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Imaging correlates of behavioral impairments: An experimental PET study in the rat pilocarpine epilepsy model

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ABSTRACT

Psychiatric comorbidities are prevalent in patients with epilepsy and greatly contribute to the overall burden of disease. The availability of reliable biomarkers to diagnose epilepsy-associated comorbidities would allow for effective treatment and improved disease management. Due to their non-invasive nature, molecular imaging techniques such as positron emission tomography (PET) are ideal tools to measure pathologic changes. In the current study we investigated the potential of [^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) and 2′-methoxyphenyl-(*N*-2′-pyridinyl)-*p*-^{18}F-fluoro-benzamidoethylpiperazine ([^{18}F]MPPF) as imaging correlates of neurobehavioral comorbidities in the pilocarpine rat model of epilepsy. Findings from rats with epilepsy revealed a regional reduction in [^{18}F]FDG uptake indicating thalamic hypometabolism. In addition, an increase in septal [^{18}F]MPPF binding was observed in rats with spontaneous recurrent seizures. Both thalamic [^{18}F]FDG and septal [^{18}F]MPPF data proved to correlate with behavioral alterations including decreases in luxury behavior such as burrowing and social interaction, and changes in behavioral patterns in anxiety tests. A correlation with seizure frequency was confirmed for thalamic [^{18}F]FDG data. Moreover, thalamic [^{18}F]FDG and septal [^{18}F]MPPF data exhibited a correlation with brain-derived neurotrophic factor (BDNF) serum concentrations, which were lowered in rats with epilepsy.

In conclusion, μPET data from rats with pilocarpine-induced epileptogenesis indicate altered septal 5-HT$_{1A}$ receptor binding. Further research is necessary assessing whether septal 5-HT$_{1A}$ receptor binding may serve as an imaging correlate of neuropsychiatric comorbidities in epilepsy patients and for severity assessment in rodent epilepsy models. In contrast, we obtained evidence that [^{18}F]FDG uptake also reflects the severity of epilepsy and, thus, might not constitute a biomarker with sufficient specificity for psychiatric comorbidities. Evidence has been obtained that BDNF might serve as a peripheral circulatory biomarker. Further experimental and clinical assessment is necessary for validation of the marker candidates.

1. Introduction

Comorbidities can significantly contribute to the burden of epilepsy with relevant consequences for the patient's quality of life (Kanner, 2013, 2016). Interestingly, the manifestation of epilepsy is bi-directionally associated with neuropsychiatric disorders (i.e. anxiety disorders, depression and autistic spectrum disorder) (Buckley and Holmes, 2016; Keezer et al., 2016). On one hand, epilepsy is associated with an increased prevalence of psychiatric comorbidities (Kanner et al., 2012; Lin et al., 2012). On the other hand, neuropsychiatric symptoms before onset of epilepsy are negatively associated with the therapeutic outcome (Hitiris et al., 2007; Pohlmann-Eden et al., 2015). It is nowadays well-accepted that seizures and neuropsychiatric symptoms can actually represent different symptoms of a shared etiology and neuronal network disease (Kanner et al., 2017; Lee et al., 2015; Pohlmann-Eden et al., 2015).

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Implications of psychiatric comorbidities need to be taken into account for an optimized individualized patient management and personalized therapeutic decisions (Kanner, 2013, 2016; Mula, 2017). Thus, objective parameters for the assessment and diagnosis of neuropsychiatric problems are of particular clinical interest (Pitkanen et al., 2016; Ravizza et al., 2017). This is especially true for patients with cognitive disabilities, which may hamper the clinical diagnosis of respective comorbidities.

Molecular neuroimaging provides opportunities to visualize alterations in brain activity patterns based on administration of [^{18}F]FDG and analysis of local glucose consumption. It is well known that major depression in patients can be associated with alterations in [^{18}F]FDG-PET data (Su et al., 2014). Regional hypometabolism has also been demonstrated in patients with temporal lobe epilepsy and a history of depression (Kondziella et al., 2007; Salzberg et al., 2006). Another parameter of interest, in the context of depression and anxiety disorders are alterations in the serotonergic system, which for instance can be assessed based on 5-HT$_{1A}$ receptor binding. Evidence has been obtained, that clinical scores of depression in temporal lobe epilepsy patients exhibit a significant inverse relation to 5-HT$_{1A}$ receptor binding (Giovacchini et al., 2005; Theodore et al., 2007). On the other hand, other studies have suggested a positive correlation between 5-HT$_{1A}$ receptor binding in different brain regions and depressive symptoms in temporal lobe epilepsy (Lothe et al., 2008).

Numerous studies have demonstrated that psychiatric comorbidities in subgroups of patients with epilepsy are reflected by behavioral alterations in various rodent epilepsy models (Minjarez et al., 2017). The most common psychiatric comorbidities in epileptic patients are anxiety, depressive and autistic spectrum disorders (Kanner, 2016; Lafrance Jr. et al., 2008). Respective behavioral symptoms in animals range from enhanced anxiety- and anhedonia-associated behavioral patterns to alterations in social interaction (Seo et al., 2013; Vrinda et al., 2017). Epilepsy models with a face validity that comprises neurobehavioral alterations reflecting those in patients may guide the validation of imaging biomarkers for clinical use. Thereby, the application of behavioral test batteries allows assessment of a correlation between a biomarker candidate and behavioral parameters under standardized conditions. The validation of imaging biomarkers for neurobehavioral alterations in experimental animals has an additional interest. There is actually an urgent need for parameters, which can guide severity assessment in chronic animal models including epilepsy models (Lidster et al., 2016; Wolfensohn et al., 2013). Valid biomarkers for severity might for instance guide recommendations for an animal-welfare based prioritization of different models, and might render a basis for the development of evidence-base refinement measures (Lidster et al., 2016).

In this study, we determined alterations in brain metabolic activity and serotonergic neurotransmission based on [^{18}F]FDG and [^{18}F]MPPF μPET in a rat post-status epilepticus model with pilocarpine-induced epilepsy development. The model has been selected based on the pronounced neurobehavioral alterations that have been repeatedly reported in this epilepsy model (Minjarez et al., 2017). The correlation of μPET data with various neurobehavioral, physiological and biochemical parameters was assessed. Behavioral data were obtained from a series of behavioral paradigms aimed to assess the neurobehavioral deficits, that have been described in patients with epilepsy. These include hyperactivity, increased anxiety, anhedonia as one symptom of depressive disorders, and autistic behavior. When selecting behavioral paradigms, we avoided the choice of paradigms that are associated with a pronounced distress situation for the animals as respective assays might have significantly affected the outcome.

Thus, the behavioral analysis focused on an assessment of anxiety- and anhedonia-associated behavior as well as changes in activity and social interaction.

2. Materials and methods

2.1. Animals and experimental groups

Female Sprague Dawley rats (180–220 g, Envigo, Netherlands) were individually housed under controlled environmental conditions (22–24 °C, 45–60% humidity) in a 12 h dark-light cycle with food and water ad libitum. Animals were kept in the absence of males. In this context, we would like to point that a vaginal swab analysis at the end of the experiment indicated that the rats exhibit an active estrous cycle with an expected number of animals in the estrous phase at one selected time point. Every animal received new bedding and 14 g of nesting material (Enviro-Dri®, Claus GmbH, Germany) once a week. 45 rats were randomly divided (randomizer.org) into three experimental groups: naïve (not implanted, n = 12), sham (implanted, n = 12) and pilocarpine-treated (implanted and treated with pilocarpine, n = 21). Rats were weighed and controlled based on severity assessment schemes including the Grimace scale (Sotocinal et al., 2011) and a modified Irwin scale (Irwin, 1968). All investigations were approved by the Committees of the government of Upper Bavaria (license number: AZ 55.2-1-54-2532-105-16) and were in line with the German Animal Welfare act and the EU directive 2010/63/EU.

2.2. Electrode implantation and SE induction

Implantation of a recording electrode was performed under general anesthesia with chloral hydrate (360 mg/kg i.p.), analgesia by meloxicam (Metacam®, Boehringer Ingelheim, Germany, 1 mg/kg, 30 min pre- and 24 h post-surgery, s.c.) and local anesthesia by bupivacaine (Bupivacain, 0.5%; Jenapharm, Germany; s.c.). A bipolar Teflon-isolated stainless-steel stimulation electrode was stereotactically implanted into the right hippocampal dentate gyrus (AP + 3.9, L + 1.7 mm and V + 4 mm, according to the atlas of Paxinos and Watson (2005)). One screw, placed above the left parietal cortex, served as ground electrode. Two additional screws and dental acrylic cement were used to anchor the entire implant. To prevent postoperative infections, rats received the antibiotic marbofloxacin (Marbofloxacin, Marbocyl, 1 mg/kg Vetoquinol, Germany) twice daily for seven days starting one day before surgery. Two rats that failed to recover from surgery were euthanized following operation. After three recovery weeks, lithium chloride (127 mg/kg i.p., Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was injected 14–16 h before pilocarpine treatment. Scopolamine methyl-bromide (1 mg/kg i.p., Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was injected 30 min before the first pilocarpine injection to minimize peripheral pilocarpine effects. In order to reduce mortality and to ensure the occurrence of SE, pilocarpine (10 mg/kg i.p., Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was injected every 30 min until the onset of SE. Injections were stopped once a rat developed SE. The total number of pilocarpine injections was limited to ten per animal. Seizure severity was evaluated based on the previously described score from Racine (1972): 1 = immobility, eye closure, ear and vibrissae twitching, salivation, sniffing, facial clonus; 2 = head nodding and mastication associated with more severe facial clonus; 3 = clonus of one forelimb; 4 = bilateral clonus accompanied by rearing; 5 = rearing and falling accompanied by generalized clonic seizures. SE onset was defined as the recurrence of at least two seizures either stage 4 or 5 within a time window of 30 min. Only rats that developed SE with generalized convulsive seizures, were used for further experiments. SE was terminated after 90 min by the administration of phenobarbital (25 mg/kg, i.p.) and diazepam (10 mg/kg, i.p., repeated up to five times every 15–20 min; Ratiopharm; Ulm, Germany). Termination of SE was confirmed by EEG recordings in addition to visual inspection. Post-SE rats were injected s.c. for two days with Ringer lactate solution and fed with baby food until they resumed normal feeding behavior. One sham rat was euthanized three months after the start of the experiment due to

tumor development, while one rat with epilepsy was euthanized before starting the black/white box test because of electrode loss. Data gathered from these rats before being euthanized was included in the analysis.

2.3. Video/EEG monitoring for spontaneous seizures

In order to detect the occurrence of spontaneous recurrent seizures (SRSs), eight weeks after pilocarpine treatment, SE rats were video/EEG monitored for 15 days (24 h/day). Sham rats were also placed in the monitoring cages and handled in parallel. Infrared light sensitive cameras, a multiple-channel PCI analog-digital converter (ABUS Security-Tech, Affing, Germany), 1-channel bioamplifiers (BioAmps, AD Instruments, Hastings, East Sussex, United Kingdom), and analog-digital converters (PowerLab/800 s, AD Instruments, Hastings, East Sussex, United Kingdom) were utilized for video-EEG recording. Video and EEG files were examined using the Digi-Protect Searcher 6.275 beta (ABUS Security-Tech, Affing, Germany) and the Chart5 for windows software (AD Instruments, Hastings, East Sussex, United Kingdom). In addition, in order to detect a possible impact of recent seizures on PET signal, rats with epilepsy were video-monitored (24 h/day) for three days up to the last PET day. The total number of generalized tonic-clonic motor seizures was recorded including information about seizure stage and duration. In addition, handling-associated seizures or seizures observed by direct observation of the rats in their home cages were noted during the entire study.

2.4. PET radiochemistry

The radiosynthesis of no-carrier-added (n.c.a.) [^{18}F]-MPPF (4-[^{18}F] fluoro-N-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)-N-(pyridin-2-yl)benzamide) was performed by a nucleophilic aromatic substitution in a fully automated process using a Elysia-Raytest module (Elysia-Raytest R&D, Straubenhardt, Germany) according to an established synthesis sequence with minor modifications (la Fougere et al., 2010). In detail, the azeotropically dried [^{18}F]F^-/Kryptofix/K_2CO_3 was reacted with 10 mg of N-(2-(4-(2-methoxyphenyl) piperazin-1-yl)ethyl)-4-nitro-N-(pyridin-2-yl)benzamide (Nitro-MPPF) (ABX, Radeberg, Germany) dissolved in 500 µl of DMSO at 180 °C for 5 min. After cooling down to 40 °C and diluting the reaction mixture with 3 ml of water, the crude reaction mixture was loaded on a SepPak C-18 light cartridge (Waters). After washing with 2 ml of water, the tracer was eluted with 1 ml of methanol/THF (3:2 *v*/v) and subsequently diluted with 1 ml of buffer (10 mM NaAc, pH 5), and purified by semipreparative HPLC (column: Kromasil C8, 250x10mm, eluent: 60% 10 mM NaAc pH 5 / 24% MeOH / 16% THF, flow: 4 ml/min). The fraction containing the product was diluted with 10 ml of phosphate buffer (0.06 M, pH 7.4) and loaded on a SepPak C-18 light cartridge. The cartridge was washed with isotonic saline and eluted with 500 µl of ethanol. Finally the ethanol was diluted with 4.5 ml of isotonic saline and sterile-filtered through a 0.22 µm membrane. Radiochemical yields were 14 ± 7% (decay corrected to the time of delivery), with a typical radiosynthesis times of 90 min. The specific activities were in the range of 1–3 Ci/µmol, and the radiochemical purities, as determined by radio-HPLC, were > 97%.

2.5. PET acquisition and reconstruction

Twenty-four rats, eight for each experimental group randomly selected (randomizer.org), were submitted to [^{18}F]FDG and [^{18}F]MPPF µPET. Small animal PET imaging was performed on a Siemens Inveon DPET scanner (Siemens Medical Solutions, Munich, Germany). Anesthesia was induced by inhalation of isoflurane (4% for induction and 2% for maintenance during preparation and scanning) supplemented with oxygen at a rate of 1 l/min. Radioligands were administered via a tail vein cannulation and rats were scanned head-to-head. For [^{18}F]FDG, rats were fasted overnight to reduce endogenous glucose levels and an emission scan of 15 min was acquired 30 min post tracer injection. The average injected radioactivity was 34.9 ± 2.7 MBq in a volume of 0.5 ml. For [^{18}F]MPPF an emission scan of 50 min was acquired 10 min post tracer injection (la Fougere et al., 2010). The average injected radioactivity was 30.6 ± 3.3 MBq in a volume of 1–2.5 ml. All emission scans were either preceded or followed by a 15 min transmission using a rotating [^{57}Co] point source. Data was acquired in list-mode and reconstructed into 1 frame for [^{18}F]FDG and 11 frames (5 × 60 s, 3 × 300 s, 3 × 600 s) for [^{18}F]MPPF. All PET data were corrected for attenuation, scatter and decay. Reconstruction was performed with four ordered-subset-expectation-maximization 3D iterations and 32 maximum-a-posteriori 3D iterations and a zoom factor of 1 resulting in a final voxel dimension of 0.78 × 0.78 × 0.8 mm.

2.6. PET processing and quantification

Image processing was completed using PMOD v3.4 software (PMOD Technologies Ltd., Zurich, Switzerland). Tracer-specific PET templates were generated through the averaging of PET images previously aligned by manual co-registration to a digital cryosection-based atlas of the rat brain (Rubins et al., 2003; Toga et al., 1995). Co-registered PET images were then spatially normalized into the space of their respective tracer-specific template by the PMOD brain normalization tool (equal modality; smoothing by 0.8 mm, non-linear warping; 16 iterations, frequency cut-off 3, regularization 1.0, no thresholding) (Overhoff et al., 2016). To facilitate the co-registration of dynamic [^{18}F]MPPF images, this processing was performed on summation images (10–60 min) and the concatenation of both transformations were then applied to the dynamic scans. Volumes of interest (VOIs) for the parietal cortex (16 mm^3), medial prefrontal cortex (13 mm^3), thalamus (22 mm^3), amygdala (23 mm^3), striatum (15 mm^3), hypothalamus (11 mm^3), cerebellum (82 mm^3) and pons (8 mm^3) were drawn manually with reference to the cryosection atlas. Threshold-based automatic VOIs were generated from [^{18}F]MPPF uptake for the hippocampus (61 mm^3) and septum (9 mm^3) due to the high specific binding in these regions. [^{18}F]FDG uptake was quantified as the standardized uptake value ratio (SUVR) by normalizing tracer uptake in target regions to that of the pons. The pons has previously been shown to be a suitable reference region in this model (Fernandes et al., 1999; Handforth and Treiman, 1995) and we confirmed that [^{18}F]FDG uptake in this region did not significantly differ between treatment groups (data not shown). One sham rat was excluded from [^{18}F]FDG analysis due to a paravenous injection. For [^{18}F]MPPF, the non-displaceable binding potential (BP_{nd}) was calculated in target regions from the entire scan period (la Fougere et al., 2010) using the Logan linear graphical method (Logan et al., 1996) with the cerebellum as a reference tissue input. For visualization purposes, images of mean tracer uptake per treatment group were generated, smoothed with an isotropic Gaussian filter (0.5 mm) and projected onto the cryosection atlas of the rat brain.

2.7. Behavioral experiments

All the behavioral tests were performed in the morning, starting 5 days after the last PET scan. The investigations and data analyses were carried out by investigators unaware of the group allocation (sham vs. epilepsy). Please note that blinding was impossible for naïve versus implanted animals during behavior investigations. Tests were run in the following order: social interaction test, burrowing, open field, black-white box, elevated-plus maze, saccharin preference and nest building. Burrowing and nest building were repeatedly assessed during the entire project. There was a three-day recovery period between social interaction and burrowing tests. Burrowing, open field, black-white box and elevated-plus maze tests were performed on four consecutive days, in the morning, while saccharin preference test took place four days after the end of elevated-plus maze test. Assessment of nest

building, burrowing and saccharin preference took place in the animal facilities, while the other tests were performed in a soundproofed test room with a video-tracking software (EthoVision, Version 8.5, Noldus, Wageningen, Netherlands) after a habituation time of 30 min. Nest complexity was scored based on pictures. All materials and devices were cleaned with 0.1% acetic acid between trials.

2.7.1. Social interaction test

Social interaction test is a sensitive method for examining autism-related behavioral deficits (Silverman et al., 2010) commonly associated with epilepsy (Buckley and Holmes, 2016). On two consecutive days of habituation, all rats were transferred to the behavioral room and placed one at a time into test cages (Makrolon Type IV) for 10 min. On the test day, rats were placed in the test arena with a weight-matched partner for 10 min. The time spent in active non-aggressive social interaction (sniffing, following and grooming the partner) was recorded in seconds, as well as the time spent in passive interaction (sitting or lying close together) or in aggressive behavior (kicking, boxing, wrestling, submitting), if present a combined score was obtained for each pair.

2.7.2. Burrowing

Burrowing behavior, together with nest building activity, are luxury behavior indicators of well-being in rodents (Jirkof, 2014). In particular, burrowing represents a sensitive method for detecting behavioral dysfunctions in rodents (Deacon, 2006) occurring in association with hippocampal damage (Deacon et al., 2002) and in stress-induced anhedonia models (Strekalova and Steinbusch, 2010) thus representing a useful tool to investigate behavioral impairments associated with epilepsy. Plastic burrowing tubes (32 cm long × 10 cm Ø × 60 mm high) were filled with 2.5 kg gravel and placed in a Makrolon Type IV cage. All rats received 4 days of training before the assessment of baseline and experimental values: on day 1, rats were placed in the empty cage for 60 min and afterwards received an empty burrowing tube for 60 min; on days 2–4, rats received a gravel-filled tube for 60 min after a habituation time of 60 min in the empty cage. Burrowing behavior was tested on day 5 (baseline), 2 weeks after surgery and 1, 4 and 16 weeks after pilocarpine treatment. As soon as the burrowing tube was placed into the cage, the latency time to start burrowing was measured. The remaining gravel in the tubes was weighed and the difference to the initial value was calculated to determine the amount of displaced gravel (Rutten et al., 2014a; Rutten et al., 2014b). For rats that did not start burrowing, time to onset of burrowing behavior was set equal to the test total duration (3600 sec).

2.7.3. Open field test

The open field test was used to assess novel environment exploration and general locomotor activity. Rats were placed in a round open field (Ø = 85 cm, 10–20 lux) facing the wall at a distance of 10 cm and observed for 10 min. The following parameters were assessed: distance moved, immobility time, immobility frequency, number of entries and time spent in center by mean of video-tracking software. Rearing behavior was manually scored by the experimenter.

2.7.4. Black-white box

The black-white box and the elevated-plus maze tests were used to assess anxiety-associated behavior. The black-white box used in this test is subdivided into two chambers: a small black compartment (39 × 20 × 39 cm, with a top) and a big white compartment (39 × 39 × 39 cm, 50 lux), connected through a poorly illuminated tunnel. The rats were placed in the white compartment, facing away from the tunnel, and the behavior (e.g. latency to enter the black box, stretching postures, time and entries in the white compartment) was recorded for 5 min.

2.7.5. Elevated-plus maze

The apparatus for the elevated-plus maze consists of a central platform (14 × 14 cm) and four arms (two open without walls and two enclosed by 29 cm high walls) 50 cm long and 14 cm wide elevated 82 cm above the ground. The rats were placed in the center of the maze facing always the same closed arm and were observed with the tracking software for 5 min to score activity and anxiety-associated behavior: total distance moved (cm), the time spent in different sections of the maze (open and closed arms), number of entries into open and closed arms, rearing and head-dip frequencies. Rats that jumped off the open arms of the maze were not included in the analysis (Walf and Frye, 2007).

2.7.6. Saccharin preference test

The saccharine preference test is used to assess anhedonia-like behavior and, more in general, depressive-like behavior. During the entire test run, two bottles were placed on both sides of the cage in order to test for a potential side preference bias. On the first day, both bottles were filled with tap water. On the second day, the bottle placed in the right side of the cage was filled with 0.1% saccharin solution (Aldrich Saccharin ≥98%, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). On the third day both bottles contained tap water, while on the fourth day only the bottle on the left side contained saccharin solution. Each bottle was filled with 500 g of liquid and the consumption over 24 h was measured (Klein et al., 2015).

2.7.7. Nest building

The complexity and the shape of the nest (1 = flat, 2 = slightly curved, 3 = deep) were scored daily at 8 a.m. to 10 a.m. for a week after the addition of new nest material to the cage (14 g Enviro-Dri®, Claus GmbH, Germany) (Van Loo and Baumans, 2004). Nest building was scored i) before implantation to determine a baseline value, ii) two week after electrode implantation to detect the impact of surgery, and iii) 1, 2 or 16 weeks after pilocarpine treatment to determine the influence of seizures on nest building behavior.

2.8. Analysis of hair corticosterone

All rats were shaved in the neck region before the start of the experiments to exclude an impact of previous stressful events on corticosterone levels. Afterwards hair was allowed to regrow and shaved again at the moment of the killing. All samples were stored protected from light at room temperature. The samples were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) (Gao et al., 2013).

2.9. Analysis of fecal corticosterone metabolites

Feces samples were collected before surgeries to determine a baseline value as well as on the day of killing. Feces samples were stored frozen at −20 °C, dried and extracted with 80% methanol. The analysis of fecal corticosterone metabolites (FCM) was carried out as described previously (Lepschy et al., 2007; Lepschy et al., 2010).

2.10. Plasma and serum analysis

For serum analysis, blood was retro-orbitally sampled under isoflurane anesthesia in the morning (9 a.m.-10:30 a.m.) the day before the killing. Blood was collected in tubes containing Aprotinin (A1153 Sigma-Aldrich, 500KIU/ml of blood) and left to coagulate for 60 min RT. Afterwards blood was centrifuged at 2000 x g for 10 min and the supernatant (serum) stored at −80 °C. For plasma analysis, blood was obtained via cardiac puncture on the day of killing, collected in EDTA-coated tubes and centrifuged at 4 °C at 1800 x g for 15 min. The supernatant (plasma) was stored at −80 °C.

Corticosterone serum levels were quantified using a commercial

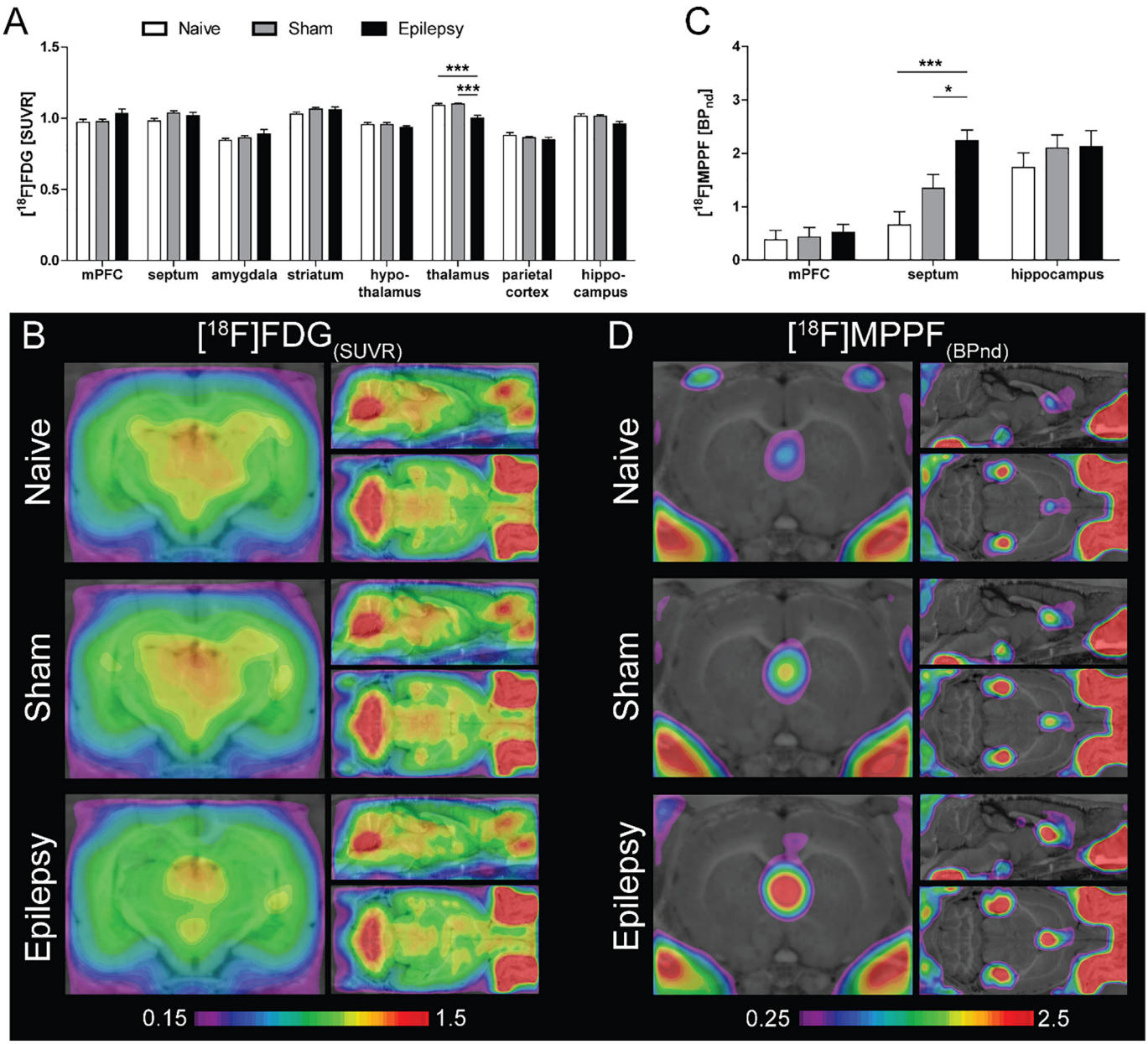

Fig. 1. Alterations in [^{18}F]FDG uptake and [^{18}F]MPPF binding. In vivo μPET quantification of glucose metabolism and 5-HT$_{1A}$ receptor binding. Rats with epilepsy demonstrated significantly reduced [^{18}F]FDG uptake in the thalamus (A) and significantly increased septal [^{18}F]MPPF binding (C) in comparison to both naïve and sham rats. Average SUVR images of [^{18}F]FDG (B) and average parametric maps of [^{18}F]MPPF binding potential (D) for each treatment group. PET images are projected on the cryosection atlas of the rat brain (greyscale) for anatomic localization.

enzyme-linked immunosorbent assays (ELISA) kit (DEV9922, Demeditec) according to the manufacturer's instructions. Optical density was determined using a microplate reader (Gen 5 microplate reader, Biotek; Gen 5 Imager Software, Biotek, Bad Friedrichshall, Germany) set to 450 nm and a four parameter logistic (4-PL) curve-fit was used to determine sample concentrations in ng/ml.

Oxytocin serum levels were quantified using a commercial ELISA kit (ADI-900-153A, Enzo Life Sciences) according to the manufacturer's instructions. To eliminate the effect of potentially interacting molecules (Leng and Sabatier, 2016), a solid phase extraction of serum samples was performed following the manufacturer's suggested protocol. Optical density was determined using the microplate reader set to 405 nm with wavelength correction set to 570 nm and a 4-PL curve-fit was used to determine sample concentrations in pg/ml.

Brain-derived neurotrophic factor (BDNF) serum concentrations (ng/ml) were measured in re-thawed samples (diluted 1:20 with sample buffer) using fluorometrictwo-site ELISA according to the manufacturer's instructions (Promega Inc., Mannheim, Germany) (Deuschle et al., 2013; Hellweg et al., 2003).

2.11. Termination of study

At the end of the project, rats were killed by an overdose of pentobarbital (600 mg/kg i.p., Narcoren, Merial GmbH, Germany), adrenal glands were sampled and weighed.

2.12. Statistics

GraphPad Prism (Version 5.04; GraphPad, San Diego, CA, USA) was used for statistical analysis. Normality of data was assessed by D'Agostino-Pearson omnibus test. To compare more than two experimental groups, one-way analysis of variance (ANOVA) was applied for

normally distributed data and a Kruskal-Wallis test for non-normally distributed data, followed, respectively, by Bonferroni or Dunn's multiple comparison post-hoc tests. Where necessary data was transformed to reach a normal distribution. Differences in p value < .05 were considered statistically significant. Values are shown as mean ± S.E.M (standard error of the mean), unless otherwise stated. Correlation matrix was calculated using R version 3.3.2. (R Core Team, 2016) and visualized by the R package "corrplot" (Wei and Simko, 2017).

3. Results

3.1. Development of SE following pilocarpine treatment

SE was induced by fractionated pilocarpine injections in 19 rats. SE development was preceded by immobility, head nodding, tremor, facial clonus, chewing, starting already after the first pilocarpine injection, and at least one clonic-tonic seizure (type 4 or 5 according to the Racine scale). SE, which was stopped after 90 min from the onset by a combination of phenobarbital and diazepam, was characterized by continuous limbic seizure activity with generalized tonic-clonic seizures. Doses of pilocarpine to induce SE ranged between 20 and 50 mg/kg (median 30 mg/kg); accordingly, time to onset of SE ranged between 60 and 140 min. One rat did not develop SE after ten injections of pilocarpine and therefore was excluded from further studies, five rats died within 48 h after SE.

3.2. Development of spontaneous recurrent seizures

Eight weeks after pilocarpine treatment, rats which developed SE were video/EEG monitored for 15 days (24 h/day). Twelve out of 13 rats exhibited seizure activity during the monitoring period. SRSs were mainly characterized by generalized convulsive activity (type 4 and 5), with few episodes of running-bouncing convulsions. Seizure frequency and duration showed large inter-individual variation. Mean seizure frequency during the entire monitoring period was 34.8 ± 13.4 with a median of 11.5 seizures (Supplementary Fig. 1A). Mean cumulative seizure duration was 1975.8 ± 797.3 s with a median of 821 s (Supplementary Fig. 1B).

3.3. Alterations in [^{18}F]FDG uptake in rats with epilepsy

Glucose metabolism in the rat brain was analyzed using [^{18}F]FDG μPET (Fig. 1A, B). Reduced [^{18}F]FDG uptake was observed in the thalamus and hippocampus of rats with epilepsy relative to both naïve (−8.9% and − 5.6%) and sham rats (−9.9% and − 5.6%). One-way ANOVA confirmed a significant effect of epilepsy induction on [^{18}F]FDG uptake in the thalamus ($F(2,20) = 16.27$, $p < .001$). Post-hoc tests indicated that thalamic [^{18}F]FDG uptake was significantly reduced in rats with epilepsy in comparison to naïve ($p < .001$) and sham rats ($p < .001$). A one-way ANOVA demonstrated a significant effect of epilepsy induction on [^{18}F]FDG uptake in the hippocampus ($F(2,20) = 3.93$, $p < .05$), however, after correction for multiple comparisons, post-hoc tests did not demonstrate significant differences between groups in this region. [^{18}F]FDG uptake was not significantly different between groups in the remaining regions investigated. During the three days prior to [^{18}F]FDG scanning, four rats with epilepsy exhibited SRSs. In these rats, [^{18}F]FDG uptake demonstrated a positive correlation with both the number of seizures and total seizure duration. This relationship was largely the result of high [^{18}F]FDG uptake in one rat which experienced the greatest number of seizures. However, these recent seizures did not affect the overall outcome of the [^{18}F]FDG uptake analyses, when this animal was removed from the analysis the group differences observed in the [^{18}F]FDG uptake in the thalamus and hippocampus remained.

3.4. Alterations of [^{18}F]MPPF binding in rats with epilepsy

5-HT$_{1A}$ receptor availability was measured using [^{18}F]MPPF μPET (Fig. 1C, D). Specific binding was observed in the hippocampus, septum and medial prefrontal cortex with negligible binding in all other regions. Increased [^{18}F]MPPF BP_{nd} was observed in the septum of rats with epilepsy relative to both naïve (+158.0%) and sham rats (+89.3%). A one-way ANOVA confirmed a significant effect of epilepsy induction on [^{18}F]MPPF BP_{nd} in this region ($F(2,21) = 11.41$, $p < .001$). Post-hoc tests revealed significantly increased septal [^{18}F]MPPF BP_{nd} in rats with epilepsy in comparison to naïve and sham rats ($p < .001$ and $p < .05$ respectively). Three rats developed SRSs in the three days preceding [^{18}F]MPPF scanning. In these rats, [^{18}F]MPPF BP_{nd} was not correlated to either the number of seizures or total seizure duration (Supplementary Fig. 2A-F).

3.5. Impact of SE and epileptogenesis on burrowing behavior

Post-SE (8 days and 4 weeks) and rats with epilepsy (15 weeks) demonstrated a significant decrease in the amount of displaced gravel (8 days: $F(2,34) = 66.76$, $p < .001$, 4 weeks: $F(2,34) = 10.28$, $p < .001$, 15 weeks: $F(2,33) = 50.55$, $p < .001$, all post-hoc comparisons with sham and naïve groups $p < .01$, Fig. 2A-C) and a significant increase in the time to onset of burrowing behavior (using log transformed data: 8 days: $F(2,34) = 48.53$, $p < .001$, 4 weeks: $F(2,34) = 18.28$, $p < .001$, 15 weeks: $F(2,32) = 217.1$, $p < .001$, all post-hoc comparisons with sham and naïve groups $p < .001$, Supplementary Fig. 3A-C). Disruptions in burrowing activity were most pronounced during the acute (8 days) and chronic phase following SE (15 weeks) with a partial recovery during the latent phase (4 weeks)($H(2) = 16.13$, $p < .001$, where significantly more gravel was displaced at 4 weeks compared to 8 days and 15 weeks post SE, Fig. 2D. $F(2,36) = 9.265$, $p < .001$, where time to onset was significantly faster at 4 weeks compared to 8 days and 15 weeks post SE, $p < .05$ Supplementary Fig. 3D).

3.6. Impact of SE-induced epilepsy development on social interaction and saccharin preference test

The social interaction test revealed a significant reduction of time spent in active interaction in pairs with epilepsy, as compared to naïve and sham pairs ($F(2,14) = 39.29$, $p < .001$, all post-hoc comparisons with epilepsy group $p < .001$, Fig. 3A). Time spent in passive interaction did not change between groups (data not shown). Only one pair of rats with epilepsy exhibited aggressive behavior. The saccharine preference test showed differences between experimental groups. Since the percentage of saccharine consumption at day 2 and day 4 was comparable in all the experimental groups, results were averaged over the two days. While all groups exhibited a pronounced preference for the saccharin solution, the percentage of consumed saccharin was significantly decreased in rats with epilepsy, as compared to both naïve and sham rats ($F(2,32) = 6.258$, $p < .001$, all post-hoc comparisons with epilepsy group $p < .05$, Fig. 3B).

3.7. Impact of SE-induced epilepsy development on anxiety-associated behavior

The black-white box and the elevated-plus maze tests were used to assess anxiety-associated behavior. The black-white box revealed significant changes in behavior between groups. We detected a decrease in the number of entries in the white box ($F(2,32) = 28.49$, $p < .001$, all post-hoc comparisons with epilepsy group $p < .001$, Fig. 4A) and in the frequency of stretching postures ($F(2,32) = 12.34$, $p < .001$, all post-hoc comparisons with epilepsy group $p < .001$, Fig. 4B) and an increase in the latency to first entrance to the black box ($F(2,32) = 10.37$, $p < .001$, all post-hoc comparisons with epilepsy

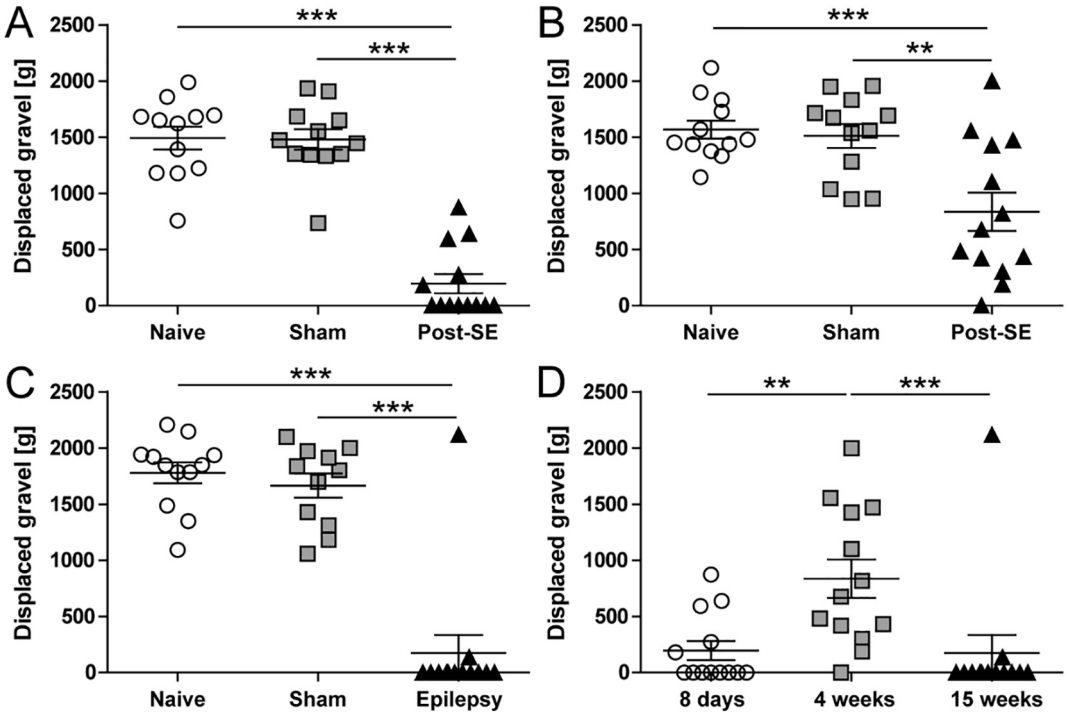

Fig. 2. Impact of SE and epileptogenesis on burrowing behavior. Burrowing behavior measured by the amount of gravel displaced during three different time points post-pilocarpine treatment. Rats exhibited reduced burrowing behavior 8 days (A), 4 weeks (B) and 15 weeks (C) post-SE in comparison to naïve and sham groups. A comparison between the performance of the rats with epilepsy between the three time points reveals a significant difference in performance (D), where during the middle time point, 4 weeks post pilocarpine treatment, the rats performed significantly better than the other two time points.

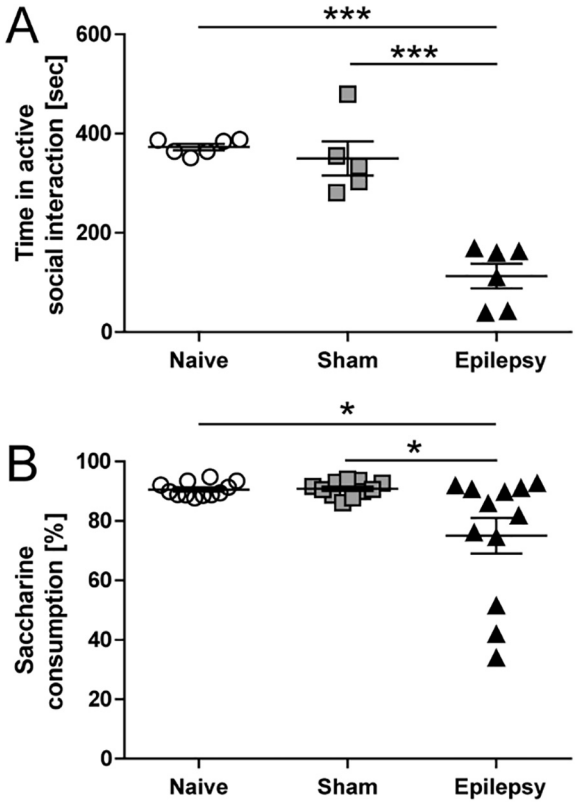

Fig. 3. Impact of SE-induced epilepsy development on social interaction and saccharin preference test. Rats with epilepsy performed significantly worse in the social interaction test (A) and the saccharine preference test (B). Rats with epilepsy spent significantly less time in active social interaction and consumed significantly less saccharine compared to both the naïve and sham treated rats.

group $p < .01$, Fig. 4C) in rats with epilepsy, as compared to both naïve and sham rats.

Since 9/12 rats with epilepsy jumped off the elevated-plus maze at least once ($F(2,32) = 19.23$, $p < .001$, all post-hoc comparisons with epilepsy group $p < .001$, Fig. 4D), the total time spent in the apparatus was not comparable between the experimental groups, and thus we could not compare behavioral parameters between groups for this test.

3.8. Impact SE and epileptogenesis on nest building activity

Nest building activity was monitored for seven days after the addition of new nest material to the cage. Pilot studies have shown that nest score increases over time, reaching a plateau at day 5 (data not shown), which represent the chosen time point to compare nest building activity between experimental groups. At 7 days post-SE, rats exhibited a significant decrease in nest scores ($H(2) = 10.17$, $p < .01$), as compared to both naïve ($p < .05$) and sham ($p < .05$) rats (Supplementary Fig. 4A). Nest scores proved to be similar between groups at later stages during the latency phase and the chronic phase following epilepsy manifestation (Supplementary Fig. 4B-C).

3.9. Impact of SE-induced epilepsy development on open field behavior

SE-induced epilepsy did not affect the time spent in center (Supplementary Fig. 5A), the frequency of entries in center (Supplementary Fig. 5B), the immobility time (Supplementary Fig. 5C), the total distance moved (Supplementary Fig. 5E) or the rearing frequency (Supplementary Fig. 5F), as compared to both naïve and sham rats. Rats with epilepsy exhibited reduced immobility phases as compared to sham rats ($F(2,32) = 4.676$, $p < .05$, epilepsy against sham rats $p < .05$, Supplementary Fig. 5D).

3.10. Impact of SE and epileptogenesis on body weight

BW was measured in all rats at the following time points: the day

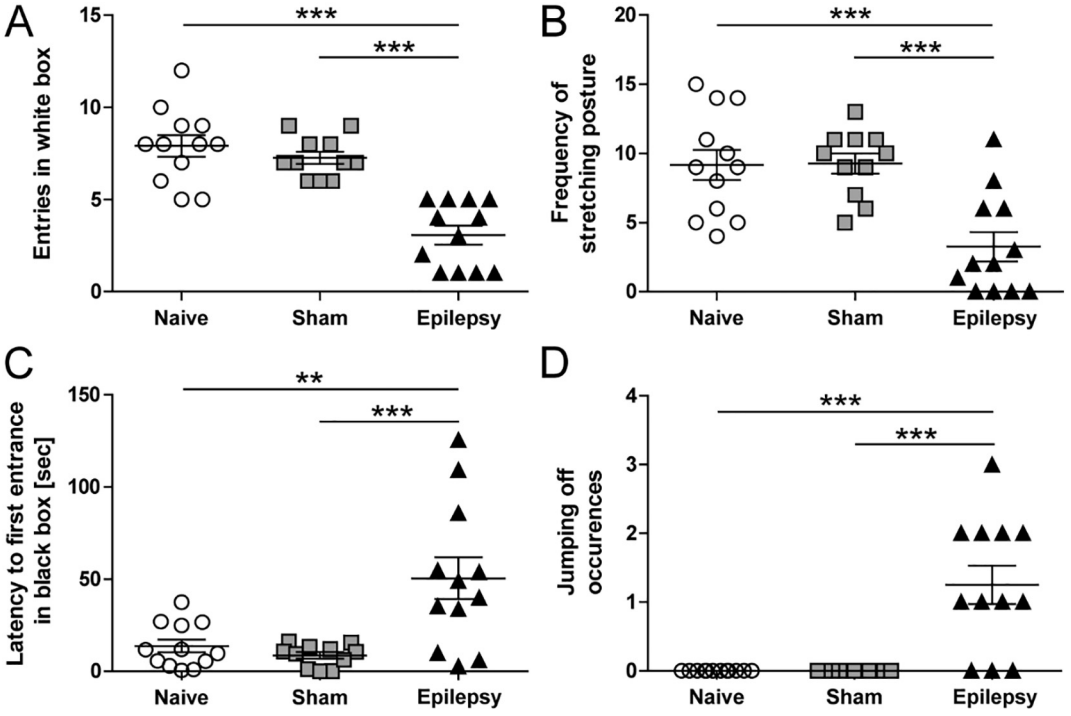

Fig. 4. Impact of SE-induced epilepsy development on anxiety-associated behavior. Anxiety-associated behavior was evaluated using black-white box and elevated-plus maze. Rats with epilepsy showed significantly worse performance in the black-white box whereby these rats made fewer entries into the white box (A), made fewer stretches into the white box (B) and were slower to enter the black box (C). The only variable where a comparison could be made in the elevated-plus maze was the number of times the animal jumped off the maze (D) where 9 out of 12 rats with epilepsy jumped off compared to none of the other groups.

before pilocarpine treatment (day −1), 2 days after (day 2), weekly for 6 weeks (day 9, 16, 23, 30, 37, 44), before PET scan (day 60), before starting the behavioral tests (day 95) and at the end of the experiment (day 115). Rats lost weight in the first two days post-SE due to seizure activity and due to sedative effects of diazepam. From that time point on, a slower weight gain was observed. At day 9 the BW between the three experimental groups became comparable, while at all subsequent time-points BW was higher in rats with epilepsy in comparison to naïve and sham rats (Supplementary Fig. 6A-B). When comparing the body weight gain of the three experimental groups at the end of the projects, a significant difference can be observed in the epilepsy group, as compared to both naïve and sham animals ($F(2,32) = 22.04, p < .001$, all post-hoc comparisons against epilepsy group $p < .001$).

3.11. Impact of SE-induced epilepsy development on physiological and biochemical parameters

In order to assess the overall impact of the experimental procedure during the whole experimental phase, adrenal gland weight was measured and corticosterone levels were analyzed in hair sampled when rats were killed. No significant changes in adrenal gland weight (Fig. 5A) and serum corticosterone levels (Fig. 5B) between experimental groups were detected. Moreover, fecal corticosterone metabolites (Fig. 5C), hair corticosterone (Fig. 5D), and oxytocin levels (Fig. 5E) were not significantly modified between groups.

Serum BDNF levels showed a significant reduction in rats with epilepsy, as compared to both naïve and sham rats ($F(2,32) = 21.36$, $p < .001$, all post-hoc comparisons $p < .01$). Interestingly, levels of BDNF were also significantly reduced in sham rats, as compared to naïve rats ($p < .05$, Fig. 5F).

3.12. Correlation matrix between PET and behavioral, physiological and biochemical measures

A Spearman cross-correlation analysis was completed considering parameters from μPET scans and from all behavioral and biochemical analyses (Fig. 6) (Full list of abbreviations are explained in Supplementary Table 1). In the following paragraph correlation coefficients are given in brackets.

Our analysis revealed a positive correlation for [^{18}F]FDG uptake in the medial prefrontal cortex and septal [^{18}F]MPPF BP_{nd} (0.66) as well as a negative correlation for thalamic [^{18}F]FDG uptake and septal [^{18}F]MPPF BP_{nd} (−0.57). When focusing on μPET data and behavioral data, stronger levels of positive or negative correlation were observed between [^{18}F]FDG uptake in different brain regions and various behavioral parameters (Fig. 6A-G). Links were particularly evident for thalamic [^{18}F]FDG data, which showed a correlation with jumping from elevated-plus maze (−0.72), social interaction (0.63, Fig. 6B), burrowing 4 weeks following SE (0.61, Fig. 6C), burrowing 8 days following SE (0.59), entries in the white box of the black-white box paradigm (0.57, Fig. 6D), difference in body weight (−0.56, Fig. 6E), immobility time in the open field (−0.56, Fig. 6F), burrowing 15 weeks post-SE (0.53), and latency to first entrance to the black box of the black-white box paradigm (−0.52, Fig. 6G). Additionally high correlations can be seen between thalamic [^{18}F]FDG uptake and, both, seizure duration (−0.76) and seizure frequency (−0.71) recording during the video/EEG monitored period.

Regarding [^{18}F]MPPF BP_{nd} the most pronounced levels of correlation were demonstrated for data from the septum, which exhibited a moderate correlation with jumping from elevated-plus maze (0.59), social interaction (−0.58), burrowing 15 weeks post-SE (−0.54), burrowing four weeks post-SE (−0.52), latency to first entrance to the black box (0.52), and entries to the white box of the black-white box paradigm (−0.50).

Additionally, we assessed the correlation between μPET and biochemical data. A moderate correlation was confirmed for hippocampal [^{18}F]FDG uptake and BDNF (0.62), septal [^{18}F]MPPF BP_{nd} and BDNF (−0.54), parietal cortex [^{18}F]FDG uptake and hair corticosterone (−0.52), striatal [^{18}F]FDG uptake and serum corticosterone (0.51), thalamic [^{18}F]FDG uptake and BDNF (0.51). Moreover, a correlation

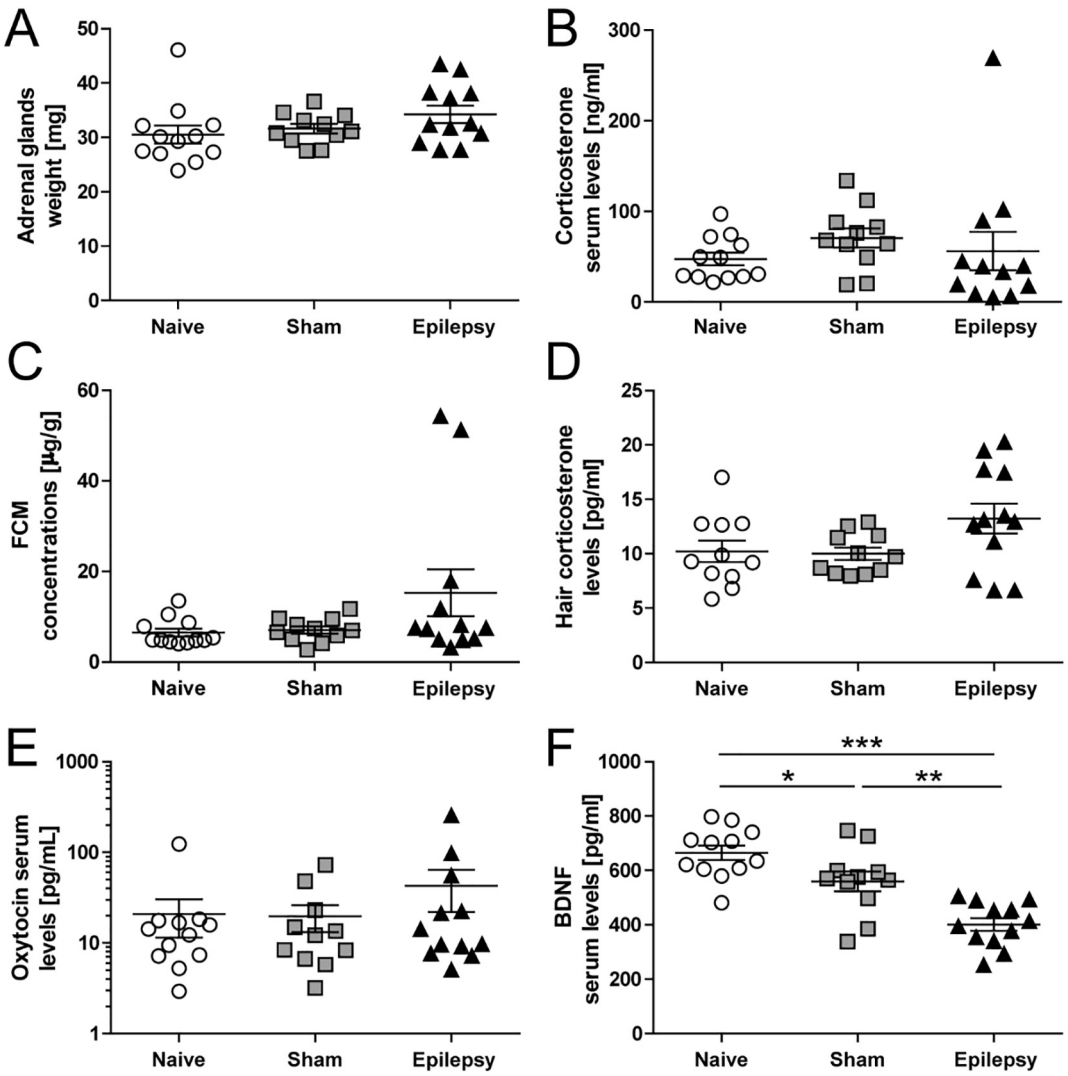

Fig. 5. Impact of SE-induced epilepsy development on physiological and biochemical parameters. Several physiological and biochemical parameters were measured during the whole experimental phase. No significant difference between rats with epilepsy and other groups were observed for the adrenal gland weight (A), hair corticosterone levels (B), fecal corticosterone metabolites (C), serum corticosterone levels (D) and oxytocin levels (note logarithmic scale) (E). BDNF levels were significantly decreased in rats with epilepsy and sham rats (F) compared to naïve control rats.

between BDNF and behavioral parameters was observed. In particular serum BDNF levels exhibited a moderate correlation with burrowing 8 days post-SE (0.53), social interaction (0.59), entries to the white box of the black-white box paradigm (0.59) and jumping from elevated-plus maze (−0.6).

Finally, the cross correlation between seizure data and all behavioral and biochemical measures was assessed, here both the total number of seizures and the total seizure duration showed a significant negative correlation with the number of entries to the center in the open field test (−0.59 and − 0.65), the number of stretching postures (−0.77 and − 0.78) and a positive correlation with the latency to first enter the black box in the black-white box (0.68 and 0.63).

4. Discussion

The current study of [^{18}F]FDG and [^{18}F]MPPF µPET imaging in a model of SE-induced epileptogenesis provided evidence for alterations in thalamic glucose metabolism and septal 5-HT$_{1A}$ receptor binding in rats with epilepsy. These metabolic and molecular alterations correlated with neurobehavioral, physiological and biochemical alterations.

Various clinical studies have described alterations and asymmetries in thalamic hypometabolism in temporal lobe epilepsy patients (Benedek et al., 2004; Chang et al., 2008; Hashiguchi et al., 2007; Khan et al., 1997; Kojan et al., 2017; Sakamoto et al., 2009). In patients the degree of hypometabolism in the thalamus correlated with the duration of epilepsy indicating that metabolic dysfunction in this brain region progresses over time with disease duration (Benedek et al., 2004). Earlier experimental µPET studies in the pilocarpine post-status epilepticus model revealed a widespread early decrease in glucose metabolism followed by a normalization and more limited hypometabolism in the chronic phase affecting cerebellum, brainstem, left striatum, left entorhinal cortex in one study and the entire limbic system in another study (Chang et al., 2008; Goffin et al., 2009). These experimental and clinical studies left the question open whether alterations in glucose metabolism just reflected neuropathological correlates of epilepsy manifestation and progression or whether these alterations can be linked with neurobehavioral alterations and psychiatric comorbidities. Salzberg and colleagues (Salzberg et al., 2006) have addressed this question and obtained evidence that depression in patients with temporal lobe epilepsy may be linked with hypometabolism in ipsilateral orbitofrontal regions. Previous PET studies pointed to a bilateral inferior frontal hypometabolism or a left temporal lobe hypometabolism in temporal lobe epilepsy patients with depressive disorder (Bromfield et al., 1992; Victoroff et al., 1994). The present µPET study in rats with

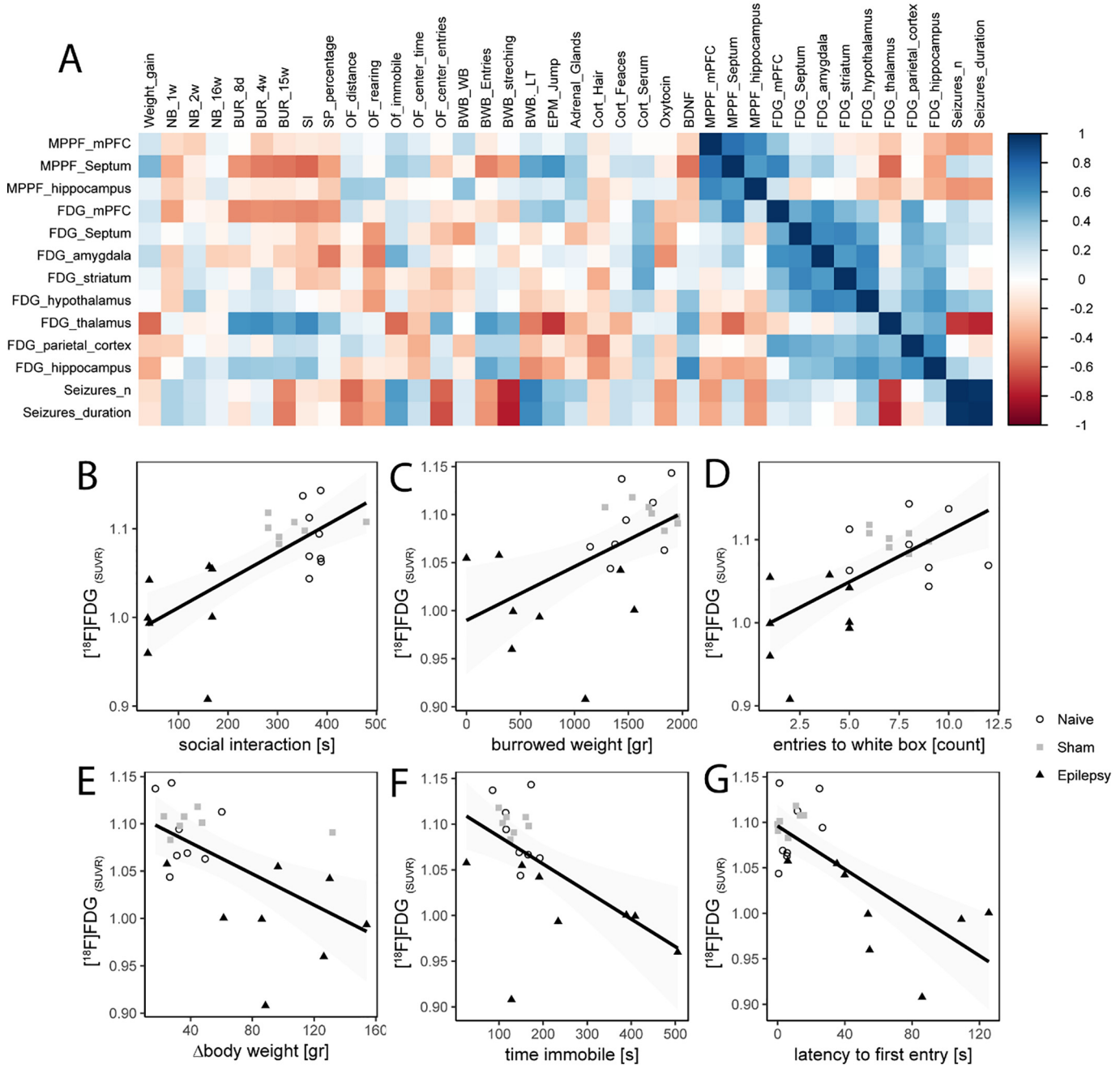

Fig. 6. Correlation matrix between PET and behavioral, physiological and biochemical measures. A: Heatmap representing a Spearman correlation matrix between the [^{18}F]FDG uptake, MPPF BP_{nd} and SRS data and all measured behavioral and biochemical variables. B-G: individual correlation between [^{18}F]FDG uptake in the thalamus and the amount spent in social interaction (B), burrowed weight 4 weeks after pilocarpine treatment (C), entries into the white box in the black-white box paradigm (D), delta weight gain (E), time spent immobile in the open field (F) and latency to first enter the black box (G).

pilocarpine-induced epilepsy development revealed significant alterations in thalamic glucose metabolism indicated by reduced levels of [^{18}F]FDG uptake. We demonstrated a correlation between thalamic hypometabolism and behavioral alterations in rats with epilepsy including decreased playful, luxurious behavior such as burrowing, decreased social interaction, and altered behavioral patterns in anxiety tests. Our data may further support the role of the thalamus as an important component in dysfunctional limbic-cortico-striato-pallido-thalamic circuits that are intensely discussed to contribute to mood disorders including depression (Price and Drevets, 2012). In this network the fact that limbic structures connect the orbital and medial prefrontal cortex with the medial segment of the mediodorsal thalamic nucleus might play a crucial role for the manifestation of neurobehavioral symptoms (Price and Drevets, 2012). In this context, it is of interest that a reduction in thalamic glucose metabolism has also been reported in rats exposed to immobilization stress or to three different models inducing depression-associated behavior (Caldecott-Hazard et al., 1988; Sung et al., 2009). However, in our study, thalamic [^{18}F]FDG uptake also exhibited a strong correlation with seizure frequency during the monitoring phase. Thus, it might just reflect the severity of the disease, being merely a function of the epileptic state, and might not serve as a specific biomarker for psychiatric comorbidities. In this context, we would like to point out that for technical reasons, we were only able to capture reliable information about generalized tonic-clonic seizures, and therefore could not consider possible additional non-convulsive seizures for the correlation analysis.

Alterations in serotonergic neurotransmission are of particular relevance for the development of psychiatric disorders (Akimova et al., 2009; Kaufman et al., 2016; Mahar et al., 2014) considering the key role of 5-HT_{1A} receptors in the feedback regulation of serotonergic signaling (Albert et al., 1996) changes in their density and expression patterns have received special attention. Our present experimental study revealed as a consequence of epilepsy development a pronounced increase of [^{18}F]MPPF binding in the septum. Interestingly, a correlation of μPET data with behavioral parameters could be confirmed for the decrease in playful, luxurious burrowing behavior, the decrease in social interaction, and altered behavior in anxiety paradigms. Thus, the epilepsy-associated rise in septal 5-HT_{1A} receptor binding seems to be linked with neurobehavioral alterations reflecting psychiatric comorbidities in patients such as anxiety and symptoms of autism spectrum disorder. On the contrary, no pronounced correlation was observed between [^{18}F]MPPF and anhedonia-associated behavior, indicating that [^{18}F]MPPF binding might not be a valid biomarker for depressive disorders associated with epilepsy. Experimental evidences have pointed out a role of septal 5-HT receptors in the regulation of neurobehavioral responses. Interestingly, 5-$HT_{1A/7}$ agonist, 8-OH-DPAT intraseptal injections increases maternal aggressive behavior (De Almeida and Lucion, 1997), regulates anxiety (De Almeida et al., 1998), and induces antidepressant-like effects (Martin et al., 1991; Schreiber and Vry, 1993), while, when injected into the lateral septum, compromises the retention of an inhibitory avoidance response in rats (Lee et al., 1992). Therefore, altered serotonergic neurotransmission in this brain region may account for the observed correlated neurobehavioral alterations.

Based on our findings the functional consequences of increased septal 5-HT_{1A} receptor signaling in temporal lobe epilepsy should be further explored in future experimental and clinical studies.

Among the biochemical markers, serum BDNF proved to be the only molecule exhibiting a significant group difference with rats with epilepsy showing reduced concentrations. BDNF changes correlated with behavioral parameters such as the decrease in burrowing behavior, the decrease in social interaction, and altered behavior in anxiety paradigms, thus being a putative biomarker candidate for specific epilepsy associated comorbidities, i.e. anxiety and autism spectrum disorders. It is well accepted that stress exposure and development of stress-induced depression-associated behavior can be associated with a lowering of BDNF serum levels (Castren and Kojima, 2017). For instance, exposure to social defeat stress in rats resulted in reduced serum BDNF in rats (Becker et al., 2015). In this study, administration of a BDNF analog interfered with the development of neurobehavioral symptoms observed following chemical SE induction (Becker et al., 2015). Our findings suggest that epileptogenesis without additional stress exposure can affect BDNF serum concentrations. Downregulation of BDNF pathway was also described in chronic cyclothiazide seizure model (Kong et al., 2014). Moreover, a comparable reduction in serum BDNF has also been reported in patients with epileptic seizures (Lafrance Jr. et al., 2010). The fact that these were patients without comorbid major depressive disorder, raises the question whether BDNF reductions in patients might just be a consequence of the epileptic disorder without a link with psychiatric comorbidities. Thus, it will be necessary to critically assess the putative validity of circulatory BDNF as a biomarker for comorbidities. Experimental studies suggest that BDNF depletion can attenuate 5-HT_{1A} receptor function (Burke et al., 2013; Hensler et al., 2007) and decrease 5-HT_{1A} receptor binding in hippocampus (Klein et al., 2010), thus indicating a positive correlation between the two factors. Our data instead suggest an inverse correlation between BDNF levels and MPPF in septum, which might reflect a reduction in endogenous 5-HT or an increased receptor density in specific brain regions, as consequence of epilepsy. Recent studies underlined a positive association between hippocampal 5-HT_{1A} receptor density and activation with disease duration, seizure frequency and memory deficits in patients with temporal lobe epilepsy (Cuellar-Herrera et al., 2014; Fonseca et al., 2017). The change in 5-HT_{1A} receptor density may also reflect a compensatory enhancement of endogenous serotonergic neurotransmission in response to prolonged epileptic seizures and reduced 5-HT_{1A} receptor activity (Assem-Hilger et al., 2010; Liew et al., 2009). On the other hand, it needs to be considered that not only increased 5-HT_{1A} density but also a reduction in endogenous 5-HT levels can explain the observed changes in [^{18}F]MPPF binding and further studies are needed to clarify this point and the inverse correlation with BDNF levels.

Our data also raise the questions whether it is worthwhile to perform molecular imaging studies in order to validate markers of psychiatric comorbidities and whether peripheral circulatory BDNF concentrations might serve as a less cost-intense and less-elaborate diagnostic biomarker. It will be necessary to further explore the validity of the different biomarker candidates in more detail in future experimental and clinical investigations. Despite the fact that a correlation with selected PET data has also been demonstrated for serum and hair corticosterone levels, our data rather argue against a value of corticosterone as a biomarker for neurobehavioral alterations as corticosterone levels did not differ among experimental groups.

As already outlined in the introduction, an early and straightforward assessment and diagnosis of neuropsychiatric comorbidities is of particular relevance for individualized patient management concepts (Kanner, 2013, 2016; Mula, 2017; Ravizza et al., 2017). As discussed by an expert group respective biomarkers may not only help to precisely and reliably diagnose psychiatric comorbidities (Ravizza et al., 2017). Taking into account that evidence exists that comorbidities may predict the outcome of epilepsy and its pharmacological management respective biomarkers may also guide the prognosis as well as therapeutic decisions (Ravizza et al., 2017). The outcome of our experimental study supports the concept to further explore imaging biomarkers assessing 5-HT_{1A} receptor binding, and additionally supports serum BDNF as a circulatory biomarker candidate.

As suggested by our findings, these imaging and biochemical biomarkers are also of interest for severity assessment in neurological models including chronic epilepsy models. In respective models behavioral alterations can significantly contribute to the burden of the experimental animals with increased levels of anxiety-associated and anhedonia-associated behavior detrimentally affecting the well-being of the laboratory rodents. Respective alterations can be explored in a laborious manner applying behavioral test batteries, which are highly sensitive to environmental and study design-related influences. Thus, reliable biomarkers of neurobehavioral alterations are of special interest for a more standardized assessment of severity of animal models. Our findings suggest elevated levels of septal [^{18}F]MPPF binding as well as serum BDNF as biomarker candidates for severity assessment. Validation of these marker candidates requires further investigations under different experimental conditions and in different animal models. Respective markers will be of value as a basis for a comparative animal-welfare based model prioritization, and for a standardized assessment of refinement measures (Lidster et al., 2016). In this context as all as regarding other conclusions it should be considered that the study design with sequential testing in different behavioral paradigms might have directly or indirectly (e.g. by an impact on seizure generation) affected the outcome.

Taken together, μPET data from rats with pilocarpine-induced epileptogenesis indicate altered septal 5-HT_{1A} receptor binding. Further research is necessary assessing whether septal 5-HT_{1A} receptor binding may serve as an imaging correlate of neuropsychiatric comorbidities in epilepsy patients and for severity assessment in rodent epilepsy models. Evidence has been obtained that BDNF might serve as a peripheral circulatory biomarker. Further experimental and clinical assessment is necessary for validation of the marker candidate.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2018.06.010.

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