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Schizophrenia-like disruptions of sensory gating by serotonin receptor stimulation in rats: Effect of MDMA, DOI AND 8-OH-DPAT

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ABSTRACT

Schizophrenia pathophysiology is associated with alterations in several neurotransmitter systems, particularly dopamine, glutamate and serotonin (5-HT). Schizophrenia patients also have disruptions in sensory gating, a brain information filtering mechanism in response to repeated sensory stimuli. Dopamine and glutamate have been implicated in sensory gating, however little is known about the contribution of serotonin. We therefore investigated the effects of several psychoactive compounds that alter serotonergic neuronal activity on event-related potentials (ERP) to paired auditory pulses. Male Sprague-Dawley rats were implanted with cortical surface electrodes to measure ERPs to 150 presentations of two 85 dB bursts of white noise, 500 ms apart (S1 and S2). Saline-treated animals suppressed the response to S2 to less than 50% of S1. In contrast, treatment with the serotonin releaser, MDMA (Ecstasy; 2.0 mg/kg), the 5-HT_{2A/2C} receptor agonist, DOI (0.5 mg/kg), or the 5-HT_{1A/7} receptor agonist, 8-OH-DPAT (0.5 mg/kg), caused an increase in S2/S1 ratios. Analysis of waveform components suggested that the S2/S1 ratio disruption by MDMA was due to subtle effects on the ERPs to S1 and S2; DOI caused the disruption primarily by reducing the ERP to S1; 8-OH-DPAT-induced disruptions were due to an increase in the ERP to S2. These results show that 5-HT receptor stimulation alters S2/S1 ERP ratios in rats. These results may help to elucidate the sensory gating deficits observed in schizophrenia patients.

KEYWORDS

Sensory gating, schizophrenia, MDMA, DOI, 8-OH-DPAT, ecstasy

1. INTRODUCTION

Serotonin has been strongly implicated in the neurobiology of schizophrenia since it was discovered that hallucinogenic 5-HT_{2A} receptor agonist drugs, such as lysergic acid diethylamide (LSD) and psilocybin, are psychotomimetic and can induce psychotic symptoms in healthy individuals (Breier, 1995; Geyer and Vollenweider, 2008; Hollister and Hartman, 1962). The current main treatments for schizophrenia are the class of atypical antipsychotic drugs, which differ from older, typical antipsychotics in that many have antagonist or partial agonist interactions with serotonin receptor subtypes in addition to antagonism of dopamine D₂ receptors (Meltzer et al., 1989; Svensson, 2003). Clinical studies have revealed changes in the density of several serotonin receptor subtypes in the brain of patients with schizophrenia (Hurlemann et al., 2008; Ngan et al., 2000). For example, 5-HT_{2A} receptor densities are reduced in the prefrontal cortex of drug-naïve schizophrenia patients (Ngan et al., 2000).

Schizophrenia patients have deficiencies in a form of information processing known as sensory gating (Olincy et al., 2010; Patterson et al., 2008). Sensory gating refers to a neural mechanism which allows for repetitive incoming sensory information to be filtered, allowing focusing on novel, relevant environmental information. Deficiencies in the ability to filter irrelevant sensory information may make it difficult for a schizophrenia patient to focus on the most important pieces of information in the environment (Light and Braff, 1999; Venables, 1964). Sensory gating dysfunction in schizophrenia patients is associated with cognitive decline and hallucinations (positive symptoms) (Cadenhead et al., 2000; Light and Braff, 1999). These deficits are familial and represent a core deficiency of central information processing that is observed in schizophrenia symptomatology (Cadenhead et al., 2005; Olincy et al., 2010).

Sensory gating has been investigated in a number of different experimental paradigms, such as suppression of the P50 or N100 event-related potential (ERP) (Boutros et al., 2009; Patterson et al., 2008), mismatch negativity (MMN) (Boutros et al., 2009) and prepulse inhibition of acoustic startle (PPI) (Light and Braff, 1999). Each of these measures investigates different aspects of the inhibitory processes that are deficient in schizophrenia patients (Braff et al., 2007; Brenner et al., 2004). Suppression of the P50 ERP (P50 gating) is measured by presenting two clicks of sound 500 ms apart and measuring the response with electroencephalography (EEG). In healthy people and animals, the ERP that occurs in response to the second click is diminished to 40-50% of the response to the first click (Boutros et al., 1991a; Light et al., 1999). However, schizophrenia patients respond more similarly to both clicks in this paired-click paradigm (Adler et al., 1982; Olincy et al., 2010; Patterson et al., 2008). Atypical antipsychotic drugs appear to be more efficacious than typical antipsychotics to reverse P50 sensory gating deficits, indicating that a mechanism beyond dopamine receptor antagonism is involved (Adler et al., 2004; Light et al., 2000; Nagamoto et al., 1996).

In rodent studies of sensory gating, the component that undergoes suppression in the paired-click paradigm occurs at a different latency and polarity (Bickford-Wimer et al., 1990) and is commonly labelled as N40 (Boutros et al., 1997) or N50 sensory gating (Adler et al., 1988; Adler et al., 1986). However the component that undergoes suppression in the paired-click paradigm can occur anywhere from 20 ms to 90 ms, depending on a number of factors including electrode placement and depth (Bickford-Wimer et al., 1990), strain of animal (Breier et al., 2010) and different auditory stimulus parameters (Boutros et al., 1997). Preclinical studies have attempted to delineate the neuronal pathways that mediate N40 gating and have implicated dopamine and

glutamate (Adler et al., 1986; Swerdlow et al., 2006), GABA (Ma and Leung, 2011), noradrenaline (Adler et al., 1988; Keedy et al., 2007) and acetylcholine (Luntzleybman et al., 1992; Stevens et al., 1995). For example, drugs that increase dopamine receptor signalling, such as the dopamine releaser, amphetamine, disrupt N40 sensory gating (Adler et al., 1986), an effect which can be attenuated by antipsychotic drugs such as haloperidol and clozapine (Adler et al., 1988; Adler et al., 1986; Joy et al., 2004). Similarly, the glutamate NMDA receptor antagonist, phencyclidine, was shown to cause a disruption of N40 sensory gating (Adler et al., 1986; Swerdlow et al., 2006).

The role of serotonin in sensory gating is less clear. The serotonin releasing drug, MDMA (ecstasy), has not been tested in the paired-click paradigm of sensory gating in humans or animals although it has been reported to disrupt sensorimotor gating in rats and mice, as measured with PPI (Bubenikova et al., 2005; van den Buuse et al., 2011). Treatment with the 5-HT_{2A/2C} receptor agonist, DOI, has produced conflicting results whereby it can reduce (Johnson et al., 1998) as well as increase (Swerdlow et al., 2006) sensory gating ratios in rats. One study has examined the effect of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, on N40 gating (Stevens et al., 2006) and found that a 0.5 mg/kg dose disrupted gating, while 0.1 mg/kg of 8-OH-DPAT had no effect when administered alone. However, this low dose ameliorated an N40 gating disruption caused by amphetamine (Stevens et al., 2006).

The aim of the present study was to further investigate the effect of direct and indirect 5-HT receptor stimulation on sensory gating. Specifically, we tested the effect of different doses of MDMA, DOI and 8-OH-DPAT in the paired-click paradigm of N40 auditory sensory gating in rats.

2. METHODS

2.1 Animals

Thirty-nine male Sprague-Dawley rats were obtained from Monash Animal Services, Monash University, Australia, and were housed in groups of 2-3 in standard rat cages. Rats were maintained on a 12-h light/dark cycle (lights on at 7:00 AM) at a constant temperature of $22 \pm 2^{\circ}\text{C}$ and had free access to standard pellet food and water. All surgical techniques, treatments and experimental protocols were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1990) set out by the National Health and Medical Research Council of Australia.

2.2 Electrode implantation

Surgery for electrode implantation was based on methodology as previously described (Breier et al., 2010; Swerdlow et al., 2006). When the rats were 250-350 g, they underwent surgery for implantation of electrodes onto the dura mater. They were anaesthetised with a mixture of 10% isoflurane and oxygen before being transferred to a stereotaxic apparatus (Stoelting Co., Wood Dale, IL, USA). Animals were positioned on a heating pad that was maintained at 37°C throughout the procedure and blunt stereotaxic ear bars were used to prevent damage to the tympanic membrane. Prior to any incision, rats were administered 5 mg/kg of the non-steroidal anti-inflammatory drug, carprofen (Rimadyl[®], Pfizer, Sandwich, Kent, United Kingdom). Through a 2 cm midline incision on the head, the recording electrode hole was drilled 4 mm posterior to bregma and 1 mm lateral to the midline. Drill holes for the earth and reference electrodes were 1 mm anterior to bregma, ± 1 mm from the midline, respectively. Three additional holes were drilled for the placement of mounting screws (PlasticsOne, Roanoke, VA, USA).

Electrodes were made of stainless steel and insulated with polyimide. The recording electrode was 0.125 mm in diameter and 5 mm long (PlasticsOne). Reference and ground electrodes were 0.25 mm diameter and 2 mm long (PlasticsOne). Prior to the beginning of surgery, all electrodes and components were sterilized in 70% ethanol for 2-3 minutes and then rinsed in saline. The proximal parts of the electrodes were inserted into a matching head plug (MS-363, PlasticsOne) and mounted onto a stereotaxic electrode holder (MH-363, PlasticsOne). The head plug ensemble could then be lowered down over the drill holes and electrode tips were carefully placed inside the drill holes, on top of the dura mater. Dental cement (Vertex-Dental, Zeist, the Netherlands) was placed around the head plug ensemble and mounting screws to firmly attach the head plug to the rat's skull and the surrounding skin was silk-sutured closed. Antiseptic cream (Betadine[®], povidone-iodine 10% w/w, Faulding Consumer, Salisbury, SA, Australia) was applied to the wound and rats were placed individually in a clean, padded and heated recovery box until they were conscious and mobile. All rats were subsequently housed individually.

2.3 ERP testing apparatus

Auditory gating was measured using a modified stimulus and acquisition system (EMG startle reflex testing system, San Diego Instruments, San Diego, CA, USA), which presented all sounds and acquired electrocorticogram (ECoG) data simultaneously. The recording chamber was a purpose-built, ventilated, electromagnetically shielded and sound-attenuating wooden box (44 cm tall x 40 cm wide x 40 cm deep). Three openings covered with fine aluminium mesh allowed air, light and sound to enter the chamber while it remained electromagnetically shielded. During testing, rats were positioned inside a clear, plexiglass cylinder (12.6 cm diameter) in the centre of the chamber and an

electronic swivel (PlasticsOne) was clamped near the ceiling so that rats could move freely. Two electromagnetically shielded speakers (frequency response predominately 55 Hz – 21 kHz; 8030A, Genelec, Finland) were positioned on both sides of the recording chamber to present sounds. Amplifier gain was set to 10,000 X and analogue high and low pass filters were set to 0.05 Hz and 300 Hz, respectively. Presentation of sounds and the recording of responses was completely automated using SRLab software (San Diego Instruments) on a laptop computer.

2.4 ERP testing

Two weeks after surgery, rats were acclimated to the testing chamber for a full testing session and the functionality of electrodes was verified. Drug trials commenced 2-3 days later. Each rat had a total of 3 test sessions after receiving a high and low dose of one of the drugs and a saline control in a pseudorandomized and repeated-measures design. Test sessions were conducted 2-3 days apart to allow for drug washout between sessions. Each test session began with a 3 minutes acclimation period of background noise (65 dB white noise). Following this, 150 trials of paired clicks (each click 85 dB, 1 ms burst of white noise; 500 ms inter-stimulus interval) were presented at random intervals between 10 and 20 seconds. Each test session went for a total of approximately 45 minutes. EEG epochs for each trial were recorded 200 ms before the first click until 500 ms after the second click (1200 ms epochs) and then stored for offline analysis.

2.5 ERP signal processing

Raw epoched data was imported into the EEGLab plugin (Delorme and Makeig, 2004) for Matlab where all 150 epochs could be visualised. Epochs were digitally filtered with a 0.1 Hz high pass filter and the baseline was removed (based on the 200 ms pre-stimulus

data). Waveform averaging for each individual rat was performed on epochs that were free of gross movement artefacts, sleep activity or amplifier saturation artefacts. The resultant average waveform for each animal was then analysed for P1 and N1 component amplitudes. The N1 component was taken as the maximum negative deflection in the 20-90 ms range following the stimulus. The P1 amplitude was measured as the most prominent peak preceding the N1 component. P1 and N1 amplitudes were measured from baseline as well as from the P1 peak to the N1 trough (P1 minus N1). N40 gating ratios were calculated by dividing the P1-N1 amplitudes that occurred in response to the second click by P1-N1 amplitudes that occurred in response to the first click (i.e. S2/S1). An S2/S1 sensory gating ratio of 0.5 or lower was considered normal gating. Gating ratios higher than 0.5 indicate a disruption of normal sensory gating. Thirteen animals were rejected from the analysis due to having gating ratios greater than 0.5 following saline (non-gaters) or because they had statistical outlier P1-N1 amplitudes, which indicates that electrode implantation surgery was unsuccessful. The final number of animals per group was n=11. In addition to average waveforms for each individual rat to obtain P1 and N1 amplitudes, a composite grand average waveform was obtained from each treatment to illustrate data quality and overall drug effects.

2.6 Drugs

MDMA (0.4 and 2.0 mg/kg, (±)-3,4-methylene-dioxymethamphetamine hydrochloride, Sigma-Aldrich, St. Louis, MO, USA), DOI (0.5 and 2.5 mg/kg, (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride, Sigma-Aldrich) and 8-OH-DPAT (0.1 mg/kg and 0.5 mg/kg, 8-hydroxy-2-dipropylaminotetralin salt; Sigma/RBI, Natick, MA), were dissolved in saline on the day of testing in an injection volume of 1 ml/kg. DOI and 8-OH-DPAT were administered by subcutaneous injection in the flank. MDMA was injected

intraperitoneally. All drug and saline injections were administered 5 minutes prior to the commencement of a test session.

2.7 Data analysis

All data are presented as mean $\pm$ standard error of the mean (S.E.M.) and were analysed with Systat 13.0 (SPSS Science, Chicago, IL, USA). P1, N1 and P1-N1 amplitudes were analysed using two-way analysis of variance (ANOVA), with repeated measures applied for trial type (S1 or S2) and drug dose. S2/S1 ratios were analysed using one-way ANOVA with repeated measures applied for drug dose. Alpha was 0.05 for all statistical analyses.

3. RESULTS AND DISCUSSION

(FIGURE 1 APPROXIMATELY HERE)

3.1 Sensory gating ratio (S2/S1)

Grand average waveforms showed clear ERPs caused by the first (S1) and second sound (S2) (Figure 1). A prominent positive peak was identified as P1 which was followed by a negative trough, labelled N1 (mean latencies $\pm$ SEM following saline treatment: 39.4 ± 1.4 ms and 73.6 ± 2.5 ms, respectively).

Treatment with MDMA significantly increased S2/S1 ratios, indicating a disruption of normal sensory gating (main effect of MDMA dose: $F(2,20)=11.4$, $P<0.001$, Figure 2a). Pairwise comparisons with saline treatment showed that S2/S1 ratios were elevated by 0.4 mg/kg ($F(1,10)=7.9$, $P=0.019$) and 2.0 mg/kg MDMA ($F(1,10)=22.4$, $P=0.001$). Furthermore S2/S1 ratios after 2.0 mg/kg MDMA were significantly higher than after 0.4 mg/kg ($F(1,10)=3.9$, $P=0.048$).

(FIGURE 2 APPROXIMATELY HERE)

Treatment with DOI also increased sensory gating ratios, indicated by a significant main effect of drug dose ($F(2,20)=3.9$, $P=0.05$, Figure 2b). Compared to saline, treatment with

the low dose of DOI (0.5 mg/kg) caused a trend for a disruption of gating ($F(1,10)=4.3$, $P=0.064$) whereas the 2.5 mg/kg dose caused a modest, but significant increase of mean S2/S1 ratios ($F(1,10)=5.1$, $P=0.046$).

Analysis of S2/S1 ratios after treatment with 8-OH-DPAT revealed a significant main effect of drug dose ($F(2,20)=9.6$, $P=0.001$). Pair-wise comparisons showed that 0.5 mg/kg 8-OH-DPAT caused increased S2/S1 ratios when compared to saline ($F(1,10)=15.4$, $P=0.003$) or 0.1 mg/kg 8-OH-DPAT ($F(1,10)=12.3$, $P=0.006$). There was no difference observed when comparing S2/S1 ratios following administration of saline or 0.1 mg/kg 8-OH-DPAT (Figure 2c).

3.2 P1 amplitude

There was a significant main effect of trial type (S1 vs. S2) on P1 amplitudes observed in all 3 cohorts of rats (MDMA: $F(1,10)=38.5$, $P<0.001$; DOI: $F(1,10)=8.7$, $p=0.015$; 8-OH-DPAT: $F(1,10)=20.4$, $P=0.001$), reflecting that P1 amplitudes in response to S1 were consistently higher than for S2 (Table 1). Grand average waveforms (Figure 1a) showed that MDMA tended to reduce P1 amplitudes to S1, however, there were no significant effects on P1 amplitudes (Table 1).

Unlike MDMA treatment, after injection of DOI, there was a main effect of drug dose ($F(2,20)=8.6$, $P=0.002$) as well as a trial type x drug dose interaction ($F(2,20)=5.7$, $P=0.011$). Pair-wise comparisons showed that compared to saline, 0.5 mg/kg DOI

reduced P1 amplitudes in response to S1 ($F(1,10)=12.4$, $P=0.005$) and had no significant effect on responses to S2 (Table 1). A dose of 2.5 mg/kg DOI caused a reduction of P1 amplitude in response to S1 ($F(1,10)=21.1$, $P=0.001$) as well as S2 ($F(1,10)=5.7$, $P=0.038$).

In contrast to DOI, treatment with 0.1 mg/kg 8-OH-DPAT increased P1 amplitudes in response to both S1 ($F(1,10)=4.9$, $P=0.051$) and S2 ($F(1,10)=5.3$, $P=0.044$) compared to saline (Figure 1c, Table 1). Treatment with 0.5 mg/kg 8-OH-DPAT caused a significant increase compared to saline in the P1 amplitude in response to S2 ($F(1,10)=9.4$, $P=0.012$) but had no effect on responses to S1. The apparently opposite effects by DOI and 8-OH-DPAT on the P1 amplitude to S2 could possibly explain why MDMA had no effect. Considering that the presumed action of MDMA is to cause serotonin release, then it follows that 5-HT_{2A}, 5-HT_{2C}, 5-HT_{1A} and 5-HT₇ receptors are all activated, in some ways mimicking co-administration of DOI and 8-OH-DPAT.

3.3 N1 amplitude

MDMA and DOI did not have any significant effects on N1 amplitudes (Table 1). In contrast, treatment with 8-OH-DPAT significantly reduced N1 amplitudes ($F(2,20)=6.6$, $P=0.006$). Further analysis showed that while 0.1 mg/kg of 8-OH-DPAT tended to reduce N1 amplitudes for both S1 and S2, there were no significant differences compared to saline or 0.5 mg/kg 8-OH-DPAT (Table 1). A dose of 0.5 mg/kg 8-OH-DPAT significantly reduced N1 amplitudes for both S1 and S2 when compared to saline ($F(1,10)=37.8$, $P<0.001$).

3.4 Peak to trough (P1-N1) amplitude

P1-N1 amplitudes are sensitive to changes in both P1 and N1 and are used to calculate the actual S2/S1 sensory gating ratios. There was a significant main effect of trial type observed in all cohorts of rats (MDMA: $F(1,10)=125.3$, $P<0.001$; DOI: $F(1,10)=12.4$, $P=0.005$; 8-OH-DPAT: $F(1,10)=34.4$, $P<0.001$), reflective of S1 amplitudes being consistently larger than S2 amplitudes. Analysis of the effect of MDMA revealed a significant trial type x drug dose interaction ($F(2,20)=6.8$, $P=0.005$) and pair-wise comparisons showed that treatment with 2.0 mg/kg MDMA abolished the difference in P1-N1 amplitudes between S1 and S2. Specifically, while P1-N1 amplitudes were significantly higher in response to S1 than to S2 following saline ($F(1,10)=127.3$, $P<0.001$) and 0.4 mg/kg MDMA ($F(1,10)=23.4$, $P=0.001$), reflective of intact sensory gating, after treatment with 2.0 mg/kg MDMA there was no difference between S1 and S2. These results may be explained by the observation that MDMA, through subtle effects on P1 or N1 values, which by themselves are not significant (see above), disrupts sensory gating by combined effects on the ERP to S1 as well as S2 (Table 1). Specifically, inspection of the data (Table 1) reveals that MDMA slightly decreased the ERP to S1 and increased the ERP to S2.

Analysis of the effect of DOI also revealed a significant trial type x drug dose interaction ($F(2,20)=4.3$, $P=0.029$), reflecting that there was a significant effect of DOI dose when responses to S1 were examined alone ($F(2,20)=5.0$, $P=0.017$), but there was no effect of

dose on the responses to S2. Pair-wise comparisons showed that, compared to saline, 0.5 mg/kg ($F(1,10)=13.6$, $P=0.004$) and 2.5 mg/kg DOI ($F(1,10)=5.6$, $P=0.04$) reduced P1-N1 amplitudes in response to S1. There was no significant difference between the low and high dose of DOI. These results suggest that DOI disrupted gating exclusively by reducing P1-N1 amplitude in response to S1. 8-OH-DPAT did not have any significant effects on P1-N1 amplitudes, which is likely due to the fact that it increased P1 amplitude and reduced N1 amplitude.

Table 1: P1 and N1 component amplitudes resulting from S1 and S2 after rats were administered saline, MDMA, DOI or 8-OH-DPAT.

	Response to S1 (μV)			Response to S2 (μV)		
	P1	N1	P1-N1	P1	N1	P1-N1
MDMA (n=11)						
Saline	101.9 $\pm$ 7.9	-4.4 $\pm$ 6.9	106.2 $\pm$ 8.8	54.6 $\pm$ 5.5 [#]	5.5 $\pm$ 4.8	49.1 $\pm$ 6.1 [#]
0.4 mg/kg MDMA	92.9 $\pm$ 13.6	-6.7 $\pm$ 8.0	99.6 $\pm$ 14.5	52.3 $\pm$ 6.3 [#]	-9.7 $\pm$ 7.6	62.0 $\pm$ 12.1 [#]
2.0 mg/kg MDMA	80.1 $\pm$ 16.4	-1.8 $\pm$ 6.6	81.9 $\pm$ 14.1	52.6 $\pm$ 9.5 [#]	-8.7 $\pm$ 3.2	61.3 $\pm$ 9.7
DOI (n=11)						
Saline	56.1 $\pm$ 12.0	-0.5 $\pm$ 11.3	56.6 $\pm$ 13.5	27.1 $\pm$ 4.2 [#]	5.7 $\pm$ 4.4	21.5 $\pm$ 6.4 [#]
0.5 mg/kg DOI	39.0 $\pm$ 11.3*	-4.6 $\pm$ 11.2	43.6 $\pm$ 12.4*	21.6 $\pm$ 4.9 [#]	2.4 $\pm$ 4.8	19.2 $\pm$ 3.2 [#]
2.5 mg/kg DOI	34.4 $\pm$ 9.2*	-4.2 $\pm$ 6.9	38.5 $\pm$ 9.5*	19.1 $\pm$ 4.2 ^{#*}	1.6 $\pm$ 2.9	17.5 $\pm$ 3.3 [#]
8-OH-DPAT (n=11)						
Saline	52.7 $\pm$ 6.6	-19.9 $\pm$ 5.6	72.6 $\pm$ 9.0	20.4 $\pm$ 2.7 [#]	-9.1 $\pm$ 2.5 [#]	29.5 $\pm$ 2.7 [#]
0.1 mg/kg 8-OH-DPAT	100.4 $\pm$ 25.6	-9.0 $\pm$ 4.9	109.4 $\pm$ 25.6	44.3 $\pm$ 10.6 ^{#*}	-2.5 $\pm$ 1.9	46.8 $\pm$ 10.3 [#]
0.5 mg/kg 8-OH-DPAT	59.5 $\pm$ 10.1	-3.8 $\pm$ 5.0	63.3 $\pm$ 10.3	34.9 $\pm$ 5.2 ^{#*}	-1.1 $\pm$ 3.0	36.0 $\pm$ 6.2 [#]

* P<0.05 compared to saline treatment, [#] P<0.05 compared to S1.

4. Discussion

Taken together, these data show that treatment with MDMA, DOI and 8-OH-DPAT all disrupted sensory gating, but by different actions on the ERP components to S1 and S2. MDMA only had minor effects on the individual P1 and N1 amplitudes to S1 or S2, but these effects combined to induce a loss of the significant difference between the P1-N1 amplitudes in response to S1 and S2 seen in controls. Moreover, in spite of these combined small changes, MDMA treatment resulted in the largest disruption of sensory gating ratio compared to the disruptions induced by DOI and 8-OH-DPAT. The DOI-induced disruption of sensory gating occurred primarily through a reduction in the P1 amplitude to S1, leaving the N1 amplitude unchanged, which resulted in smaller P1-N1 amplitudes. Compared to a low dose, a high dose of 8-OH-DPAT also disrupted sensory gating but appeared to do so via a different mechanism, involving increases in P1 in response to S2 and a reduction of N1 in response to both S1 and S2. The resulting changes in P1-N1 amplitudes significantly increased S2/S1 ratios.

A consistent finding in this study was that, when S2/S1 ratios were significantly increased after 2.0 mg/kg MDMA, 2.5 mg/kg DOI and 0.5 mg/kg 8-OH-DPAT, the ERP amplitude to S1 was reduced compared to saline. Schizophrenia patients also have reductions in the response to S1 compared to healthy controls (Boutros et al., 1991b; Patterson et al., 2008). This adds validity to the drug-induced impairments of sensory gating as a rodent model for schizophrenia-like deficits, however it indicates that these deficits could be detected using a single stimulus. This would not reflect a 'gating' phenomena as such, but rather a reduced level of reactivity to the first

stimulus (Swerdlow et al., 2006). However a recent meta-analysis revealed that the effect size for detection of schizophrenia-like deficits of P50 suppression is much greater when the second stimulus is used (Chang et al., 2011). Effect sizes between patients and controls were greatest for S2/S1 ratios and P50 amplitudes caused by S2, while effect sizes when using the P50 amplitudes caused by S1 alone were small. These authors concluded that deficits of sensory gating in schizophrenia patients reflect “defective inhibition of redundant input rather than an abnormal response to novel stimuli” (Chang et al., 2011). If deficient suppression of the P50 ERP is to be used as an endophenotype for schizophrenia then the second stimulus is crucial. In light of the present results, limited information would be obtained if only the responses to S1 were considered.

MDMA activates all 5-HT receptor subtypes indirectly, by causing 5-HT release. Therefore, our results suggest that an increase in synaptic levels of 5-HT may induce disruption of sensory gating. The effect of DOI and the high dose of 8-OH-DPAT suggest an involvement of 5-HT_{2A/2C} and postsynaptic 5-HT_{1A/7} receptors in this effect, albeit via differential changes in the waveform components. Further experiments combining MDMA treatment with 5-HT_{2A}, 5-HT_{2C}, 5-HT_{1A} and 5-HT₇ receptor antagonists, respectively, are required to confirm this. Overall, both indirect and direct stimulation of 5-HT receptors, particularly 5-HT_{2A/2C} and 5-HT_{1A/7} receptors, appears to cause sensory gating deficits in rats that are similar to those observed in schizophrenia patients. Some atypical antipsychotic drugs have antagonist or partial agonist affinity at 5-HT_{2A} and 5-HT_{1A} receptors. Therefore, it is possible that the improvements in sensory gating ratios that have been observed in

schizophrenia patients treated with atypical, but not typical antipsychotic drugs, are mediated by activity at 5-HT_{2A} and 5-HT_{1A} receptors (Adler et al., 2004; Becker et al., 2004; Light et al., 2000). In order to investigate this, further experiments are required to investigate the effects of typical and atypical antipsychotics on 5-HT mediated disruptions of sensory gating.

It should be noted, that despite MDMA being a potent releaser of 5-HT, it cannot be ruled out that a part of its action on sensory gating is by secondary effects on dopaminergic and noradrenergic neurons. MDMA induces the release of both of these catecholamines, although to a lesser degree than it does 5-HT (de la Torre et al., 2004; Fitzgerald and Reid, 1990; Gold et al., 1989). Therefore, the MDMA-induced disruption of sensory gating might be partly due to the release of dopamine and/or noradrenaline, which have been shown to potently modulate sensory gating on their own at high doses, particularly dopamine. Further studies where MDMA treatment is combined with selective 5-HT_{2A}, 5-HT_{2C}, 5-HT_{1A} or 5-HT₇ receptor antagonists, or co-administration of MDMA with a dopamine receptor antagonist, such as haloperidol, could determine which 5-HT or dopamine receptors are required for MDMA to disrupt sensory gating.

DOI has had variable effects on N40 sensory gating in previous studies. Swerdlow et al. (2006) found that subcutaneous administration of 1.0 mg/kg of DOI caused an increase of S₂/S₁ ratios, which agrees with the present study. However, Johnson et al. (1998) found that intraperitoneal administration of DOI caused a reduction in gating ratios and furthermore reported that treatment with 2.5 mg/kg DOI

ameliorated an N40 gating deficit induced by amphetamine. It is unlikely that the different route of administration caused this discrepancy between studies, however this cannot be ruled out. Additionally, rats in the Johnson et al. study (1998) underwent 10 baseline testing sessions prior to drug trials, whereas in the Swerdlow et al. (2006) study and the present study they did not. Therefore, DOI may be sensitive to the level of habituation to the recording chamber and this may have an effect on 5-HT_{2A/2C} receptor-induced disruptions of N40 sensory gating. Thus, hallucinogens, such as DOI, may disrupt sensory gating more in a novel or stressful environment. Previously it has been proposed that 5-HT₂ receptor activation by DOI may be beneficial for the treatment of schizophrenia (Johnson et al., 1998), however data from the present study as well as Swerdlow et al., (2006) disagree with this notion and suggest that 5-HT_{2A/2C} receptor agonism would more likely be detrimental to the symptomatology of schizophrenia.

The effect of the 5-HT_{1A/7} receptor agonist, 8-OH-DPAT, on sensory gating ratios was associated with a different action on waveform components compared to DOI and MDMA. Thus, unlike the other drugs, 8-OH-DPAT affected the N1 component in response to S1 as well as S2 and increased rather than decreased P1 in response to S1. 5-HT_{1A} receptors are expressed presynaptically on raphe neuron cell bodies as well as postsynaptically in a number of brain regions such as the prefrontal, mesiotemporal and occipital cortices (Larsson et al., 1990; Moses-Kolko et al., 2011). Activation of 5-HT_{1A} autoreceptors causes the reduction of 5-HT synthesis and release and it is tempting to explain the differential action of 8-OH-DPAT compared to MDMA or DOI on an involvement of such an autoreceptor mechanism. However,

at this point, this is purely speculative and further experiments are needed to investigate any differential involvement of 5-HT_{1A} autoreceptors or postsynaptic receptors in sensory gating. Such experiments could involve local administration of 8-OH-DPAT into the raphe nuclei or by studying the effect of 5-HT_{1A} receptor ligands with preferential action on autoreceptors or postsynaptic receptors.

CONCLUSIONS

These results suggest that 5-HT receptors, especially 5-HT_{2A/2C} and 5-HT_{1A/7} receptors, modulate ERPs to repeated auditory stimulation. This study showed that by increasing levels of 5-HT indirectly, or by directly activating 5-HT_{2A/2C} receptors or 5-HT_{1A/7} receptors, S₂/S₁ ERP ratios were increased. These changes are reminiscent of the abnormal sensory gating processes that occur in schizophrenia patients.

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FIGURE LEGENDS

Figure 1:

Grand average ERP waveforms following administration of saline or MDMA (panel a, $n=11$), DOI (panel b, $n=11$) or 8-OH-DPAT (panel c, $n=11$). S1 and S2 occurred at 0 ms and 500 ms, respectively. P1 and N1 components are indicated for saline treatment in panel a). An additional 50 Hz low-pass filter was applied for display purposes.

Figure 2: Mean ($\pm$ S.E.M.) S2/S1 sensory gating ratios following administration of a) MDMA ($n=11$), b) DOI ($n=11$) or c) 8-OH-DPAT ($n=11$). * $P<0.05$ for difference with saline. # $P<0.05$ for difference between 0.1 mg/kg and 0.5 mg/kg 8-OH-DPAT.

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Figure 1

Figure 2

HIGHLIGHTS

- MDMA (ecstasy), DOI and 8-OH-DPAT caused deficits in a paired-pulse sensory gating paradigm in rats
- Activation of 5-HT_{2A} or 5-HT_{1A} receptors had different effects on the ERP waveform components
- New insight into mechanisms involved in schizophrenia sensory gating deficits and the action of atypical antipsychotic drugs

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