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ORIGINAL ARTICLE

Chronic Nicotine Exposure Alters Metabotropic Glutamate Receptor 5: Longitudinal PET Study and Behavioural Assessment in Rats

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Abstract

Using positron emission tomography (PET), a profound alteration of the metabotropic glutamate receptor 5 (mGluR5) was found in human smoking addiction and abstinence. As human PET data either reflect the impact of chronic nicotine exposure or a pre-existing vulnerability to nicotine addiction, we designed a preclinical, longitudinal study to investigate the effect of chronic nicotine exposure on mGluR5 with the novel radiotracer [^{18}F]PSS232 using PET. Twelve male dark Agouti rats at the age of 6 weeks were assigned randomly to three groups. From day 0 to day 250 the groups received 0 mg/L, 4 mg/L, or 8 mg/L nicotine solution in the drinking water. From day 250 to 320 all groups received nicotine-free drinking water. PET scans with [^{18}F]PSS232 were performed in all animals on days 0, 250, and 320. To assess locomotion, seven tests in square open field arenas were carried out 72 days after the last PET scan. During the first four tests, rats received 0 mg/L nicotine and for the last three tests 4 mg/L nicotine in the drinking water. After 250 days of nicotine consumption [^{18}F]PSS232 binding was reduced in the striatum, hippocampus, thalamus, and midbrain. At day 320, after nicotine withdrawal, [^{18}F]PSS232 binding increased. These effects were more pronounced in the 4 mg/L nicotine group. Chronic administration of nicotine through the drinking water reduced exploratory behaviour. This preliminary longitudinal PET study demonstrates that chronic nicotine administration alters behaviour and mGluR5 availability. Chronic nicotine administration leads to decreased [^{18}F]PSS232 binding which normalizes after prolonged nicotine withdrawal.

Keywords Nicotine · mGluR5 · PET · [18F]PSS232 · Addiction

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Introduction

Reducing the public health burden caused by chronic tobacco smoking requires a better understanding and treatment of nicotine addiction (Centers for Disease Control and Prevention, 2005). Interdisciplinary research has identified altered brain glutamate signaling as an important neurobiological factor mediating substance-related disorders, including nicotine addiction (Kalivas 2009; Koob and Volkow 2016). In particular, metabotropic glutamate receptors of the subtype 5 (mGluR5) have attracted considerable interdisciplinary research interest due to their anatomical distribution, molecular interactions, and the wide range of available options for their pharmacological modulation and assessment (Chiamulera et al. 2017). mGluR5 is densely expressed in addiction-related brain networks (Ametamey et al. 2006; Ametamey et al. 2007; Ferraguti and Shigemoto 2006). It interacts with other receptors mediating the reinforcing and addictive properties of nicotine, NMDA, and dopamine D2 receptors (Beggiato et al.

2016; Conn et al. 2005). Preclinical investigations with the wide range of highly specific mGluR5 agents available provided important insights into their function and interactions (Emmitte 2017; Pomierny-Chamiolo et al. 2014). mGluR5 antagonists reduce the nicotine-induced release of dopamine in the nucleus accumbens (Tronci and Balfour 2011). Systemic administration of negative allosteric modulators (NAMs) of mGluR5 reduce self-administration of nicotine in rodents (Mihov and Hasler 2016; Olive 2009; Pomierny-Chamiolo et al. 2014). Intracranial delivery of the mGluR5 NAM 2-methyl-6-(phenylethynyl)-pyridine (MPEP) into the nucleus accumbens and ventral tegmental area dose-dependently reduced nicotine self-administration in rats (D'Souza and Markou 2011). These preclinical findings received further support from in vivo positron emission tomography (PET) studies in humans with the mGluR5-specific radioligand [^{11}C]ABP688 (Ametamey et al. 2006). Smokers and ex-smokers displayed a global reduction in mGluR5 binding (Akkus et al. 2013). PET investigations in persons with schizophrenia and cocaine consumers further corroborated the smoking-associated decrease in mGluR5 binding (Akkus et al. 2017; Hulka et al. 2014). Moreover, long-term ex-smokers exhibited higher mGluR5 binding than recent ex-smokers (Akkus et al. 2016). These findings suggest that tobacco smoking downregulates expression of mGluR5, whereas mGluR5 binding normalizes after prolonged abstinence. Hulka et al. (2014) found a robust correlation between nicotine abstinence and mGluR5 density in smokers indicating for the first time that mGluR5 density normalized with prolonged abstinence. However, the correlative nature of these findings allows alternative explanations, such as the lower mGluR5 binding found in smokers reflecting a predisposition for nicotine addiction, rather than the effect of chronic nicotine exposure (Akkus et al. 2013). Preclinical studies have investigated the causal relationship between nicotine exposure and mGluR5 (Higa et al. 2017; Kane et al. 2005; Pistillo et al. 2016). Overall, these studies yielded mixed findings regarding mGluR5 protein levels and mGluR5 mRNA expression in brain tissue samples after administration of saline or nicotine over a short period of time, i.e. 3 to 28 days (Higa et al. 2017; Kane et al. 2005; Pistillo et al. 2016). To circumvent limitations caused by short nicotine exposure, postmortem, or cross-sectional evaluations, we designed a longitudinal pharmacological PET study using the mGluR5 specific radiotracer [^{18}F]PSS232 (Sephton et al. 2015). We assessed mGluR5 binding in vivo at baseline, after 250 days of nicotine exposure, and after 70 days of subsequent withdrawal in the same subjects. To mimic the chronic non-invasive nicotine self-administration in tobacco smokers, we administered nicotine via the oral route with the drinking water (Galli and Wolffgramm 2011). To assess an established addiction-related behaviour, we designed a nicotine behavioural sensitisation paradigm, which was carried out after termination of the last PET scan (Hamilton et al. 2012; Ludwig et al. 2008). Behavioural sensitisation refers to the increasing motor response to repeated drug exposure (Robinson and Berridge 1993). Behavioural sensitisation is proposed to reflect neuroplastic changes in addiction-associated brain circuits. The findings of this preliminary study should help to clarify whether mGluR5 levels reflect a simple consequence of nicotine consumption or represent a precondition of nicotine dependence.

Material and Methods

Animals and Experimental Design

Animal husbandry and experiments were carried out in accordance with the Swiss legislation on animal welfare and were approved by the Veterinary Offices of the Canton Zurich and Bern, Switzerland. Male Dark Agouti (DA/OlaHsd) inbred rats at the age of 6 weeks (Harlan, Füllinsdorf, Switzerland) were randomly assigned to one of the three groups of four rats each. Figure 1 and Supplementary Table S1 illustrate the experimental design. Animals were allowed to adapt for at least 1 week at a 12/12-h light/dark cycle with standard chow and nicotine-free drinking water ad libitum. At the end of the habituation phase (−)-nicotine (free-base, Sigma, N3876, Schnelldorf, Germany) was added to the drinking water at concentrations of 0 mg/L (group 1, 4 rats), 4 mg/L (group 2, 4 rats), or 8 mg/L (group 3, 4 rats in the first and second PET scans and 3 rats in the third PET scan). The respective pH values were 8.1, 8.1, and 8.2. In all groups water bottles were replaced twice a week. Thus, fresh nicotine was added to the drinking water of the treatment groups twice weekly to avoid nicotine decomposition.

Three series of PET/CT scans were performed (Fig. 1). The first PET/CT scan was conducted before day 0 (total $n = 12$). The second PET/CT scan was carried out after 250 days of chronic exposure to either nicotine-containing or nicotine-free drinking water (chronic nicotine, total $n = 12$). The third PET/CT scan was acquired after another 70 days (total $n = 11$), during which all groups received water (withdrawal). One rat in the group 3 (8 mg/L nicotine) died after the second PET scan and was excluded from the analyses of the effects of nicotine treatment and subsequent nicotine withdrawal on [^{18}F]PSS232 binding (total $n = 11$).

Behavioural testing was carried out in a single-blinded manner such that the experimenter was not aware of the group pretreatment. During the first four open field-behavioural tests (days 394–413) all rats received drinking water without supplements. One rat, pretreated with nicotine-free water, was excluded from group 1 due to aggressive behaviour, resulting in a total animal cohort of $n = 10$. To induce an acute treatment via subcutaneous (s.c.) injection of nicotine, all rats were adapted to this handling and received s.c. 0.9% NaCl

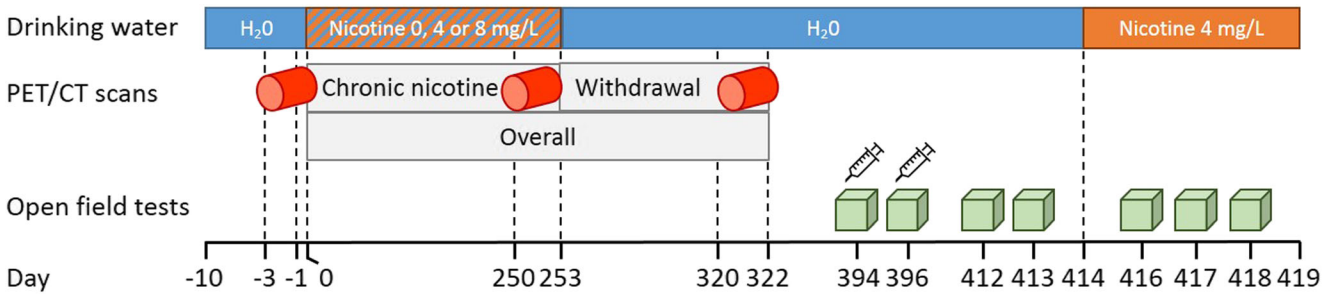

Fig. 1 Time course of the experimental setup. All dark Agouti rats were housed with normal drinking water ad libitum until the first PET/CT scan (red cylinders) was performed at days − 3 to − 1. From days 0–253 the three groups of rats received either 0, 4 or 8 mg/L nicotine in the drinking water (chronic nicotine administration phase). The second PET/CT scan was performed at the end of the nicotine consumption phase from day 250–253. From day 253–322 all three groups received normal drinking water (withdrawal phase) and the last PET/CT scan was performed within days 320–322. Behavioural tests in an open field arena (green cuboids) were conducted at the seven indicated time points. In the first four measurements all rats received nicotine-free water and in the last three measurements 4 mg/L nicotine in the drinking water. Before the first two measurements rats received subcutaneous injection of 0.9% NaCl indicated by syringes

(1 mg/kg) on day 394. Two rats received s.c. 0.9% NaCl (1 mg/kg) on day 396 after which s.c. treatment stopped. The behavioural sensitisation design was replaced by a non-invasive procedure. After a pause of 16 days, open field testing continued for two consecutive days (day 412, 413), however, without injections ($n = 10$). On the following day (day 414), drinking water was supplemented with 4 mg/L nicotine for all animals. Following 2 days on which the animals remained in their home cages, open field testing was continued for 3 consecutive days (days 416, 417, 418). Animals were euthanized on day 418, after completing data acquisition in all animals.

Metabolite Study

We tested whether chronic nicotine exposure influences the metabolism of [^{18}F]PSS232. After chronic nicotine exposure for 250 days, one rat (326.4 g) pretreated with 8 mg/L nicotine and another rat pretreated with 0 mg/L nicotine (336.2 g) were injected with 482.5 and 498.8 MBq (6–8 nmol) [^{18}F]PSS232, respectively. After 5, 15, 30, and 45 min or 10, 20, 30, and 40 min blood was collected from the tail artery and centrifuged at 4800×*g* for 5 min, 4 °C. Blood plasma was transferred into a new tube and mixed with an equal volume of ice-cold acetonitrile (Sigma). After protein precipitation by centrifugation (4800×*g*, 5 min, 4 °C), supernatants were analysed by radio-TLC. For the calculations, we assumed equal extraction efficiency for parent radiotracer and radiometabolites.

In Vivo PET/CT Imaging

In vivo PET/CT scans were performed with a calibrated Super Argus scanner (Sedecal, Madrid, Spain). Rats were anesthetized with isoflurane, respiratory rate and temperature were controlled during the whole scan period (SA Instruments, Inc., Stony Brook, USA). Three groups (0, 4, and 8 mg/L nicotine) of four rats were each injected with [^{18}F]PSS232 (14–30 MBq, 0.1–1.7 nmol) via tail vein and scanned for 60 min in dynamic list mode. After PET acquisition, animals underwent a CT scan for anatomical orientation. Each rat underwent three PET/CT scans in total. The first PET scan was undertaken at the beginning of the study (day − 3 to − 1), the second after the nicotine administration phase (day 250–253) and the last scan after the withdrawal phase (day 320–322). PET data were reconstructed in user-defined time frames with a voxel size of 0.3875 × 0.3875 × 0.775 mm^3 by 2-dimensional-ordered subsets expectation maximisation (2D-OSEM). Random and single but no attenuation correction was applied. Image files were analysed with PMOD 3.6 software (PMOD Technologies Ltd., Zurich, Switzerland). For calculation of distribution volume ratios (DVRs) in regions of interest, specific brain regions were defined on the bases of the rat MRI T2 template (provided with PMOD software). The specific brain regions are depicted in Supplementary Fig. S1. The cerebellum was used as a reference region given the negligible specific binding in this brain region. DVRs were calculated from area-under-the-curves as described recently (Müller Herde et al. 2015).

Behavioural Data Acquisition

Locomotion was assessed in square open field arenas (50 cm × 50 cm × 50 cm, Noldus Information Technology, Wageningen, Netherlands) under controlled light conditions. Data was acquired and processed with EthoVision XT 10.1 (Noldus Information Technology, Wageningen, Netherlands). In EthoVision the arenas were digitally subdivided into a centre zone and a circumventing border zone. The centre zone was defined as a square placed at the centre of the open field with a surface area equal to 25% of the total open field surface area. The border zone was defined as the remaining area of the open field. Movement artefacts were removed by 2 cm minimum distance moved filter. Data acquisition was carried out with subject size-filtering set in the range 350–8000 pixels.

Trial duration was set to 40 min under automatic trial start, triggered by detection of the animal in the arena for three contiguous seconds. In five instances trial start was premature due to light artefacts. In these cases, short sequences were appended to the trials to compensate for the premature trial start, and the light artefacts were removed by retracking with subject size filtering set to the range 2000–8000 pixels. In addition, on the first day of testing for two animals from the group pre-treated with 4 mg/L nicotine in the drinking water the trials were started approximately 30–90 s after the animals were placed in the open field, such that behaviour during these 30–90 s was not recorded. In one case, the trials of both simultaneously tested animals were restarted after about 40 s in order to remove a chip of bedding from the home cage that had been attached to one of the animals and the first 40 s were discarded.

We intended to implement a standard behavioural sensitisation protocol throughout. However, this protocol could not be carried out as planned due to the aggressive behaviour of the animals. On the second day we stopped s.c. treatment and replaced it by giving placebo/nicotine through drinking water. Specifically, all animals received nicotine-free drinking water in the first 5 days. Thereafter, they received nicotine in the drinking water (Supplementary Table S1).

Statistical Analysis

We chose mixed-effects modelling to account for interindividual variance and brain structure-heterogeneity in [^{18}F]PSS232 DVR values, and multiple testing issues (Barr et al. 2013; Bates et al. 2015). We fitted a mixed-effects model with [^{18}F]PSS232 DVR as a dependent variable, a between-subject fixed-effects factor group (levels: placebo, 4 mg/L, and 8 mg/L), a within-subject fixed-effects factor day (levels: 0, 250, 320), an interaction group-by-day, a random effect of subject, and a random effect of brain structure (caudate-putamen (striatum), cingulate cortex, cortex, and hippocampus). Analysis was carried out with the package "afex" in the statistical software R, Version 3.2.3, using Type III sums of squares (Supplementary Methods). We supplemented this analysis by a nonparametric analysis of longitudinal data, as implemented in the package "nparLD" for R (Brunner et al. 2002) (Supplementary Methods). This statistical procedure does not assume a normal distribution and yields a nonparametric measure of effect, the relative treatment effect, ranging from 0 to 1 (Supplementary Figs. S3, S7, S9) (Brunner et al. 2002). Analysis was constrained to the abovementioned four brain regions since these regions represented a cluster of highly intercorrelated [^{18}F]PSS232 DVR values (Spearman's rho in the range 0.87–0.94, all corresponding p values < 0.01), whereas correlations with the DVR values for the midbrain and thalamus were lower (Supplementary Fig. S2, Supplementary Table S2). A total of 11 animals were included in the analysis of the PET data (4 pretreated with water, 4 pretreated with 4 mg/L, and 3 pretreated with 8 mg/L). A total of 10 animals were included in the analysis of the behavioural data (3 water-pretreated, 4 pretreated with 4 mg/L nicotine, 3 pretreated with 8 mg/L nicotine).

Results

Influence of Oral Nicotine Intake on Drinking Behaviour, Body Weight, and In Vivo Metabolism of [18F]PSS232

Figure 2 depicts the drinking behaviour as volume of fluid intake (Fig. 2A) and the dose of nicotine intake (Fig. 2B). Drinking behaviour did not differ between groups during nicotine exposure (Fig. 2A, $F_{2,92} = 1.839$, $p = 0.1648$). The body weights of the three groups increased between day 0 and 250 in accordance with the normal body weight gain of Dark Agouti rats (Fig. 2C, $F_{1,28} = 1635$, $p < 0.001$). Nicotine at 4 or 8 mg/L added to the drinking water had no influence on the body weight of the rats both during the nicotine and withdrawal phase (Fig. 2C, $F_{1,28} = 0.0036$, $p = 0.953$). Descriptive data did not indicate a significant influence of chronic nicotine exposure on [^{18}F]PSS232 metabolism (Fig. 2D). The percentage of intact compound of total radioactivity in blood plasma of the rats with and without nicotine treatment was comparable and in agreement with former data from male Wistar rats (Fig. 2D) (Müller Herde et al. 2015). Altogether, chronic nicotine exposure did not significantly alter fluid consumption, body weight, or radiotracer metabolism.

Distribution Volume Ratio of mGluR5 in Different Brain Regions Before Nicotine Consumption

Data from the baseline scans revealed a clear pattern of correlations between mGluR5 DVR for different brain regions (n = 12, Supplementary Fig. S2, Supplementary Table S2). Whereas the striatum (caudate/putamen), hippocampus, cingulate cortex, and cortex formed a cluster of highly intercorrelated values (Spearman's rho in the range 0.87–0.94, all corresponding p values < 0.01), correlations between DVR in the thalamus or midbrain and other brain structures were lower. Based on these results, we focused our statistical DVR-analysis on the abovementioned brain regions.

Influence of Nicotine Intake on mGluR5 Distribution

The amount of [^{18}F]PSS232 administered did not differ between the three different time points of the PET measurements (day 0, 22.1 ± 4.5 MBq, 2.7 ± 0.7 nmol/kg; day 250, 21.7 ± 4.4 MBq, 2.1 ± 0.5 nmol/kg; day 320, 20.8 ± 4.5 MBq, 2.2 ± 0.8 nmol/kg). Moreover, mean [^{18}F]PSS232 radioactivity

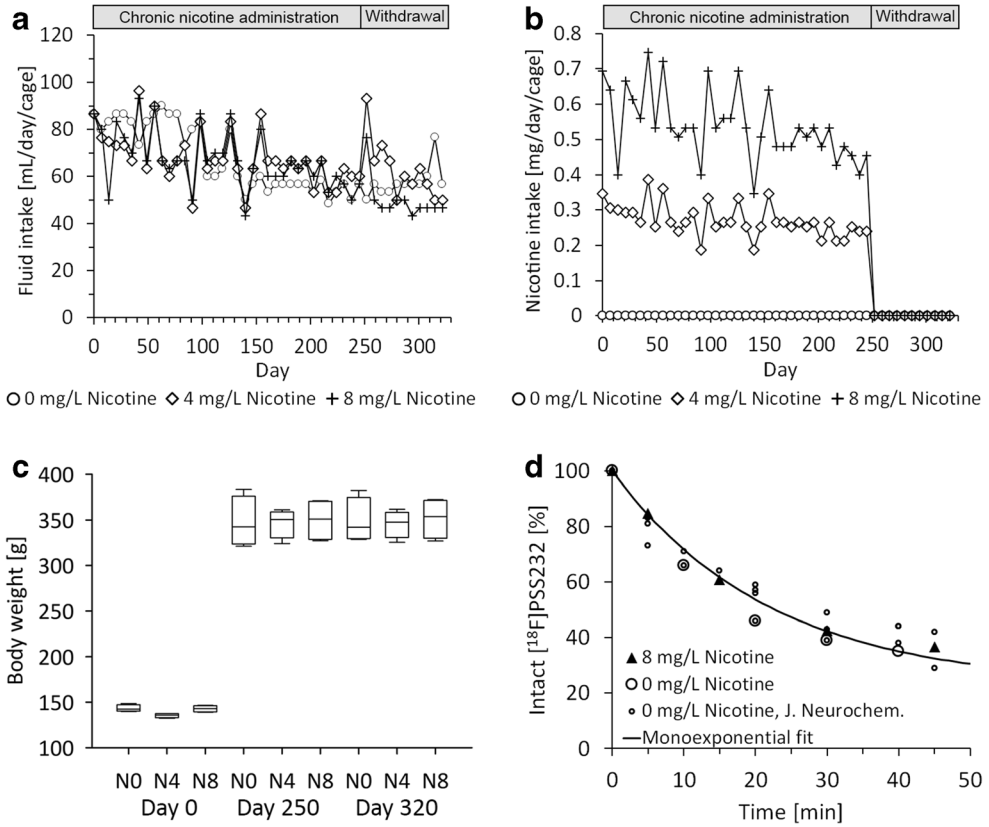

Fig. 2 **a** Fluid intake (mL/day) and **b** nicotine dose (mg/day) for the three groups (0, 4 and 8 mg/L nicotine, $n = 1$ cage per group of 4 animals each) over the whole PET imaging experiments from day 0 to 322. **c** Box plot of the body weights of Dark Agouti rats in the three groups treated with either 0 ($n = 4$), 4 ($n = 4$) and 8 mg/L ($n = 4$) nicotine and scanned with [^{18}F]PSS232. The boundaries of the box indicate the 25th and 75th percentile, respectively. Solid line graphs the median. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Body weights within group a ($F_{2,12} = 3.176$, $p = 0.078$) or b ($F_{5,24} = 0.34$, $p = 0.883$) are not significantly different. Body weights between group a and b are significantly different ($F_{1,28} = 1635$, $p < 0.001$). **d** Metabolic fate of [^{18}F]PSS232 at different time points post radiotracer injection (intact [^{18}F]PSS232 of total ^{18}F-radioactivity in plasma). Rats were housed at 0 ($n = 1$) or 8 mg/L ($n = 1$) nicotine for 253 days. Both data sets are in agreement with the data from our former study with male Wistar rats (Müller Herde et al. 2015). The respective data are added for comparison (squares). Solid line, monoexponential fit to all data (half-life 15.2 min, steady-state ratio at 22.6%)

administered over the course of the entire study did not differ between groups (0 mg/L, 21.5 ± 5.3 MBq, 1.9 ± 0.6 nmol/kg; 4 mg/L, 21.2 ± 3.7 MBq, 2.0 ± 0.4 nmol/kg; 8 mg/L, 25.1 ± 2.3 MBq, 2.5 ± 0.6 nmol/kg). Figure 3 shows DVRs comparing the influence of nicotine at different time points for each rat. We found a dose-dependent effect of nicotine treatment over time (Fig. 3). Mixed-effects analysis yielded a non-significant effect of group ($F_{2,11.10} = 0.17$, $p = 0.85$), a significant effect of day ($F_{1,115} = 27.54$, $p < 0.01$) and a significant interaction day-by-group ($F_{2,115} = 3.42$, $p = 0.04$) (Supplementary Table S3). Nonparametric longitudinal data analysis yielded a similar pattern of results, with a non-significant effect of group ($p = 0.76$), a significant effect of brain region ($p < 0.01$), a significant effect of day ($p < 0.01$), and a trend for a day-by-group interaction ($p = 0.06$) (Supplementary Table S4, Supplementary Fig. S3). These findings reflect an overall increase in DVR from day 0 to day 320 for striatum, hippocampus, cingulate cortex, and cortex across all three groups. Nicotine treatment resulted in decreased DVRs at day 250 compared with treatment with nicotine-free water in the striatum, hippocampus, thalamus, and midbrain. The effects of chronic nicotine exposure were stronger in the 4 mg/L than in the 8 mg/L treatment group, whereas there was almost no effect in the 8 mg/L treatment group. After subsequent exposure to nicotine-free water for 70 days, the group pretreated with 4 mg/L nicotine showed a marked recovery with DVRs reaching or surpassing those in nicotine-naïve controls. Importantly, mixed-model fitting and nonparametric analysis of longitudinal data converged, showing that our results are consistent for different statistical procedures. Supplementary Figs. S4–S6 and Supplementary Tables S5–S10 show the results of comprehensive post-hoc comparisons. To corroborate our region-wise findings, we calculated average voxel-wise DVRs within each group and plotted the results as DVR brain maps (Fig. 4). The average axial, sagittal, and coronal PET images clearly demonstrated a decrease in DVR in the 4 mg/L group from day 0 to day 250 as well as a recovery at day 320 (Fig. 4).

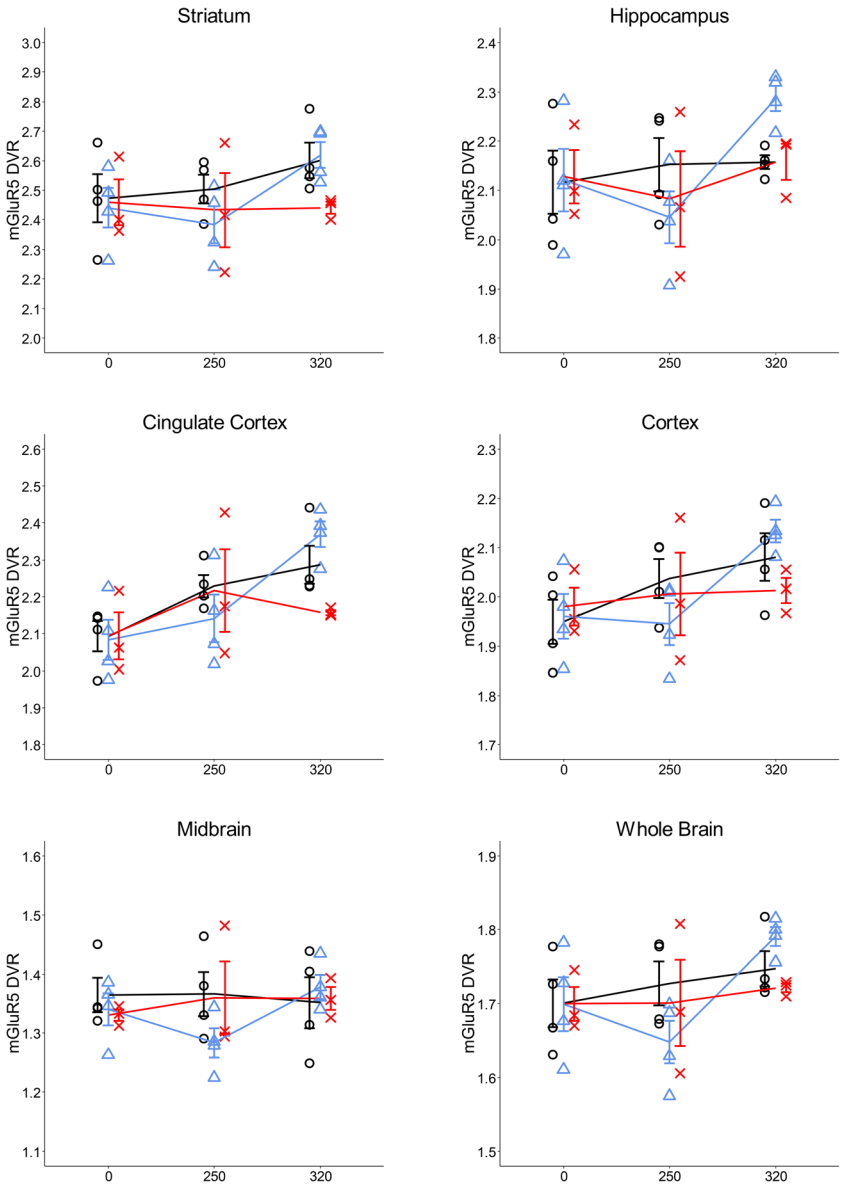

Fig. 3 Influence of nicotine on mGluR5 distribution volume ratios (DVRs) in different rat brain regions. Rats received three PET scans, on day 0, 250 and 320, respectively. After a baseline scan (day 0) the rats received either nicotine-free drinking water (black symbols, $n = 4$), drinking water containing 4 mg/L nicotine (blue symbols, $n = 4$) or 8 mg/L nicotine (red symbols, $n = 3$). After the second PET measurement (day 250) all rats received nicotine-free drinking water and were subjected to a follow-up scan on day 320. Plotted lines connect the average of each group over the PET scans, whiskers indicate standard error of the mean

Behavioural Data

After the first subcutaneous injection of 1 mg/kg saline, exploratory behaviour, reflected by total locomotion, was reduced in chronically nicotine-pretreated animals (Fig. 5). Correspondingly, a one-way analysis of variance with total locomotion as a dependent variable and a fixed effect of dose (0 mg/L, 4 mg/L, 8 mg/L) yielded a significant main effect of nicotine treatment ($F_{2,7} = 6.05$, $p = 0.03$, Supplementary Table S11). This effect was larger in the group pretreated with 4 mg/L than in the group pretreated with 8 mg/L nicotine solution: average distance moved was 1324 cm for controls (range 1173–1429), 529 cm for 4 mg/L-treated rats (range 289–989), and 887 cm for 8 mg/L-treated rats (range 446–1150). Further ANOVAs revealed no significant effect of chronic nicotine pre-treatment on locomotion in the centre, centre time, and centre entries on the first day of behavioural testing (all $p > 0.05$, Supplementary Table S11). These results received further support from nonparametric analyses. Kruskal-Wallis tests showed a significant effect of chronic nicotine pre-treatment on total distance moved ($p = 0.03$), and no significant effects on the other behavioural measures (all $p > 0.05$, Supplementary Table S12). Overall, nonparametric and parametric tests converged to show reduced locomotion in animals chronically exposed to nicotine on the first day of behavioural testing. Post-hoc exploratory analyses with parametric and nonparametric tests showed no between group-differences, no effect of nicotine administration during behavioural testing, and no interaction between the effects of chronic nicotine/water administration for 250 days prior to

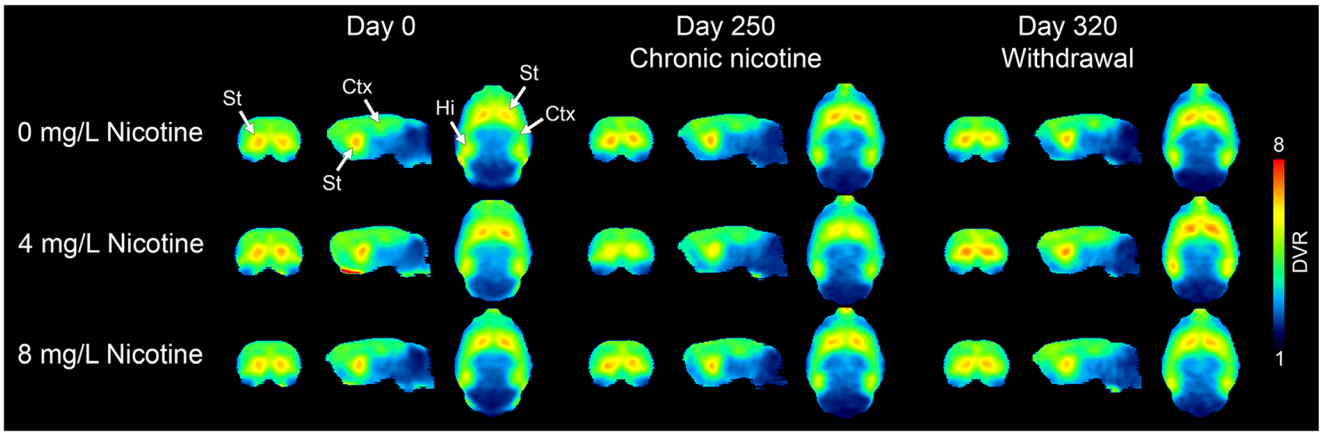

Fig. 4 Averaged [^{18}F]PSS232-PET images (DVR) at the investigated nicotine doses and time points as indicated. DVR images are shown in three anatomical orientations and were averaged from 4 scans for the 0 mg/L and 4 mg/L nicotine group and from 3 scans for the 8 mg/L nicotine group. Highest accumulation of [^{18}F]PSS232 is seen in the striatum, hippocampus and cortex at day 0 in all groups. [^{18}F]PSS232-DVR decreased in the striatum and hippocampus after chronic administration of 4 mg/L nicotine (day 250) and increased after withdrawal (day 320). The quantitative DVR are shown in Fig. 3. St, striatum; Ctx, cortex; Hi, hippocampus

behavioural testing and subsequent nicotine/water administration during behavioural testing (all p values > 0.05, Supplementary Tables S13–S16, Fig. 5, Supplementary Figs. S7–S9).

Discussion

The present preliminary investigation revealed an increase in mGluR5 DVR over 1 year in all male Dark Agouti rats.

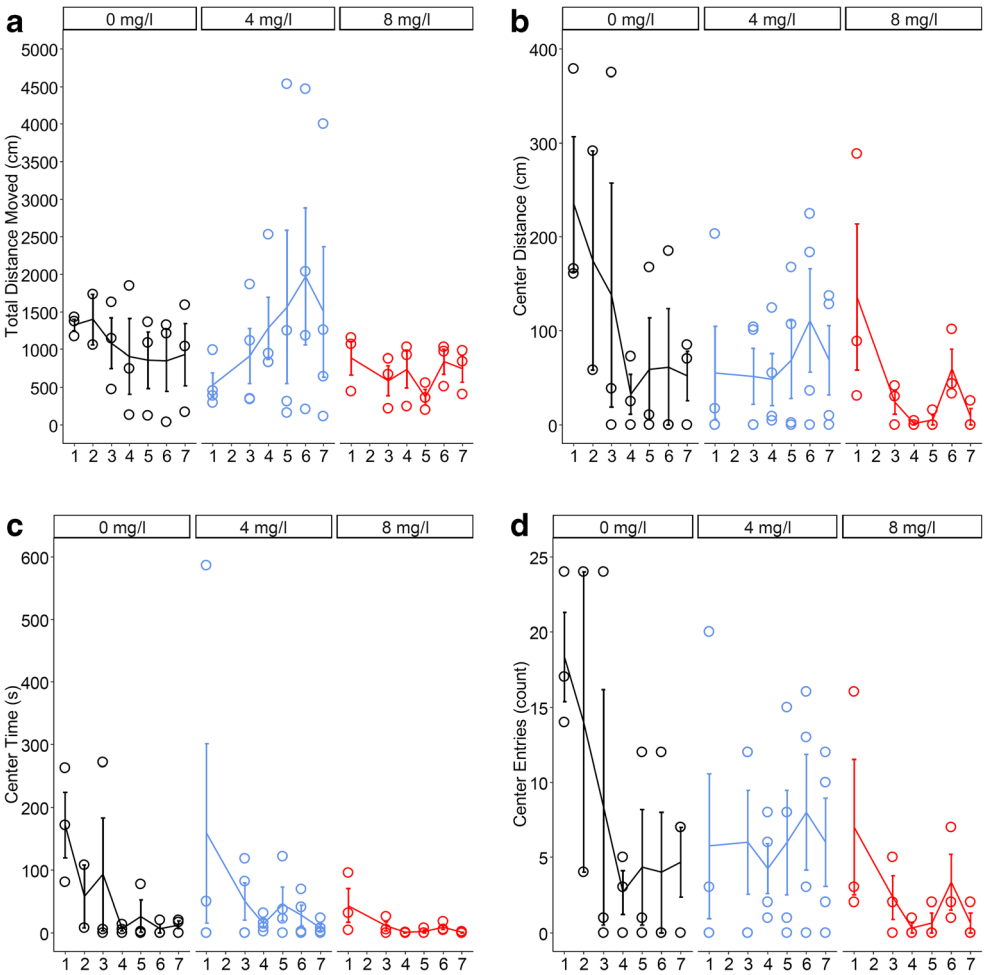

Fig. 5 Behaviour in the open field over 7 days of testing (days 394–418). Panels depict **a** the total distance moved, **b** centre distance, **c** centre time, and **d** centre entries for rats chronically exposed to water (placebo, black symbols, $n = 3$), a nicotine solution of 4 mg/L (blue symbols, $n = 4$), or a nicotine solution of 8 mg/L (red symbols, $n = 3$). Lines and whiskers indicate mean ± standard error of the mean

Chronic nicotine administration induced a transient decrease in this development that reversed to control values after prolonged withdrawal. After long-term withdrawal, nicotine-pretreated animals showed reduced exploratory behaviour in an open field test, compared to nicotine-naïve controls. The observed preliminary effects of chronic nicotine exposure on both mGluR5 and exploratory behaviour were strongest in animals treated with 4 mg/L and negligible in those treated with 8 mg/L nicotine.

To our knowledge, this is the first longitudinal mGluR5 PET study in rats. We found an increase in mGluR5 binding over 320 days in rats that were 6 weeks old at the time of the first PET measurement. This adds new evidence to the existing literature that mostly reports decreasing mGluR5 levels in older age (Catania et al. 2007; Domenici et al. 2003; Leurquin-Sterk et al. 2016; Menard and Quirion 2012; Romano et al. 1995; Romano et al. 2002; Tsamis et al. 2013; Wijetunge et al. 2008; Yu et al. 2000). PET studies in humans with the mGluR5 tracer [^{11}C]ABP688 reported a non-significant association between mGluR5 availability and age (age range 22–77 years) or no relationship between mGluR5 availability and age (DuBois et al. 2016; Leurquin-Sterk et al. 2016). A series of experiments in rats and mice showed lower mGluR5 levels in older animals (i.e., 24 months, reflecting 30 years of human age) (Catania et al. 2007; Domenici et al. 2003; Romano et al. 1995; Romano et al. 2002; Wijetunge et al. 2008). Of note, all of these studies had a cross-sectional design, and were mainly post-mortem. Our study significantly extends the available literature on mGluR5 development with the first longitudinal investigation that followed the same cohort of subjects through an extended period. We found an increase in mGluR5 availability across three consecutive measurements over 320 days. We also showed that chronic nicotine exposure alters this development.

We have previously shown a global reduction in mGluR5 DVR in smokers, as compared with non-smokers, and a less pronounced mGluR5 DVR reduction in ex-smokers (Akkus et al. 2013; Akkus et al. 2016). These findings are consistent with the hypothesis that chronic nicotine exposure reduces mGluR5, and that prolonged abstinence normalizes abnormal mGluR5 (Hulka et al. 2014). However, the correlational nature of these results in humans does not allow a causal interpretation. They might reflect an mGluR5-related predisposition to nicotine addiction, or the effect of chronic nicotine exposure (Akkus et al. 2013). Previous work investigating the causal effect of nicotine on mGluR5 in rodents partly supports the latter notion (Higa et al. 2017; Kane et al. 2005; Pistillo et al. 2016). A study in mice showed a trend towards mGluR5 downregulation after 33 days of continuous nicotine administration via an osmotic pump (Higa et al. 2017). The authors reported that this effect was only present in animals still under nicotine treatment and rapidly disappeared in animals tested 4 h after nicotine withdrawal (Higa et al. 2017). Another investigation in mice studied the effects of continuous nicotine administration over 14 days via microinfusion (Pistillo et al. 2016). Compared with nicotine-naïve controls, nicotine-treated mice displayed higher mGluR5 levels on the last day of nicotine administration in the midbrain, but not in the striatum and the prefrontal cortex (Pistillo et al. 2016). Mice that received the same pretreatment and underwent 1, 4 or 14 days of withdrawal after nicotine microinfusion did not differ significantly from nicotine-naïve controls (Pistillo et al. 2016). Both studies showed a recovery of mGluR5 after withdrawal from nicotine exposure, supporting our hypotheses (Akkus et al. 2013). Taken together, both studies suggest a non-linear relationship between nicotine exposure and its effects on mGluR5: longer exposure (33 days) downregulated mGluR5, whereas the shorter exposure (14 days) rather increased mGluR5. A study in rats yielded further support for this non-linear relationship (Kane et al. 2005). In rats that received daily multiple nicotine or saline injections for 3, 7 or 14 days, mGluR5 protein levels and mRNA expression in the amygdala were higher in nicotine-treated than in nicotine-naïve animals after 3 days of treatment but not after 7 or 14 days of treatment (Kane et al. 2005). In the same study, mGluR5 mRNA and protein levels in the ventral tegmental area and the nucleus accumbens did not differ between nicotine- and saline-treated animals irrespective of treatment duration (Kane et al. 2005). Overall, the findings of previous preclinical investigations suggest a non-linear relationship, such that brief nicotine exposure increases, whereas prolonged nicotine treatment decreases mGluR5 availability. Of note, mGluR5 measurements in these studies were post-mortem and cross-sectional, rather than longitudinal. In contrast, in the present preliminary study, we investigated the causal effect of chronic nicotine exposure and subsequent withdrawal on mGluR5 in a longitudinal manner. We found that nicotine induces a transient decrease in the developmental trajectory of mGluR5 binding. However, the effects of nicotine treatment were stronger in the group that received the lower dose of 4 mg/L than in the group that received the higher dose of 8 mg/L, where we found almost no effect of both nicotine exposure and withdrawel.

A series of previous investigations examined the relationship between chronic nicotine consumption and explorative behaviour in rodents. In rats, locomotor activity increased during nicotine administration through drinking water over 50 days, and this effect was stronger with increasing nicotine dose (Kita et al. 1985). Another study in rats supported and extended these findings by showing that locomotion increased over 98 days of nicotine administration and rapidly normalized after the onset of withdrawal (Welzl et al. 1988). The findings in rats imply that the effects of prolonged nicotine exposure on locomotion depend on the duration of nicotine withdrawal. Studies carried out in mice further support this notion. Chronic nicotine exposure through the drinking water increased locomotion while still under nicotine treatment (Caldarone et al. 2008). After 50 days of chronic nicotine

administration with the drinking water mice displayed increased locomotor activity that normalized after withdrawal (Gaddnas et al. 2000). Mice treated with nicotine through the drinking water for 4 or 7 weeks followed by a subcutaneous injection of saline after 24 h or 48 h of withdrawal did not show altered locomotion as compared with nicotine-naïve mice that also received saline injections (Pietila et al. 1998). Most of these studies examined locomotor activity during or shortly after the end of nicotine treatment. We found decreased locomotion in nicotine-pretreated rats after an extended period of withdrawal from nicotine. Our findings might reflect the long-term effects of chronic nicotine exposure, rather than acute withdrawal symptoms.

In our preliminary study, both the neural and behavioural effects of chronic nicotine treatment were stronger in the low-dose than in the high-dose group. From a statistical point of view, this finding might reflect the high effect size variance in small samples. Meta-analysis research regards effect size as a random variable (Fan and Konold 2010). The variance of this random variable increases with smaller sample sizes (Fan and Konold 2010). Thus, even in the presence of a linear dose-response relationship, small sample sizes are less likely to reliably show this dose-response relationship than large samples. An alternative explanation holds that there is no linear dose-response relationship. In our PET study in smokers and ex-smokers, we found no relationship between the amount of nicotine consumption and mGluR5 binding (Akkus et al. 2013). Rodent studies suggested stronger effects of nicotine after a brief exposure than after a longer exposure and suggested that shorter durations of continuous exposure might elevate, whereas prolonged continuous exposure rather decreases mGluR5 levels (Higa et al. 2017; Kane et al. 2005; Pistillo et al. 2016). Other studies applying chronic nicotine administration also showed a non-linear dose-response relationship between nicotine treatment and behavioural and neurochemical outcome measures (Kita et al. 1985; Welzl et al. 1988). Overall, our preliminary investigation and other studies emphasize the distinction between acute and chronic exposure and show that chronic nicotine treatment does not have a linear dose-response relation.

In animal studies, nicotine is generally administred in the drinking water, while human studies are conducted with smokers where nicotine is inhaled. After oral administration, about 30% of the nicotine is absorbed readily in the mouth and crosses all biological membranes including the blood-brain barrier. The remaining 70% of nicotine reaching the stomach and intestine is facing fist-pass metabolism in the liver (Collins et al. 2012; Matta et al. 2007; Nesil et al. 2011). Plasma nicotine concentrations similar to smokers (7–40 ng/mL) can be achived if nicotine is administered chronically and orally in doses of 10 mg/L and higher (Collins et al. 2012).

The low sample sizes we used are a major limitation of this study. They increase the influence of individual outliers, reduce the overall statistical power and limit the generalizeability of our findings. We calculated nonparametric tests in addition to parametric tests, to address the possible impact of deviations from normality on our findings. Nonparametric and parametric analyses converged, showing that our findings cannot be explained by the influence of deviations from normality on parametric testing in small samples.

The main strengths of the present investigation are the long duration of nicotine exposure and the longitudinal assessment of mGluR5 within the same individuals. Both the expected and novel findings of our study underline the importance of prolonged exposure in ecologically valid models of a disorder with a chronic and potentially life-long course such as nicotine addiction.

In conclusion, we demonstrated for the first time marked reduction of mGluR5 density after nicotine administration that is reversed after nicotine withdrawal in the same individuals. These preliminary results support the concept that mGluR5 density is reversibely affected by exposure to nicotine and could, therefore, serve as a target for the development of smoking cessation agents. Importantly, such agents could aid the cessation of both smoking and alcohol abuse, as both involve a dysfunction of mGluR5, co-occur and interact to produce severe neurotocixity (Akkus et al. 2018; Manavalan et al. 2017; Marszalek-Grabska et al. 2018).

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Compliance with Ethical Standards

Conflict of Interest G. Hasler received consulting fees and/or honoraria within the last three years from Eli Lilly, Janssen, Lundbeck, Otsuka, Schwabe, Servier and Takeda. Adrienne Herde, Yoan Mihov, Stefanie Krämer, Linjing Mu, Antoine Adamantidis, and Simon Ametamey have no conflicts of interest to report.

Ethical Approval Animal husbandry and experiments were carried out in accordance with the Swiss legislation on animal welfare and were approved by the Veterinary Offices of the Canton Zurich and Bern, Switzerland.

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