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Differential Role of Serotonergic Projections Arising from the Dorsal and Median Raphe Nuclei in Locomotor Hyperactivity and Prepulse Inhibition

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While an involvement of brain serotonin systems in schizophrenia has been suggested by many studies, the relative role of different serotonergic projections in the brain remains unclear. We therefore examined the effects of selective brain serotonin depletion on psychotropic drug-induced locomotor hyperactivity and prepulse inhibition, two animal models of aspects of schizophrenia. Pentobarbital-anesthetized (60 mg/kg, i.p.) male Sprague–Dawley rats were stereotaxically microinjected with 1 μl of a 5 μg/μl solution of the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) into either the dorsal or median raphe nucleus. At 2 weeks after the surgery, rats with dorsal raphe lesions did not show changes in psychotropic drug-induced locomotor hyperactivity, but displayed partial disruption of prepulse inhibition. In contrast, rats with median raphe lesions showed significant enhancement of phencyclidine-induced, but not amphetamine-induced locomotor hyperactivity and a marked disruption of prepulse inhibition. These results provide evidence for differential involvement of serotonergic projections in locomotor hyperactivity and prepulse inhibition. This study may help to explain the role of different serotonin projections in the brain in the pathophysiology of schizophrenia.
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INTRODUCTION

The dopamine hypothesis of schizophrenia originally proposed that overactivity of the subcortical dopaminergic system in the brain is responsible for at least some of the symptoms of the disorder (Carlsson and Lindqvist, 1963; Harrison, 1999; Rossum, 1966). However, it is becoming increasingly clear that altered activity of the serotonin system, interacting with dopaminergic systems, may also be an important factor in the pathophysiology of schizophrenia (Abi-Dargham *et al*, 1997; Kapur and Remington, 1996; Roth and Meltzer, 1995). Post-mortem studies have shown significant alterations in serotonin systems in schizophrenia. For example, serotonin transporter affinity was reduced in the ventral hippocampus while the density of serotonin receptors, particularly of the 5-HT_{2A} subtype, was reduced in the frontal cortex of schizophrenic subjects (Dean, 2000; Laruelle *et al*, 1993). Furthermore, 5-HT_{1A} receptor density was increased in the prefrontal and temporal cortices of schizophrenic subjects, independent of antipsychotic drug treatment (Hashimoto *et al*, 1991). Several of the newer atypical antipsychotic drugs display high affinity for serotonin receptor subtypes as well as dopamine D_2 receptors; these drugs display a lower incidence of extrapyramidal side effects and are more effective for the treatment of symptoms of schizophrenia, particularly negative symptoms and cognitive impairment, than antipsychotics that rely on dopaminergic blockade alone (Josselyn *et al*, 1997; Meltzer, 1989; Roth and Meltzer, 1995).

In the brain, interactions of dopamine and serotonin systems are present at different anatomical levels. These interactions are mediated by different serotonin receptor subtypes affecting different aspects of dopaminergic function (Kapur and Remington, 1996). Central serotonergic neurons project to various cortical and limbic structures and innervate virtually the entire brain (Abi-Dargham *et al*, 1997). Serotonin-producing neurons are found predominantly in the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) in the brainstem (Azmitia and Whitaker-Azmitia, 1995). The DRN projects to the frontal cortex, ventral hippocampus, and striatal regions (Adell and Myers, 1995; McQuade and Sharp, 1997), while the MRN projects to the dorsal hippocampus and cingulate cortex (Mokler *et al*, 1998; Thomas *et al*, 2000). Several brain regions, such as the

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hypothalamus, substantia nigra, and nucleus accumbens, are innervated by both nuclei (Abi-Dargham *et al*, 1997; Kapur and Remington, 1996).

Animal behavioral models have been used to assess the functional importance of serotonergic projections in at least some symptoms of schizophrenia. The two most widely used models are psychotropic drug-induced locomotor hyperactivity and prepulse inhibition. Psychotropic drugs, such as amphetamine and phencyclidine, can induce abnormal behaviors in animals and mimic certain aspects of psychotic disorders in humans (Geyer and Markou, 1995). Amphetamine, an indirectly acting sympathomimetic, causes increased dopamine release from presynaptic terminals (Seiden *et al*, 1993) as well as noradrenaline and serotonin release (Kuczenski *et al*, 1995; Rothman *et al*, 2001). While all three transmitters may contribute to the behavioral effects of amphetamine (Kuczenski *et al*, 1995; Rothman *et al*, 2001), it has been shown in rats that hyperlocomotion induced by treatment with this drug is critically dependent upon intact subcortical dopamine activity in the nucleus accumbens (Kelly *et al*, 1975). Similar brain regions are activated by amphetamine in humans and are implicated in psychosis (Drevets *et al*, 2001; Laruelle *et al*, 1996). Also, the doses of amphetamine used in animal studies are similar to those effective in humans. In contrast, phencyclidine is a drug that interferes with multiple neurotransmitter systems (Contreras *et al*, 1987). Phencyclidine acts as a noncompetitive antagonist at the ion channel associated with the *N*-methyl-D-aspartate (NMDA) glutamate receptor, but also facilitates dopaminergic and serotonergic transmission (Javitt and Zukin, 1991; Martin *et al*, 1998a). In animal studies, phencyclidine increased locomotor activity which may involve 5-HT_{2A} receptors, but not 5-HT_3 or 5-HT_{1A} receptors (Gleason and Shannon, 1997). Similar mechanisms are activated in humans by phencyclidine and other NMDA receptor antagonists, such as ketamine (Duncan *et al*, 2001; Pradhan, 1984). Prepulse inhibition of acoustic startle is an operational measure of sensorimotor gating, which is disrupted in patients with schizophrenia and other mental illnesses (Geyer *et al*, 1990; Swerdlow and Geyer, 1998). The prepulse inhibition-acoustic startle reflex model in rats offers a unique opportunity to assess attentional and information processing deficits in schizophrenia since modulation of startle is similar between mammalian species (Braff and Geyer, 1989).

Early studies utilizing electrolytic lesions of the raphe nuclei have shown that in MRN-lesioned rats locomotor activity was markedly increased, whereas DRN lesions had no such effect (Albinsson *et al*, 1996; Jacobs *et al*, 1974). Electrolytic lesions of the MRN furthermore resulted in enhanced effects of amphetamine on locomotor activity (Asin and Fibiger, 1983). However, electrolytic lesions of the raphe nuclei are not specific to serotonin neurons, and by causing general neuronal destruction, including nonserotonergic cells and fibers of passage, these lesions may affect other behavioral and physiological functions (Andrade and Graeff, 2001). In contrast, studies using the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) have shown more selective effects on serotonin-containing neurons (Gately *et al*, 1986; Jonsson, 1980). Microinjection of 5,7-DHT into the DRN resulted in significant serotonin depletion in the striatum, while MRN lesions caused significant serotonin depletion in the hippocampus, but not in the striatum (File and Deakin, 1980; Gogos and Van den Buuse, 2000; Thomas *et al*, 2000). One previous study showed that 5,7-DHT lesions of the MRN did not affect amphetamine-induced locomotor hyperactivity (Asin and Fibiger, 1983). However, in this study, the effect of phencyclidine was not tested and the role of the DRN was not investigated, nor were lesion effects on prepulse inhibition assessed. Combined neurotoxic lesions of the DRN and MRN cause disruption of prepulse inhibition but have no effect on basal startle reactivity (Fletcher *et al*, 2001). Similarly, generalized depletion of serotonin by combined treatment with the serotonin synthesis inhibitor *p*-chlorophenylalanine (PCPA) and the serotonin releaser D-fenfluramine nearly abolished prepulse inhibition (Prinssen *et al*, 2002). However, these latter studies have not addressed psychotropic drug-induced locomotor hyperactivity, nor do they provide an insight into the differential involvement of different serotonergic projections arising from the two raphe nuclei.

The aim of the present study was therefore to investigate the effects of selective serotonergic lesions of the DRN or MRN on locomotor hyperactivity and prepulse inhibition. We hypothesized that serotonergic projections arising from these two raphe nuclei are important regulators of motor behavior and prepulse inhibition, but that the role of these two projection systems differs.

MATERIALS AND METHODS

Subjects

A total of 36 male Sprague–Dawley rats (Department of Pathology, University of Melbourne), weighing 250–300 g at the time of surgery, were used. The animals were housed under standard conditions in groups of two to three, with free access to food and water. They were maintained on a 12 h : 12 h light/dark cycle (lights on at 0700) at a constant temperature of 21°C. Prior to the surgical procedure, the animals were handled each day over 5 days. The experimental protocol and surgical procedures were approved by the Animal Experimentation Ethics Committee of the University of Melbourne, Australia.

Drugs and Solutions

D-amphetamine sulfate (Sigma Chemical Co., St Louis, MO, USA) and phencyclidine HCl (PCP, Sigma) were dissolved in 0.9% saline solution and injected subcutaneously (s.c.) in the nape of the neck. Desipramine HCl (Sigma) was dissolved in distilled water and injected intraperitoneally (i.p). All doses are expressed as the weight of the salt and administered in an injection volume of 1 ml/kg of body weight. The neurotoxin 5,7-DHT (Sigma) was dissolved in 0.1% ascorbic acid (BDH, Kilsyth, VIC, Australia) in saline to prevent oxidation of the neurotoxin.

Surgical Procedure

Rats were pretreated with 20 mg/kg of desipramine, 30 min prior to lesions, to prevent the destruction of noradrenergic

neurons by 5,7-DHT (Jonsson, 1980), and anesthetized with sodium pentobarbitone (60 mg/kg i.p., Rhone Merieux, QLD, Australia). The rat was mounted in a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) with the incisor bar set at –3.3 mm (Paxinos and Watson, 1998). The skull surface was exposed and a small hole was drilled. A 25-gauge stainless-steel cannula, that was connected to polyethylene tubing and attached to a 10 μl glass syringe mounted in an infusion pump (UltraMicroPump, World Precision Instruments, Sarasota, FL, USA), was lowered into the DRN or MRN. Stereotaxic coordinates were used to determine the location of the raphe nuclei (Paxinos and Watson, 1998). With the bregma as zero and with the stereotaxic arm at 25°, the coordinates were: DRN: 8 mm posterior, 2.9 mm lateral, and 6.8 mm ventral of the bregma; MRN: 8 mm posterior, 3.7 mm lateral, and 8.8 mm ventral of the bregma (Paxinos and Watson, 1998). A measure of 1 μl of 5 μg/μl of 5,7-DHT was infused over a period of 2 min. Sham-operated controls underwent the same surgical procedure and received an equal volume of vehicle solution containing ascorbic acid. The animals were s.c. administered with 5 mg/kg of carprofen (Heriot AgVet, Rowville, VIC, Australia), a nonsteroidal, anti-inflammatory analgesic, to reduce postoperative inflammation and discomfort. Rats were placed on a heat pad until recovered from anesthesia. After surgery, rats were allowed to recover for 2 weeks, during which they were handled and health checks were made two to three times a week.

Apparatus

Locomotor activity was monitored using eight automated photocell cages ($31 \times 43 \times 43$ cm, $h \times w \times l$, ENV-520, MED Associates, St Albans, VT, USA). The position of the rat at any time was detected with 16 infrared sources and sensors on each of the four sides of the monitor. Several types of behavioral responses were recorded including distance moved, ambulation, stereotypy, and rearing. Rearing was detected by an additional row of photobeams above the rat. Small, repetitive beam breaks within a virtual box of 4×4 beams around the rat were recorded as stereotypic counts. Ambulatory counts consisted of consecutive interruption of at least four beams within a period of 500 ms. Locomotor activity data were expressed as cumulative 30 min data.

Prepulse inhibition testing was performed using four automated startle chambers (SR-LAB, San Diego Instruments, San Diego, CA, USA) consisting of clear Plexiglas cylinders, 9 cm in diameter, resting on a platform inside a ventilated, sound-attenuated, and illuminated chamber. Whole-body startle responses of the animal in response to acoustic stimuli caused vibrations of the Plexiglas cylinder, which were then converted into quantitative responses by a piezoelectric accelerometer unit attached underneath the platform. Percentage prepulse inhibition was calculated as $100 \times \{[\text{pulse-alone trials} - (\text{prepulse} + \text{pulse trials})]/(\text{pulse-alone trials})\}$ (Geyer *et al*, 1990).

Experimental Design

The study included 12 DRN-lesioned rats, 12 MRN-lesioned rats, six DRN sham-lesioned rats, and six MRN sham-lesioned rats. As the two groups of sham-lesioned rats showed no significant difference, the data obtained from them were pooled to yield a sham-operated group of 12 animals. For example, the total distance moved during the 30 min before amphetamine injection was 4139 ± 505 in DRN sham-lesioned rats and 3725 ± 862 in MRN-lesioned rats. The total distance moved during the 90 min after amphetamine injection was 16789 ± 2521 and 16854 ± 2145, respectively.

Behavioral tests were performed starting 2 weeks after the surgery, each session including random numbers of DRN- and MRN-sham-operated rats and DRN- and MRN-lesioned rats. Two locomotor activity tests were performed with 3–4 days intervals to prevent habituation due to repeated testing and to allow clearance of the drugs. Rats were treated with 0.5 mg/kg of amphetamine or 2.5 mg/kg of phencyclidine in random order. These doses were chosen on the basis of preliminary dose–response experiments. During the experiments, the rats were placed in the locomotor photocell cages for 30 min, to establish baseline locomotor activity and allow habituation to the test environment, after which they were injected and locomotor activity recorded over a further 90 min. In untreated or saline-injected rats, locomotor activity tends to be very low after the first 30 min (not shown).

Prepulse inhibition measurements were carried out in the same group of control and lesioned rats 1 week after the locomotor activity tests. A single prepulse inhibition session lasted for about 45 min and consisted of high- and low-intensity stimulus combinations with a continuous background noise of 70 dB. The session started and ended with a block of 10 pulse-alone trials. These blocks, together with 20 randomly presented pulse-alone trials during the prepulse inhibition protocol, were used to calculate basal startle reactivity and startle habituation. Prepulse inhibition was assessed by random presentation of 115 dB pulses combined with 10 of each of prepulse-2, -4, -8, -12 and -16. For example, a prepulse-8 (PP8) was a 20 ms prepulse of 8 dB above the background noise, that is, 78 dB, followed 100 ms later by a 40 ms 115 dB pulse (Van den Buuse, 2003; Van den Buuse and Eikelis, 2001). The session also included 10 'no pulse' trials.

Histology

After completion of the behavioral experiments, rats were deeply anesthetized with sodium pentobarbitone (60 mg/kg i.p., Rhone Merieux) and transcardially perfused with 0.9% NaCl solution, followed by a fixative solution containing 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer. Brains were removed from the skull, postfixed in the same fixative solution for 1 h, and then stored in 20% sucrose overnight at 4°C. The next day, the brains were blocked into a rostral and caudal block and frozen for immunohistochemistry and histological assessment of the accuracy of the injection sites. Sections of the region of the raphe nuclei (20 μm) were cut on a cryostat into two parallel series and mounted on gelatin-coated glass slides. Sections from one series were stained with cresyl violet (ProSciTech, Thuringowa, QLD, Australia), dehydrated, cleared with xylene, coverslipped, and examined microscopically to verify the appropriate location of the tips of the infusion cannula. Sections from the other series were used for immunohistochemistry.

Immunohistochemical Detection of Serotonin Transporter Protein

Brain sections of both lesioned and control groups were simultaneously processed as follows (Legutko and Gannon, 2001). Slides with 20 μm sections of the region of the raphe nuclei were washed three times in 0.1 M phosphate-buffered saline (PBS). In order to inhibit endogenous peroxidase activity, sections were incubated with 0.3% hydrogen peroxide (H_2O_2) in methanol for 30 min. Sections were then washed in the PBS and incubated in 10% normal goat serum in PBS for 45 min to block nonspecific staining. Subsequently, the sections were incubated overnight at 4°C with a PBS solution containing 0.3% Triton X-100, 2% normal goat serum and rabbit polyclonal antibody against the rat serotonin transporter (anti-SERT) (1 : 5000, Oncogene Research Products, San Diego, CA, USA). The next day, following three 5 min washes in PBS, sections were incubated for 60 min in biotinylated goat-anti-rabbit secondary antibody (1 : 100, Vectastain-Elite ABC kit, Vector Laboratories, Burlingame, CA, USA), appropriately diluted in PBS containing 2% normal goat serum. After incubation for another 60 min with an avidin–biotin–peroxidase complex (Vectastain-Elite ABC kit), the sections were treated with 0.02% 3,3′-diaminobenzidine tetrahydrochloride (DAB) in PBS for 10 min and then 0.03% H_2O_2 in PBS was added for a further 5 min. The reaction was terminated by three washes in buffer solution. For control sections, the protocol was identical, except that the primary antibody was omitted and sections were incubated with a primary diluent only. Following immunohistochemical procedures, the sections were air-dried, dehydrated through an alcohol series, cleared in xylene, and coverslipped with DPX mountant (ProSciTech). Tissue sections were examined with a light microscope and the relative optical density (ROD) of SERT-positive staining in the raphe nuclei in each group was assessed using a SCION IMAGE system (Scion Corporation, Frederick, MA, USA) to give an indication of the extent of cell body loss following microinjection of 5,7-DHT. The average optical density values obtained from six sections where the primary antibody was omitted (nonspecific binding), were subtracted from optical density values obtained from each of the sections from the experimental animals (total binding), yielding values of specific binding. Average values from individual animals were subsequently averaged by group and analyzed statistically.

Data Analysis

Data were expressed as the mean ± the standard error of the mean (SEM). All data were analyzed with analysis of variance (ANOVA) with repeated measures where appropriate, using the statistical software package SYSTAT 9.0 (SPSS Inc., Chicago, IL, USA). In the locomotor activity experiments, data were summed in 30 min blocks and these blocks were used to assess main effects of lesion type (Group) and treatment with amphetamine or phencyclidine (Drug) and interactions between these factors. In this analysis, the Drug effect was a repeated-measures factor. In the prepulse inhibition experiments, factors were Group and Habituation (four blocks of 10 startle responses) or Group and Prepulse (five different prepulse intensities), where Habituation and Prepulse were repeated-measures factors. After calculating ANOVAs including all three surgery groups, subsequent pairwise ANOVAs were performed where needed. For optical density measurements, one-way ANOVA was used, followed by *post hoc* Bonferroni-corrected *t*-test comparison. A '*p*-value' of $p < 0.05$ was considered to be statistically significant.

RESULTS

Histology: Injection Sites

Inspection of cresyl violet-stained brain sections revealed that all but one of the rats had the tip of the infusion cannula situated within the boundaries of the DRN or MRN, respectively, as delineated by the Paxinos and Watson rat brain atlas (Paxinos and Watson, 1998). Behavioral data from the animal with the misplaced cannula were rejected from the analysis. Representative examples of the area in which the injections were localized are shown in Figure 1.

Effects of 5,7-DHT Lesions on Baseline Locomotor Activity

Baseline locomotor activity was assessed during 30 min in the photocell cage prior to drug administration. When expressed as total distance moved, neither DRN- nor MRN-lesioned rats showed differences in baseline activity levels compared to the sham-operated controls (Figure 2). Other baseline behavioral parameters, such as ambulatory counts or stereotypy counts, were also not significantly different between control and lesioned rats (data not shown).

Effects of 5,7-DHT Lesions on Activity Responses to Amphetamine and Phencyclidine

When comparing the time course of locomotor activity after amphetamine injection (Figure 2), there was a main effect of Drug ($F_{3,96} = 25.5$, $p < 0.001$), reflecting the hyperactivity induced by this treatment. However, the lack of a main effect of Group or a Drug × Group interaction suggested that this response did not differ between sham-operated rats or rats with DRN lesions or MRN lesions (Figure 2).

In contrast to amphetamine treatment, the locomotor hyperactivity induced by phencyclidine treatment (main effect of Drug, $F_{3,96} = 22.9$, $p < 0.001$) was significantly different between the lesion groups (main effect of Group, $F_{2,32} = 13.0$, $p < 0.001$, Group × Drug interaction, $F_{6,96} = 4.6$, $p < 0.001$). Pairwise ANOVAs revealed a significantly greater phencyclidine response in MRN-lesioned rats compared to sham-operated rats (main effect of Group, $F_{1,21} = 17.2$, $p < 0.001$, Group × Drug interaction, $F_{3,63} = 6.1$, $p = 0.001$), but no difference between DRN-lesioned rats and sham-operated controls (Figure 2). The phencyclidine-induced hyperactivity, expressed as the total distance moved in the 90 min following injection minus baseline values obtained in the first 30 min, was 147% greater in MRN-lesioned rats (18952 ± 2615) than in controls (7655 ± 1010) and 137% greater than in DRN-lesioned rats (7990 ± 1225).

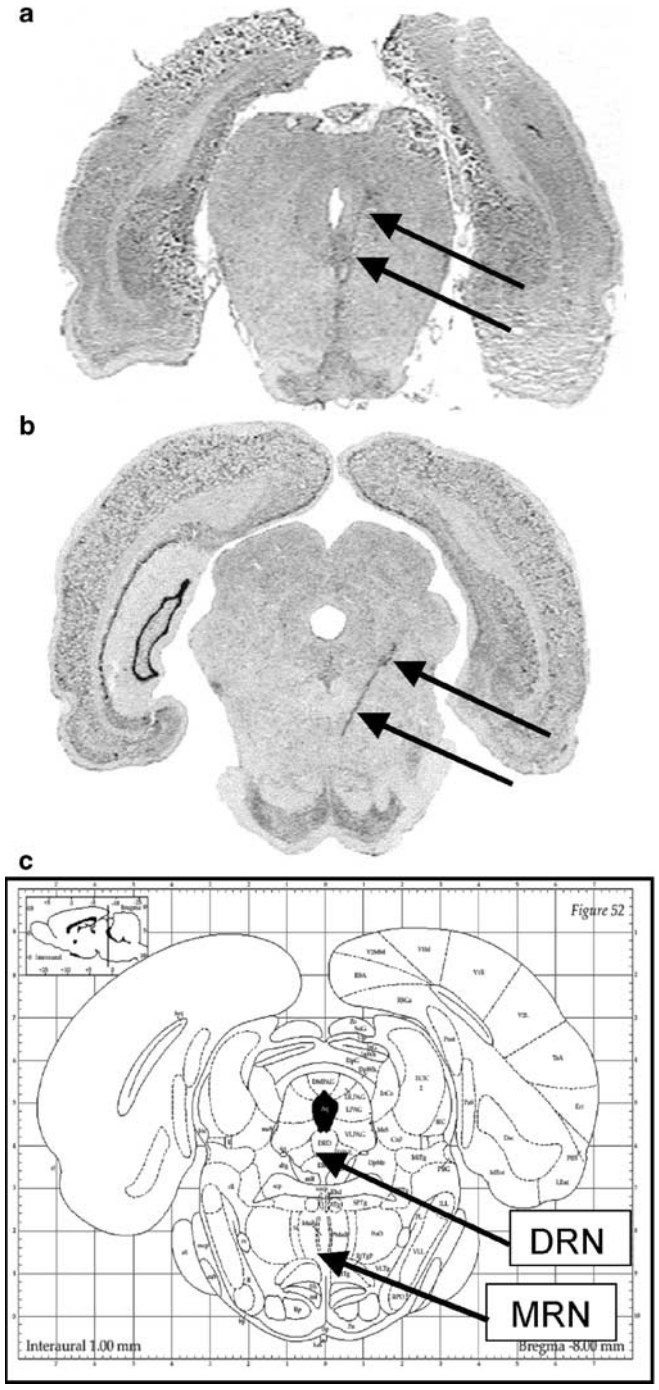

Figure 1 Representative photomicrographs of the injection sites in the DRN (panel a) and MRN (panel b) in cresyl violet-stained brain sections. Panel (c) illustrates the diagram of a section of the rat brain showing the location of DRN and MRN (Paxinos and Watson, 1998).

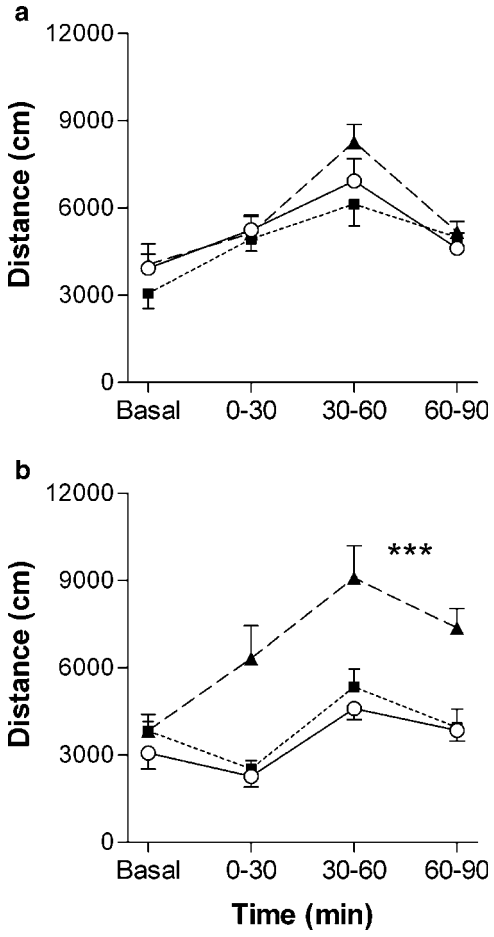

Figure 2 Time course of the effects of 5,7-DHT lesions on locomotor hyperactivity induced by s.c. injection of 0.5 mg/kg of amphetamine (a) or 2.5 mg/kg of phencyclidine (b). Locomotor hyperactivity is expressed as the distance moved (cm) ± SEM, for sham-operated ($n = 12$, ○), DRN-lesioned ($n = 12$, ■), and MRN-lesioned rats ($n = 11$, ▲). ***$p < 0.001$ for the difference between responses in MRN-lesioned rats and control rats as indicated by ANOVA.

Effect of 5,7-DHT Lesions on Startle Response, Startle Habituation, and Prepulse Inhibition

The average startle amplitude, derived from the four blocks of pulse-alone trials, was significantly increased in the DRN-lesioned group compared to sham-operated controls, whereas there was a trend towards an increase in MRN-lesioned rats (Figure 3). ANOVA indicated a significant main effect of Group on startle amplitude ($F_{2,32} = 4.1$, $p = 0.027$). *Post hoc* analysis with Bonferroni adjustment showed significant enhancement of startle amplitude in DRN-lesioned ($p = 0.023$), but not MRN-lesioned rats.

When comparing habituation data from DRN-lesioned rats and sham-operated controls, there was a significant Habituation × Group interaction ($F_{1,22} = 11.4$, $p = 0.003$). Inspection of the data revealed that DRN-lesioned rats showed increased startle amplitudes, particularly in the first block, and a rapid decline of startle amplitudes between the first and second block. MRN-lesioned rats did not show changes in startle habituation (Figure 3).

In all groups, an increase in prepulse intensity led to reduction of the startle response, and consequently enhanced percentage inhibition. Thus, there was a significant main effect of prepulse intensity for all groups (not shown). When comparing all three groups, there was a significant main effect of Group ($F_{2,32} = 7.1$, $p = 0.003$) and a significant Group × Prepulse interaction ($F_{4,128} = 2.7$, $p = 0.009$). Further ANOVAs were conducted on data from MRN-lesioned rats and sham-operated controls and on data from DRN-lesioned rats and sham-operated controls. In the MRN-lesioned group, prepulse inhibition was significantly

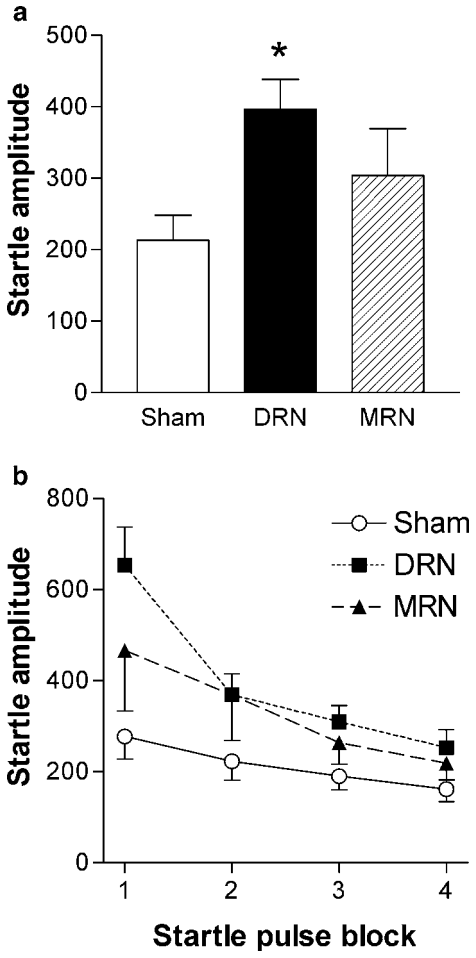

Figure 3 Effect of sham surgery ($n=12$) or 5,7-DHT lesions of the DRN ($n=12$) or MRN ($n=11$) on startle. Panel (a) illustrates the effects on basal startle reactivity. Data are expressed as the average startle amplitude of the startle responses to 40 pulse-alone trials ± SEM. *$p=0.023$ for the difference between sham- and DRN-lesioned rats as indicated by *post hoc* analysis with Bonferroni adjustment. Panel (b) represents startle habituation expressed as mean startle amplitudes ± SEM for each of the four blocks of ten 115 dB pulses. ANOVA indicated a significant difference between sham- and DRN-lesioned rats ($p=0.003$).

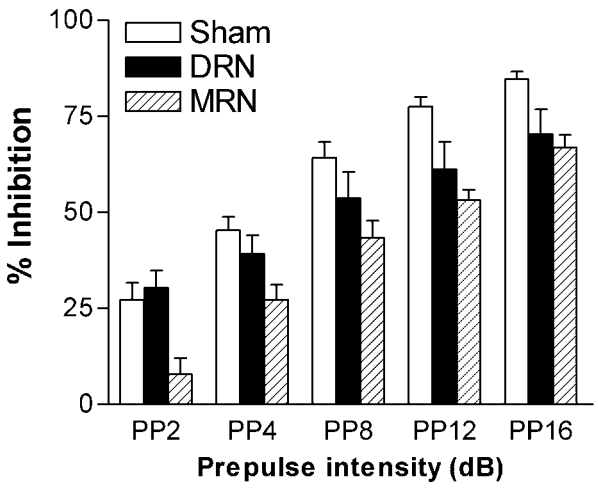

Figure 4 The effect of 5,7-DHT lesions on prepulse inhibition of the acoustic startle. The prepulse inhibition is expressed as % inhibition ± SEM, for sham-operated ($n=12$), DRN-lesioned ($n=12$), and MRN-lesioned rats ($n=11$) at different prepulse intensities. ANOVA indicated a main effect of lesion when comparing sham- and MRN-lesioned rats, $p<0.001$, and a prepulse × lesion interaction, $p=0.003$, when comparing sham- and DRN-lesioned rats. PPI was significantly reduced in MRN-lesioned rats at all prepulse intensities, but only at PP12 and PP16 in DRN-lesioned rats.

disrupted and this effect was not dependent on the prepulse intensity (Figure 4). ANOVA using Group (MRN lesion *vs* sham) as the between-subject factor and prepulse intensity as the within-subject factor, revealed a significant main effect of Group ($F_{1,21}=42.7$, $p<0.001$), but no Group-× Prepulse interaction. Further analysis revealed that PPI was significantly reduced in MRN-lesioned rats at all individual prepulse intensities (Figure 4). In contrast to locomotor hyperactivity experiments, where no effect of DRN lesions was observed, with these lesions rats displayed significant disruption of prepulse inhibition and this effect appeared to be dependent on the prepulse intensity (Figure 4). ANOVA using Group (DRN lesion *vs* sham) as the between-subject factor and prepulse intensity as the within-subject factor, revealed a significant Group × Prepulse intensity interaction ($F_{4,88}=4.3$, $p=0.003$), but no significant main effect of Group. Inspection of the data showed that the effect of DRN lesions was greater at higher prepulse intensities (Figure 4). Further analysis revealed that the group difference was significant for PP12 and PP16.

Immunohistochemical Detection of Serotonin Transporter Protein

In sham-operated rats, the DRN and MRN could be clearly observed as areas of higher densities of staining against the background (Figure 5). In lesioned groups, this staining had completely disappeared (Figure 5). Control sections, on which the primary antibody was omitted, showed little staining (Figure 5).

Analysis of ROD of SERT-immunoreactivity in the area of the DRN of sham-operated, DRN-lesioned, and MRN-lesioned groups revealed significant differences between the groups ($F_{2,24}=40.3$, $p<0.001$). *Post hoc* analysis with Bonferroni-corrected *t*-test showed a significantly lower density in the DRN of DRN-lesioned rats compared to either of the other groups (Figure 6). The density of SERT-positive staining in the MRN was also significantly different between the groups ($F_{2,24}=91.7$, $p<0.001$). *Post hoc* analysis showed a significantly lower density in the MRN of MRN-lesioned rats compared to values found in either of the other groups (Figure 6).

DISCUSSION

There is accumulating evidence to suggest the importance of brain serotonergic activity in the development and symptoms of schizophrenia (Abi-Dargham *et al*, 1997; Harrison, 1999; Kapur and Remington, 1996; Meltzer, 1995). However, it is unclear where in the brain the modulatory action of serotonin is most important. We therefore assessed the effect of 5,7-DHT lesions of either the DRN

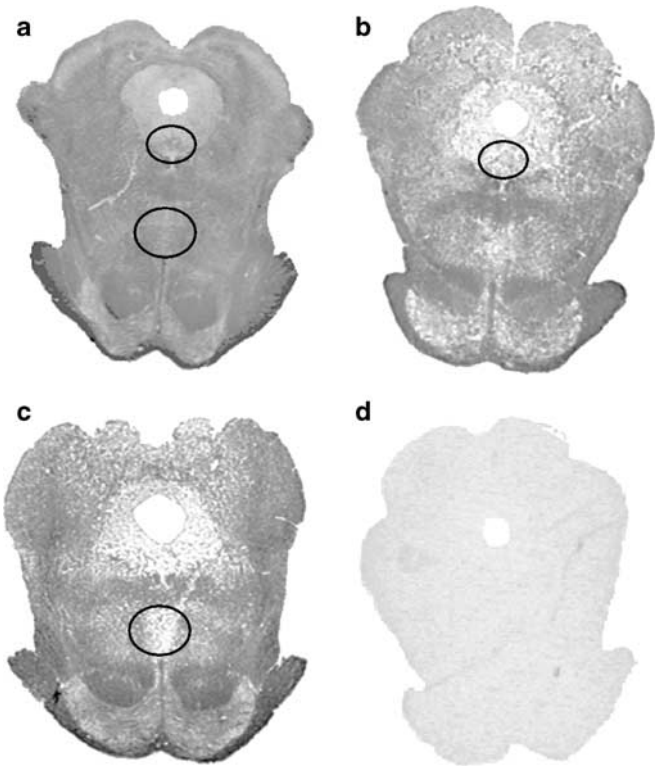

Figure 5 Representative photomicrographs of SERT immunoreactivity in the raphe nuclei region of a sham-operated rat (a), a DRN-lesioned rat (b), and an MRN-lesioned rat (c). Note that sections were taken at different levels along the anterior–posterior axis. Panel (d) shows nonspecific binding after omission of the primary antibody.

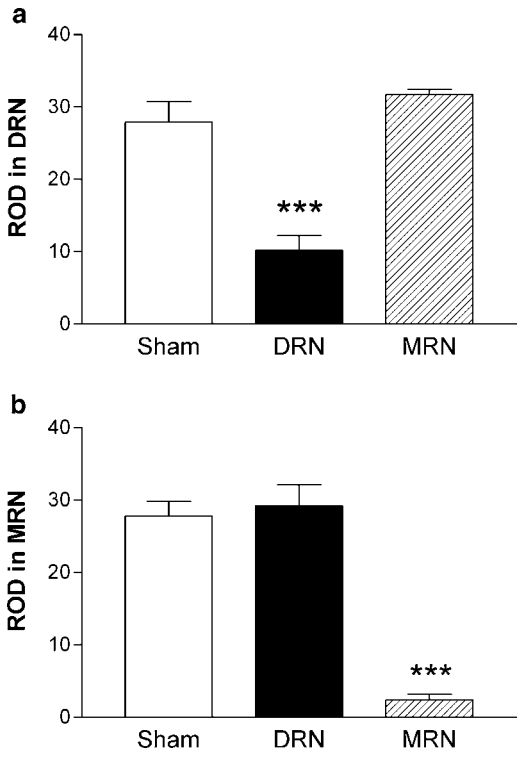

Figure 6 ROD of SERT immunoreactivity in the DRN (a) and MRN (b) after sham surgery or 5,7-DHT microinjection into the DRN or MRN. Data are expressed in arbitrary ROD units ± SEM. Data were analyzed with ANOVA and *post hoc*, Bonferroni-corrected *t*-test. $^{*}p<0.05$ for the difference with sham-operated controls.

or MRN in animal models of aspects of schizophrenia: locomotor hyperactivity induced by amphetamine or phencyclidine, and prepulse inhibition. The most important findings in this study were that: (1) phencyclidine-induced locomotor hyperactivity was increased in MRN-lesioned rats compared to sham-operated controls; (2) prepulse inhibition was disrupted in both DRN- and MRN-lesioned groups, but the effect was more marked in MRN-lesioned rats; (3) startle amplitude was significantly enhanced in DRN-lesioned rats.

Psychotropic drug-induced locomotor hyperactivity and prepulse inhibition of the acoustic startle response in rats are useful animal models to study serotonin–dopamine interactions and their implications in the symptomatology of psychiatric disorders (Geyer and Markou, 1995; Meltzer, 1989; Sams-Dodd, 1998; Swerdlow and Geyer, 1998). One shortcoming of the lesion approach used in this study is that it is not certain by which forebrain region the effect of the lesions on phencyclidine-induced locomotor hyperactivity and prepulse inhibition is mediated. The distribution of serotonergic projections arising from the DRN and MRN is strikingly different (Azmitia and Whitaker-Azmitia, 1995). While it is therefore likely that the effect of MRN lesions on phencyclidine-induced hyperlocomotion and prepulse inhibition is localized in a region that receives predominant serotonergic innervation from the MRN, such as the dorsal hippocampus (Mokler *et al*, 1998), it could also be mediated by a region that receives innervation from both nuclei such as the nucleus accumbens, substantia nigra, or hypothalamus (McQuade and Sharp, 1997). The MRN lesion may have simply led to a greater serotonin depletion than the DRN lesion in such structures receiving a common input from both nuclei. Further studies, using 5,7-DHT microinjection in forebrain areas, are needed to localize the effect of the raphe lesions to one of their projection areas. These studies are currently underway.

The observation that serotonin depletion had no significant effect on baseline locomotor activity is consistent with previous observations (Asin and Fibiger, 1983; Gately *et al*, 1986). While Jacobs *et al* (1974) reported that electrolytic lesions of the MRN caused elevated baseline locomotor activity above that of rats receiving DRN lesions or sham lesions, more recent studies have shown that a marked reduction of serotonin release from the DRN or MRN does not have an effect on basal activity (Thomas *et al*, 2000). Since baseline locomotor activity in these experiments is essentially a form of novelty-induced exploratory hyperactivity, these results could indicate that DRN and MRN lesions do not have an effect on the degree of anxiety when the animal is exposed to novelty. However, other behavioral tests, such as the plus maze, are required to confirm this.

The observation that MRN lesions lead to a markedly enhanced effect of phencyclidine on locomotor activity could be explained in a number of ways. Firstly, serotonergic projections arising from the MRN could exert a tonic inhibitory role on brain structures involved in the expression of phencyclidine-induced hyperlocomotion, such as

the dorsal hippocampus or nucleus accumbens. Grace and colleagues have proposed a 'tonic/phasic model' where reduction in tonic dopamine activity leads to negative symptoms and an increase in phasic dopamine activity is associated with positive symptoms and psychotic episodes (Grace, 1995). In this model, subcortical dopaminergic activity can be modulated by inputs from limbic structures such as the hippocampus, amygdala, and frontal cortex, thus allowing a 'gating' of sensory information appropriate to the behavioral context that the subject is in (Grace, 1995; Moore *et al*, 1999). Removal of inhibitory serotonergic projections into limbic areas could then allow the enhancement of the effect of phencyclidine observed in our study because of altered gating in the nucleus accumbens. Previous studies support such an inhibitory serotonergic role, for example, administration of 5-HT_{2C} receptor antagonists enhances phencyclidine-induced locomotion (Hutson *et al*, 2000). Interestingly, it has been shown that in patients with chronic schizophrenia and first-episode psychosis there are structural changes in the hippocampus that are present from the onset of the illness (Velakoulis *et al*, 1999).

A modified version of this model is also possible. It has been shown that acute administration of phencyclidine activates dopamine release in the prefrontal cortex and nucleus accumbens (Jentsch *et al*, 1998) and also stimulates serotonin release in the dorsal hippocampus and frontal cortex (Martin *et al*, 1998a, b). While the stimulation of locomotor activity by phencyclidine appears to be independent of dopamine release in the nucleus accumbens (Carlsson *et al*, 2001), it is possible that serotonin release from pathways originating in the MRN is needed for the normal expression of phencyclidine-induced hyperactivity. Carlsson has proposed that glutamatergic control of motor activity involves both stimulatory ('accelerator') and inhibitory ('brake') inputs onto other brain pathways involved in motor control (Carlsson *et al*, 2001). We hypothesize that phencyclidine-induced activation of serotonergic projections from the MRN forms part of the 'brake' loop that normally limits its effect. Removing the serotonergic component of this feedback loop then leads to exaggerated motor stimulation through the 'accelerator' component, as seen in our experiments. In this model, it is also clear that dopaminergic activity itself is not necessarily altered by MRN lesions, rather serotonergic and glutamatergic modulation of this activity. Thus, it is not surprising that no changes were found in the effect of amphetamine on locomotor activity in either DRN- or MRN-lesioned rats. Furthermore, this is consistent with previous studies in rats that were treated intraventricularly with this neurotoxin (Gately *et al*, 1985) or after electrolytic lesions of the MRN (Asin and Fibiger, 1983).

The primary acoustic startle circuit in the rat is relatively simple (Davis *et al*, 1982), but its activity can be influenced by different pharmacological treatments, such as dopamine receptor agonists, serotonin receptor agonists, and NMDA-receptor antagonists (Geyer, 1998; Kretschmer and Koch, 1998; Swerdlow *et al*, 1991). Several studies have indicated that a reduction in brain serotonin levels is associated with an increased sensitivity to various sensory stimuli (Davis and Sheard, 1974; Davis *et al*, 1980). This could explain why DRN-lesioned rats showed enhanced startle responses to acoustic stimuli. Although the effect of the MRN lesions on startle amplitude was not significant, there was clearly a trend towards increased startle reactivity. This finding could indicate that serotonin in brain structures innervated by both DRN and MRN is responsible for normal regulation of startle reactivity.

In prepulse inhibition experiments, MRN-lesioned rats displayed significant disruption of prepulse inhibition at all prepulse intensities, while disruption of prepulse inhibition in the DRN-lesioned group was seen particularly at higher prepulse intensities. Using a different prepulse inhibition paradigm, robust disruption of prepulse inhibition has also been found in rats with combined 5,7-DHT lesions of the DRN and MRN or after treatment with the tryptophan hydroxylase inhibitor PCPA (Fletcher *et al*, 2001; Prinssen *et al*, 2002). Prepulse inhibition is modulated by a neuronal circuit consisting of cortico-limbic brain structures wherein the nucleus accumbens plays an important role (Geyer *et al*, 1990; Koch and Schnitzler, 1997; Swerdlow and Geyer, 1998). In our study, by selectively lesioning one of the two raphe nuclei, we were able to distinguish brain regions involved in serotonergic regulation of prepulse inhibition and the startle reflex. The fact that the DRN lesions caused disruption of prepulse inhibition only at higher prepulses, could suggest that different brain structures may be involved in the regulation of high *vs* low prepulse intensities. Brain structures that receive predominant serotonergic input from the DRN, such as the frontal cortex, ventral hippocampus, or striatum (McQuade and Sharp, 1997), may be involved in responses to higher prepulse intensities, while brain structures innervated by the MRN, such as the dorsal hippocampus and cingulate cortex (Mokler *et al*, 1998), could be involved in responses to a broader range of prepulse intensities.

In conclusion, our results provide neuroanatomical support for differential involvement of different serotonergic projections in psychotropic drug-induced locomotor hyperactivity and prepulse inhibition. Furthermore, these results could indicate an important role of serotonergic inputs into limbic areas, such as the hippocampus, possibly through an interaction with glutamatergic activity in the forebrain, in the regulation of subcortical dopaminergic activity, and some symptoms of schizophrenia.

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