European Journal of Pharmacology 794 (2017) 257–269

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural pharmacology

The 5-HT$_{1A/1B}$-receptor agonist eltoprazine increases both catecholamine release in the prefrontal cortex and dopamine release in the nucleus accumbens and decreases motivation for reward and "waiting" impulsivity, but increases "stopping" impulsivity

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ARTICLE INFO

Chemical compound studies in this article:
Eltoprazine (PubChem CID: 65853)

Keywords:
Eltoprazine
5-HT$_{1A/1B}$-receptor
Microdialysis
Monoamines
Prefrontal cortex
Impulsivity

ABSTRACT

The 5-HT$_{1A/1B}$-receptor agonist eltoprazine has a behavioral drug signature that resembles that of a variety of psychostimulant drugs, despite the differences in receptor binding profile. These psychostimulants are effective in treating impulsivity disorders, most likely because they increase norepinephrine (NE) and dopamine (DA) levels in the prefrontal cortex. Both amphetamine and methylphenidate, however, also increase dopamine levels in the nucleus accumbens (NAc), which has a significant role in motivation, pleasure, and reward.

How eltoprazine affects monoamine release in the medial prefrontal cortex (mPFC), the orbitofrontal cortex (OFC), and the NAc is unknown. It is also unknown whether eltoprazine affects different forms of impulsivity and brain reward mechanisms.

Therefore, in the present study, we investigate the effects of eltoprazine in rats in the following sequence: 1) the activity of the monoaminergic systems using in vivo microdialysis, 2) motivation for reward measured using the intracranial self-stimulation (ICSS) procedure, and finally, 3) "waiting" impulsivity in the delay-aversion task, and the "stopping" impulsivity in the stop-signal task.

The microdialysis studies clearly showed that eltoprazine increased DA and NE release in both the mPFC and OFC, but only increased DA concentration in the NAc. In contrast, eltoprazine decreased 5-HT release in the mPFC and NAc (undetectable in the OFC). Remarkably, eltoprazine decreased impulsive choice, but increased impulsive action. Furthermore, brain stimulation was less rewarding following eltoprazine treatment. These results further support the long-standing hypothesis that "waiting" and "stopping" impulsivity are regulated by distinct neural circuits, because 5-HT$_{1A/1B}$-receptor activation decreases impulsive choice, but increases impulsive action.

1. Introduction

The 5-HT$_{1A/1B}$-receptor agonist eltoprazine is a relatively "old" drug that was originally developed as a serenic drug (Sybesma et al., 1991a, 1991b; De Boer, Koolhaas, 2005). Recently, PsychoGenics Inc. has used their SmartCube®, a high-throughput behavioral platform for detecting therapeutic efficacy, for comparing the behavioral profile of eltoprazine with those from their proprietary reference drug database (Alexandrov et al., 2015). Interestingly, it was shown that eltoprazine has a drug signature that resembles that of a variety of psychostimulant drugs (amphetamine, methylphenidate, and modafinil) and the norepinephrine (NE) reuptake inhibitor atomoxetine (Alexandrov et al., 2015). What all of these drugs have in common, despite the different working mechanisms, is that they increase NE and/or dopamine (DA) in the prefrontal cortex (Solanto, 1998), and enhance cognition and reduce impulsivity (Arnsten and Pliszka, 2011). In addition, ampheta-

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http://dx.doi.org/10.1016/j.ejphar.2016.11.024
Received 19 September 2016; Received in revised form 3 November 2016; Accepted 16 November 2016
Available online 17 November 2016
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mine and methylphenidate also increase DA levels in the nucleus accumbens (NAc) and therefore are frequently abused for recreational purposes (i.e., to get high) (Stoops et al., 2003; Pierce and Kalivas, 1997). There is a growing body of literature that recognizes the importance of impulsivity as both a psychological construct and an endophenotype underlying ADHD and drug abuse (Urcelay and Dalley, 2012).

Different categories of impulsivity exist. 1) Impulsive action or"-stopping" impulsivity is the inability of individuals to stop a response that has already been initiated. This type of impulsivity can be measured with the stop-signal task (Dalley et al., 2011; Evenden, 1999). 2) Impulsive choice or"waiting" impulsivity as the inability to wait for a large reward over an immediate small reward. This tendency can be measured with delay-aversion/delay-discounting paradigms (Bari and Robbins, 2013; Sonuga-Barke et al., 1992). These different types of impulsivity probably have discrete underlying neural circuits, in which the medial prefrontal cortex (mPFC), the orbitofrontal cortex (OFC), and the nucleus accumbens (NAc) play an important role (Dalley et al., 2011).

Recent years have seen a renewed interest in eltoprazine, because this specific 5-$HT_{1A/1B}$-receptor agonist counteracts l-DOPA-induced dyskinesias in Parkinson's (Svenningsson et al., 2015). This suggests that eltoprazine also affects the dopaminergic system. It is widely accepted that the serotonergic and dopaminergic system are closely interconnected and exert regulatory control over each other (for review see: Assié et al., 2005; Diergaarde et al., 2008; Fink and Göthert, 2007a, 2007b). Thus, investigating the role of 5-$HT_{1A/1B}$-receptors and monoamine release on impulsivity is of special interest. The 5-$HT_{1A/1B}$-receptors may alter dopamine function and other neurotransmitters in complex ways, because they function both pre- and postsynaptically.

The objective of this paper is to investigate the effects of eltoprazine on the release-profile of 5-HT, NE and DA and their metabolites in the mPFC, OFC and NAc in rats. Both DA and 5-HT are involved in reward-related processes related to impulsivity (Kranz et al., 2010). We therefore also assessed the motivation for reward using an intracranial self-stimulation (ICSS) procedure. In addition, we examined the effects of eltoprazine on impulsive choice and impulsive action, as measured by the delay-aversion task and the stop-signal task, respectively.

2. Material and methods

2.1. Compounds

Eltoprazine (1-[2,3-dihydro-1,4-benzodioxin-5-yl]-piperazine hydrochloride, synthesized by Psychogenics Inc, USA), has high affinitiy for the 5-HT_{1A} and 5-HT_{1B} receptor subtypes (K_i =40 and 52, respectively) (Schipper et al., 1990). Eltoprazine was dissolved in 0.9% NaCl and administered intraperitoneally (i.p.) in a volume of 2 ml/kg. Doses were 0, 1, and 3 mg/kg eltoprazine in both the microdialysis- and ICSS experiments and 0, 0.25, 0.5, and 1 mg/kg eltoprazine in the impulsivity tests. In all experiments, the drugs or vehicle (NaCl) were administered 30 min before testing.

2.2. Animals

Male Wistar rats (in total 116) obtained from Harlan (The Netherlands), weighing 125–175 g on arrival. Seventy-two animals were used for the microdialysis experiment (mPFC: n=24; OFC: n=24; NAc: n=24); 16 for intracranial self-stimulation; 12 for delay-aversion and finally, and 16 for the stop-signal task. The subjects were randomly divided over the different experimental groups. Animals weighed between 250 and 360 g at the time of microdialysis experiments, when they were ca. 10–12 weeks of age. During impulsivity testing, the rats weighed between 350–500 g and were ca. 4–6 months of age. The rats were socially housed, four per cage. For the microdialysis experiments, animals were housed singly directly after surgery until the experiment the next day. All animals were kept on a 12 h light/dark cycle with lights on between 6:00 A.M. and 6:00 P.M., and rooms were temperature (21 ± 2 °C) and humidity (50 ± 10%) controlled. Food and water were available ad libitum except during ICSS training and the delay-aversion task, during which they received ca. 75% of their ad libitum intake. All experiments were approved by the Ethical Committee for Animal Research of Utrecht University, The Netherlands. During the experiments every effort was made to minimize animal pain, distress and discomfort.

2.3. Surgery

Rats were anesthetized by inhalation of isoflurane gas (2–3%), mixed with nitrous oxide and oxygen and animals were placed in a stereotaxic instrument (Kopf, David Kopf Instruments). Lidocaine hydrochloride (2%) was applied in the incision as a local anesthetic. All animals received Rimadyl (5 mg/kg, subcutaneously) for pain relief.

In the microdialysis experiment Cuprophan microdialysis probes were implanted in the mPFC (MAB 4.7., 3 mm CU), the OFC (MAB 4.6., 2 mm CU), and the NAc (MAB 4.7., 2 mm CU) of rats as part of three separate cohorts. For the mPFC, the incisor bar was lowered to the coordinates at −3.3 mm, AP: +3.2 mm, ML: +0.8 mm, DV: −4.0 mm from bregma and skull. The incisor bar was lowered to coordinates of the OFC at −3.3 mm, AP: +3.2 mm, ML: +2.5 mm, DV: −6.2 mm from bregma and skull. For the NAc, the incisor bar was lowered to the coordinates −3.3 mm, AP: +1.5 mm, ML: +1.8 mm, DV: −8.4 mm from bregma and skull (Paxinos, 2007). Probes were anchored with three screws and dental cement on the skull. After microdialysis probe implantation, animals were housed individually until the end of the experiment. For the intracranial self-stimulation (ICSS) experiments, bipolar ICSS electrodes (Plastics One, cut to 11 mm in length) were implanted into the lateral hypothalamus (LH). Coordinates were AP: −0.5 mm from bregma; ML: ± 1.7 mm; DV: −8.3 mm from dura. The incisor bar was adjusted to 5 mm above the interaural line (Pellegrino et al., 1979). Electrodes were anchored with four screws and dental acrylic on the skull.

2.4. Microdialysis experiment

One day after surgery, microdialysis experiments were carried out in awake, freely moving animals. Although 1 d after surgery the animals may not be fully recovered from the operation, most neuroscientists (including our group) perform microdialysis experiments within the optimal window of 24–48 h after probe insertion (Westerink et al., 1987). Microdialysis probes produce gliosis extending 200–300 µm from the track by 3–7 days after implantation, which is not observed 1 d after probe implantation (Hascup et al., 2009; Benveniste and Diemer, 1987). In line with this observation, it has also been shown that astrocytes around the guide cannula and microdialysis probe increase over time and this may clog the microdialysis membrane (Georgieva et al., 1993). Conducting microdialysis experiments immediately after probe insertion, however, are not recommended, because probe insertion is well known to cause localized tissue damage that compromises the blood-brain barrier to small molecules, but is re-established after 24 h (Benveniste et al., 1987; Morgan et al., 1996; Hascup et al., 2009; Benveniste and Hüttemeier, 1990).

The tubing was pre-rinsed with Ringer solution (147 mM NaCl, 2.3 mM KCL, 2.3 mM $CaCl_2$, and 1 mM $MgCl_2$) with use of a KdScientific Pump 220 series (USA) at constant flow rate of 20 µl/h for approximately 14 h. before every experiment. At the beginning of the test day animals were connected to a dual channel swivel (type 375/D/22QM), which allowed them to move freely. During microdialysis, the pump rate was set at 1.5 µl/min. Two h after connection, ten 30-min samples were manually collected in vials containing 15 µl of 0.1 M acetic acid and frozen at −20 °C. At the end of the test day samples were transferred to −80 °C until analysis with HPLC. After two h of

baseline samples, animals were injected with eltoprazine (1 and 3 mg/kg, 2 ml/kg i.p.) or vehicle (0.9% NaCl), and samples were collected for an additional 3 h.

2.5. Histology

Immediately after the experiments, animals were decapitated under gas anesthesia (isoflurane gas (2–3%), mixed with nitrous oxide and oxygen); brains were removed and kept for probe placement verification in 4% paraformaldehyde for at least three days. Data were discarded in cases where the microdialysis probe was not in the correct brain area.

2.6. HPLC analysis

Samples were analyzed with HPLC with electrochemical detection for norepinephrine (NE), dopamine (DA) and serotonin (5-HT) and 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxy-indoleacetic acid (5-HIAA) simultaneously by an Alexys 100 LC-EC system (Antec, The Netherlands), as described previously (Korte-Bouws et al., 1996). The system consisted of two pumps, one auto sampler with a 10-port injection valve, two columns, and two detector cells. DA and 5-HT were separated and detected by column 1 (ALF 105 C18 1×50 mm, 3 µm particle size) in combination with detector cell I. Column 2 (ALF 115 C18 1×150 mm, 3 µm particle size), in combination with detector cell II, separated and detected NE, DOPAC, HVA and 5-HIAA. The mobile phase for column 1 consisted of 50 mM phosphoric acid, 8 mM KCL, 0.1 mM EDTA (pH 6.0), 12% Methanol and 500 mg/L 1-Octanesulfonic acid, sodium salt (OSA). The mobile phase for column 2 consisted of 50 mM phosphoric acid, 50 mM citric acid, 8 mM KCl, 0.1 mM EDTA (pH 3.2), 10% methanol and 500 mg/L OSA. Both mobile phases were pumped at 50 µl/min. Samples were kept at 8 °C during analysis. From each microdialysis sample 5 µl was injected simultaneously onto each column. The neurotransmitters were detected electrochemically using µVT-03 flow cells (Antec Leyden, The Netherlands) with glassy carbon working electrodes. Potential settings were for DA and 5-HT +0.30 V versus Ag/AgCl and for NE and metabolites +0.59 V versus Ag/AgCl. The columns and detector cells were kept at 35 °C in a column oven. The chromatogram was recorded and analyzed using the Alexys data system (Antec, The Netherlands). The limit of detection was 0.03 nM (signal/noise ratio 3:1). Baseline concentrations are set at 100% and all drug effects are presented as percentage change from these baselines.

2.7. Behavioral tests

2.7.1. ICSS behavior: measurement of brain reward mechanisms

All ICSS experiments were performed in eight sound-attenuating operant cubicles (Med Associates Inc., interior: l x b x h=35.6 cm x 55.9 cm x 38.1 cm) with a grid floor and a wheel manipulandum on one of the sides. The implanted electrode was connected to an electrical stimulator through a swivel and bipolar connector cable (Plastics One), ensuring unrestrained movement throughout the ICSS procedure. A constant current stimulator (Med Associates Inc.) was used for electrical stimulation. The stimulator was connected to a computer running MED-PC IV software (Med Associates, Inc.) controlling all stimulation settings, programs and recording of data. All the animals were initially trained to turn the wheel manipulandum on a fixed ratio schedule of reinforcement, in order for the animals to make the association that turning the wheel results in electrical stimulation. In this training phase, each quarter turn of the wheel resulted in an electrical stimulus with a duration of 500 ms. After several successful training sessions, the rats were trained on a discrete-trial current-threshold procedure according to the procedure described earlier in following citations (Markou and Koob, 1992; Kenny, 2007). All ICSS current thresholds were expressed as a percentage of an animals' own pre-drug test of that day. Animals were tested according to a crossover within-subjects design in which all animals received all drug doses in random order with a one-week interval between doses.

2.7.2. Delay-aversion task: measurement of "waiting" impulsivity

The delay-aversion task used in this study was adapted from Cardinal and colleagues (Cardinal et al., 2000), and performed as described previously with some adaptation (Van den Berg et al., 2006a, 2006b) (Fig. 5B). Training for the delayed-reward task took approximately 2 months. In a session consisting of 6 blocks of 8 trials, rats had a choice between a nosepoke hole which, if the rat poked its nose in the hole, delivered a single food reward instantaneously, and a second nosepoke hole that delivered four food rewards, but after a delay. In the first block, this delay was 0 s, but each block the delay was increased until a maximum of 60 s in the final block (0 s, 5 s, 10 s, 20 s, 30 s, 60 s). To make sure that the rats had actually sampled both choices, the first two trials of each block were forced trials in which only one of the nosepoke holes was illuminated. Both nosepoke holes were illuminated once in the forced trials, and the order of presentation was determined randomly. The remaining 6 choice trials were used to calculate a preference ratio for each delay.

2.7.3. Stop-signal task: measurement of "stopping" impulsivity

The stop-signal task in the current study was adapted from Eagle and Robbins (2003). Animals were placed in the skinnerbox for 60 min or until they had completed 200 trials. Training for the stop-signal task took approximately four months. Sessions were divided into blocks, which consisted of several successful go-trials 1–3, determined randomly), and a concluding stop-trial. Lever extensions and food rewards were signaled by the illumination of a light above the lever or feeder tray. At the start of a go-trial, the left lever was extended for a maximum of 60 s. A response on the left lever resulted in the retraction of that lever and extension of the right lever for a limited amount of time (the limited hold period), during which the animal had to make a response to receive a food reward. If the animal failed to respond within the limited hold period, an omission was scored, and the animal received a timeout. Stop-trials were similar to go-trials, except for a 400 ms tone that was presented either 0 ms, 500 ms, 600 ms, 700 ms or 800 ms before the expected response on the second lever. On stop-trials, animals had to inhibit their response on the second lever for the entire limited hold period to receive a food reward. Failure to do so resulted in a timeout. During timeouts, the houselight was extinguished for 5 s. After the timeout period, the inter-trial interval (ITI) commenced automatically. The duration of the limited hold period was determined for rats individually, and was based on their previous performance. The limited hold period was calculated as the mean reaction time between pressing the left lever and the right lever plus 150–300 ms, and ranged between 850 ms and 1500 ms in total. We were especially interested in the proportion correct responses in the go-trials and in the stop-trials (Fig. 5C).

2.8. Statistical analysis

Microdialysis data and ICSS thresholds were analyzed independently, with use of repeated measures ANOVA with 'time' as within-subjects factor and 'treatment' as between-subjects factor. Greenhouse-Geisser corrected values were used in case of non-sphericity of the data (see ε values). When a significant time x treatment interaction was found, each time point was analyzed by one-way ANOVA. The Area Under the Curve (AUC) was calculated to better visualize the overall changes after eltoprazine administration. AUC was calculated using the trapezoid algorithm.

Go-trials and corrected stops in the stop-signal task were analyzed using oneway-ANOVA. Delay-aversion task data were reduced to the Area Under the Preference Curve (AUPC). This area reflects a theory-neutral index of inhibition in the delay-aversion task (Myerson et al.,

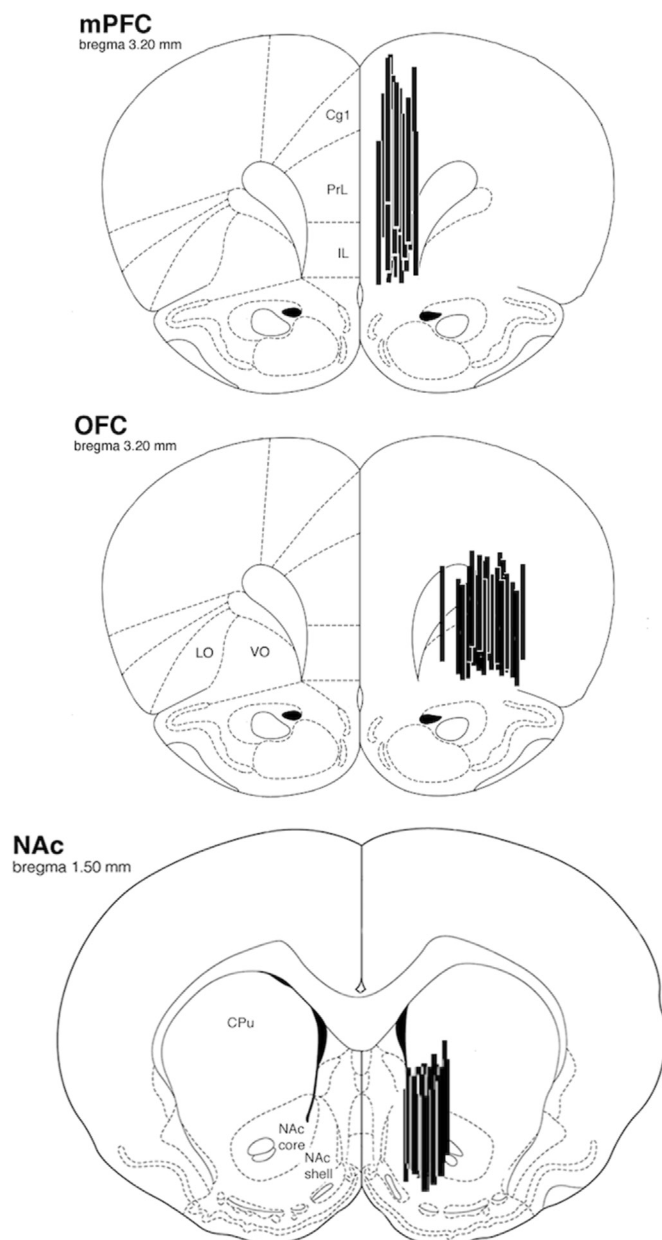

Fig. 1. Location of the microdialysis probes in the medial prefontal cortex (mPFC), the orbitofrontal cortex (OFC) and the nucleus accumbens (NAc). The schematic drawing adapted from Paxinos (2007) represents the tracts of the dialysis membranes (2 mm for both OFC and NAc and 3 mm for mPFC). Abbreviations: anterior cingulate cortex (Cg1); prelimbic cortex (PrL); infralimbic cortex (IL); lateral orbital cortex (LO); ventral orbital cortex (VO); caudate-putamen (CPu).

2001). The AUPC was analyzed with a repeated measures ANOVA.

In all cases where ANOVAs were significant, post-hoc comparisons were made with the Dunnett's test with the vehicle as control category. The results are expressed as the mean ± standard deviation of the mean. A P-value of < 0.05 was considered to indicate statistical significance.

3. Results

3.1. Microdialysis

3.1.1. Location of the microdialysis probes

The data of 4 animals were discarded because the microdialysis probe was not in the mPFC. The other 20 probes were located in anterior cingulate cortex (Cg1), prelimbic cortex (PrL) and/or infralimbic cortex (IL) (see Fig. 1.) All 24 microdialysis probes were located in the lateral orbital cortex (LO) or ventral orbital cortex (VO), as part of the OFC (see Fig. 1.). All 24 microdialysis probes were located in the NAc shell or core. Verification of the location of the electrodes in the lateral hypothalamus was not needed, because only animals that were performing self-stimulation were used.

3.1.2. Monoamine and metabolite concentrations in mPFC

The mean (± S.E.M.) absolute extracellular baseline concentrations in the mPFC were for DA: 0.61 ± 0.04 nM; for NE: 0.69 ± 0.03 nM; for 5-HT: 0.16 ± 0.01 nM; for DOPAC: 17.36 ± 1.30 nM; for HVA: 31.29 ± 2.66 nM, and; for 5-HIAA: 127.26 ± 6.18 nM. The baseline was set at 100% and all drug effects are presented as percentage change from baseline.

Eltoprazine (1 and 3 mg/kg) increased both DA ($F_{(3,48)}$=33.09, P < 0.001 and ε=0.469) and NE ($F_{(4,60)}$=17.03, P < 0.001 and ε=0.590). This is also reflected in the associated significant area under the curve (AUC) calculations (Fig. 1). The effects of eltoprazine on DA were more pronounced than the effects on NE. Eltoprazine (1 and 3 mg/kg) produced large significant decreases in 5-HT concentrations ($F_{(4,62)}$=7.53, P < 0.001 and ε=0.647). All metabolites followed the significant changes in concentrations of their neurotransmitter: DOPAC ($F_{(3,49)}$=27.06, P < 0.001 and ε=0.515), HVA ($F_{(2,42)}$=20.47, P < 0.001 and ε=0.413); 5-HIAA ($F_{(3,43)}$=61,66, P < 0.001 and ε=0.420). In Fig. 2, the significant changes per time point are presented. Highly significant (at least P < 0.01) time x treatment interactions were found for DA, NE, 5-HT, DOPAC, HVA, and 5-HIAA concentrations in the mPFC.

3.1.3. Monoamine and metabolite concentrations in OFC

Mean (± S.E.M.) absolute extracellular baseline concentrations in the OFC were for DA: 0.19 ± 0.01 nM; for NE: 0.26 ± 0.02 nM; for DOPAC: 15.93 ± 1.90 nM; for HVA: 40.10 ± 2.89 nM, and; for 5-HIAA: 102.45 ± 4.12 nM. 5-HT was undetectable in microdialysate from the OFC, because the concentrations were below detection range.

Eltoprazine (1 and 3 mg/kg) increased both DA ($F_{(3,60)}$=22.25, P < 0.001 and ε=0.474) and NE ($F_{(3,56)}$=9.49, P < 0.001 and ε=0.587). This is also reflected in the associated significant area under the curve (AUC) calculations (Fig. 2). Because 5-HT concentrations in the OFC were non-detectable, no responses to eltoprazine could be measured. All metabolites of DA and NE followed the significant changes in concentrations of their neurotransmitter: DOPAC ($F_{(3,49)}$=27.06, P < 0.001 and ε=0.267), HVA ($F_{(2,42)}$=20.47, P < 0.001 and ε=0.318), respectively. Also the metabolite 5-HIAA concentrations significantly decreased ($F_{(2,43)}$=116.84, P < 0.001 and ε=0.342). In Fig. 3, the significant changes per time point are presented. Highly significant (at least P < 0.01) time x treatment interactions were found for DA, NE, DOPAC, HVA and 5-HIAA concentrations in the OFC.

3.1.4. Monoamine and metabolite concentrations in NAc

Mean (± S.E.M.) absolute extracellular baseline concentrations in the NAc were for DA: 2.82 ± 0.15 nM, for NE: 0.32 ± 0.05 nM, for 5-HT: 0.11 ± 0.01 nM, for DOPAC: 607.77 ± 37.82 nM, for HVA: 278.12 ± 20.73 nM, and; for 5-HIAA: 234.41 ± 9.62 nM. The baseline was set at 100% and all drug effects are presented as percentage change from baseline.

Eltoprazine (1 and 3 mg/kg) significantly increased DA ($F_{(2,43)}$=14.6, P < 0.001 and ε=0.343). This is not reflected in the associated significant area under the curve (AUC) calculations (Fig. 3), probably because increases are only observed at t=30 and t=90 min. No effect of eltoprazine on NE concentrations was observed. Eltoprazine (1 and 3 mg/kg) produced large significant decreases in 5-HT concentrations ($F_{(3,66)}$=42.77, P < 0.001 and ε=0.524). The following metabolites changed significantly: DOPAC ($F_{(2,30)}$=26.29, P < 0.001 and ε=0.323); HVA ($F_{(2,48)}$=42.97, P < 0.001 and ε=0.383); 5-HIAA ($F_{(3,69)}$=318.33, P < 0.001 and ε=0.545). This is also reflected in the

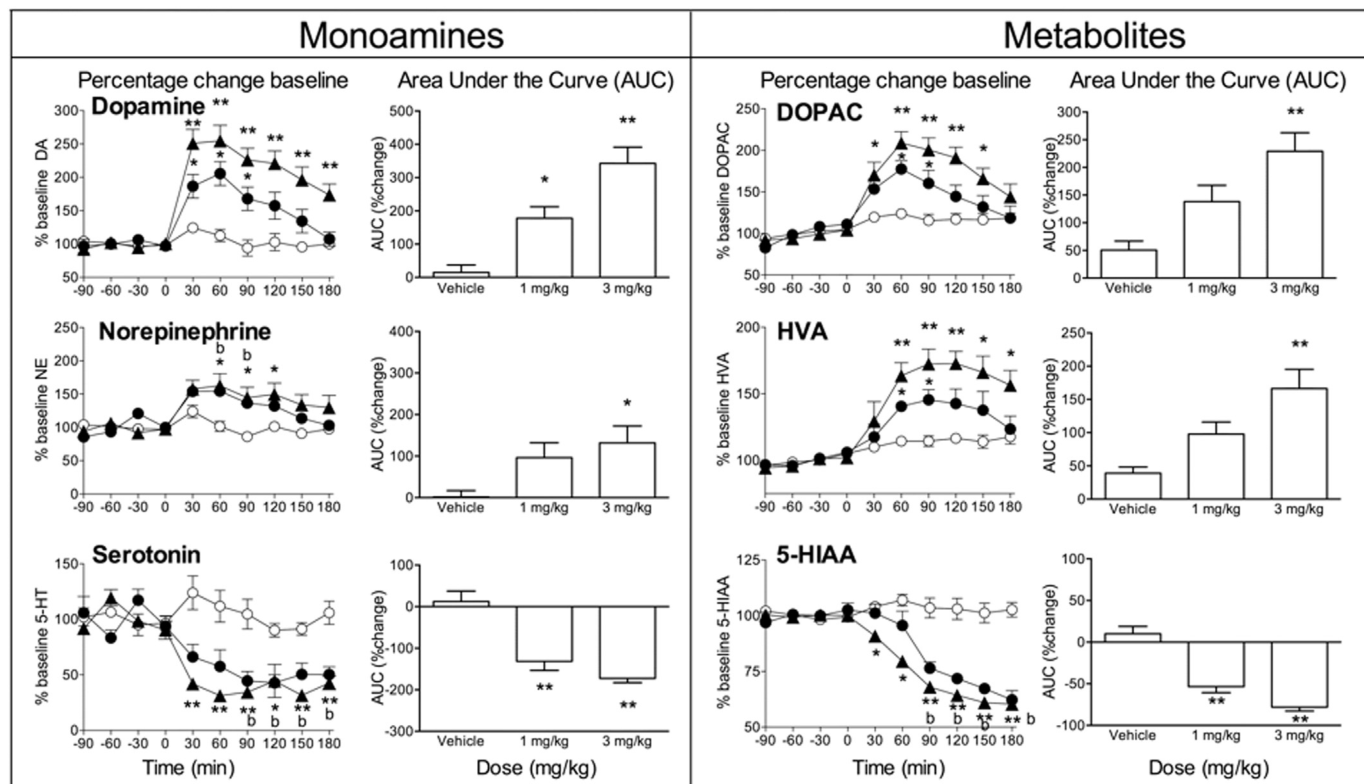

Fig. 2. Microdialysis in medial prefrontal cortex (mPFC). The four time points from −90 to 0 min represent baseline samples. At t=0 a single injection of eltoprazine was given and 30-min samples were taken up to 3 h after injection. Bars represent the area under the curve (AUC). * P < 0.05, ** P < 0.001 and b indicates that for both doses of eltoprazine the same significant change was observed.

associated significant area under the curve (AUC) calculations and the significant changes per time point are presented (Fig. 4). Significant (at least P < 0.05) time x treatment interactions were found for 5-HT, DOPAC, HVA and 5-HIAA concentrations in the NAc.

3.2. Behavioral tests

3.2.1. ICSS behavior

Fig. 5A shows that eltoprazine significantly increased ICSS thresholds as shown by a significant main effect of treatment ($F_{(2,16)}$=3.794, P < 0.05). Furthermore, a post-hoc analysis revealed that the increase in thresholds was only significantly increased by the lowest dose of eltoprazine (1 mg/kg; P < 0.05) compared to the vehicle, while the highest dose (3 mg/kg) did not differ significantly from the vehicle. A significant main effect of time ($F_{(2,32)}$=23.742, P < 0.001) was found, indicating an increase in thresholds over time. No significant time x treatment interaction was found.

3.2.2. Delay-aversion task

Fig. 5B displays the choice preference for eltoprazine and the area under the preference curve (AUPC) for each of the dosages. A high AUPC in the delay-aversion task corresponds to low delay-aversion. Eltoprazine significantly increased the AUPC, and thus significantly lowered impulsivity in the delay-aversion task ($F_{(3,33)}$=4.9, P=0.007). All dosages of eltoprazine significantly increased the AUPC (p < 0.005).

3.2.3. Stop-signal task

Fig. 5C displays data of go-trials and stop-trials. Performance in stop-trials was significantly and dose-dependently impaired by the administration of eltoprazine ($F_{(3,27)}$=7.3, P < 0.001). Post-hoc tests showed that performance was significantly worsened at all dosages (P < 0.05). Eltoprazine had no effect on performance in the go-trials.

Furthermore, no significant differences were found between the response latencies of the different treatment groups, indicating that eltoprazine administration did not influence the reaction time (RT) of the animals (data not shown).

4. Discussion

The microdialysis studies clearly showed that eltoprazine (1 and 3 mg/kg) increased DA and NE concentration in two different regions of the prefrontal cortex (mPFC and OFC), but only increased DA concentration in the NAc. In contrast, eltoprazine (1 and 3 mg/kg) decreased 5-HT release in the mPFC and NAc (undetected in the OFC). Eltoprazine (1 mg/kg, but not the higher 3 mg/kg dose) increased stimulation thresholds on the ICSS task, indicating that the stimulation was less rewarding following eltoprazine treatment (i.e. a state of hypohedonia). Eltoprazine (1 mg/kg and lower doses) lowered “waiting” impulsivity on a delay-aversion task, but increased “stopping” impulsivity on a stop-signal task. In the following, the various working mechanisms are discussed.

4.1. Involvement of $5\text{-}HT_{1A}$- and $5\text{-}HT_{1B}$-receptor in 5-HT release, brain reward function, and impulsivity

4.1.1. Serotonin release

Eltoprazine decreased 5-HT levels in both mPFC and NAc. This was expected, because eltoprazine acts as an agonist on inhibitory G protein-coupled (Gi/Go) $5\text{-}HT_{1A}$- and $5\text{-}HT_{1B}$-receptors (Bouhelal et al., 1988; Schipper et al., 1990; Seuwen et al., 1988) to inhibit firing of dorsal (DR) and median raphe (MR) nuclei by activation of somatodendritic and terminal autoreceptors respectively (Sijbesma

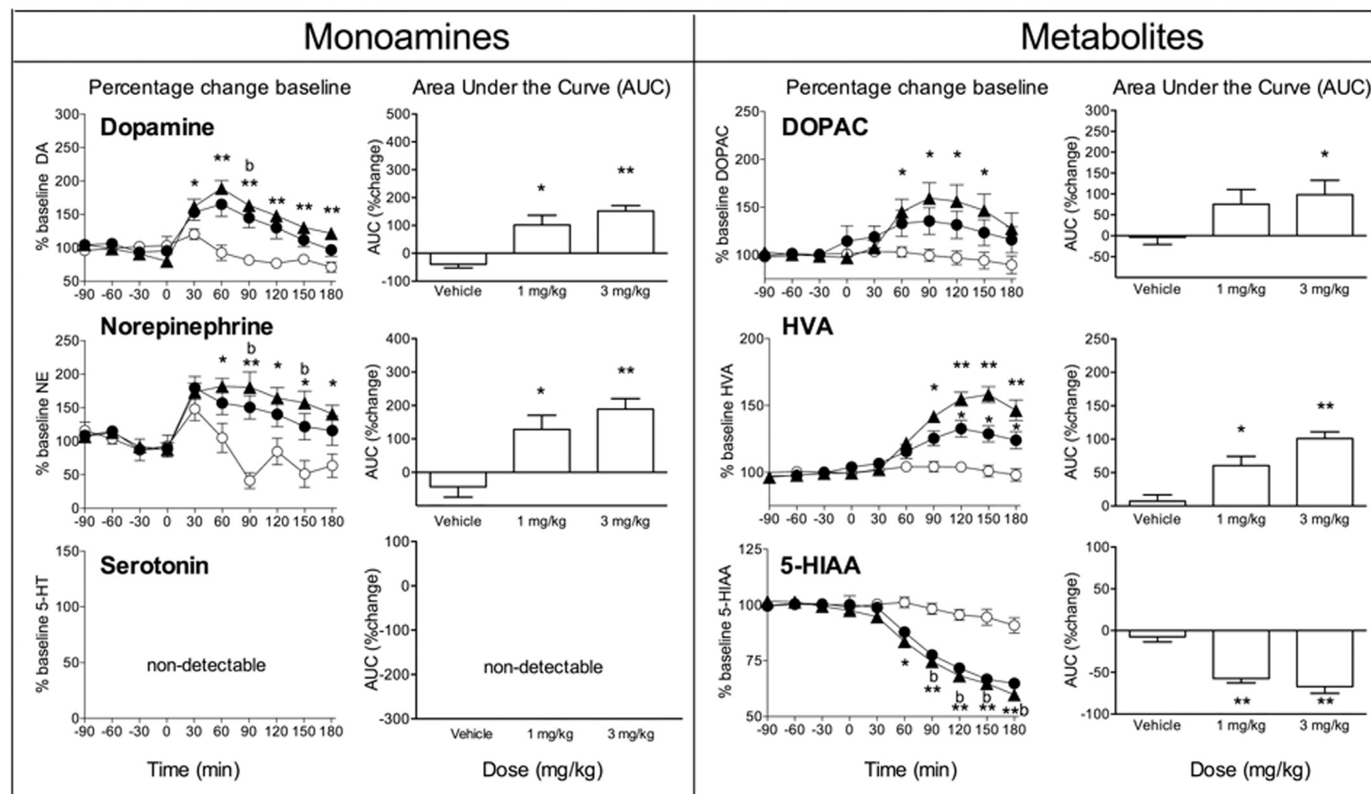

Fig. 3. Microdialysis in orbitofrontal cortex (OFC). The four time points from −90 to 0 min represent baseline samples. At t=0 a single injection of eltoprazine was given and 30-min samples were taken up to 3 h after injection. Bars represent the area under the curve (AUC). * $P < 0.05$, ** $P < 0.001$ and b indicates that for both doses of eltoprazine the same significant change was observed.

et al., 1991a, 1991b; Sprouse and Aghajanian, 1987). This reduces 5-HT levels in many postsynaptic areas (Adell et al., 2001; Blier and Ward, 2003; Casanovas et al., 1999). Surprisingly, 5-HT levels were below detection limit in the OFC. One explanation may be the use of different microdialysis probes with different lengths in the present study. Because the OFC is a smaller brain area than the mPFC in which to insert a microdialysis probe, a probe of 2 mm was used in the OFC, in contrast to a 3 mm probe in the mPFC. It is well known that a shorter microdialysis probe results in a lower recovery than a longer one (Chefer et al., 2009). In agreement with this explanation, we observed a decrease in 5-HIAA after eltoprazine treatment (due to the high 5-HIAA concentrations, it can be measured more easily than 5-HT), suggesting a decreased activity of the serotonergic system.

4.1.2. Serotonin and brain reward

Eltoprazine at a dose of 1.0 mg/kg, but not the 3.0 mg/kg dose, increased ICSS thresholds, suggesting an inhibitory influence on brain reward systems. The observed lack of effect of the highest dose on ICSS was not caused by non-specific effects such as sedation. This is in agreement with earlier findings that systemic administration of 8-OH-DPAT, probably via stimulation of postsynaptic 5-HT_{1A}-receptors, increased ICSS thresholds reflecting a decreased rewarding effect (Ahn et al., 2005). Similarly, activation of postsynaptic 5-HT_{1B}-receptors reduced brain stimulation reward (Harrison et al., 1999; Hayes et al., 2009). Thus, postsynaptic 5-HT_{1A}- and 5-HT_{1B}-receptors play an important role in the reduction of brain reward. In contrast, specific local activation of presynaptic 5-HT_{1A}-autoreceptors in the dorsal raphe (DR) and median raphe (MR) nuclei decreased ICSS thresholds reflecting an increased rewarding effect (Ahn et al., 2005; Fletcher et al., 1995; Harrison and Markou, 2001). Altogether, it is suggested that eltoprazine (1.0 mg/kg) reduces brain stimulation reward via postsynaptic 5-$HT_{1A/1B}$-(hetero)receptor activation. In addition, it is speculated that at a higher dose of eltoprazine (3.0 mg/kg) the stronger inhibition of 5-HT release due to presynaptic 5-HT_{1A}-autoreceptor stimulation might counteract this effect.

Thus, the balance between presynaptic 5-HT_{1A}-autoreceptor/postsynaptic 5-$HT_{1A/1B}$-(hetero)receptor activation may be crucial to the final observed effects on brain reward and ICSS.

4.1.3. Serotonin and “waiting” impulsivity

In the present study, the partial 5-$HT_{1A/1B}$-receptor agonist eltoprazine (0.25, 0.5 and 1.0 mg/kg, i.p.) decreased impulsive choice in the delay-aversion task. This is in agreement with earlier findings from our lab that 0.5 mg/kg eltoprazine reduced impulsive choice (Van den Bergh et al., 2006a, 2006b). To understand these data, one has to realize that systemic administration of eltoprazine can bind to presynaptic 5-$HT_{1A/1B}$-autoreceptors in the raphe nuclei and to postsynaptic 5-$HT_{1A/1B}$-(hetero)receptors.

The observed decrease in “waiting” impulsivity, as measured by decreased impulsive choice, can be explained by eltoprazine's effects on postsynaptic 5-HT_{1A}-receptors, because the full 5-HT_{1A}-receptor agonist 8-OH-DPAT (administered postsynaptically into the OFC) has been shown to decrease impulsive choice (Yates et al., 2014), whereas the partial 5-HT_{1A}-receptor agonists buspirone, ipsapirone or flesinoxan (preferentially acting presynaptically) increase impulsive choice (Bizot et al., 1999; Van den Bergh et al., 2006a, 2006b; Blasio et al., 2012). This might explain why previously it was shown that only the highest dose of 8-OH-DPAT (1.0 mg/kg, i.p.) increased impulsive choice, whereas the lower doses (0.1 and 0.3 mg/kg i.p.) did not affect impulsive choice (Winstanley et al., 2005). In addition, activation of 5-$HT_{1A/1B}$-autoreceptors have been shown to reduce DR neuron firing that lowers 5-HT levels and increases “waiting” impulsivity (Miyazaki

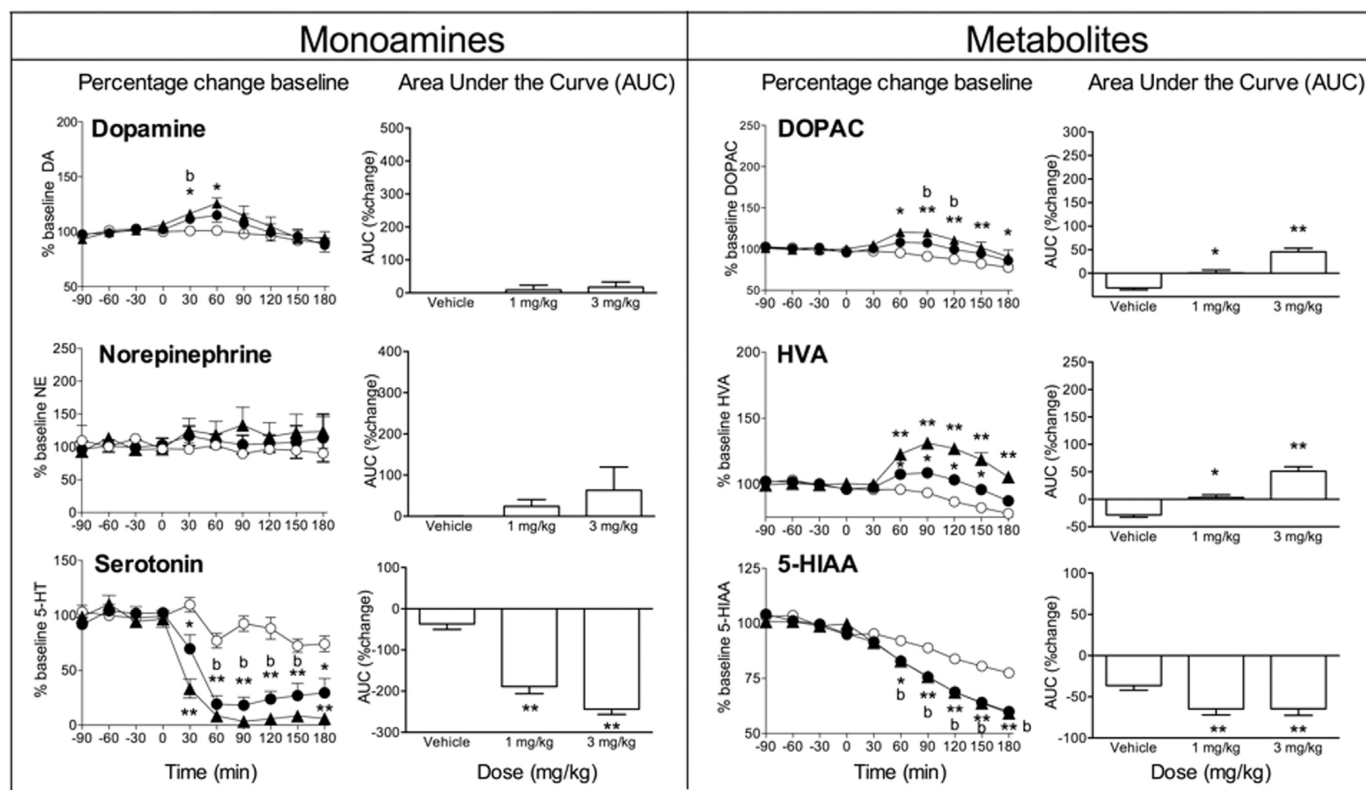

Fig. 4. Microdialysis in nucleus accumbens (NAc). The four time points from −90 to 0 min represent baseline samples. At t=0 a single injection of eltoprazine was given and 30-min samples were taken up to 3 h after injection. Bars represent the area under the curve (AUC). * $P < 0.05$, ** $P < 0.001$ and b indicates that for both doses of eltoprazine the same significant change was observed..

et al., 2012a, 2012b; Bizot et al., 1999; Mobini et al., 2000; Wogar et al., 1993). In addition, cessation of waiting was associated with a drop in 5-HT neuron firing in the DR, preceding the exit from reward sites (Miyazaki et al., 2011), whereas increased raphe 5-HT neuron firing facilitates waiting behavior in the prospect of forthcoming rewards (Miyazaki et al., 2011, 2012; Miyazaki et al., 2014; Fonseca et al., 2015; Ranade et al., 2014).

Animals prefer small over large rewards when the delays preceding large rewards exceed an individual tolerance limit. Behavioral research has shown that the animal's choice is guided by the subjective value of reward, which is a function of reward amount and waiting (Kable and Glimcher, 2009). The subjective value of delayed reward is reduced along a hyperbolic discounting function (Ainslie, 1975), i.e. longer delays make an option less attractive, because a delayed delivery is associated with waiting costs. Single cell recordings in different areas of the PFC revealed that neural activation reflected the temporal devaluation of the anticipated reward during impulsive decision-making (Kalenscher et al., 2005). Against this background, our observation that eltoprazine increases ICSS thresholds, and thus induces a hypohedonic state, fits perfectly with our results that stimulating $5\text{-HT}_{1A/1B}$-receptors decreases impulsive choice in the delay-aversion task. Therefore, we can speculate that eltoprazine discounts the hedonic value of immediate reward by activating prefrontal $5\text{-HT}_{1A/1B}$-receptors, thereby inducing a behavioral shift towards an increased tolerance for periods of waiting.

4.1.4. Serotonin and "stopping" impulsivity

In both human and rat studies, it has clearly been shown that 5-HT is not involved in "stopping" impulsivity, because neither 5-HT depletion, 5-HT lesions, nor partial 5-HT_{1A} -receptor agonists have any effect (Chamberlain et al., 2006; Chamberlain and Sahakian, 2007; Bari et al., 2009; Clark et al., 2005; Eagle et al., 2009). However, 5-HT is strongly implicated in action restraint, i.e., excitatory and inhibitory influences on premature responding via 5-HT_{2A}- and 5-HT_{2C} -receptors, respectively (Fletcher et al., 2007, 2011). Thus, neither 5-HT_{1A} -receptors nor 5-HT_{1B} -receptors are directly involved in impulsive action.

4.2. Involvement of 5-HT_{1A}- and 5-HT_{1B}-receptor in DA release, brain reward function, and impulsivity

4.2.1. Dopamine release

Eltoprazine increased dopamine levels in mPFC, OFC and NAc. The increased dopaminergic activity may be explained as follows:

a). The activation of presynaptic 5-HT_{1A}-autoreceptors in the raphe nuclei increase the firing rate of DA neurons in the ventral tegmental area (VTA), which consequently increases DA release in the postsynaptic projection areas of the VTA (Arborelius et al., 1993; Chen and Reith, 1995; Lejeune and Millan, 1998).

b). A high density of postsynaptic 5-HT_{1A}-receptors can be observed in the mesocortical dopaminergic systems, but not in the dorsal striatum and only sparsely in the NAc (Pompeiano et al., 1992), whereas postsynaptic 5-HT_{1B}-receptors are especially present in the mesolimbic DA system. This is supported by previous evidence that 5-HT_{1B}-receptor activation in the NAc produces an increase in phasic DA release (Hållbus et al., 1997; Sari, 2004; Yan and Yan, 2001).

c). Another explanation can be the activation of 5-HT_{1A}-receptors located on GABAergic interneurons in the mPFC, resulting in a disinhibition of glutamatergic pyramidal neurons projecting to the VTA (Ago et al., 2003; Amargós-Bosch et al., 2004; Díaz-Mataix et al., 2005; Sakaue et al., 2000; Santana et al., 2004) resulting in increased phasic DA release in the PFC (Arborelius et al., 1993; Ichikawa et al.,

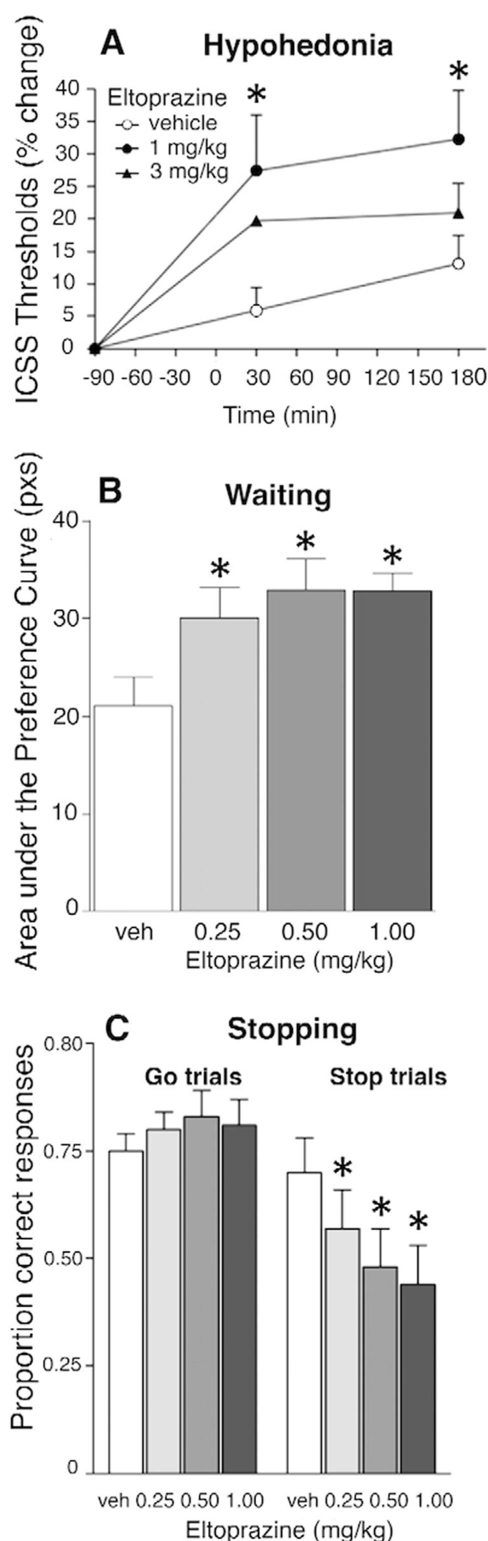

Fig. 5. A. Intracranial Self-Stimulation. Eltoprazine (1 mg/kg) increased intracranial self-stimulation (ICSS) thresholds. B. Impulsive choice in delay-aversion task. Eltoprazine increased the area under the preference curve, reflecting increased preference for waiting for large reward. C. Impulsive action in stop-signal task. Eltoprazine impaired performance in the stop-signal task, decreasing the number of correct stops in the stop-signal task. * $P < 0.05$.

2001; Rasmusson et al., 1994; Rollema et al., 2000). A similar mechanism has been proposed for 5-HT$_{1B}$-receptors in the mPFC (Iyer and Bradberry, 1996). However, high concentrations of 5-HT$_{1A}$-receptor agonists may overcome the increase in DA concentrations by directly activating pyramidal 5-HT$_{1A}$-receptors in the mPFC and reducing the excitatory output to VTA (Díaz-Mataix et al., 2006).

d). The activation of postsynaptic 5-HT$_{1A}$-receptors on GABAergic interneurons in the VTA exerts an inhibitory tone on these suppressive interneurons (Sumiyoshi et al., 2014), thereby indirectly stimulating the dopaminergic neurons in the VTA and increasing DA levels in its projection areas, including the NAc, mPFC and OFC (Carr, Sesack, 2000; Geisler and Wise, 2008; Iderberg et al., 2015). A similar mechanism has been described for postsynaptic 5-HT$_{1B}$-receptors (Boulenguez et al., 1996; O'Dell and Parsons, 2004; Yan et al., 2004).

4.2.2. Dopamine and brain reward function

Early research demonstrated that rats will work (press a lever or turn a wheel) for direct electrical stimulation of certain brain regions, including the lateral hypothalamus (Olds and Milner, 1954). Recently, it was shown that phasic stimulation of DA neurons located in the VTA was sufficient for the acquisition and maintenance of vigorous ICSS behavior and that stimulation of the dopaminergic VTA projection (passing the lateral hypothalamus) to the NAc was itself sufficient to support ICSS (Witten et al., 2011; Adamantidis et al., 2011; Steinberg et al., 2014). Remarkably, this ICSS behavior driven by optical stimulation of DA neuron somata in the VTA was attenuated by intra-NAc injections of D1 or D2 receptor antagonists, suggesting the involvement of both DA receptors (Steinberg et al., 2014). Therefore, it is not surprising that multiple drugs, including the psychostimulants methylphenidate and amphetamine, which increase DA levels in the NAc, also lower ICSS thresholds (Leith and Barrett, 1976; Lin et al., 1999; Cryan et al., 2003; Kenny et al., 2003; Kenny, 2007; Korte et al., 2015). This stimulatory effect of psychostimulants on brain reward systems represents an important source of positive reinforcement that motivates habitual consumption (Volkow et al., 2001; Kenny, 2007), and explains why psychostimulants are gaining popularity in humans for their hedonic properties, but also abuse potential (Teter et al., 2006).

Notably, in the present study, eltoprazine produced a small but significant increase in DA levels in the NAc, which can be explained by activation of postsynaptic 5-HT$_{1A/1B}$-receptors (see above: 4.2.1.), as well as increasing ICSS thresholds, suggesting an inhibitory influence on brain reward systems. Other research has also found that despite an increase in accumbal DA, several 5-HT$_{1A}$- and 5-HT$_{1B}$-receptor agonists decrease brain reward as measured by ICSS thresholds (see above: Section 4.1.2). In contrast, a growing body of evidence indicates that 5-HT$_{1B}$-receptor agonists enhance cocaine reinforcement (Parsons et al., 1998; Pentkowski et al., 2009, 2013), facilitate cocaine-induced locomotor hyperactivity (Przegaliński et al., 2002), and enhance cocaine place conditioning (Cervo et al., 2002), which is in line with the higher DA in the NAc. However, stimulation of 5-HT$_{1B}$-receptors also reduces cocaine-and sucrose-seeking behavior (Pentkowski et al., 2009; Acosta et al., 2005), which fits with the reduced brain stimulation reward. Recently, it was suggested that the role of 5-HT$_{1B}$-receptors in the behavioral effects of cocaine might vary depending on the stage of the addiction cycle, with a facilitatory role during periods of ongoing drug use (i.e., maintenance phase), and an inhibitory role during extended abstinence (Pentkowski et al., 2014). Future studies are needed to elucidate the neural mechanisms of precisely how 5-HT$_{1A/1B}$-(hetero)receptor activation may oppositely affect mesocorticolimbic DA release, brain stimulation reward, and drug reward-related behaviors over time.

4.2.3. Dopamine and "waiting" impulsivity

In the present study, eltoprazine produced a small increase in DA in the NAc, which was associated with reduced "waiting" impulsivity. Psychostimulants (e.g., amphetamine and methylphenidate) and specific DA reuptake inhibitors (DRI) enhance dopaminergic neurotransmission and reduce impulsive choice (Wade et al., 2000; van Gaalen et al., 2006; Baarendse and Vanderschuren, 2012). Thus, increased

dopaminergic neurotransmission is often associated with longer waiting for delayed rewards. In contrast, reduced DA levels in the NAc core, NAc shell and mPFC have been observed in rats exhibiting high levels of impulsive choice (Diergaarde et al., 2008). Often, the best experiments come from nature itself. When rats become infected with the parasite *Toxoplasma gondii*, they exhibit a reduced avoidance of predator odours. This behavioral change is likely to increase transmission of the parasite from rats to cats. Importantly, the *Toxoplasma gondii* infected rats make more impulsive choices, manifested as delay-aversion in an intertemporal choice task (Tan et al., 2015). Furthermore, *Toxoplasma gondii* infection lowers DA concentration in the NAc core, but not in the NAc shell (Tan et al., 2015). Thus, an increase in DA release in the NAc may indeed reduce "waiting" impulsivity.

4.2.4. Dopamine and "stopping" impulsivity

Surprisingly, eltoprazine dose-dependently increases impulsive action, whereas based on previous research it is known that 5-HT is not directly involved in "stopping" impulsivity. Furthermore, the role of DA receptors is not clear. Some reports suggest that D_1- and D_2-receptors play only a minor role in "stopping" impulsivity, because the mixed D_1/D_2-receptor antagonist cis-flupenthixol had no effect on action cancellation (Eagle et al., 2007). Nevertheless, evidence is starting to emerge that DA-receptors in the dorsomedial striatum (DMStr) modulate SSRT, suggesting the importance of this brain region in inhibitory control (Eagle et al., 2011). It is of note that the NAc core does not seem to be involved (Eagle et al., 2011). In more detail, tonic activation of D_1-receptors in the DMStr are supposed to increase "stopping" impulsivity, whereas D_2 receptors, probably via subthalamic nucleus (STN), may oppose D_1-receptor-mediated disinhibition (Eagle et al., 2011). Additionally, D_1/D_2-receptors in other brain areas may be important. For example, microinfusion of a D_1-receptor antagonist into the OFC decreased impulsive action in highly impulsive rats, but not in less impulsive rats (Winstanley et al., 2010). It also has been shown in humans using fMRI that the anterior lateral OFC is activated during response inhibition (Horn et al., 2003).

4.3. Involvement of $5\text{-}HT_{1A}$- and $5\text{-}HT_{1B}$-receptor in NE release, brain reward function, and impulsivity

4.3.1. Norepinephrine release

Eltoprazine increased the NE release in both mPFC and OFC, but not in the NAc. This is in agreement with other studies showing that activation of postsynaptic $5\text{-}HT_{1A}$-heteroreceptors (Chen and Reith, 1995; Suzuki et al., 1995) increases phasic NE release (Hajós-Korcsok and Sharp, 1996) in the mPFC (Gobert et al., 1998), hippocampus, and hypothalamus (Hajós-Korcsok and Sharp, 1996; Done and Sharp, 1994). $5\text{-}HT_{1A}$-receptors are located on GABAergic interneurons in the proximity of the locus coeruleus (LC), and therefore deserve special attention (Hajós-Korcsok and Sharp, 1999). The LC does not contain GABAergic interneurons like the VTA and the raphe nuclei. The center of the LC is composed of a homogenous compact cluster of noradrenergic neurons. Aston-Jones et al. (2004) nicely showed that GABAergic neurons are located in the pericerulear dendritic zone of the LC (peri-LC). These GABAergic neurons are located dorsomedial to the LC nucleus, and opto-stimulation of this area drastically inhibited LC neuronal firing frequency (Jin et al., 2016). These data suggest that GABAergic interneurons in the peri-LC may inhibit LC neurons as well as part of the local neuronal circuitry in the LC. Therefore, it is speculated that eltoprazine activates the $5\text{-}HT_{1A}$-receptors on GABAergic interneurons receptors in the peri-LC, thereby reducing the release of GABA and consequently disinhibiting LC neurons, in turn producing higher NE levels. This is in agreement with our results that elroprazine increases NE levels in mPFC and OFC, but not in NAc, because LC neurons project heavily to the entire cortical mantle, including PFC and primary sensory and motor areas, but not to the striatum or NAc (Berridge and Waterhouse, 2003). No evidence was found for the involvement of the brain's $5\text{-}HT_{1B}$-receptors in NE release.

4.3.2. Norepinephrine and brain reward function

There is no evidence that NE plays a direct role in mediating the rewarding effects of psychostimulants, such as amphetamine and methylphenidate (Weinshenker and Schroeder, 2007). In agreement, the selective NE reuptake inhibitor atomoxetine, which does not increase DA in the NAc, lacks abuse potential and unlike the psychostimulants, atomoxetine is not a controlled substance (Banaschewski et al., 2004). Furthermore, it has been shown that the NE-reuptake inhibitors reboxetine (Korte et al., 2015) and nomifensine (Schaefer and Michael, 1992) do not affect ICSS thresholds, suggesting that an increase in brain NE levels does not directly increase brain reward.

An indirect role of NE in reward, however, cannot be excluded. It has been speculated that the DA projections, from the VTA to the NAc and PFC, increase reward and receive NE innervation that modulate reward (Weinshenker and Schroeder, 2007). Evidence supporting this has shown that NE in the mPFC is critical for amphetamine-induced reward and DA release in the NAc (Ventura et al., 2003 J. Neurosc.). In addition, genetically engineered mice unable to synthesize NE because of a targeted disruption of the dopamine β-hydroxylase (DBH) gene appear totally blind to morphine reward, which can be restored by viral restoration of DBH expression (Olson et al., 2006). There is a growing body of evidence that the activity of LC/NE neurons reflects both expected reward and action, suggesting that the NE system is critical in effortful situations, in which mental and physical challenges require a high level of energy to be completed (Bouret and Richmond, 2015; Varazzani et al., 2015).

4.3.3. Norepinephrine and "waiting" impulsivity

Previously, it was shown that the specific NE reuptake inhibitor atomoxetine or desimipramine did not affect impulsive choice (Baarendse and Vanderschuren, 2012; van Gaalen et al., 2006). No evidence for a role played by NE in impulsive choice has been found in the literature. In support of this, it was recently shown that neither pretreatment with the NE α1 receptor agonist phenylephrine, nor pretreatment with the NE α2 receptor agonist guanfacine into the mPFC or OFC, had an effect on impulsive choice (Pardey et. al., 2013).

4.3.4. Norepinephrine and "stopping" impulsivity

There is growing evidence supporting that NE is the most important neurotransmitter in the mediation of "stopping" impulsivity (Eagle et al. 2008a, 2008b). Novel or intense stimuli activate the locus coeruleus (LC) to release NE in its projection areas, such as the hippocampus and PFC (Loughlin et al., 1986), which are associated with arousal and the orienting response (Aston-Jones and Bloom, 1981). This increase in NE can facilitate sensory processing, enhance cognitive flexibility and executive function in the PFC, and promote offline memory consolidation in order to prepare the organism for a reorientation and an adaptive behavioral response (Sara and Bouret, 2012; Bouret and Sara, 2004; Sara, 2009). Previously, and similar to humans, the existence of a general behavioral inhibition network in the rat brain has been proposed, including OFC, Basolateral Amygdala (BLA), dorsomedial striatum (DMStr), STN and hippocampus (Eagle et al., 2008a, 2008b; Aston-Jones and Cohen, 2005). The OFC could be a potential target for the NE-dependent improvements in SSRT, because in clinical studies the NRI atomoxetine activates the right inferior frontal cortex (RIFG), which is a key structure in the control circuitry of SSRT (Chamberlain et al., 2009; Eagle et al., 2008b). Interestingly, the RIFG has functional similarities to the rat OFC in terms of its involvement in SSRT (Chamberlain et al., 2009). In agreement with this, the selective NRI atomoxetine administered via microinfusion into the rat OFC mediates beneficial effects in the stop-

signal task (Bari et al., 2011).

The mechanism by which NE exerts its action is rather complex, because the ultimate effect highly depends on NE concentration and NE response shape. Catecholamines (both NE and DA)exert “Inverted-U” dose-dependent effects on PFC working memory function, whereby either too little NE/DA activity or too much NE/DA (e.g., stress) impairs PFC function (Arnsten and Pliszka, 2011; Berridge and Arnsten, 2013). Stimulants such as methylphenidate and amphetamine may act in the prefrontal pyramidal neurons to enhance signal strength by increasing NE, and reducing noise by increasing DA, thereby reducing symptoms of inattention, hyperactivity, and impulsivity in ADHD (Arnsten, 2007; Ramos and Arnsten, 2007; Vijayraghavan et al., 2007; Goldman-Rakic, 1996; Stahl, 2010). The beneficial effects of NE occur at postsynaptic α2A-receptors on the dendritic spines of PFC pyramidal cells (Arnsten, 2009a, 2009b). Surprisingly, eltoprazine dose-dependently increases impulsive action, despite the fact that eltoprazine enhances both NE and DA activity in the OFC. Therefore, alternative explanations have to be found. For instance, eltoprazine itself may activate both 5-HT_{1A}- and 5-HT_{1B}-receptors on neurons in the OFC, thereby inhibiting the neuronal activity of the OFC and consequently increasing impulsive action, despite the increase in both extracellular NE and DA levels.

4.4. Limitations of the present study

It is important to note that we only studied acute effects of eltoprazine. It is well known that chronic treatment with 5-HT_{1A}-receptor agonists or SSRIs result in desensitization of the 5-HT_{1A}-autoreceptor in the raphe nuclei (Blier and De Montigny, 1994), whereas postsynaptic 5-$HT_{1A/B}$-receptors may be more resistant to desensitization after chronic treatment (Blier and De Montigny, 1994; De Vry, 1995; Assié et al., 2006). Another issue is the use of normal experimental animals instead of hyperimpulsive animals that may have altered brain functions. Future studies are needed to elucidate the neural mechanisms of how 5-$HT_{1A/1B}$-receptor activation may oppositely affect mesocorticolimbic DA release, brain stimulation reward, and drug reward-related behaviors over time.

5. Conclusions

The behavioral neurobiological mechanisms involved in eltoprazine's actions may be explained as follows: eltoprazine activates both 5-HT_{1A}- and 5-HT_{1B}-receptors, probably on GABAergic interneurons in the VTA and 5-HT_{1A}-receptors on GABAergic interneurons in the peri-LC. Consequently, eltoprazine via indirect disinhibiting mechanisms phasically increases DA levels in NAc, mPFC and OFC and increases NE levels in mPFC and OFC, respectively. However, it cannot be excluded that additionally, postsynaptic 5-HT_{1A}- and 5-HT_{1B}-receptors on neurons in the OFC are involved in the observed changes in impulsivity.

In summary, the finding that 5-$HT_{1A/1B}$-receptor activation decreased impulsive choice, while increasing impulsive action further supports the long-standing hypothesis that “waiting” and “stopping” impulsivity are regulated by distinct neural circuits.

Conflicts of interest

In the past, we have received payments from PsychoGenics Inc. USA for other studies. Co-author Berend Olivier has worked for PsychoGenics Inc. from April 1999 until January 2001 and afterwards as an advisor until 2010. In 2014, Amarantus Bioscience Holdings Inc. acquired the rights to eltoprazine from PsychoGenics. Currently, Berend Olivier is a consultant for Amarantus, which has eltoprazine as phase 2 drug in its pipeline for ADHD and for Parkinson's Disease levodopa-induced dyskinesia. The other authors declare that they have no conflict of interest.

Acknowledgements

The present study was fully supported by Utrecht University.

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