity however) (Kristufek et al. 2002; Schmitt et al. 2003). Therefore, increased OCT3 expression (which possibly results in extended 5-HT uptake driven by the OCT3) may act as a compensatory mechanism to decrease

the elevated extracellular 5HT concentrations in 5HTT-deficient mice, thereby compensating for the loss of the major re-uptake mechanism.

Schildkraut and Mooney (2004) proposed that OCT3 blockade might show antidepressant properties, similar to monoamine reuptake inhibitors. In accordance with this proposal, Kitaichi et al. (2005) demonstrated that antisense knockdown of OCT3 decreased immobility time in the forced swimming test, thus thought to mimic antidepressant effects. These results suggest that extracellular monoamines such as 5-HT and noradrenalin might be increased by decreased availability of OCT3. If this is correct, increased 5-HT by a reduction of OCT3 binding sites might show anxiolytic effects since many papers have shown the anxiolytic properties of 5-HT receptor agonists such as buspirone (Faludi 1994; Scholz and Lhotka 1991).

These data implicate that human OCT3 might as well contribute to behavioural domains thought to be driven by serotonin and noradrenalin, such as anxiety and depression. In this respect, investigation of gene \times gene interactions—more specifically, 5HTT (or NET) \times OCT3 interactions—seems intriguing and are under way in our laboratory. Recently, mutations and a non-synonymous substitution (Met370Ile) of the human orthologue of the OCT3 gene, the EMT, have been identified in patients with OCD (Lazar et al. 2008).

Due to the possible involvement of OCT3 in serotonin homeostasis and the hypothesis that the activity of the central serotonergic system is inversely correlated with impulsivity and aggression (Amara and Kuhar 1993; Pattij and Vanderschuren 2008; Pihl and LeMarquand 1998; Serretti et al. 2006), we proposed that OCT3 $-/-$ mice might display decreased aggressive behaviour in the resident-intruder test as also found in 5HTT knockout mice (Holmes et al. 2002). Our data however did not indicate an involvement of OCT3 in aggression, which might be due to the fact that OCT3 seems to have a pronounced role in the hippocampus (see above) and not in brain areas which are more specifically implicated in the control of aggression and impulsivity. Cognitive impairment, probably underlying the less anxious phenotype, were not present in knockout mice as evidenced by the Morris water maze test, and thus could not account for the behavioural effect of gene disruption. While spatial learning is generally thought to be a hippocampal function, it is not modulated to a great extent by the serotonergic and noradrenergic systems which might explain this negative finding.

In conclusion, our study thus suggests that the complex behavioural profile observed in OCT3-deficient mice may serve as a valuable tool to understand anxiety disorders and their underlying neurobiological mechanisms and might become a novel molecular target to treat diseases related to monoaminergic neuronal systems.

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