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ORIGINAL INVESTIGATION

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Effects of acute and chronic buspirone on impulsive choice and efflux of 5-HT and dopamine in hippocampus, nucleus accumbens and prefrontal cortex

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Abstract *Rationale:* Reduced central serotonin (5-HT) activity has been associated with impulsive choice behaviour, but there is no consensus about the precise nature of these effects. Behavioural and neurochemical effects of 5-HT_{1A} agonists such as buspirone depend critically on the dose and the duration of treatment. We thus undertook a parametric study of the effects of acute and chronic buspirone on the performance on a test of delayed gratification, as well as on the efflux of serotonin and dopamine (DA) in cortical and subcortical regions in rats. *Objectives:* Three experiments examined (i) the effects of acute buspirone on impulsive choice and how such effects were modified by prior chronic exposure to buspirone; (ii) the effects of chronic buspirone on impulsive choice; (iii) the effects on impulsive choice of a selective 5-HT_{1A} antagonist, WAY-100635 tested alone and in combination with buspirone; (iv) the effects of chronic and acute buspirone on 5-HT and DA efflux in anaesthetised rats. *Methods:* In experiment 1, rats previously trained on the delayed gratification task were tested with acute buspirone (0.5, 1 and 2 mg/kg). The same rats were then treated with chronic buspirone (1 mg/kg/day) over the next 65 days, and the effects of acute buspirone (1 mg/kg) re-determined at 20, 45 and 65 days of chronic treatment. In experiment 2, two groups of rats trained on the delayed gratification task were treated either with saline or buspirone (1 mg/kg/day) continually for 65 days before being tested with acute buspirone (1 mg/kg), WAY-100635 (0.08 mg/kg), or a combination of the two drugs. In experiment 3, rats received the same regimen of buspirone dosing as in experiment 2, before receiving in-vivo microdialysis for 5-HT and DA in the ventral hippocampus, nucleus accumbens and medial prefrontal cortex. *Results:* Acute buspirone dose dependently increased the choice for the small, immediate reinforcer (impulsive choice) but the effects of 1 mg/kg were reversed on chronic administration of buspirone. This increased choice of the large, delayed reinforcer, which was not accompanied by any changes in baseline (non-drugged) performance, was blocked by the 5-HT_{1A} receptor antagonist WAY-100635. The chronic buspirone regimen did not alter buspirone-evoked reductions in 5-HT efflux in hippocampus but did lead to a differential effect of acute buspirone in medial prefrontal cortex, with the chronic buspirone and saline groups exhibiting decreases and increases in efflux, respectively. There were no systematic changes in DA efflux under any condition. *Conclusions:* These findings show that the effects of acute buspirone on impulsive choice are reversed following chronic treatment and are mediated by 5-HT_{1A} receptors, and suggest, in addition, that the behavioural effects may involve changes in 5-HT functioning in medial prefrontal cortex.

Keywords Delayed reinforcement · 5-HT · Dopamine · Buspirone · 5-HT_{1A} receptors · WAY-100635 · Microdialysis · Chronic regime · Hippocampus · Accumbens · Prefrontal cortex

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Introduction

Clinical and experimental evidence implicates the central serotonin (5-HT) systems in 'impulsivity' (Linnoila et al. 1983; Soubrié 1986), the tendency to act without foresight. Impulsivity is manifest in several ways, for example, as enhanced, and generally inappropriate, anticipatory responding in reaction time tasks, as a failure to cancel pre-potent responses, for example in 'go/no go'

tests, and also in choice paradigms in which there is temporal or probabilistic discounting of reward. For example, delayed gratification requires the temporal discounting of reinforcement, as occurs in the choice between an immediate, small reward and a large delayed reward. Selection of the immediate, small reward has frequently been taken as a sign of impulsivity, while selection of the large, delayed reward has been taken as evidence of 'self-control', in rats (Evenden and Ryan 1996; Richards et al. 1997; Evenden 1999), pigeons (Ainslie 1975) and humans (Rodriguez and Logue 1988). There is, however, some disagreement in the extent to which the central 5-HT systems are implicated in such temporal discounting and, hence, in impulsivity. For example, experiments on temporal discounting in rats in which 5-HT has been depleted using the neurotoxin 5,7 dihydroxytryptamine have reported shifts towards choice of immediate rewards (Wogar et al. 1993; Ho et al. 1998; Bizot et al. 1999; Mobini et al. 2000), although the effects have tended to be weak and transient and only present under certain conditions. In a recent study, such 5-HT depletion produced an attenuation of the effects of amphetamine to promote the choice of the large delayed reward, but had no effect per se (Winstanley et al. 2003a).

Buspirone, which inhibits 5-HT cell firing (Sprouse and Aghajanian 1987) via activating somatodendritic 5-HT_{1A} autoreceptors [and thus consequently reduces 5-HT neurotransmission in forebrain projection areas (Hamon et al. 1998)], has also been found to increase the frequency of choice for immediate reward in rats in a T-maze procedure (Bizot et al. 1999) consonant with an inhibitory role of central 5-HT on impulsive choice. However, in an appetitive operant paradigm, the same group also reported a negative result (Charrier and Thiebot 1996). In this study, we tested the effects of buspirone in an operant temporal discounting task, using a modification (Cardinal et al. 2000) of the original test described by Evenden and Ryan (1996).

In clinical terms, the effects of buspirone when used as an anxiolytic, do not appear unless a relatively long-term course of treatment (usually between 3 weeks and 4 weeks) has been undertaken, this time course resembling that of an antidepressant rather than of a typical anxiolytic (Goa and Ward 1986). The precise mechanism that underlies this therapeutic action remains to be determined, but it may involve desensitisation of the somatodentritic 5-HT_{1A} autoreceptor following long-term treatment (Blier and De Montigny 1994). Thus, another aim of the present experiments was to determine whether impulsive choice exhibited a similar pharmacological lability by examining the effects of long-term treatment with buspirone on temporal discounting, both with respect to changes in baseline, non-drugged performance and the response to further, acute administrations of buspirone. As buspirone has significant affinity for the dopamine (DA) D_2 receptor (McMillen et al. 1983), we also examined the pharmacological specificity of any effects by testing whether they were antagonised by WAY-100635, a selective 5-HT_{1A} antagonist (Corradetti et al. 1998).

Previous work by Sharp et al. (1993) has indicated that the neurochemical effects of chronic buspirone putatatively underlying its slow onset anxiolysis probably do not occur in the ventral hippocampus. However, the delayed gratification choice task implicates striatal and prefrontal regions (Cardinal et al. 2001; Kheramin et al. 2002). Thus, in a final experiment we utilised simultaneous microdialysis of three brain regions (ventral hippocampus, nucleus accumbens and prefrontal cortex) in anaesthetised rats to assess possible changes in response to acute buspirone in 5-HT and DA systems following chronic buspirone treatment.

Materials and methods

Subjects

A total of 38 male Lister Hooded rats (Harlan Olac, Bicester, UK), aged approximately 4 months and weighing 300–350 g at the start of the experiment, were used. Eight animals took part in experiment 1, sixteen in experiment 2 and fourteen in experiment 3. All animals were housed in groups of three and in a temperature- and humidity-controlled holding facility at the Babraham Institute on a 12-h/12-h light/dark cycle (light on at 0700 hours). Water was available ad libitum, but food was restricted to that earned during the test [average of 150, Formula P 45 mg sucrose pellets (P.J. Noyes Company, Lancaster, UK)] and 20 g per rat standard rodent chow (SDS, Witham, Essex, UK) at the end of the test. Testing took place between 0800 hours and 1800 hours with individual animals tested at the same time each day where possible. All animals used in the studies were treated in accordance with the U.K. Animals (Scientific Procedures) Act 1986.

Apparatus

Four identical operant conditioning aluminium chambers were used (30×24×30 cm; Modular Test Chamber ENV-007, Med Instruments Inc., Georgia, Vt., USA). Each chamber was fitted with a 2.8-W overhead house-light and two retractable levers with a 2.8-W stimulus light above each lever. Between the two levers was an alcove fitted with a tray light, an infrared photodiode to detect head entry (nose-pokes), and a tray into which the sucrose pellets were delivered. The chambers were enclosed within sound-attenuating boxes fitted with fans to provided air circulation. The apparatus was controlled by software written by R. N. Cardinal in Arachnid (Paul Fray Ltd., Cambridge), running on an Acorn Archimedes series computer.

Behavioural shaping and training procedures

Rats were introduced to the restricted feeding regimen 1 week prior to training and gradually reached 90% of their free-feeding weight. Initial behavioural shaping consisted of magazine training, whereby the animals learned that food was available in the magazine. This training stage took 20 min per day for 2 days. The rats were then trained under a fixed-ratio 1 schedule to a criterion of 50 presses in 30 min, first for the left lever and then for the right. This stage took about 3 days. The rats then moved on to a simplified version of the full task. The session began with the levers retracted and the operant chamber in darkness. Every 40 s, a trial began with illumination of the house light and the tray light. The subject was required to make a nose-poke response within 10 s, or the current trial was aborted and the chamber returned to darkness. If the subject made a nose-poke within this time limit, the tray light was extinguished and a single lever was presented. If the rat failed to respond on the lever (i.e. press the lever) within 10 s, the lever was retracted and the chamber darkened; but, if it responded, the house light was switched off and a single pellet was delivered immedi-

ately. The tray light was illuminated until the rats collected the pellet (or a 10-s collection time limit elapsed). In every pair of trials, the left lever was presented once and the right lever once, though the order within the pair of trials was random. The fixed trial length increased to 70 s once the rats reached the criterion of 50 successful trials out of a total 60 trials per session. There was no programmed delay in this training stage. The ITI used in this stage was shorter than for the final task, and was increased in a step-wise manner to approximate the long ITI (100 s) of the final schedule.

After attaining criterion performance at the fixed trial length of 70 s, the subjects were shifted to the final schedule where the delays and small versus large rewards were introduced, the overall trial length being set at 100 s. The task was based on the 'no cue' condition used by Cardinal et al. (2000). Each trial began with the illumination of the house light and the tray light. The rat was required to make a nose poke response, ensuring that it was centrally located at the start of the trial (latency to nose-poke was designated the initiation latency). If the rat did not respond within 10 s of the start of the trial, the chamber was reset to the inter-trial state (darkness with the levers retracted) until the next trial began and the trial was scored as an omission. If the rat made a successful nose-poke, the tray light was switched off and one or both levers were presented (dependent on forced-choice trial or free-choice trial, respectively). One lever was designated the 'delayed' lever, the other 'immediate' lever in a counterbalanced left/right order. If the rat did not respond within 10 s of lever presentation, the chamber was reset to the inter-trial state until the next trial began and the trial was recorded as an omission. When a lever was chosen, both levers were withdrawn and followed by either the delivery of one pellet immediately, or four pellets after a period of delay, dependent on whether the choice was made on the 'immediate' or 'delayed' lever, respectively. Multiple pellets were delivered 0.5 s apart and the time from the delivery of the first pellet until a nose-poke occurred was recorded as the collection latency. The tray light was switched on during the collection latency. If the rat did not collect the food within 10 s of its delivery, the tray light was switched off and the chamber was then in the inter-trial state and remained so until the next trial.

A session consisted of five blocks with systematically varied delays across the session. Each block began with two forced-choice trials followed by 10 free-choice trials. For forced-choice trials, only one lever was presented so there was no 'choice' (one trial for each lever, in randomised order); whereas on free-choice trials, both levers were presented. The 'forced choice' trials were designed to ensure that rats were informed about the given delay at the start of each block (and had the opportunity to sample both the small and large reinforcers). Delays for each block were 0, 10, 20, 40 and 60 s, respectively. As trials began every 100 s, the total length of a session was 100 min. The rats were tested in one session per day.

Experiment 1. Effects of buspirone before and after chronic buspirone regimen

Acute buspirone challenge

A series of tests with the 5-HT_{1A} receptor agonist buspirone (Sigma, UK) were carried out after the subjects had attained stable baseline performance. Eight rats received a sequence of sub-cutaneous (s.c.) injections of vehicle (0.9% saline solution) or buspirone (dissolved in the same vehicle) and administered in a volume of 0.1 ml/100 g body weight, 40 min prior to testing. The doses used were 0.5, 1.0 and 2.0 mg/kg (free base) and the injection order of doses was based on a Latin-Square design. Each test session was preceded by two control sessions on which no injections were given.

Chronic buspirone regimen

After the initial phase of acute testing with buspirone, all eight rats were then moved to a chronic buspirone regimen. A separate three stage, chronic injection schedule was employed (cumulatively, 25, 20 and 20 days respectively, so that at the end of the chronic treatment regime the drug was given for a total of 65 days). In each injection stage, rats received subcutaneous injections of buspirone (1 mg/kg/day) between 1400 hours and 1600 hours. No testing on the operant procedure occurred during these injection periods. After each injection stage, rats were re-trained to stable baselines and received test sessions following the injection of acute buspirone (1 mg/kg) or vehicle. Each rat received either drug or vehicle sessions counterbalanced across rats, and with two control sessions in-between. No more than 7 days separated each injection stage.

Experiment 2. Effects of acute and chronic buspirone with and without WAY-100635

Sixteen rats were trained up as for experiment 1 but were then randomly assigned either to 'chronic buspirone treatment' (1 mg/kg/day continuously for 65 days) or 'chronic saline treatment' with saline injected in parallel to the buspirone subjects over the same time period (a control group needed to assess the effects of repeated injections per se). Following the chronic regimens and the re-establishment of stable baselines, a series of test sessions was carried out to examine the effects of WAY-100635 injected alone and in combination with acute buspirone. WAY-10065 (0.08 mg/kg) was administered 50 min and 1 mg/kg buspirone 40 min prior to testing. Drug effects were compared with saline and the saline/saline, drug/saline and drug/drug combinations were administered using a Latin-Square design.

Experiment 3. Effects of acute and chronic buspirone on 5-HT and DA efflux in the ventral hippocampus, nucleus accumbens and medial prefrontal cortex

A further 14 rats were trained up and subjected to the continuous 65-day chronic regimen as in experiment 2. Seven subjects were given buspirone and seven subjects chronic saline. These groups went for surgery to assess basal and buspirone-evoked 5-HT and DA efflux in ventral hippocampus, nucleus accumbens and medial prefrontal cortex, using in-vivo microdialysis methods with simultaneous sampling from each brain area.

Microdialysis methods

Surgical procedure. Animals were anaesthetised intraperitoneally with urethane (1.5 g/kg) and mounted in a stereotaxic apparatus (Kopf Instruments, USA) with the incisor bar set at +5.0 mm above the interaural line. Microdialysis probes (0.5 mm o.d., Microbiotech, Sweden) were implanted in the core region of the nucleus accumbens (1 mm membrane length), the ventral hippocampus and the medial prefrontal cortex (2 mm membrane length). Stereotaxic coordinates were based on Pellegrino et al. (1979). The coordinates used were: (i) nucleus accumbens (core region): anteroposterior +3.4 mm from bregma; lateral ±1.5 mm from the midline; and dorsoventral −7.6 mm from the dural surface (ii) hippocampus: anteroposterior −4.0 mm from bregma; lateral ±4.4 mm from the midline; and dorsoventral −8.0 mm from the dural surface; and (iii) medial prefrontal cortex: anteroposterior +4.8 mm from bregma; lateral ±2.5 mm from the midline; and dorsoventral −4.0 mm from the dural surface. Body temperature was maintained using a homeothermic heated blanket (Harvard Apparatus, UK) at 37°C. At the end of each experiment, brains were stored in 4% paraformaldehyde for 24 h and then transferred to a 30% sucrose solution before sectioning and staining with Cresyl Violet to determine probe placements.

Microdialysis sampling and high-performance liquid chromatography methods. Dialysate samples were collected for 150 min to establish baseline values, following which buspirone (1 mg/kg, s.c.) was given. The experiment ended 330 min after the commencement of sampling with a total of 15 dialysate samples collected for analysis (3 baseline and 12 post-drug samples at 15-min intervals). A Krebs-Ringer solution (pH 7.4) was made up with Milli-Q

(Waters, Milford, MA, USA) deionised water (resistivity, 18.2 M μcm^{-1}) containing (in mM): NaCl 138, $CaCl_2$ 1.5, $NaHCO_3$ 11, KCl 5, $MgCl_2$ 1, and NaH_2PO_4 1. This solution was passed through the probes at 1.5 μl/min using a syringe pump (CMA-100; CMA Microdialysis, Sweden). Samples were collected from the outflow tube of the probe into 500-μl Eppendorf tubes containing 2 μl 10% acetic acid at 15-min intervals using a fraction collector (CMA-142; CMA Microdialysis, Sweden). A liquid switch (CMA-110; CMA Microdialysis, Sweden) was placed between the syringe pump and the probe to switch between perfusion solutions without introducing air bubbles into the probe. The dead space between the switch and the end of the outflow tubing at the end of the probe was calculated to be 10 μl, and this was taken into account when switching between solutions. Collected samples (25 μl) were frozen (−70°C) immediately upon collection and subsequently used for high-performance liquid chromatography (HPLC) analysis.

5-HT and DA and were quantified by means of HPLC with electrochemical detection (LC4B with a Unijet cell and a 6-mm glassy carbon cell at +0.65 V; Bioanalytical Systems, West Lafayette, IN, USA). The mobile phase was NaCl 10 mM, sodium acetate 85 mM, octane sulphonic acid 150 mg/ml, EDTA 100 mg/l and 4% methanol (pH 4.5) and a flow rate of 210 μl/min was used. The column was a C18 reversed-phase Spherisorb (S3ODS2; Waters, UK). A CMA-200 refrigerated micro-injector (CMA Microdialysis, Sweden) was used to inject the samples. The detection sensitivity was 100–200 pM.

Statistical analysis

The data were analysed using the SAS program (Software Release 6.09, NC, USA) supported by the computer system of University of Cambridge. Behavioural data were analysed using analysis of variance (ANOVA), with one between-subject factor, chronic treatment (chronic saline or buspirone) and several within-subject factors; delay (delay on the large reward response), treatment days (days of chronic buspirone dosing), drug (acute administration of buspirone and/or WAY-100635) and dose (dose of acute buspirone). Post-hoc comparisons were made using the Newman-Keuls analysis, following significant ($P<0.05$) main effects or interactions. For the dialysis data, it was confirmed that the mean concentrations of substances measured in the three control samples taken before a challenge did not differ significantly using a repeated-measures ANOVA. Dialysis data were analysed using ANOVA with one between-subjects factor, chronic treatment, and one within-subject factor, sampling bin (microdialysis sample).

For all data where appropriate, the standard error of the difference between means (SED) was used as an index of variation. SEDs were taken from the error terms for the interaction between the multiple factors. As the SED can be used as the denominator for post-hoc statistical comparisons with Student's *t*-test, it is an appropriate comparator for the visual evaluation of differences between two data points (Cochran and Cox 1957; Howell 1997).

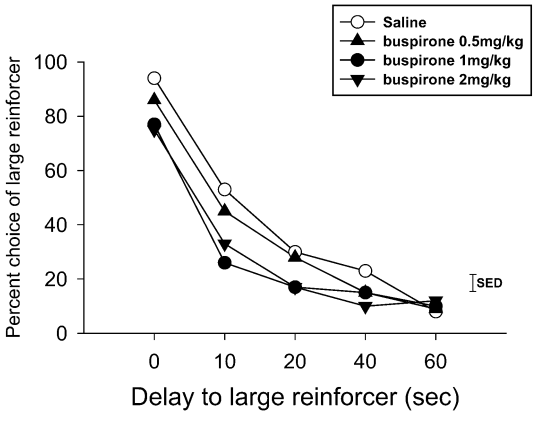

Fig. 1 Experiment 1. The figure shows dose-dependent effects of acute buspirone s.c. on the percentage choice for the large, delayed reinforcer. Values are presented as means. The *vertical bar* represents the standard error for the difference between means (SED) taken from the error terms for the interaction between the factors dose and delay. The relevant formulae are given in Cochran and Cox (1957). As the SED could be used as the denominator for post-hoc comparisons with Student's t-test, it is an appropriate comparator for the visual evaluation for the difference between two mean values

Results

Behavioural effects of buspirone on the delayed gratification task

Experiment 1. Effects of acute buspirone

The effects of systemic buspirone (0.5, 1 and 2 mg/kg) given to drug naive animals on choice behaviour in the delayed gratification task, are shown in Fig. 1. Buspirone,

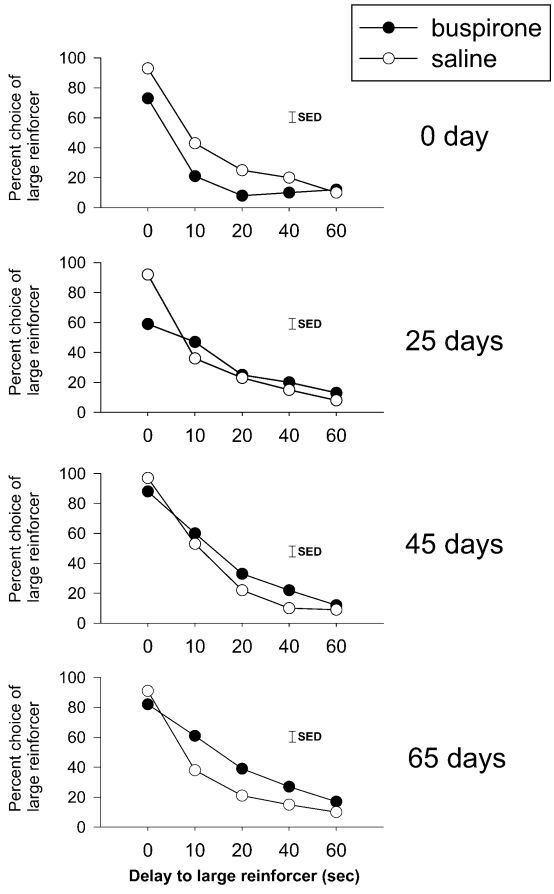

Fig. 2 Experiment 1. The figure shows the effects of acute buspirone (1 mg/kg) s.c. following 0, 25, 45 and 65 days of chronic buspirone treatment on the percentage choice for the large, delayed reinforcer. Each rat experienced acute buspirone or saline control in a counterbalanced manner over two sessions separated by two control sessions. Values are presented as means. The *vertical bar* represents the standard error for the difference between means (SED) taken from the error terms for the interaction between the factors chronic treatment and delay. See legend of Fig. 1 for further details

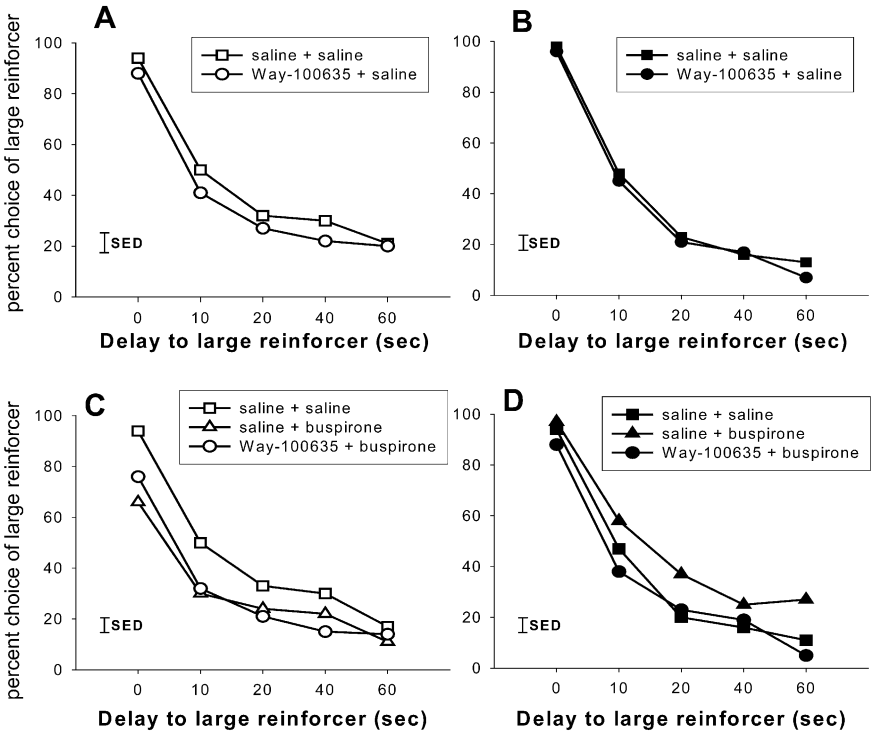

Fig. 3 Experiment 2. Effects of WAY-100635 (0.08 mg/kg) alone (**A**, **B**) and in combination with acute buspirone (1 mg/kg, s.c.; **C**, **D**) on percentage choice for the large, delayed reinforcer. *Open symbols* refer to the chronic saline group and *filled symbols* to the chronic buspirone group. Values are presented as means. The *vertical bar* represents the standard error for the difference between means (SED) taken from the error terms for the interaction between the factors drug and delay. See legend of Fig. 1 for further detail

dose dependently reduced responding for the large, delayed reward in favour of the smaller, immediate reward. The ANOVA of the data showed significant main effects of dose ($F_{3,21}$=4.0, P<0.05) and delay ($F_{4,28}$=4.0, P<0.001) but no dose × delay interaction ($F_{12,82}$=1.01, n.s.). Post-hoc comparisons of the data by Newman-Keuls confirmed that the effects of dose were mainly attributable to differences between saline versus 1 mg/kg and saline versus 2 mg/kg. The statistical analyses confirmed that, in drug naive animals, buspirone increased preference for the smaller, immediate reward but in a delay-independent manner.

Experiment 1. Effects of acute buspirone following chronic buspirone

Figure 2 shows the effects of an acute 1-mg/kg dose of buspirone after 0, 25, 45 and 65 days of daily buspirone dosing on choice behaviour. The data indicated a progressive shift from an initial acute drug-induced preference for the small, immediate reward to an acute drug-induced preference for larger, delayed reward. This was reflected in the ANOVA by a significant drug × treatment days interaction ($F_{3,18}$=3.49, P<0.05). Subsequent post-hoc analyses showed that the interaction was mainly attributable to differences at 0 days and 65 days of chronic buspirone treatment. The data were further analysed to determine whether the changes in responsivity to acute buspirone were accompanied by changes in the acute response to saline (indicative of a general change in behaviour due to the repeated injections involved in the chronic dosing regimen per se). For the saline data, there was no main effect of treatment days ($F_{3,20}$=0.18, n.s.), or treatment days × delay interaction ($F_{12,79}$=1.39, n.s.). In contrast, the treatment-days effect for buspirone was highly significant ($F_{3,20}$=7.81, P<0.01). Together, the data indicate that the reversal of effects of acute buspirone with chronic buspirone dosing were independent of any underlying changes in baseline, un-drugged, responding.

Experiment 2. Effects of acute WAY-100635 following chronic buspirone and saline (continuous 65-day regimen)

The effects of WAY-100635 (0.08 mg/kg), a selective 5-HT_{1A} antagonist, on choice behaviour in the chronic saline and buspirone regimens are shown in Fig. 3A and B, respectively. Behaviour was indifferent to the administration of WAY-100635 alone, in both the chronic buspirone (main effect of drug, $F_{1,7}$=3.8, n.s.) and chronic saline groups (main effect of drug, $F_{1,7}$=1.4, n.s.).

Experiment 2. Effects of acute co-administration of WAY-100635 and buspirone following chronic buspirone and saline (continuous 65-day regimen)

The effects of acute co-administration of WAY-100635 and acute buspirone on choice behaviour in the chronic saline and buspirone groups are illustrated in Fig. 3C and Fig. 3D, respectively. In both groups there were main effects of drug (chronic saline, $F_{2,14}$=5.61, P<0.05; chronic buspirone, $F_{2,14}$=3.82, P<0.05) but the pattern of drug effects were different. In the chronic saline animals, post-hoc analysis of the main effects indicated that the difference was in the acute saline treatment with no difference between the acute buspirone or buspirone +

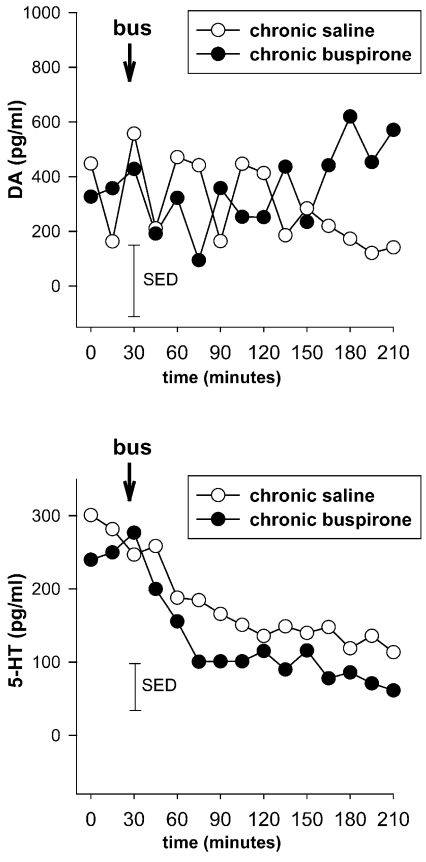

Fig. 4 Experiment 3. The figure shows the effects of chronic buspirone treatment on extracellular dopamine (DA; *upper panel*) and serotonin (5-HT; *lower panel*) in the ventral hippocampus prior to and following acute challenge with buspirone (*bus*; 1 mg/kg s.c.) at the time shown by the *arrow*. Values are presented as means. The *vertical bar* represents the standard error for the difference between means (SED) taken from the error terms for the interaction between the factors chronic treatment and sampling bin. See legend of Fig. 1 for further details

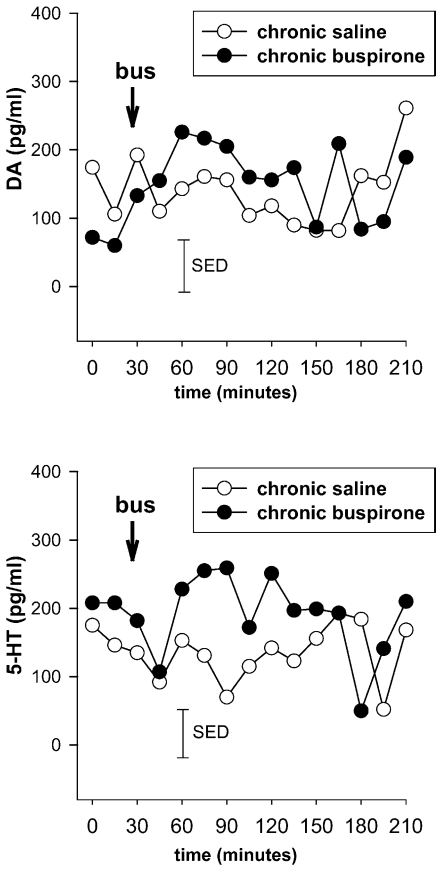

Fig. 5 Experiment 3. The figure shows the effects of chronic buspirone treatment on extracellular dopamine (DA; *upper panel*) and serotonin (5-HT; *lower panel*) in the nucleus accumbens prior to and following acute challenge with buspirone (*bus*; 1 mg/kg s.c.) at the time shown by the *arrow*. Values are presented as means. The *vertical bar* represents the standard error for the difference between means (SED) taken from the error terms for the interaction between the factors chronic treatment and sampling bin. See legend of Fig. 1 for further details

WAY-100635 treatments. In the chronic buspirone animals, the difference was in the acute buspirone treatment with no difference in the acute saline or buspirone + WAY-100635 treatments. These results indicate that the effects of acute buspirone could be reversed by WAY-100635 following chronic buspirone, but not chronic saline treatment.

Neurochemical effects of buspirone

Experiment 3. Effects of chronic buspirone and saline (continuous 65-day regimen) on efflux of 5-HT and DA

Ventral hippocampus. Chronic buspirone had no effects on basal levels of either 5-HT or DA in ventral hippocampus. Administration of acute buspirone had no systematic effects on DA efflux (sampling bin, $F_{14,110}$=0.90, n.s.; chronic treatment, $F_{1,11}$=0.02, n.s.) but did lead to a significant reduction in 5-HT levels (sampling bin, $F_{14,110}$=3.61, P<0.01) that was common to both chronic saline and chronic buspirone groups (chronic treatment, $F_{1,11}$=1.67, n.s.; Fig. 4).

Nucleus accumbens. Chronic buspirone had no effects on basal levels of either 5-HT or DA in nucleus accumbens. Administration of acute buspirone had no significant effects on DA efflux but did lead to a trend for higher levels of 5-HT in the chronic buspirone group, which failed to reach significance at P<0.05 (chronic treatment, $F_{1,9}$=2.17, n.s.; Fig. 5).

Medial prefrontal cortex. Chronic busprirone had no effects on basal levels of either 5-HT or DA in the medial prefrontal placement. Again, acute buspirone had no significant effects on DA efflux but did alter 5-HT levels, with the drug decreasing 5-HT in the chronic buspirone animals but increasing 5-HT levels in the chronic saline

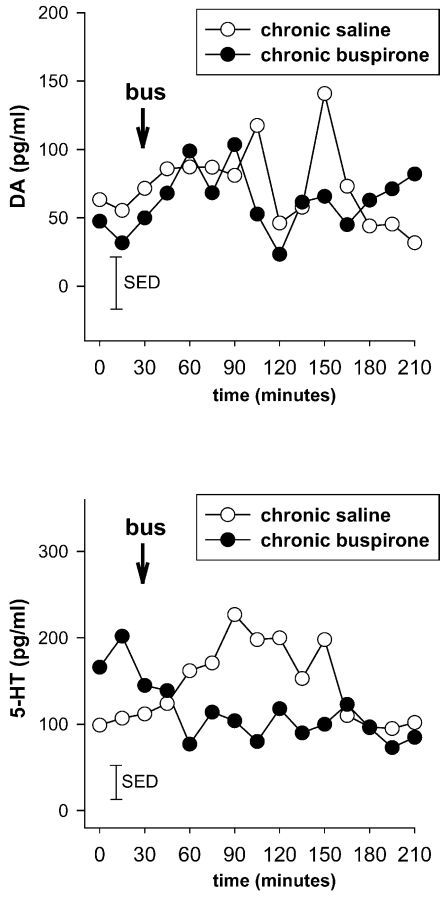

Fig. 6 Experiment 3. The figure shows the effects of chronic buspirone treatment on extracellular dopamine (DA; *upper panel*) and serotonin (5-HT; *lower panel*) in the medial prefrontal cortex prior to and following acute challenge with buspirone (*bus*; (1 mg/kg s.c.) at the time shown by the *arrow*. Values are presented as means. The *vertical bar* represents the standard error for the difference between means (SED) taken from the error terms for the interaction between the factors chronic treatment and sampling bin. See legend of Fig. 1 for further details

group (chronic treatment × sampling bin, $F_{9,108}$=2.66, P<0.01; Fig. 6).

Discussion

These experiments have shown that the widely employed anxiolytic drug buspirone, a non-selective 5-HT_{1A} receptor partial agonist, has differential effects on choice behaviour in a temporal discounting paradigm measuring impulsive choice, depending on previous chronic exposure to the drug. Acute buspirone given to rats without a history of chronic exposure to the drug increased the preference for small immediate rewards, whereas following chronic buspirone treatment, the acute effects were expressed instead as an enhanced preference for large, delayed rewards. This enhanced preference for large, delayed reward was blocked by the 'silent' 5-HT_{1A} receptor antagonist WAY-100635, indicating mediation by the 5-HT_{1A} receptor. In neurochemical terms, acute buspirone reduced extracellular levels of 5-HT in the hippocampus, irrespective of chronicity of treatment. By contrast, acute buspirone only decreased 5-HT in the prefrontal cortex following chronic treatment, actually increasing levels of 5-HT in controls. There were no significant effects on extracellular DA levels. These findings will be discussed in terms of the role of the central 5-HT system in impulsive behaviour and the possible clinical implications.

Acute buspirone effects: naive animals without drug history

Acute buspirone dose dependently reduced the frequency of choice for the large reward, suggesting that the tolerance for the anticipated delay had been reduced, leading to impulsive-like behaviour. Such effects clearly cannot be explained by an anxiolytic action, as enhanced choice of the large reward would be predicted through an attenuation of the aversive effects of delay. A motor disinhibition account would make no clear predictions in this choice paradigm. The effects also occurred in a dose-range (0.5–2 mg/kg), much smaller than the dose (10 mg/kg) reported to alter working memory in a three-choice water escape task (Bass et al. 1992), making it unlikely that the drug simply impaired the association between responding on the large reward lever and the reward itself over the ensuing delay period, particularly as the effects of acute buspirone were themselves delay independent. The mechanisms underlying the delay-independent nature of the reduced choice for the large delayed reward under buspirone remain to be established; they may possibly be consistent with the drug affecting how reward magnitude influences choice behaviour.

Impulsive behaviour has long been suggested to be related to the dysfunction of central 5-HT systems, reduced 5-HT function generally being found to reduce waiting capacity or lead to behavioural disinhibition (for review, see Soubrié 1986). Buspirone is a partial agonist at 5-HT_{1A} post-synaptic receptors, but a full agonist at 5-HT_{1A} pre-synaptic receptors (Hjorth and Carlsson 1982). Via the activation of 5-HT_{1A} somatodendritic autoreceptors, buspirone inhibits 5-HT cell firing (Sprouse and Aghajanian 1987) and, thereby, reduces 5-HT neurotransmission (Hamon et al. 1988). This implies a net decrease of 5-HT transmission when administered acutely and so accounts for the impulsive-like behaviour found in the present experiments. Consonant with this hypothesis were the additional findings of reduced extracellular levels of 5-HT in the hippocampus and prefrontal cortex in response to acute buspirone (experiment 3).

The effects of acute buspirone are consistent with some results reported for 8-OH-DPAT, a full agonist at 5-HT_{1A} receptors. At the lower doses of 8-OH-DPAT that could be expected to reduce 5-HT neurotransmission, choice behaviour shifts towards immediate but small reward, with larger doses of 8-OH-DPAT producing the

opposite effects (Poulos et al. 1996). Evenden and Ryan (1999) also found that a very high dose of 8-OH-DPAT could produce opposite effects, but within the same session; initial impulsivity but later enhanced preference for large, delayed reinforcement. By contrast, Bizot (1999) consistently found enhanced preference for the large, delayed reward in a maze choice test at most doses tested of 8-OH-DPAT, in marked contrast to effects of partial 5-HT_{1A} agonists, including buspirone. They concluded that the variations in response to these full and partial 5-HT_{1A} agonists might be accounted for by their respective efficacies at pre- and post-synaptic receptors. Although activation of the pre-synaptic 5-HT_{1A} somatoautoreceptor reduces 5-HT neurotransmission, stimulation of the post-synaptic 5-HT_{1A} receptor presumably enhances 5-HT function (for review, see De Vry 1995). In other words, the effects of 5-HT_{1A} receptor ligands on choice behaviour may largely depend on their net impact on 5-HT neurotransmission.

It is possible that the 'impulsive' effects of acute buspirone are not mediated by 5-HT_{1A} auto-receptors, especially as it was not possible to antagonise the impulsive choice behaviour using a dose of the selective 5-HT_{1A} receptor antagonist WAY-100635 that was effective in blocking the qualitatively different effects of acute buspirone emerging after chronic treatment. The failure of WAY-100635 to antagonise some effects of buspirone, as well as other 5-HT_{1A} receptor agonists is not unprecedented. Indeed, in their comprehensive study, Bizot et al. (1999) found that WAY-100635 also failed to counteract the effects of the specific 5-HT_{1A} receptor agonist 8-OH-DPAT. Moreover, several other behavioural, endocrine and electrophysiological effects of 5-HT_{1A} receptor agonists are not blocked by this receptor antagonist (De Vry 1995; Bizot et al. 1999). Therefore, despite this lack of confirmatory evidence, we do not think it wise to reject completely the hypothesis that the effects of acute buspirone might be mediated by its pre-synaptic actions to reduce 5-HT neurotransmission. It seems unlikely that such impulsive behaviour would result from the known DA D_2 receptor blocking actions of buspirone (McMillen et al. 1983; Algieri et al. 1988; Piercey et al. 1994), although this remains a possibility. There was no evidence in experiment 3 of either acute or chronic effects of buspirone on DA levels either in the nucleus accumbens or medial prefrontal cortex. Nonetheless, it is plausible that other actions of acute buspirone in drug-naive animals, such as its effects on noradrenergic cell firing (Piercey et al. 1994) and noradrenaline efflux in the prefrontal cortex (Dalley et al. 1996) and hippocampus (Done and Sharp 1994) might mediate the increased impulsivity.

Chronic buspirone effects

One of the major new findings of the present experiments was that the effects of acute buspirone were shifted towards an enhanced preference for large, delayed rewards following chronic buspirone treatment. These effects occurred on a stable baseline, as there were no evident shifts in the preference/delay function caused by repeated treatment with buspirone itself over the 65 days of treatment. Moreover, when experiment 1 was repeated with the addition of a chronic saline treatment control group (experiment 2), again there were no baseline shifts evident in either chronic treatment group. These findings allow us to conclude that whatever neurochemical changes may result from chronic buspirone treatment, these do not affect impulsive choice behaviour per se.

Following the chronic buspirone regimen, it is hypothesised that the drug loses its capacity to desensitise the somatodendritic 5-HT_{1A} autoreceptors (Blier and De Montigny 1990). Therefore, it is postulated that the long-term administration of buspirone leads to an enhanced activation of postsynaptic 5-HT_{1A} receptors, which becomes evident upon acute treatment with a 5-HT_{1A} receptor agonist. The strengthening of net 5-HT function presumably accounts for the reversal of the effects of buspirone found in the present experiment towards enhanced preference for the large, delayed reward. It is also consistent with the additional mechanism proposed by De Vry (1995) that the post-synaptic 5-HT_{1A} receptors actually become sensitised following chronic treatment with 5-HT_{1A} agonists. These mechanisms may underlie the delayed onset of clinical action of these drugs (to be discussed further below). This action of acute buspirone in the chronically treated rats was effectively blocked by WAY-100635, which agrees with most previous findings (Evenden and Ryan 1999; but see also Bizot et al. 1999) implicating mediation of these effects of acute buspirone by 5-HT_{1A} receptors.

Other possible receptor or neurochemical adaptations occur following chronic treatment with 5-HT_{1A} agonists which might explain the altered effects of buspirone, although few, if any, studies have examined such effects over such a lengthy period (65 days) of chronic treatment. For example, there is no biochemical evidence for increases in post-synaptic 5-HT_{1A} receptor numbers, although there is some evidence for downregulation of cortical 5-HT_2 receptors (see review by De Vry 1995). The latter is consistent with some evidence of reductions in certain tests of impulsivity following 5-HT receptor antagonists such as ketanserin (Passetti et al. 2003) and MDL-100, 907 (Winstanley et al. 2003b), although Evenden and Ryan (1999) and J. Talpos, L.S. Wilkinson, and T.W. Robbins (unpublished observations) could detect no such effects of systemic ritanserin or ketanserin, respectively, on a delayed gratification paradigm similar to that used here.

Neurochemical findings with in vivo microdialysis

There has been no consistent evidence that chronic treatment with 5-HT_{1A} agonists alone produces large changes in extracellular 5-HT in such targets of the dorsal and median raphé respectively as the striatum and

hippocampus (De Vry 1995). The present findings are largely consistent with that conclusion, while extending it to include the medial prefrontal cortex and nucleus accumbens.

The present study showed within the ventral hippocampus that both the chronic buspirone and the control groups exhibited a decrease in 5-HT levels in response to acute buspirone. Since the hippocampus receives its 5-HT innervation from the median raphé nucleus (for review, see Jacobs and Azmitia 1992), it is possible that, via the activation of somatodentritic 5-HT_{1A} autoreceptor in the median raphé nucleus, buspirone inhibits 5-HT cell firing (Sprouse and Aghajanian 1987) and, therefore, reduces 5-HT transmission in the ventral hippocampus (Hamon et al. 1988). Further, our findings imply that the desensitisation of the somatodendritic 5-HT_{1A} autoreceptor following repeated treatment with a 5-HT_{1A} agonist does not occur in the median raphé-ventral hippocampal pathway, a conclusion which is consistent with that of another study employing microdialysis by Sharp et al. (1993). These findings are evidently difficult to reconcile with the classical view that the chronic treatment desensitises the 5-HT autoreceptors. The similarity in the neurochemical response to buspirone in the hippocampus in two groups that exhibit opposite behavioural effects of buspirone implies that the hippocampus is not implicated in these particular actions of buspirone.

We observed quite different changes in the neurochemical sequelae of chronic buspirone treatment in the nucleus accumbens and, even more strikingly, in the medial prefrontal cortex, where acute buspirone induced an increase in extracellular 5-HT in saline-treated animals while having the opposite effect of reducing 5-HT levels following chronic buspirone. By contrast, chronic buspirone led to a non-significant increase in 5-HT in the nucleus accumbens. Nevertheless, these findings are all still at variance with the densensitisation hypothesis.

Given the behavioural findings that the chronic buspirone group manifested a choice preference towards delayed large rewards in response to acute buspirone, this implies that lower levels of extracellular 5-HT in the prefrontal cortex (and higher levels in the nucleus accumbens) are more conducive to self-control. This is in line with findings of Dalley et al. (2002a, 2002b) that a lower level of 5-HT efflux in prefrontal cortex, as determined using microdialysis, was associated with lower levels of premature responding in a 5-choice serial reaction time task. Moreover, lower 5-HT utilisation indices in rat frontal cortical tissue were also found to correlate with lower rates of premature responding (Puumala et al. 1998). Taken together, these results suggest that the 5-HT innervation of the prefrontal cortex seems to function in a quite different manner from that in other forebrain regions in the behavioural inhibitory functions implicated in self-control.

It remains to be explained whether the dependence of the response to acute buspirone following chronic buspirone treatment on 5-HT_{1A} receptors can be related to the observed fluctuations in extracellular 5-HT. The most parsimonious account is of a sensitisation of 5-HT_{1A} receptors resulting from chronic buspirone, possibly restricted to post-synaptic sites (this possibility being favoured by De Vry 1995) with the changes in 5-HT levels being only secondary. This would lead to a net enhancement in serotoninergic transmission in the prefrontal cortex and possibly other sites such as the nucleus accumbens, following acute buspirone, which reduces impulsive choice in a 5-HT_{1A} receptor-dependent manner.

Clinical implications

The finding that chronic buspirone treatment completely reversed the behavioural effects of the drug in this delayed gratification/impulsivity paradigm is consistent with the delayed onset of clinical action of buspirone, which usually takes 3–4 weeks to achieve its anxiolytic effect (Rickels et al. 1988; Murasaki and Miura 1992). The delayed onset of action and lack of sedative effects of buspirone is more reminiscent of serotonin selective reuptake inhibitors (SSRIs), rather than a typical benzodiazepine anxiolytic. In fact, buspirone may also act as an antidepressant at relatively high doses (Robinson et al. 1989; Fabre 1990) and augments the effect of the SSRI fluoxetine when used at a lower dose (Markowitz et al. 1990), particularly in treatment of patients with combined anxiety and depression (i.e., "neurotic depression"). However, it should be noted that the therapeutic effects of SSRIs are thought to depend on elevations in extracellular 5-HT in the prefrontal cortex, rather than the reductions observed here following chronic buspirone (Sharp et al. 1997). While apparently rendering less likely the possibility that a unitary action on the central 5-HT system underlies the chronic effects of both 5-HT_{1A} agonists and SSRIs, it remains possible that impulsive behaviour as studied in the present operant temporal discounting paradigm is only of marginal relevance to anxiety and depression, despite relating to other clinical disorders described in the Introduction. However, the finding that the drug combats impulsive choice is consistent with both its anxiolytic actions (reducing the aversiveness of delay) and also a putative important antidepressant effect (reducing impulsivity, as may be important when treating suicide). Our data are most consistent with mediation of these effects on impulsivity via the prefrontal cortex (or possibly nucleus accumbens) rather than the hippocampus. The extent to which the prefrontal cortex and related areas also mediates the anxiolytic and anti-depressant effects of buspirone remains to be determined.

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