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ORIGINAL PAPER

Traumatic Brain Injury Elicits Similar Alterations in α7 Nicotinic Receptor Density in Two Different Experimental Models

Peter-Georg Hoffmeister · Cornelius K. Donat · Martin U. Schuhmann · Cornelia Voigt · Bernd Walter · Karen Nieber · Jürgen Meixensberger · Reinhard Bauer · Peter Brust

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Abstract Traumatic brain injury (TBI) is a major cause of death and disability worldwide, especially in children and young adults. Previous studies have shown alterations in the central cholinergic neurotransmission after TBI. We therefore determined α7 nicotinic acetylcholine receptor (nAChR) densities in newborn piglets and adult rats after experimental TBI. Thirteen newborn piglets (post-TBI survival time: 6 h) underwent fluid percussion (FP) injury ($n = 7$) or sham operation ($n = 6$). Furthermore, adult rats randomized into three groups of post-TBI survival times (2, 24, 72 h) received controlled cortical impact injury (CCI, $n = 8$) or sham operation ($n = 8$). Brains were frozen, sagittally cut and incubated with the α7-specific radioligand [^{125}I]α-bungarotoxin for autoradiography. In injured newborn piglets, decreased α7 receptor densities were observed in the hippocampus (−38%), the hippocampus CA1 (−40%), thalamus (−30%) and colliculus superior (−30%). In adult rats, CCI decreased the receptor densities (between −16 and −47%) in almost any brain region within 2 and 24 h. In conclusion, widespread and significantly lowered α7 nAChR densities were demonstrated in both TBI models. Our results suggest that a nearly similar TBI-induced decrease in the α7 density in the brain of immature and adult animals is found, even with the differences in species, age and experimental procedures. The alterations make the α7 nAChR a suitable target for drug development and neuroimaging after TBI.

Keywords Traumatic brain injury · α7 nAChR · Autoradiography · Cholinergic system · α-Bungarotoxin

Peter-Georg Hoffmeister and Cornelius K. Donat contributed equally to this study.

P.-G. Hoffmeister · C. K. Donat (✉) · P. Brust
Institute of Radiopharmacy, Research Site Leipzig, Research Center Dresden-Rossendorf, Permoserstraße 15, 04318 Leipzig, Germany
e-mail: c.donat@fzd.de

M. U. Schuhmann · C. Voigt · J. Meixensberger
Department of Neurosurgery, University of Leipzig, Leipzig, Germany

B. Walter
Department of Neurosurgery, Zentralklinik Bad Berka, Bad Berka, Germany

B. Walter · R. Bauer
Institute of Molecular Cell Biology, Friedrich-Schiller-University, Jena, Germany

K. Nieber
Institute of Pharmacy, University of Leipzig, Leipzig, Germany

Abbreviations

ACh	Acetylcholine
AChE	Acetylcholine esterase
AChR	Acetylcholine receptor(s)
CCI	Controlled cortical impact
ChAT	Choline acetyltransferase
FP	Fluid percussion
mAChR	Muscarinic acetylcholine receptor(s)
nAChR	Nicotinic acetylcholine receptor(s)
PET	Positron emission tomography
PSL	Photostimulated luminescence
TBI	Traumatic brain injury
vAChT	Vesicular acetylcholine transporter

Introduction

Traumatic brain injury (TBI) is a complex injury with a broad spectrum of symptoms and disabilities. In the

paediatric age group, TBI remains the number one cause of death (Keenan and Bratton 2006). Despite an increased knowledge that paediatric TBI is distinct from the injuries in adults, there are still serious deficiencies in our understanding of paediatric TBI (Jankowitz and Adelson 2006; Kochanek 2006).

Growing evidence exists that altered cholinergic activity is involved in long-term sequelae after TBI (Arciniegas et al. 1999; Arciniegas 2003; Salmond et al. 2005) and often causes devastating attentional disorders (Whyte et al. 1996). The cholinergic neurotransmission mediated by acetylcholine (ACh) as a neurotransmitter and mainly via $\alpha4\beta2$ nicotinic acetylcholine receptors (nAChR) is highly important for cognitive processes of attention, consciousness, memory (McAllister 1992) and cortical plasticity (Rasmusson 2000). Cholinergic transmission plays a central role in brain development. Therefore, the influence of paediatric TBI on the cholinergic system in the developing brain is of special interest and importance (Slotkin 2004). Stabilization of basal ganglia function by early light deprivation, for instance, may accelerate recovery from TBI-induced attention deficits in adult animals (Vargo et al. 1999).

It is known from adult animals that TBI is followed by various alterations in the cholinergic neurotransmission. An increased ACh turnover (Saija et al. 1988), up to 700% increased cortical choline levels (Scremin et al. 2006), reduced choline uptake (Dixon et al. 1994), reduced choline acetyltransferase (ChAT) activity (Gorman et al. 1996), increased expression of the vesicular acetylcholine transporter (vAChT) after 2–4 weeks (Ciallella et al. 1998; Shao et al. 1999), time-dependent reductions in vAChT density from 2 h up to 3 days (Donat et al. 2008), and reduced AChE activity in the hippocampus, but increased activity in the basal forebrain (Donat et al. 2007) have previously been described. Additionally, a reduction in the density of $\alpha4^*/\alpha3^*$ nAChR and muscarinic acetylcholine receptors (mAChR) was found by different authors (DeAngelis et al. 1994; Donat et al. 2008; Lyeth et al. 1994). The hippocampal brain regions of adult rats seem especially sensitive. There is a loss of $\alpha7$ nAChR by 30–50% within 1 h after controlled cortical impact (CCI) (Verbois et al. 2000, 2002).

The $\alpha7$ nAChR assembles as a homopentamer composed of five $\alpha7$ subunits (Rangwala et al. 1997). This receptor subtype is expressed on neurons, macrophages, microglia and astrocytes (Shytle et al. 2004; Wang et al. 2007). The $\alpha7$ nAChR is functionally differentiated from other neuronal nAChR by a high affinity binding domain for α-bungarotoxin (Drisdel and Green 2000; Orr-Urtreger et al. 1997). Neuronal $\alpha7$ nAChR modulate the release of other neurotransmitters (e.g. glutamate) (Radcliffe and Dani 1998), fast synaptic transmission (Orr-Urtreger et al. 1997) and, besides $\alpha4\beta2$ nAChR, cognitive functions (Levin 2002). The $\alpha7$ nAChR also plays an important role in inflammation (de Jonge and Ulloa 2007), a fact that becomes increasingly important for the treatment of TBI (Cederberg and Siesjo 2010). An involvement of the $\alpha7$ nAChR in TBI-induced disturbances in cognitive brain functions has been discussed. Moreover, the $\alpha7$ nAChR has a very high calcium permeability, which could contribute to excitotoxic damage after excessive activation following TBI. In addition, other authors reported very fast changes in $\alpha7$ receptor density after experimental TBI (Verbois et al. 2002), which indicates a relevance of this receptor for possible imaging with positron emission tomography (PET). Taken together, this makes the $\alpha7$ nAChR an interesting target in TBI research. Therefore, in this study we investigated the density of the $\alpha7$ nAChR after TBI. To evaluate the effect of species and age, we performed the experiments with adult rats and newborn pigs.

Materials and Methods

The experimental protocols were approved by the local ethic committee and were compliant with national regulations for animal research.

Rat Model (Controlled Cortical Impact)

Forty-eight male, adult (55–70 days of age) Sprague–Dawley rats (in-house breeding of the University of Leipzig) weighing 250–350 g were assigned to three groups. These groups had survival times of 2, 24 and 72 h. Each group consisted of 16 rats that were randomly assigned to serve as controls or to receive trauma. Prior to the experiments, animals were kept in social groups under controlled conditions of temperature and humidity and subjected to a 12/12 h dark/light cycle. After surgery, animals were housed in individual cages with nutrition ad libitum.

Surgical procedures and injury model were previously described in detail (Donat et al. 2007, 2008). Briefly, rats underwent surgery to produce a unilateral 6 mm circular craniotomy centred over the motor cortex (3.5 mm posterior, +4.0 mm lateral to bregma) under anaesthesia with fentanyl (0.005 mg/kg, Janssen, Germany), midazolam (2 mg/kg, Ratiopharm, Germany) and medetomidine (0.15 mg/kg, Pfizer, Germany). A focal TBI (CCI model) to the left cortex was induced with a 5-mm-diameter electromagnetically driven rounded impactor striking the exposed dura for 100 ms at a velocity of 4 m/s and a depth of 2 mm. Immediately after CCI, the bone flap was placed in the craniotomy and fixed with acrylic resin (TechnoVit®

3040, Heraeus Kulzer, Germany). The incision was sutured and anaesthesia antagonized with a subcutaneous injection of a mixture of naloxone (0.12 mg/kg, Ratiopharm, Germany), flumazenil (0.2 mg/kg, Roche, Germany) and atipamezole (0.75 mg/kg, Pfizer, Germany). Two, 24 and 72 h after CCI, rats were lightly anaesthetized with isoflurane (5%) and subsequently decapitated. After decapitation, the brain was quickly removed. Hemispheres were separated sagittally and immediately frozen in −35°C 2-methylbutane for at least 30 s.

Pig Model (Lateral Fluid Percussion Injury)

Thirteen crossbreed female piglets were used (Deutsches Edelschwein, 7.7 ± 1.2 days old, 3.1 ± 0.3 kg body weight). Surgical interventions and the employed model were described previously (Donat et al. 2010a, b). Animals were initially anaesthetized with 1.3% isoflurane. Temperature was maintained at 38.3 ± 0.2°C using infrared lamps controlled by a rectal thermal probe. All pigs were mechanically ventilated (Servo Ventilator 900C, Siemens-Elema, Solna, Sweden) and paralysed with pancuronium bromide (0.2 mg kg^{-1} h^{-1}). A craniotomy was performed, centred between lambda and bregma over the left parietal cortex to fix the fluid percussion adapter. After surgery, all animals rested for 60 min. Baseline physiological values were recorded. Subsequently, seven randomly chosen piglets were subjected to lateral fluid percussion (FP) injury. A self-made device designed according to (Sullivan et al. 1976) was used. Briefly, the fluid percussion adapter was connected to a transduced housing and this in turn connected to the fluid percussion device. One end of the device was connected to the transducer housing and the other end had a metal piston mounted on O-rings. The longitudinal piston movement during FP was measured with a precision of 6.3 µL. The exposed end of the piston was covered with a rubber pad. The entire system was filled (air bubble-free) with physiological solution. FP-TBI was produced by allowing a 1.85 kg pendulum to strike the Plexiglas plug and generate a hydraulic pressure transiently travelling through the device and impacting upon the dura overlying the brain (the surface area amounted to 113.1 mm^2). The severity of the injury was recorded on a storage oscilloscope. It achieved 3.8 ± 0.3 atmospheres (TBI group). The six animals of the sham group received all experimental procedures except FP-TBI administration and served as control animals. Six hours after the last measure, animals were sacrificed. A 30% potassium chloride solution was injected intracardially, the whole brain was quickly removed from the skull, the hemispheres were separated sagittally and then immersed in −50°C 2-methylbutane for at least 2 min.

Tissue Preparation

Sagittal whole-brain sections, ipsilateral to trauma (rat: 12 µm, pig: 20 µm) were cut with a cryostat microtome (MICROM, Walldorf, Germany), entirely mounted onto untreated glass slides [rat: 25 × 75 mm (3 slices); pig: 45 × 75 mm, (2 slices) Neolab, Heidelberg, Germany], dried at room temperature and stored at −28°C for at least 3 days. The piglet brain had to be sectioned with greater thickness to allow the preparation of sagittal whole-brain slices. Consequently, the greater number of receptors per brain slice was taken into account by normalizing the amount of bound tracer to the amount of protein included in the regions of interest.

In vitro Autoradiography

In vitro autoradiography of [^{125}I]Tyr^{54}-α-bungarotoxin (BTX) (Perkin Elmer, USA, specific activity: 76 Ci/mmol) was performed according to published methods (Sparks and Pauly 1999; Terry et al. 2005) with minor modifications. Briefly, thaw-mounted sections were dried for 15 min at room temperature and pre-incubated in assay buffer (50 mM TRIS–HCl, pH 7.4, 0.1% bovine serum albumin) for 30 min at room temperature. Slices were then incubated for 120 min at room temperature in assay buffer containing the ligand. Non-specific binding was determined in the presence of 3 mM nicotine. At the end of the incubation, the slides were washed three times in 50 mM TRIS–HCl (pH 7.4, 4°C) for 20 min, rinsed for 30 s in 5 mM TRIS–HCl (pH 7.4) and dipped in ice-cold ultrapure water for 10 s. Thereafter, the slides were air-dried for 20 min, dehydrated over phosphorus pentoxide for 3 days and exposed to BAS-MS2325 imaging plates (Fuji Film, Tokyo, Japan) together with ^{125}I standards (Micro scales, Amersham, UK). Plates were analysed using a BAS-1800 II system BioImaging Analyzer (Fuji Film, Tokyo, Japan). Quantitative analysis of the scan data was performed by computer-assisted microdensitometry (Aida 2.31, Raytest, Germany). Irregular regions of interest were drawn over the selected areas of the brain. The brain regions of interest were confirmed by Nissl staining on adjacent sections.

To determine the radioligand affinity (K_D) to α7 nAChR, rat brain sections were incubated with serial dilutions of [^{125}I]α-BTX (0.1–15 nM). Non-linear regression analysis was performed based on Michaelis–Menten-type kinetics: $f(x) = (B_{max} * x)/(K_D + x)$, where x represents the concentration of the radioligand and B_{max} the maximal receptor occupancy.

Statistical Processing

All brain regions of interest were determined in every single animal (within-subject design). Afterwards, the

corresponding values of the groups were processed yielding an averaged value of receptor binding [fmol [^{125}I]α-BTX/mg protein]. Differences between trauma and control groups were tested using a two-tailed Student's t test for homoscedastic variables with significance levels of $\alpha = 0.05$ and 0.005.

Results

Rat Model (Controlled Cortical Impact)

Saturation analysis provided an approach to estimate the whole-brain affinity of the radioligand to the α7 nAChR. We determined a K_D of 0.9 ± 0.5 nM. Using 5 nM incubation solutions of [^{125}I]α-BTX in our experiments, a receptor occupancy of about 85% will be achieved. Therefore, the calculated radiotracer binding [fmol/mg] is a reasonable estimate of the α7 nAChR density. Table 1 summarizes α7 nAChR densities in various brain regions of adult rats at 2, 24 and 72 h after sham operation and CCI, respectively. In the 2 h control group, the highest α7 nAChR densities (>100 fmol/mg) were observed in the superior colliculus, hypothalamus, dentate gyrus and orbital cortex (Fig. 1a). Moderate densities (50–100 fmol/mg) were present in the cortical areas, forebrain, hippocampus and cerebellum. Two hours after CCI, 13 out of 15 regions exhibited significant reductions (Fig. 2). Twenty-four hours after CCI, we detected the most prominent decreases in α7 nAChR densities (reduction of 21–47% compared with control: significance in 14 out of 15 regions, with nine regions significantly lower compared with the 2 h group). Seventy-two hours after CCI, significantly lowered receptor densities were found in 7 out of 15 brain regions. The rest of the investigated regions had nearly adapted to the values of the sham animals.

In sham-operated animals, the α7 nAChR densities remained stable over time in most brain regions. However, in a few regions (orbital and visual cortex, nucleus accumbens, thalamus and pons), a significant decline was observed, whereas the dentate gyrus exhibited a significant increase.

Pig Model (Fluid Percussion Injury)

Figure 1b shows an autoradiograph of the [^{125}I]α-BTX binding in the pig brain. Values of [^{125}I]α-BTX binding in 22 regions of the newborn piglet's brain are listed in Table 2. In newborn pigs subjected to TBI, the colliculus superior (−26%) including stratum superficiale (−30%), the nucleus lateralis dorsalis (−31%) of the thalamus, the nucleus centralis medialis (−32%) of the thalamus and the hippocampus (−38%) including cornu ammonis 1 (CA1) (−40%) exhibited statistically significant declines in α7 receptor density compared with the control group. The other investigated brain regions showed a tendency of declined α7 density, except the pons, which exhibited a statistically not significant increase in α7 receptor density (+29%).

Table 1 α7 nAChR density [fmol/mg protein] in various brain regions at different times after controlled cortical impact in the rat (n = 8/group)

Region of interest	2 h			24 h			72 h		
	Sham (control)	Trauma	% control	Sham (control)	Trauma	% control	Sham (control)	Trauma	% control
Caudate putamen	44.1 ± 5.5	39.7 ± 6.7	90.0	41.5 ± 4.7	28.8 ± 5.2	69.4**	41.2 ± 1.9	37.2 ± 8.0	90.3
Cerebellum	52.1 ± 4.6	35.4 ± 5.1	67.9**	46.7 ± 7.3	28.4 ± 4.2	60.8**	47.4 ± 3.0	37.4 ± 5.1	78.9*
Colliculus superior	136.9 ± 8.0	112.0 ± 8.7	81.8**	138.8 ± 10.7	96.5 ± 4.6	69.5**	134.3 ± 10.6	114.0 ± 8.3	84.9
Colliculus superior stratum superficiale	201.9 ± 6.1	163.6 ± 11.1	81.2**	198.8 ± 8.3	150.2 ± 12.0	75.6**	204.4 ± 24.1	173.3 ± 10.7	84.8
Corpus callosum	33.1 ± 3.9	23.7 ± 3.7	71.6**	31.5 ± 2.8	16.9 ± 4.3	53.7**	32.1 ± 1.4	24.8 ± 4.1	77.6*
Dentate gyrus	126.0 ± 19.9	111.7 ± 22.7	88.7	143.9 ± 24.9	112.3 ± 13.7	78.0*	149.5 ± 7.0	140.5 ± 32.4	94.0
Hippocampus	86.4 ± 6.6	72.8 ± 9.5	84.3*	89.8 ± 11.8	63.3 ± 5.7	70.5**	89.9 ± 4.2	77.8 ± 7.5	86.5*
Hippocampus CA1	80.5 ± 3.9	67.5 ± 6.1	83.9**	82.8 ± 6.3	58.3 ± 3.0	70.4**	83.6 ± 5.0	65.8 ± 6.4	78.7*
Hypothalamus	133.1 ± 22.5	108.4 ± 14.5	81.4*	123.6 ± 27.0	97.5 ± 13.7	78.9	124.6 ± 13.3	106.8 ± 6.7	85.7
Motor cortex	84.8 ± 7.1	55.3 ± 8.4	65.2**	77.2 ± 3.8	45.8 ± 5.5	59.3**	80.8 ± 2.6	61.2 ± 5.7	75.7**
Nucleus accumbens	54.8 ± 3.9	40.0 ± 4.7	73.0**	48.1 ± 5.4	33.4 ± 8.1	69.4*	46.1 ± 6.3	38.8 ± 6.3	84.2
Orbital cortex	102.4 ± 9.1	75.0 ± 8.9	73.2**	75.6 ± 8.3	56.2 ± 11.2	74.3*	75.7 ± 5.3	63.2 ± 7.5	83.5
Pons	44.0 ± 4.8	29.6 ± 5.3	67.3**	39.0 ± 7.7	34.4 ± 3.5	88.2*	38.1 ± 3.3	28.5 ± 3.2	74.8**
Thalamus	58.0 ± 5.0	42.8 ± 5.9	73.8**	48.4 ± 4.6	32.8 ± 3.8	67.8**	48.5 ± 4.1	40.6 ± 6.3	83.7
Visual cortex	82.4 ± 4.3	65.0 ± 3.7	78.9**	76.9 ± 7.6	53.5 ± 5.2	69.6**	76.5 ± 2.9	58.8 ± 7.8	76.9*

Data are mean ± SD. Significance tested with the Student's t test. * $P < 0.05$; ** $P < 0.005$

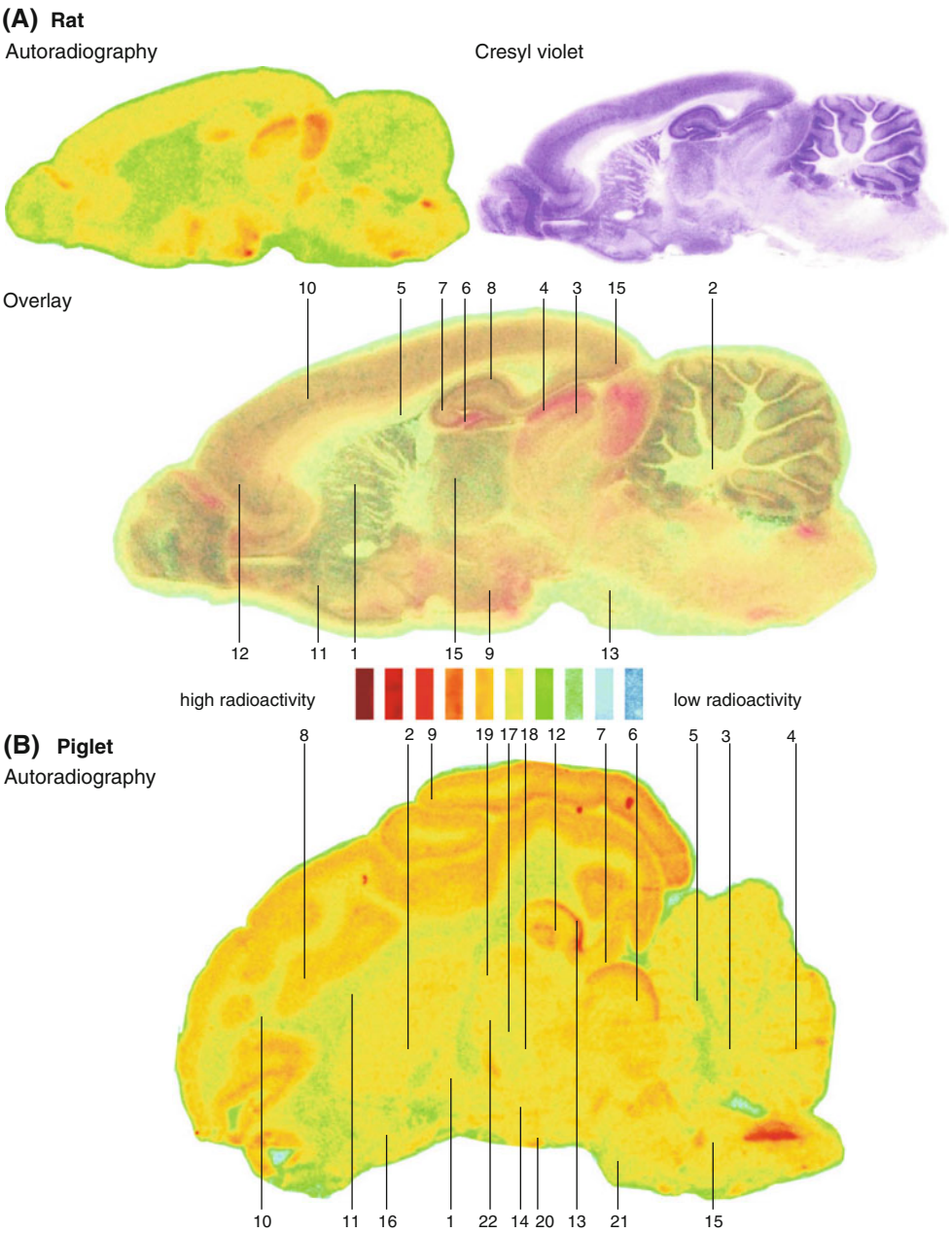

Fig. 1 **a** Autoradiography of an adult rat brain slice (trauma); *red* (*dark*) areas represent high, *yellow*/*green* (*bright*) areas represent low binding of [^{125}I] α-BTX. Adjacent Nissl-stained rat brain slice and overlay with investigated brain regions. 1. Caudate Putamen; 2. Cerebellum; 3. Colliculus superior; 4. Colliculus superior stratum superficiale; 5. Corpus callosum; 6. Dentate gyrus; 7. Hippocampus; 8. Hippocampus CA1; 9. Hypothalamus; 10. Motor cortex; 11. Nucleus accumbens; 12. Orbital cortex; 13. Pons; 14. Thalamus; 15. Visual cortex **b** Autoradiography of a newborn piglet brain slice (trauma); *red* (*dark*) areas represent high, *yellow*/*green* (*bright*) areas represent low binding of [^{125}I]α-BTX. 1. Area hypothalamica anterior; 2. Caudate putamen; 3. Cerebellum; 4. Cerebellum, grey matter; 5.Cerebellum, white matter; 6. Colliculus superior; 7. Colliculus superior stratum superficiale; 8. Cortex; 9. Cortex, grey matter; 10. Cortex, white matter; 11. Corpus callosum; 12. Hippocampus; 13. Hippocampus CA1; 14. Hypothalamus; 15. Medulla; 16. Nucleus accumbens; 17. Nucleus centralis medialis; 18. Nucleus dorsalis medialis; 19. Nucleus lateralis dorsalis; 20. Nuclei mammillaris; 21. Pons; 22. Thalamus. (Color figure online)

Discussion

The binding of [^{125}I]α-BTX to α7 nAChR was investigated by quantitative autoradiography in adult rats and newborn pigs after experimental TBI. This radioligand is highly selective for α7 nAChR (Alexander et al. 2006). A moderate affinity (K_D = 50 nM) to β3/β3 subunits of $GABA_A$ receptors has been described (McCann et al. 2006). The β3-subunit mRNA of the $GABA_A$ receptor is widely expressed in the central nervous system, especially in the forebrain (Wisden et al. 1992). However, the subunit composition with β3/β3 interface has not been described in the brain so far. [^{125}I]α-BTX binds to α1 and α7–α10 subunits but not to α2–α6 subunits (Alexander et al. 2006; Gotti and Clementi 2004). Of α1-, α7-, α8-, α9- and α10-containing nAChR, only the α7 nAChR is present in the mammalian brain (Gotti and Clementi 2004). We determined a high [^{125}I]α-BTX affinity to α7 nAChR in the adult rat brain (0.9 nM). This value corresponds to previously reported data from various studies (Alexander et al. 2006; Clarke et al. 1985; Verbois et al. 2002).

The cell population, which is mainly involved in the reduction in α7 nAChR density, cannot be determined in this study because quantitative macroscopic autoradiography, as used in these experiments, is unable to differentiate between cell types. As reported in previous studies, α7 nAChR are

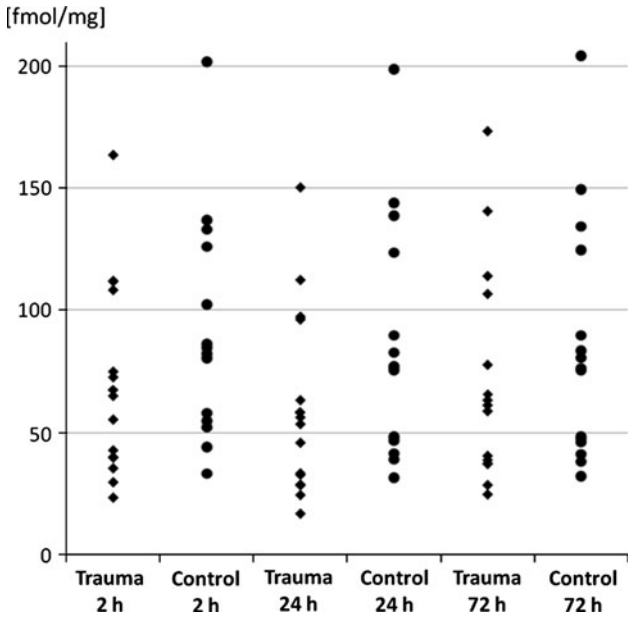

Fig. 2 [^{125}I]α-BTX binding in investigated rat brain regions displayed as mean values of the trauma and control animals. Each *symbol* represents a specific brain region

expressed not only on neurons but also on peripheral macrophages (Wang et al. 2003), central microglia (Shytle et al. 2004) and astrocytes (Wang et al. 2007). Activation of this α7 nAChR attenuates the release of inflammatory mediators (Wang et al. 2004). Nimmerjahn and co-workers identified central microglial cells as housekeepers in the adult mouse brain, which are activated near injuries (Nimmerjahn et al. 2005), including TBI (Kelso et al. 2006). Activated microglial cells are assumed to support neuroprotection after brain injury (Shein et al. 2008). Presumably, the microglia-mediated neuroprotection, at least partly, is due to microglia-associated α7 nAChR (Suzuki et al. 2006). Therefore, the α7 nAChR is likely to be essential to cholinergic control of inflammation (Wang et al. 2003). It can be hypothesized that cholinergic hypofunction, including lowered α7 nAChR density, causes increased inflammation after TBI and thus may contribute to neurodegenerative processes (Carnevale et al. 2007); for a review see (Conejero-Goldberg et al. 2008; Rosas-Ballina and Tracey 2009). In this study, we regard the reported changes in [^{125}I]α-BTX binding to reflect general alterations in α7 nAChR densities.

Alterations in α7 nAChR Densities in Rat Brain After CCI Injury

The exceptionally high α7 nAChR densities in the regions of superior colliculus, hypothalamus and dentate gyrus are in accordance with previous studies of α7 nAChR distribution in rats (Miller et al. 1982; Tribollet et al. 2004). CCI caused a widespread and significant decrease in α7 nAChR

Table 2 α7 nAChR density [fmol/mg protein] 6 h after fluid percussion injury in the newborn pig (Sham: $n = 6$; Trauma: $n = 7$)

Region of interest	Sham (control)	Trauma	% control
Area hypothalamica anterior	22.9 ± 11.0	17.2 ± 12.7	75.1
Caudate putamen	21.6 ± 12.5	14.3 ± 8.0	66.2
Cerebellum	16.7 ± 9.5	11.2 ± 8.9	67.1
Cerebellum, grey matter	24.3 ± 8.8	21.8 ± 11.9	89.7
Cerebellum, white matter	6.3 ± 5.2	5.5 ± 4.3	87.3
Colliculus superior	50.5 ± 10.7	37.5 ± 13.7	74.3*
Colliculus superior stratum superficiale	90.5 ± 16.3	63.5 ± 21.9	70.2**
Cortex	32.0 ± 18.0	24.9 ± 10.6	77.8
Cortex, grey matter	46.7 ± 19.9	37.6 ± 15.0	80.5
Cortex, white matter	12.5 ± 12.7	9.4 ± 6.3	75.2
Corpus callosum	8.1 ± 9.7	5.7 ± 4.0	70.4
Hippocampus	49.8 ± 16.2	31.0 ± 14.4	62.2**
Hippocampus CA1	115.6 ± 30.5	69.2 ± 31.3	59.9**
Hypothalamus	22.6 ± 12.4	17.8 ± 11.1	78.8
Medulla	28.1 ± 22.6	16.8 ± 11.6	59.8
Nucleus accumbens	17.8 ± 8.8	12.8 ± 9.9	71.9
Nucleus centralis medialis	29.0 ± 8.3	19.6 ± 10.5	67.6*
Nucleus dorsalis medialis	31.1 ± 8.3	23.7 ± 10.0	76.2
Nucleus lateralis dorsalis	33.9 ± 13.3	23.4 ± 12.2	69.0*
Nuclei mammillaris	30.7 ± 21.4	24.1 ± 17.2	78.5
Pons	11.8 ± 12.9	15.2 ± 15.1	128.8
Thalamus	29.2 ± 8.3	22.4 ± 11.5	76.7

Data are mean ± SD. Significance tested with the Student's t test. * $P < 0.05$; ** $P < 0.005$

densities in most of the brain regions compared with the controls (13 out of 15 regions after 2 h, 14 regions after 24 h and seven regions after 72 h). As the α7 density reached its lowest values 24 h after CCI and increased until 72 h after CCI, this pattern of α7 nAChR kinetics suggests the *post*-traumatic activation of a compensatory mechanism. The processing of control groups for every survival time confirmed that the measured alterations are directly associated with trauma-triggered events. Our findings support previous studies in isoflurane-anaesthetized rats that showed a decrease in [^{125}I]α-BTX binding in 10 brain regions (among them, seven of the hippocampal formation) between 1 and 72 h after CCI (Verbois et al. 2000, 2002).

Also, the presented alterations in α7 nAChR are in accordance with our earlier results, where we found changes in cholinergic markers after CCI (Donat et al. 2008). It seems that there are mechanisms that cause a loss of cholinergic receptors after experimental TBI. This could be attributed to an increased activity of the AChE (Donat et al. 2007). Such an increase in turn could be caused by an excessive release of ACh indirectly found by other authors (Scremin et al. 2006).

In the present study, sagittal instead of coronal brain slices were used. This allowed us to include regions anterior and posterior to the hippocampus. The hippocampal dentate gyrus had its minimal α7 nAChR density already 2 h *post*-injury. This may be attributed to its exceptionally high sensitivity (Förster et al. 2006), which has been shown before (Verbois et al. 2002). Furthermore, excitatory afferent neurons terminate in dentate gyrus, a region of post-natal neurogenesis (Förster et al. 2006). Both aspects provide an explanation for the high susceptibility to alterations in the cholinergic system and excitotoxic effects.

Surprisingly, we detected time-dependent alterations in the brain of control animals. The present data suggest two possible explanations for this pattern. First, the sham operation may result in a microtrauma. Given the high susceptibility of the α7 nAChR to TBI (Verbois et al. 2002) even that slight injury caused during sham operation may lead to a reduction in α7 receptor density. Scremin and coworkers described a similar effect after a sham operation including a craniotomy (Scremin et al. 2006). This was the reason for us to just cut the scalp of the control animals and suture the laceration afterwards. Second, the time-dependent alterations in the sham groups may be related to the applied anaesthesia. Midazolam and fentanyl are known to decrease the ACh release in the hippocampus and at pontine cholinergic terminals, respectively (Imperato et al. 1993; Mortazavi et al. 1999). The antidotes flumazenil and atipamezole are known to increase the ACh release in rats (Moor et al. 1998; Tellez et al. 1997). In either case, the described alterations in the control groups confirm the exceptional susceptibility of α7 nAChR for disturbances in the brain (Verbois et al. 2000, 2002). Nonetheless, the reductions in α7 density that were detected especially at 24 h after trauma are unambiguous and can be hardly assigned entirely to an effect of anaesthetics.

Alterations in α7 nAChR Densities in Newborn Pig Brain After Lateral Fluid Percussion Injury

Newborn pigs underwent a lateral FP injury as described in previous publications (Donat et al. 2010a, b). Compared to the CCI technique, the FP method causes a diffuse and more widespread injury to the brain (McIntosh et al. 1989; Prins and Hovda 2003).

Six hours after FP injury, the decline in α7 density was not only measurable throughout the brain, but was significantly reduced in regions essential for memory and attention: the hippocampus and thalamus. The relatively high variability shown in Table 2 may be attributed to individual differences in the α7 nAChR expression perhaps caused by the crossbreed origin of the piglets. Another possible explanation for high variability may be provided by certain differences in reaction of the immature brain to TBI: the immature brain is evolving rapidly, undergoing the so-called brain growth spurt (Dobbing and Sands 1979). During this period, it is highly sensitive and vulnerable to disturbances (Dobbing 1982). The cholinergic system in general (Dwyer et al. 2009; Lauder and Schambra 1999) and the α7 nAChR in particular (Broide et al. 1995; Dwyer et al. 2008) are believed to play an important role in cortical development, which *in toto* possibly accounts for individual differences.

Remarkably, reduction in α7 density occurred not only in brain regions close to the trauma site but also in regions that are spatially distant to the trauma site (for example thalamus and colliculus superior). As for the FP injury applied in our pig model, these remote regions may be partly influenced in a direct manner by the shockwave, which originates at the FP device and traverses the pig brain. As for the focal CCI injury in the rat model, only brain regions close to the trauma site can be damaged directly. Therefore, significant reductions in α7 density in remote rat brain regions have to originate from an indirect mechanism on the cellular/neuronal level. Excitotoxic neurodegeneration and apoptotic neurodegeneration are known effects of trauma to the developing brain (Bittigau et al. 2004) and provide a possible explanation for trauma site-distant reductions in α7 density.

To date, the authors are not aware of other data on α7 nAChR in newborn pig brain and thus on involvement of α7 nAChR in immature TBI. We found significant decreases in α7 nAChR density in newborn pig brains. The affected regions and the extent of the decreases are, at least at 6 h post-injury, similar to those found in adult rats.

Therefore, it seems that α7 nAChR is influenced by TBI not only in adult (Verbois et al. 2002) but also in immature animals.

Considering the fact that the gyrencephalic pig brain is structurally much closer to the human brain than the lissencephalic rat brain (Duhaime 2006; Duhaime et al. 2000), this provides evidence that α7 nAChR is possibly affected in a similar way in paediatric TBI.

These findings are in line with our earlier findings of cholinergic changes in newborn piglets after FP injury. No significant changes in other nAChR (α4*/α3*) were observed (Donat et al. 2010a) contrary to the reductions found in adult rats (Donat et al. 2008). Also besides decreased nAChR density, mAChR also showed significant reductions (Donat et al. 2010a). It can be hypothesized that there is a common TBI-triggered pathophysiological mechanism that causes the loss of cholinergic receptors in rats and pigs [for example increases in AChE activity without loss of cholinergic neurons (Donat et al. 2007)]. However, this remains highly speculative because of the mentioned differences in methodology.

Adult and Immature TBI: Similarities and Differences

Age is still seen as a predictor of functional recovery after human TBI, although consensus does not exist. Several authors suggested a better recovery of children at an early age (Levin et al. 1992) due to the plasticity of the immature brain. Despite the postulated enhanced capacity for functional recovery, the immature brain appears to be more vulnerable to injuries, at least after severe injury in humans (Feickert et al. 1999; Mazzola and Adelson 2002), with potentially more drastic consequences for the cognitive development (Koskiniemi et al. 1995). However, a recent experimental study with pigs of different age showed that the size of a cortical lesion increases with age (Missios et al. 2009). Therefore, in experimental studies on immature TBI, the age of the animals is an important factor, because it determines roughly the comparable age group of children. Regarding electroencephalography, ChAT activity and synapse formation, a rat of post-natal days 12–13 can be compared with a human full-term newborn (Romijn et al. 1991). Therefore, newborn pigs are far more suitable animal models of immature TBI than rodents, because their physiological parameters and brain development are much closer to those of human infants (Duhaime 2006). Biomechanical properties of the injury, the species and the age complicate the comparison of injury types and severities. This is also the case in the present study. However, the immature brain shows unique responses to TBI due to structural differences in the brain. This includes increased water content of the tissue often causing diffuse brain swelling (Adelson and Kochanek 1998), lower antioxidant defences, which contributes to oxidative stress (Bayir et al. 2006) and increased cerebral blood volume, which is associated with brain swelling. Additionally, neurotransmitter systems also show different reactions to TBI: severe FP-TBI induces an upregulation of dopaminergic activity in the mesotelencephalic dopaminergic system of newborn, but not juvenile piglets (Walter et al. 2004). There is also an age-dependent susceptibility of the brain to excitotoxicity. Activation of NMDA receptors in the immature brain induces a higher calcium influx, compared with the adult brain (Burnashev et al. 1992). Although we did not investigate excitotoxicity in our study, it seems likely that excitotoxic effects are associated with this ion channel due to the exceptionally high Ca^{2+} influx mediated by the α7 nAChR on activation (Burnashev 1998).

In conclusion, we showed significant decreases in α7 nAChR densities in adult rats as well as in immature pigs, each injured with a different model of TBI. This indicates a common pathophysiological mechanism in TBI that causes a loss of α7 nAChR and therefore influencing excitotoxic damage, inflammation and cognitive impairment after brain injury. It might provide a valuable target for trauma research and imaging with PET.

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