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BEHAVIOURAL CHARACTERIZATION OF THE NOVEL GAMMA-AMINOBUYTRIC ACID (B) ($GABA_B$) RECEPTOR POSITIVE MODULATOR GS39783 (N, N'- DICYCLOPENTYL-2-METHYLSULFANYL-5-NITRO-PYRIMIDINE-4,6-DIAMINE): ANXIOLYTIC-LIKE ACTIVITY WITHOUT SIDE-EFFECTS ASSOCIATED WITH BACLOFEN OR BENZODIAZEPINES

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Abbreviations

GABA = γ-aminobuytric acid; GS39783 = N,N'- DICYCLOPENTYL-2-METHYLSULFANYL-5-NITRO-PYRIMIDINE-4,6-DIAMINE; CDZ = Chlordiazepoxide; [^{35}S]GTPγS = guanosine 5'-*O*-(3-[^{35}S]thio)triphosphate); ANOVA = analysis of variance

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Abstract

The role of γ-aminobuytric acid (B) ($GABA_B$) receptors in various behavioural processes has been largely defined using the prototypical $GABA_B$ receptor agonist baclofen. However, baclofen induces sedation, hypothermia and muscle relaxation, which may interfere with its use in behavioural paradigms. Although there is much evidence for a role of the inhibitory neurotransmitter GABA in the pathophysiology of anxiety the role of $GABA_B$ receptors in these disorders is largely unclear. We recently identified GS39783 as a selective allosteric positive modulator at $GABA_B$ receptors. The aim of the present studies was to broadly characterize the effects of GS39783 in well-validated rodent models for motor activity, cognition and anxiety. The following tests were included; locomotor activity in rats and mice, rotarod and traction tests (including determinations of core-temperature) in mice, passive avoidance in mice and rats, elevated plus maze in rats, elevated zero maze in mice and rats, stress-induced hyperthermia in mice, and pentobarbital and ethanol-induced sleep in mice. Unlike baclofen and/or the benzodiazepine chlordiazepoxide, GS39783 had no effect in any of the tests for locomotion, cognition, temperature or narcosis. Most interestingly GS39783 had anxiolytic-like effects in all the tests used. Overall, the data obtained here suggest that positive modulation of $GABA_B$ receptors may serve as a novel therapeutic strategy for the development of anxiolytics, with a superior side-effect profile to both baclofen and benzodiazepines.

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γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain and hence GABAergic neurotransmission regulates many physiological and psychological processes. There are two classes of GABA receptors: ionotropic GABA receptors, including $GABA_A$ and $GABA_C$ receptors, and metabotropic $GABA_B$ receptors (Barnard et al., 1998; Bormann, 2000; Bowery et al., 2002). The $GABA_B$ receptor is a heterodimer made up of two subunits, $GABA_{B(1)}$ and $GABA_{B(2)}$ both necessary for $GABA_B$ receptors to be functionally active (Calver et al., 2002). Pharmacological studies with baclofen, the prototypical highly selective $GABA_B$ receptor agonist, have pointed to a role for $GABA_B$ receptors in epilepsy, cognition, pain, gastroesophageal reflux disease and addiction (Bowery et al., 2002). Baclofen has been in clinical use for the treatment of spasticity for over 30 years (Brogden et al., 1974). However, it is this muscle relaxing property, together with the compound's sedative and hypothermic effects, which limits its widespread use as a tool in behavioural pharmacological studies.

Allosteric, positive modulation of metabotropic receptors is a newly identified phenomenon, providing novel means for the pharmacological manipulation of G-protein-coupled receptors acting at a site apart from the orthosteric binding region of the receptor protein (Soudijn et al., 2002; Jensen and Spalding, 2004). Allosteric modulators are therefore thought to offer a number of potential pharmacological improvements when compared to the use of conventional agonists, as has been demonstrated for modulators acting at ligand-gated ion channels (Costa, 1989). Modulators at the $GABA_A$ receptors are used therapeutically; for example, benzodiazepines amplify the action of the endogenous neurotransmitter GABA at the $GABA_A$ receptor. More recently, novel positive allosteric modulators of the $GABA_B$ receptor have been identified (Urwyler et al., 2001; Urwyler et al., 2003). In analogy to the effects of benzodiazepines on $GABA_A$ receptors, we hypothesize that $GABA_B$ receptor positive modulators might represent therapeutically superior drugs compared with full $GABA_B$ receptor agonists with respect to undesired side effects.

The $GABA_B$ receptor positive modulator GS39783 (N,N'- dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine) has of late been characterized *in vitro* (Urwyler et al., 2003). GS39783 potentiates both the potency and maximal efficacy of GABA-stimulated guanosine 5'-*O*-(3-[^{35}S]thio)triphosphate

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([^{35}S]GTPγS) binding to membranes from a $GABA_{B(1b/2)}$-expressing Chinese hamster ovary cell line, but does not stimulate [^{35}S]GTPγS binding itself. Potentiation of $GABA_B$ receptor responses by GS39783 are also observed using native $GABA_B$ receptor preparations from rat brain. Furthermore, like baclofen GS39783 suppresses paired pulse inhibition in rat hippocampal slices. This effect is reversed by the competitive $GABA_B$ receptor antagonist CGP55845A and is produced most likely by enhancing the effect of synaptically released GABA at presynaptic $GABA_B$ receptors. In functional GTPγS binding assays, positive modulation by GS39783 was observed to be $GABA_B$ receptor selective, as functional responses from a related receptor, the mGluR2, were not enhanced by the compound (Urwyler et al., 2003). Furthermore, 1μM GS39783 was inactive in binding assays on more than 30 different receptor proteins (G protein-coupled receptors, transporters and ion channels, unpublished data.

Although GABAergic neurotransmission is long known to play a critical role in anxiety, data on the specific role of $GABA_B$ receptors in anxiety are limited and rather variable (see (Millan, 2003). This is largely because investigators relied on using baclofen for such an analysis, a compound having only a narrow efficacy window before confounding side-effects are manifested in anxiety paradigms (Dalvi and Rodgers, 1996). Renewed interest in the role of $GABA_B$ receptor in anxiety has recently emerged; $GABA_{B(1)}$ deficient mice were found to be more anxious than their wildtype counterparts (Mombereau et al., 2004). With the availability of GS39783, we now have a new tool to assess the role of $GABA_B$ in anxiety disorders. In the present studies, we broadly characterized GS39783 in behavioural paradigms where full agonists, such as baclofen, have been shown to induce alterations; these include models of motor performance, cognition and body-temperature. Furthermore, we characterize the effects of GS39783 in a number of well validated animal models of anxiety and compare its efficacy and side-effect profile with that of the benzodiazepine chlordiazepoxide.

Materials and Methods

Animals

All animals were purchased from Iffa Crédo, L'Arbresle, France. The animals had access to water and food ad libitum and were experimentally naïve. Rats and mice were separately housed in macrolon cages (42 x 26 x 15 cm or 55 x 33 x 19 cm; n = 4 per cage for rats; n = 10-15 per cage for mice) in a temperature controlled room under artificial illumination. Lights were maintained on a 12 h light/dark cycle (lights on 06:00-06:30 depending on experiment). Male OF1/IC mice (18 – 35g) were used in all mouse studies with the exception of the passive avoidance test where male CD-1 mice (25-35 g) were used and rotarod-ethanol study where BALB/c mice (25 - 30 grams) were used. Male Sprague Dawley rats (160 - 180 g) were used for locomotor activity and elevated zero maze larger animals (250 - 300 g) were used for passive avoidance, whereas male Wistar rats (180-200 g) were used for elevated zero maze. All studies were performed according to methods approved by the Veterinary Authority of the City of Basel

Drugs

All drugs were made up fresh prior to use. For all studies GS 39783 (Novartis), l-baclofen (Novartis) and chlordiazepoxide hydrochloride (Sigma, St. Louis) were dissolved in 0.5% methylcellulose (Vehicle) solution as a fine suspension (GS 39783). They were applied p.o. in a volume of 1 ml/kg to rats and 10 ml/kg to mice unless otherwise noted.

Statistics

A Kruskal-Wallis analysis of variance (ANOVA) was used followed by the Mann Whitney U test (Bonferoni corrected) for rotarod test. The Fischer exact test was used (Bonferoni corrected) for the traction test. As appropriate a repeated or single ANOVA was used followed by either Dunnett's or Fisher's Posthoc test for locomotor activity, temperature, passive avoidance, elevated zero maze, elevated plus maze, stress induced hyperthermia and ethanol-induced rotarod impairment. For rat locomotor activity, plus maze and zero maze the effects of comparator compound (baclofen or chlordiazepoxide were against control was compared using a Student's t-test.

Effects of GS39783 on rotarod and traction test

Rotarod test: The rotarod apparatus consists of a cylinder subdivided into five available mice positions of each 6 cm in diameter, which is positioned 30 cm above the table and rotates at a speed of 12 rpm (Dunham and Miya, 1957). The mice were placed singly on the cylinder. One the day before the start of the experiment animals were trained to stay on the rotarod for 300 seconds. Mice that failed to learn the test or did not reach the criterion (300 sec endurance) were excluded from the study. During the test day the length of time each mouse remained on the cylinder (= 'endurance time', maximal score 300 sec) was measured 1, 3, 6 and 24 hours after application of a test compound or vehicle.

Traction: The animal is suspended by the front paws from a horizontal wire. The test is successfully completed when the animal is able to touch the wire with at least one hind-paw within 5 seconds (Boissier and Simon, 1960). The animals were tested 1, 3, 6 and 24 hours after application immediately prior to the rotarod test (see above). Treatments: Mice received oral administrations of GS39783 (0.1, 1, 10, 50, 100 or 200 mg/kg, p.o.), l-baclofen (2.5 or 5 mg/kg, p.o.), chlordiazepoxide (5 or 10 mg/kg, p.o.) or vehicle (0.5% methylcellulose, p.o.).

Effects of GS39783 on core body temperature

Body temperature: Rectal temperature was measured to the nearest 0.1 ^{o}C by an ELLAB instruments thermometer (Copenhagen, Denmark) via a lubricated thermistor probe (2 mm diameter) inserted 20 mm into the rectum while the mouse was hand held near the base of the tail. The probe was left in place until steady readings were obtained (approx 15 seconds). The animals were tested -1, 0, 1, 2 and 4 hours after application with a test compound or vehicle *Treatments:* Mice received oral administrations of GS39783 (25, 50, 100 or 200 mg/kg, p.o.), l-baclofen (5, 10 or 15 mg/kg, p.o.), or vehicle (0.5% methylcellulose, p.o.).

Effects of GS39783 on locomotor activity

a) Rats

Recording device: Horizontal locomotor activity was assessed in transparent Plexiglas boxes (dimensions: 19x31x16 cm) and activity was detected and registered using the TSE Moti system (TSE, Bad Homburg, Germany) which is based on the registration of infrared light beam interruptions along the x, y and z axes, as

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caused by an animal's movements (Spooren et al., 2000); data were directly stored into the computer. *Experimental procedures:* Rats received injections with GS39783 (doses 0.1, 1, 10, 100, 200 mg/kg, p.o.), L-baclofen (5 mg/kg, p.o.) or vehicle (0.5% methylcellulose, p.o.) and were immediately returned to their home cages for 60 minutes.

b) Mice

Recording device: See above for rats *Experimental procedures:* Mice received injections with GS39783 (doses 1, 3, 10, 30, 100, mg/kg, p.o.), baclofen (1, 3, 10 mg/kg, p.o.) or vehicle (0.5% methylcellulose, p.o.) and also were immediately returned to their home cages for 60 minutes. Subsequently, they were individually placed in plexiglas boxes (as for rats) and their spontaneous locomotor activity was registered for the next 30 minutes.

Effects of GS39783, baclofen and chlordiazepoxide on passive avoidance behaviour

a) Mice

Experimental Procedure: Starting on the day before the training trial and continuing throughout the experiment they were housed singly in the experimental room. The apparatus and procedure were as previously described in detail (Venable and Kelly, 1990). Briefly for the training trial mice were gently placed into the light side of the two-compartment trough-shaped apparatus. The door to the dark compartment was opened and a button pressed to initiate timing by the computer. When the mouse broke a photocell beam located 10.5 cm into the dark compartment, the latency from opening the door to the animal breaking the beam (step-through latency) was automatically recorded and a Campden Instruments 521 C Shock Source was automatically activated. This resulted in the application of a footshock (0.5 mA rectangular current waves) between the stainless steel plates, which comprised the dark compartment. This ended when the mouse escaped back to the light compartment or after 5 seconds elapsed, whichever came first. If an animal did not enter the dark compartment within 150 sec on the training trial it was removed from the apparatus without receiving any shock, and was excluded from the retention test. The retention test was carried out on the following day and the same procedure was followed except that the shock generator was switched off. *Drugs:* GS39783 (1, 3, 10, 30, 100 mg/kg) or

chlordiazepoxide (5, 10, 20, 40 mg/kg) were applied p.o. 60 min before the training trial. l-Baclofen (1, 3 mg/kg) was administered s.c. 30 min before the training trial.

b) Rats

Since more pronounced effects of GS39783 emerged in the rat version of the elevated zero maze compared with mouse test, we also sought to clarify if undesired effects would also emerge in rats even though none were present in mice. Therefore we tested the effects of GS39783 on passive avoidance. *Experimental Procedure:* On the day before the experiment rats were housed singly in the experimental room and were handled twice for 30-60 sec each. The passive avoidance apparatus for rats was trough-shaped like that for mice (see above), and was exactly as previously described (Venable and Kelly, 1990). *Drugs:* GS 39783 (25, 50, 100 mg/kg), l-baclofen (1, 3 mg/kg) and chlordiazepoxide (20, 40 mg/kg) or vehicle (0.5% methylcellulose).

The effects of GS39783 on behaviour in the elevated zero maze test

a) Mice

Apparatus: The apparatus was a 5.5-cm-wide circular track constructed of grey plexiglas with an inside diameter of 34 cm, a mid-track circumference of approximately 121 cm, and an elevation of 40 cm. It consisted of two open quadrants with a raised, 2-mm edge and two closed quadrants with walls 11 cm high. As the zero maze has no central area, the animal must be in either an open or a closed part of the arena. *Experimental Procedure:* After oral drug administration (60 min. prior test), the animals were returned to their home cage. Mice were placed in one of the closed quadrants designated as the starting quadrant and were allowed to investigate the zero maze for a period of 5 min. During this time, an observer scored mice on several anxiety-related variables as identified in previous studies (Shepherd et al., 1994; Tarantino et al., 2000) These included time spent in both open and closed quadrants, number of transitions between quadrants, latency to leave the closed quadrant, stretchings (elongated body posture with at least snout over open/closed divide) into open quadrant, rearings, head-dips. *Drugs:* GS39783 (3, 10, 30 mg/kg, p.o.), chlordiazepoxide (10 mg/kg p.o.) or (vehicle 0.5% methylcellulose) were administered 60 min before the test.

b) Rats

Apparatus: The maze is essentially a larger version of the mouse test and consists of a grey Plexiglas annular platform (105 cm diameter, 10 cm width) elevated to 65 cm above floor and divided equally into four quadrants. Two opposite quadrants were enclosed by grey Plexiglas walls (high: 27 cm for the outer and 20 cm for the inner wall) while the remaining two opposite quadrants were surrounded by a small border (1 cm high). The apparatus was illuminated by white light (150-190 lux). *Experimental Procedure:* After oral drug administration, the animals were returned to their home cage. After an appropriate pre-treatment time (60 min) rats were individually placed into a closed section. A five-minute trial was performed and between subjects the maze was thoroughly cleaned with Thedra (Thedra, Zwingen, Switzerland). Unlike the mouse test, the different parameters, (the time spent in open quadrants, the distance-ratio (expressed as the distance travelled in open quadrants / total distance travelled) and the total distance travelled), were automatically recorded and analyzed by means of an in-house developed video-tracking system and quantified with appropriate software. Briefly, an ordinary black and white camera mounted 2 m above the zero-maze was connected to a frame grabber (Data Translation, Marlboro, USA, type DT3155). Every second the digitized frame was compared with the previously stored frame, whereby the pixels with altered intensity were identified and used to compute the position of the animal and the distance travelled. In addition, the number of stretched attend postures (elongated body posture with at least snout over open/closed divide) into the open quadrants and the number of head dips were scored manually by an experimenter sitting in the room. Trials were performed between 11:30 - 16:30 hr. Unlike the mouse version of the test the latency to exit the closed quadrant was not analysed due to high variability of this parameter in rats (Chaperon, unpublished observations). *Drugs:* Rats were treated with GS39783 (3, 10, 30 mg/kg, p.o.), chlordiazepoxide-HCl (10 mg/kg p.o.) or vehicle (distilled water with 1% Tween 80) 60 min before the test, in a volume of 2 ml/kg.

Effects of GS39783 in the elevated plus maze test of anxiety

Apparatus: The elevated plus-maze consists of two open arms (40 x 12 cm) and two enclosed arms (40 x 12 x 20 cm) which all extend from a common central platform (12 x 12 cm). The configuration forms the shape of a

plus sign, with similar arms arranged opposite to one another and the apparatus is elevated 60 cm above the floor on a central pedestal. The maze is made from grey Plexiglas. The grip on the open arms is facilitated by inclusion of a small raised edge (0.25 cm) around their perimeter. *Drug treatment and experimental procedure*: The method was adopted from Handley and Mithani (1984). Rats were randomly allocated to one of the treatments. Animals were transported from the housing room to the laboratory at least one hour before testing. Following oral drug administration rats were individually housed in macrolon cages (22 x 16 x 14 cm) and after 60 minutes placed onto the central platform facing an enclosed arm. An eight-minute trial was performed and between subjects the maze was thoroughly cleaned with Thedra (Thedra, Zwingen, Switzerland). Direct registrations were made by an observer sitting close to the maze and the following conventional parameters were used: number of open and closed arm entries (arm entry defined as all four paws entering an arm) and time spent on open arms (excluding the central platform), distance traveled on open arms, grooming time and the number of rearings. Additionally, the ratio of open: total arm entries were calculated which takes into account any potential confounding motor effects of compounds. Animals from the different treatment-groups were alternatively tested and trials were performed between 08:30 hr - 12:30 hr, i.e. within the first half of the light phase. *Drug Treatment:* Rats were treated with GS39783 (doses: 0.1, 1, 10 or 100 mg/kg p.o.), chlordiazepoxide (10 mg/kg p.o.), or vehicle (0.5% methylcellulose) (n=15 per group).

The effects of GS39783 on stress-induced hyperthermia test of anxiety

. *Experimental Procedure:* Mice were singly housed in smaller macrolon cages (26 x 21 x 14 cm) 24 hours before testing The test procedure for the modified stress-induced hyperthermia was adopted from Van der Heyden et al. (Van der Heyden et al., 1997) and is based on the original description of stress-induced hyperthermia by Lecci et al.(Lecci et al., 1990): rectal temperature was measured to the nearest 0.1 °C by an ELLAB instruments thermometer (Copenhagen, Denmark) via a lubricated thermistor probe (2 mm diameter) inserted 20 mm into the rectum while the mouse was hand held near the base of the tail. The probe was left in place until steady readings were obtained (approx. 15 seconds). Stress-induced hyperthermia was assessed as follows: The core-temperature of each mouse was measured twice. The second measurement (T2) was 15

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minutes after the first measurement (T1), which served as the basal value for each condition. The dependent variable, i.e. the stress-induced hyperthermia, was defined as the difference between T2 - T1. T1 was used to evaluate whether the test-compound by itself would have a potential effect on basal body temperature. *Drug Treatment:* Sixty minutes before measuring T1, the animals received an oral administration of GS39783 (doses 0.01, 0.1, 1 3, 10 or 30 mg/kg), or vehicle (0.5% methylcellulose).

The effects of GS39783 on ethanol- induced sedation and narcosis and on pentobarbital-induced narcosis

(a) Rotarod studies

Experimental procedures: The rotarod test was carried out as described in Expt 1. During the test day the length of time each mouse remained on the cylinder (= 'endurance time', maximal score 300 sec) was measured 1, 2, and 4 hours after application of GS39783 or vehicle. Treatments: Mice received oral administrations of GS39783 (1, 3, 10, 30, p.o.), chlordiazepoxide (10 mg/kg, p.o.) or vehicle (0.5% methylcellulose, p.o.). Thirty minutes later mice received an injection of ethanol (20%) or vehicle. The dose of ethanol was chosen based on dose-finding studies showing that this concentration had threshold effects on motor impairment (Cryan unpublished observations).

(b) Narcosis Studies

Experimental Procedure: Sleep was induced by either 50 mg/kg i.p. pentobarbital or by 10 ml/kg i.p. of a 40% ethanol-solution (4 g/kg) and quantified by assessing the period between the loss of the righting reflex and its return. A cut-off at 150 min. was applied and those mice which did not fall asleep at all or died (maximally n = 1 per group in the present study) were excluded. Doses of pentobarbital and ethanol were chosen based on previous dose-finding studies (Gentsch unpublished observations), which demonstrated that these doses caused a reliable but sub-maximal sleep to allow for prolongations of the duration of sleep by test-compounds within the 150 sec cut-off period. *Pentobarbital-induced sleeping-time*: Two separate studies were performed; groups of n=15 mice were orally pretreated with (a)1, 10 or 100 mg/kg or (b) 0.3, 3 or 30 mg/kg p.o. GS39783, respectively. Beside the vehicle-treated group (0.5% Methylcellulose) an additional group was pretreated, per

experiment, with 7.5 mg/kg chlordiazepoxide and served as 'positive' standard. Sixty minutes later all mice were treated intraperitoneally with 50 mg/kg pentobarbital (Fluka, Buchs, Switzerland).

Ethanol-induced sleeping-time: Groups of n=15 mice were orally pretreated with vehicle (0.5% Methylcellulose) or with 1, 10 or 100 mg/kg p. GS39783 or 7.5mg/kg chlordiazepoxide. Sixty minutes later all mice were treated intraperitoneally with 4 g/kg ethanol. (Merck, Darmstadt, Germany).

Results

Effects of GS39783 on rotarod and traction test

***Rotarod test*:** GS39783: ANOVA indicated a significance 1 hour after application only ($p < 0.01$). However, posthoc tests found no significance at any dose level (Table.1). L-baclofen: ANOVA indicated a significance 1 hour after application only. The posthoc test indicated that endurance performance was significantly reduced at the dose of 5 mg/kg l-baclofen (Table.1). Chlordiazepoxide: The ANOVA revealed no significant effect ($p = 0.056$) but 1 hour after application rotarod endurance was significantly reduced at the dose of 5 mg/kg as compared to vehicle (Table.1).

***Traction*:** GS39783: All animals treated with any dose of GS39783 (0.1 - 200 mg/kg, p.o.) were successful in the traction test at all time points (Table.1). L-baclofen: the number of successful animals in the traction test was significantly reduced at 1 hour after application of 2.5 and 5 mg/kg ($p < 0.5$ and $p < 0.01$, respectively). Chlordiazepoxide: Following both 5 and 10 mg/kg p.o. chlordiazepoxide all animals were successful in the traction test at all time points (Table.1).

There were no significant effects of any drug at other time-points tested (data not shown)

Effects of GS39783 on core body temperature

Repeated measures ANOVA revealed a significant effect of drug [$F(7,88) = 27.10$, $p < 0.001$], a significant effect of time [$F(4, 352) = 52.97$, $p < 0.001$] and a significant drug treatment x time interaction [$F(28, 352) = 25.90$, $p < 0.001$]. Posthoc analysis revealed that all doses of baclofen (5-15 mg/kg) significantly lowered core body temperature one and two hours following administration, whereas GS39783 did not modify temperature at

any time-point tested. The effects of baclofen on temperature had dissipated four hours following injection (see Figure 1).

Effects of GS39783 on locomotor activity

a) Rats:

GS39783 at doses of 0.1, 1, 10, 100 or 200 mg/kg had no effect on spontaneous locomotor activity in rats. In contrast, a highly significant effect of l-baclofen (5 mg/kg, p.o.) on activity ($p < 0.001$) was found (see Figure 2A).

b) Mice:

ANOVA revealed a significant effect of drug treatment on locomotor activity in mice [$F(8,63)=4.31$, $p = 0.001$]. Posthoc revealed that GS39783 did not affect the locomotor activity (all doses tested). In contrast, l-baclofen induced a marked hypolocomotion at 3 mg/kg and 10 mg/kg ($p< 0.01$ and $p < 0.001$, respectively) (see Figure 2B).

Effects of GS39783 and baclofen on passive avoidance behaviour

a) Mice:

When mice received GS 39783 (1-100 mg/kg) orally 60 min before the training trial it had no effect on latencies to step-through into the dark (shock) compartment on either the training trial or retention test. In all groups a high degree of learning was produced as indicated by prolonged step-through latencies in the retention test. In contrast, chlordiazepoxide, while not affecting latencies in the training trial significantly reduced latencies in the retention test [$F(4,35) = 4.80$, $p = 0.003$], with both the 20 mg/kg and the 40 mg/kg groups differing significantly from the vehicle group [$p < 0.05$, $p < 0.01$ respectively, 2-tailed Dunnett's test]. In the l-baclofen experiment one animal did not enter the shock compartment within the 150-sec limit, and was therefore excluded from the retention test. Like chlordiazepoxide, baclofen did not affect latencies in the training trial but significantly reduced latencies in the retention test [$F(2,26)=4.80$, $p = 0.003$], with both the 20

mg/kg and the 40 mg/kg groups differing significantly from the vehicle group ($p < 0.05$, $p < 0.01$ respectively). (See Figure 3)

b) Rats

Two animals (one from the 3 mg/kg baclofen group and one from the 40 mg/kg chlordiazepoxide group) failed to enter the dark compartment within the 150-sec limit on the training day, and were excluded from the experiment. One-way ANOVA of training latencies showed no significant effect of treatment group. However there was a significant effect of treatment group ($F(6,59)=4.04$, $p = 0.002$) on retention test latencies, with both chlordiazepoxide groups showing shorter latencies than the control group ($p < 0.05$ and $p < 0.01$ for the 20 and 40 mg/kg groups respectively) (Figure 4).

The effects of GS39783 on behaviour in the elevated zero maze test of anxiety

(a) Mice

Latency: The ANOVA indicated that a significant effect of treatment [$F(5,63) = 2.82$; $p = 0.023$] (Figure 5a). Post hoc revealed a decrease of latency to enter in open quadrants only in chlordiazepoxide (10 mg/kg) treated mice (Figure 5a). *Time spent in open quadrants:* As shown in Fig 4b, ANOVA [$F(5,63) = 10.36$; $p < 0.001$] revealed that treatment with chlordiazepoxide (10 mg/kg) increased time spent in open quadrant. In contrast, GS39783 had no effect on this parameter (Figure 5b). *Number of line crossing:* ANOVA revealed a significant effect of dug treatment on the number of line crossings during the test [$F(5,63) = 19.79$; $p < 0.001$]. Only chlordiazepoxide (10 mg/kg), not GS39783, increased this parameter ($p < 0.001$) (data not shown). *Number of stretched attend postures:* As indicated by the ANOVA [$F(5,63) = 13.89$; $p < 0.001$], treatment with GS39783 induced an overall effect on number of stretched attend posture (Figure 5c). Posthoc tests revealed that GS39783 at 10 mg/kg and 30 mg/kg significantly decreased the number of stretched attend posture ($p = 0.004$ and $p = 0.001$). Chlordiazepoxide (10 mg/kg) also significantly decreased this parameter ($p < 0.001$). *Number of head dips:* ANOVA indicated an inter-group difference for the number of head dips [$F(5,63) = 5.622$; $p < 0.01$]. Posthoc comparisons revealed that GS39783 had no effect on the number of head dips. Chlordiazepoxide (10 mg/kg) induced a significant increase in the number of head dips ($p< 0.001$) (data not shown). *Number of*

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rearing: The ANOVA indicated that treatment significantly modified the number of rearings [$F(5,63) = 4.162$; $p = 0.002$]. Posthoc tests revealed that GS39783 at 30 mg/kg significantly increased the number of rearing ($p= 0.024$). Chlordiazepoxide (10 mg/kg) also significantly increased this parameter ($p< 0.001$) (data not shown).

(b) Rats

Time spent in open quadrants: As shown in Fig 6a, ANOVA [$F(3,36) = 5.09$; $p < 0.01$] revealed that treatment with GS39783 induced an overall effect on the time spent in open quadrants. Subsequent analysis indicated that all tested doses of GS39783 (3, 10, 30 mg/kg) produced a significant increase in the time spent in open quadrants ($p < 0.001$, $p < 0.01$). As expected for a positive standard, chlordiazepoxide (10 mg/kg) also significantly increased the time spent in open quadrants ($p< 0.01$). *Total distance traveled:* ANOVA revealed no significant effect of GS39783 on the total distance traveled during the test. In contrast, chlordiazepoxide (10 mg/kg) significantly increased this parameter ($p< 0.01$) (Figure 6d). *Distance-ratio:* As indicated by the ANOVA [$F(3,36) = 3.02$; $p < 0.05$], treatment with GS39783 induced an overall effect on the distance-ratio. Posthoc tests revealed that GS39783 at 3 mg/kg significantly increased the distance-ratio ($p < 0.01$) and that the dose of 30 mg/kg tended to raise this parameter ($p = 0.066$). Chlordiazepoxide (10 mg/kg) also significantly increased the distance-ratio ($p< 0.01$). *Number of stretched attend postures:* ANOVA indicated an intergroup difference for the number of stretched attend postures [$F(3,36) = 2.91$; $p < 0.05$]. Posthoc comparisons revealed that GS39783 significantly reduced the number of stretched attend postures at the doses of 3 and 30 mg/kg ($p < 0.05$). chlordiazepoxide (10 mg/kg) also induced a significant decrease in the number of stretched attend postures ($p< 0.001$). *Number of head dips:* The ANOVA indicated that GS39783 significantly modified the number of head dips [$F(3,36) = 5.945$; $p < 0.01$]. Posthoc tests revealed that GS39783 at 3 and 30 mg/kg significantly increased the number of head dips ($p = 0.001$ and $p < 0.01$, respectively). Chlordiazepoxide (10 mg/kg) also significantly increased this parameter ($p< 0.001$)(see Figure 6c).

Effects of GS39783 in the elevated plus maze test of anxiety

Ratio: ANOVA indicated a highly significant effect for GS39783 on ratio ($F = 5.849$, $p < 0.001$): all tested doses of GS39783, i.e. 0.1, 1, 10 or 100, induced a significant increase in ratio as compared to vehicle (Fig. 7a).

Chlordiazepoxide (10 mg/kg), i.e. the positive standard, also significantly increased the ratio ($p < 0.001$; Fig. 7a). Total arm entries: The ANOVA indicated no significant effect for GS39783 on the total number of arm entries. Chlordiazepoxide (10 mg/kg), significantly increased the number of open arm entries ($p < 0.01$; Fig. 7b). *Time on open arms:* The ANOVA indicated a highly significant effect for GS39783 on the time spent on open arms ($F=5.868$, $p < 0.001$): all tested doses of GS39783, i.e. 0.1, 1, 10 or 100, induced a significant increase in time on open arms as compared to vehicle (Fig. 7c). Chlordiazepoxide (10 mg/kg) also significantly increased the time spent on open arms ($p < 0.001$; Fig. 7c). *Open arm entries:* The ANOVA indicated a highly significant effect for GS39783 on the number of open arm entries ($F = 4.708$, $p < 0.01$): all tested doses of GS39783, i.e. 0.1, 1, 10 or 100, induced a significant increase in number of open arm entries as compared to vehicle (Fig. 7d). Chlordiazepoxide (10 mg/kg), also significantly increased the number of open arm entries ($p < 0.001$; Fig. 7d). *Distance traveled on open arms:* The ANOVA indicated a highly significant effect for GS39783 on the distance traveled on open arms ($F = 5.185$, $p < 0.001$): all tested doses of GS39783, i.e. 0.1, 1, 10 or 100, induced a significant increase in distance traveled on open arms as compared to vehicle. Chlordiazepoxide (10 mg/kg), also significantly increased the distance traveled on open arms ($p < 0.001$; data not shown).

The effects of GS39783 on stress-induced hyperthermia test of anxiety

ANOVA revealed a significant effect of treatment on the magnitude of the SIH response [$F(7, 136) = 14.068$, $p < 0.001$]. Posthoc analysis revealed that the positive control chlordiazepoxide (10 mg/kg, p.o.) and GS39783 (0.1, 1, 10, 30 mg/kg) significantly reversed the SIH response (Figure 8b). Analysis of basal temperature (T1) revealed a significant effect of treatment on core body temperature [$F(7, 136) = 3.11$, $p < 0.01$]. Posthoc analysis revealed that chlordiazepoxide induced a slight but significant increase in body temperature. In confirmation of experiment 2, GS39783 at any dose tested did not alter baseline body temperature (Figure 8a).

The effects of GS39783 on ethanol induced sedation and narcosis and on pentobarbital-induced sleep

(a) Rotarod studies

All of the animals were successfully trained on the rotarod and completed the test prior to injection. Repeated measures ANOVA detected a significant effect of drug treatment [$F(5,108) = 34.407$, $P < 0.001$]; a significant effect of ethanol administration [$F(1,108) = 37.422$, $P < 0.001$]; a significant drug treatment x ethanol administration [$F(5,108) = 29.081$, $P < 0.001$]; a significant effect of time [$F(2,216) = 33.520$, $P < 0.001$]; and a significant time x drug treatment x ethanol administration [$F(10,216) = 9.165$, $P < 0.001$. Posthoc analysis revealed that only animals treated with chlordiazepoxide and ethanol in combination had a marked reduction in endurance on the rotarod. This was maximal one hour following compound administration but was persistent at two hours post injection. There was no significant effect four hours following administration. These data demonstrate that there is no deleterious interaction between GS39783 and ethanol (See Figure 9).

(b) Narcosis Studies

Irrespective of whether using pentobarbital or ethanol as sleep-inducer, GS39783 did not cause any significant change in the sleep-duration up to the 100 mg/kg p.o. dose tested. The lack of effect in the 2nd experiment with pentobarbital also ruled out that the trend (not significant) seen following 10 mg/kg GS39783 in the 1st study, was meaningful. In contrast, chlordiazepoxide in all three experiments significantly prolonged the time spent asleep ($p < 0.05$ (Dunn's test vs. vehicle-group, following significant ANOVA on ranks (in all three studies). These data points to superiority of GS39783 over chlordiazepoxide, with regard to its lack of hypnotic potential at anxiolytic doses, and/or its potential to interact with alcohol (Table 2).

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Discussion

In these studies we sought to behaviourally characterize the prototypical $GABA_B$ receptor modulator GS39783, in paradigms for assessing motor activity, body temperature, cognition and anxiety. Our data demonstrate that GS39783 is devoid of many of the effects associated with either full $GABA_B$ receptor agonists and/or benzodiazepines and additionally it displays an anxiolytic profile in a number of rodent models. Taken together, these studies clearly demonstrate that $GABA_B$ receptors play a major role in the modulation of behaviours relevant to anxiety and suggest that positive modulation is a novel approach to probe a role for $GABA_B$ receptors in behavioural processes.

In tests of motor ability (rotarod, locomotor activity) GS39783 is devoid of any sedation compared with baclofen or the anxiolytic agent chlordiazepoxide at doses far above what is active in other behavioural models. Complimentary data were also obtained using the beam balance test of fine motor behaviour and loaded grid test of muscle strength/relaxation (unpublished observations). This demonstrates that use-dependent activation of $GABA_B$ receptors fails to compromise locomotor ability and that GS39783 is not a muscle relaxant. Further, these data strongly suggest that the therapeutic benefits of positive modulators may be greater than that of full agonists for the treatment of disorders were sedation and muscle relaxation may be undesired side-effects. However, these data also suggest that $GABA_B$ receptor positive modulators may not be useful treatments for disorders associated with spasticity, whereas baclofen has been therapeutically successful in this indication for over 30 years (Brogden et al., 1974). GS39783 also differed from baclofen in that it had no intrinsic influence on body temperature, whereas baclofen induces a marked hypothermia. This lack of sedation, muscle relaxation and hypothermia positions GS39783 as a very useful tool for investigating $GABA_B$ receptor function in various behavioural and physiological paradigms.

The full agonist baclofen and the positive modulator GS39783, also differed on their influence on cognitive function. In the mouse passive avoidance cognition paradigm baclofen disrupted the performance at doses as low as 1 mg/kg whereas GS39783 did not have any deleterious effects on cognitive performance in the test. Further, the anxiolytic chlordiazepoxide also negatively affected passive avoidance behaviour. In the rat

version of the test GS39783, unlike chlordiazepoxide, was without effect on cognitive performance. Interestingly, no significant deleterious effects of baclofen on cognitive function were seen at the doses tested in the rat passive avoidance test. Reasons for this are presently unclear. Higher doses of baclofen maybe required to disrupt performance in the rat test but we were precluded from using higher doses due to the sedative side effects of baclofen emerging (Kelly unpublished observations) (also see Figure 2). Overall, these data are consistent with previous reports demonstrating cognitive impairing effects of both baclofen and chlordiazepoxide on passive avoidance behaviour (Swartzwelder et al., 1987; Castellano et al., 1989; Zarrindast et al., 2002). Further, they exemplify the better side-effect profile of $GABA_B$ receptor positive modulators over that of full agonists and benzodiazepine anxiolytics

Recently we have shown that mice lacking the $GABA_B$ receptors exhibit an increase in anxiety behaviours (Mombereau et al., 2004), suggesting that activation of $GABA_B$ receptors may decrease anxiety. However, previous data investigating $GABA_B$ mechanisms in anxiety are limited and rather variable; this is largely because investigators relied on using baclofen for such analysis. Baclofen has a narrow efficacy window before confounding side-effects are manifested in anxiety paradigms, e.g. in the elevated plus maze it enhanced time spent in the center of the maze i.e. neither in the open arms or closed arms which is uninterpretable in terms of influencing anxiety (Dalvi and Rodgers, 1996). Nonetheless, baclofen has demonstrated anxiolytic-like effects in a number of tests. It reduced separation induced-calling by mouse pups (Nastiti et al., 1991) and enhanced punished drinking in rats (Ketelaars et al., 1988; Shephard et al., 1992) and had an anxiolytic-like response to novelty in a T-Maze (Quintero et al., 1985). Further, baclofen also reversed the anxiogenic response induced by withdrawal from chronic diazepam or alcohol treatment (File et al., 1991; File et al., 1992; Andrews and File, 1993). Clinically baclofen reversed the anxiety associated with alcohol withdrawal (Addolorato et al., 2002) and post traumatic stress (Drake et al., 2003) and panic disorder (Breslow et al., 1989).

Because of the abovementioned data on baclofen we tested GS39783 in a variety of anxiety models. We first tested the effects of GS39783 in a mouse elevated zero maze because it has been reported that baclofen enhanced time in the center of the more widely-used elevated plus maze in mice (Dalvi and Rogers, 1996). The

zero maze has no central area, so the animal must be in either an open or a closed part of the arena, which obviates these potential problems (Shepherd et al., 1994). GS39783 had a mild anxiolytic effects in the test (non-significant increases in time spent in open quadrants; significant reduction in stretch-attend postures) and these effects were not as robust as the anxiolytic chlordiazepoxide. We recently reproduced these mild effects in another strain of mouse, BALB/c (Mombereau et al., 2004). Therefore, we assessed whether these effects of GS39783 were species specific; by testing this compound in the rat version of the test. Interestingly, in the rat elevated zero maze, GS39783 had a much more pronounced anxiolytic-like profile (increased time in open quadrants; reduction in stretch-attend postures and increase in head dips); similar to that of chlordiazepoxide. It is unlikely that a pharmacokinetic explanation underlies the more marked effects in rat versus mouse elevated zero maze, as GS39783 is very effective in the mouse SIH test (Fig 8) and mouse light-dark box test (Mombereau et al., 2004). Nonetheless, these data prompted us to test GS39783 in the more commonly used test of anxiety behaviour, the elevated plus maze in the rat. In this test GS39783 has an anxiolytic profile similar to the benzodiazepine chlordiazepoxide (increased time in open arms; number of entries onto open arms and the ratio of open to closed arm entries). Further, evidence for anxiolytic-like potential of GS39783 arises from the fact that it attenuates stress-induced hyperthermia in mice, albeit not as markedly as chlordiazepoxide. This paradigm gives a parametric analysis of the physiological response to anxiety (in this case the anticipatory anxiety caused by an acute stressor) and has been validated extensively as a preclinical paradigm useful to detect conventional and putative anxiolytics (Spooren et al., 2002; Olivier et al., 2003). In addition to the data presented here we also have recently shown that GS39783 (at doses similar to that employed here) has anxiolytic effects following acute administration in the light-dark box anxiety test (Mombereau et al., 2004) and that these effects persist over 21 days, suggesting that there is no obvious tolerance to its anxiolytic effects.

In order to be effectively marketed as an anxiolytic any new chemical entity must show a superior side-effect profile to benzodiazepines. In addition to sedation, cognitive effects and tolerance, one of the primary problems associated with benzodiazepine use is the potentially deleterious interaction with ethanol (Hollister, 1990; Tanaka, 2002). Therefore, we compared the effects of GS39783 with chlordiazepoxide on ethanol-

induced changes in two different paradigms- rotarod and sleeping time. To confirm that the effect was not simply pharmacokinetic, we also assessed the effects of both classes of compound on pentobarbital-induced sleep. Chlordiazepoxide at doses that was without effects itself significantly enhanced the ability of ethanol to inhibit motor performance on a rotarod and potentiated both ethanol and pentobarbital induced sleeping time. On the other hand GS39783 was without effect on either of these parameters.

In conclusion, our data suggest that the $GABA_B$ receptor positive modulator GS39783 has a different behavioural profile compared to that of full agonists, which engender it as a more favorable tool for behavioural or physiological analyses. Further, $GABA_B$ receptor positive modulators may be a novel avenue for the development of an innovative class of anxiolytic agent devoid of many of the serious side-effects associated benzodiazepines. Furthermore, it is tempting to speculate that these positive modulators may be a useful and innovative therapeutic strategy for certain disorders, such as addiction and nicotine dependence, where there is ample evidence that $GABA_B$ receptors play a role, yet baclofen's side-effects have thus far limited major therapeutic advances (Brebner et al., 2002; Cryan et al., 2003).

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Footnotes

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Figure Legends

Figure 1. The effects of GS39783 and baclofen on core body temperature of mice. (A) Effect of acute $GABA_B$ positive modulators (25, 10, 100, 200 mg/kg; p.o) and (B) l-baclofen (5, 10, 15 mg/kg; p.o.) on core body temperature in mice (n = 12). Values are means ± SEM. *,***groups that differed significantly to vehicle treated animals ($P < 0.05$, and $P< 0.001$, respectively).

Figure 2. The effects of GS39783 and baclofen on locomotor activity in rats and mice. (A) Effect of acute $GABA_B$ positive modulators (0.1, 1, 10, 100, 200 mg/kg; p.o) and l-baclofen (BAC) (5 mg/kg; p.o.) on locomotor activity in rats (n = 15-16) compared with vehicle (VEH) treated animals. (B) Effect of acute $GABA_B$ positive modulators (1, 3, 10, 30, 100 -mg/kg; p.o) and L-Baclofen (1, 3, 10mg/kg; p.o) on locomotor activity in mice (n = 8) compared with vehicle (VEH) treated animals. Values are means ± SEM. *,**,***groups that differed significantly to vehicle treated animals ($P < 0.05$, $P < 0.01$ and $P< 0.001$, respectively).

Figure 3. The effects of GS39783, baclofen and chlordiazepoxide on passive avoidance behaviour in mice. $GABA_B$ positive modulator GS39783 (1, 3, 10, 30, 100 mg/kg; p.o.) didn't affect latencies to step-through into the dark during training (A) and retention (B) sessions compared with vehicle (VEH) treated animals. Conversely, l-baclofen (1, 3 mg/kg; s.c.) and chlordiazepoxide (20, 40 mg/kg; p.o.) treatment induced a decrease of latencies to step-through into the dark during retention session (D and F respectively) compared with vehicle (VEH) treated animals, but not in training session (C and E respectively) compared with vehicle (VEH) treated animals. GS39783 experiment: n =10 per treatment group. Baclofen experiment: n = 10 per treatment group. Chlordiazepoxide experiment: n = 8 per treatment group. All values represent mean values, with vertical lines indicating one SEM. *,**groups that differed significantly to vehicle treated mice ($p < 0.05$ and $P< 0.01$, respectively).

Figure 4. The effects of GS39783, baclofen and chlordiazepoxide (CDZ) on passive avoidance behaviour in rats. The $GABA_B$ positive modulator GS39783 (10, 30, 100 mg/kg; p.o.) and l-baclofen (1, 3 mg/kg; p.o.) didn't affect latencies to step-through into the dark during training (A) and retention (B) sessions compared with vehicle (VEH) treated animals. However, chlordiazepoxide (20, 40 mg/kg; p.o.) treatment induced a decrease of latencies to step-through into the dark during retention session (A), but not in training session (B) compared with vehicle (VEH) treated animals. (n= 7-11). *, ** = groups that differed significantly to vehicle treated mice ($p < 0.05$ and $P< 0.01$, respectively).

Figure 5. The effects of GS39783 and chlordiazepoxide on behaviour in the mouse elevated zero maze. (A) Latency Only chlordiazepoxide (CDZ) significantly decreased the latency to enter into the open quadrants compared with vehicle (VEH) treated animals. (B) Time in open Only chlordiazepoxide (CDZ) significantly decreased the latency to enter into the open quadrants compared with vehicle (VEH) treated animals. (C) Stretched Attend Postures Both the $GABA_B$ positive modulator GS39783 and chlordiazepoxide (10 mg/kg, p.o.) decreased the number of stretched attend postures compared with vehicle (VEH) treated animals. $n = 12$ per treatment group. All values represent mean values, with vertical lines indicating one SEM. **,*** groups that differed significantly to vehicle treated mice ($p < 0.01$ and $p < 0.001$, respectively).

Figure 6. The effects of GS39783 and chlordiazepoxide on behaviour in the rat elevated zero maze (A) Time open Values represent mean (± SEM) of time (s) spent in open quadrants during the 5 min test. Drugs were administered p.o. 60 min before the test (n=9/10 rats per group). * $p < 0.05$; *** $p < 0.001$ versus vehicle (VEH); Fisher's posthoc tests after ANOVA. ## $p < 0.01$ versus vehicle; Student's t-test. (B) Stretch-Attend Postures Values represent mean (± SEM) of number of stretched attend postures made by animals during the 5 min test. Drugs were administered p.o. 60 min prior the test (n=9/10 rats per group). * $p < 0.05$ versus vehicle (VEH); Fischer's posthoc tests after ANOVA. ### $p < 0.001$ versus vehicle (VEH); Student's t-test. (C) Head

dips Values represent mean (± SEM) of number of head dips made by animals during the 5 min test. Drugs were administered p.o. 60 min prior the test. ** $p < 0.01$; *** $p < 0.001$ versus vehicle (VEH); Fisher's posthoc tests after ANOVA. ### $p < 0.001$ versus vehicle; Student's t-test. (D) Total distance travelled Values represent mean (± SEM) of total distance (cm) travelled during the 5 min test. Drugs were administered p.o. 60 min before the test (n=9/10 rats per group). ** $p < 0.01$ versus vehicle; Fisher's posthoc tests after ANOVA. ## $p < 0.01$ versus vehicle (VEH); Student's t-test.

Figure 7. The effects of GS39783 and chlordiazepoxide on behaviour in the rat elevated plus maze. Both the effects of acute $GABA_B$ positive modulator GS39783 (0.1, 1, 10, 10mg/kg; p.o.) and chlordiazepoxide (CDZ) (10 mg/kg, p.o.) affected (A) the ratio, (C) the time spent in open arms and (D) number of open arm entries compared with vehicle (VEH) treated animals. In contrast, only chlordiazepoxide (10 mg/kg; p.o.) increased the total number of arm entries (B) compared with vehicle (VEH) treated animals. $n = 15$ per treatment group. All values represent mean values, with vertical lines indicating one SEM. *,**,***groups that differed significantly to vehicle treated rats ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively).

Figure 8. The effects of GS39783, and chlordiazepoxide on stress-induced hyperthermia in mice. (A) The effects of GS39783, chlordiazepoxide (CDZ) or vehicle (VEH; methylcellulose) on basal temperature 60 minutes following injection Values represent mean and s.e.m. (n= 12-24 animals). *** = $P \leq 0.001$ versus control group (Vehicle) (Dunnett's posthoc test after ANOVA). (B) SIH was assessed as the difference in body temperature 15 minutes after stress (the taking of rectal temperature is the stressor) compared to baseline temperature which was determined 60 min after subjects were treated (p.o.) with GS39783, chlordiazepoxide (CDZ) or vehicle (VEH, methylcellulose). Values represent mean and s.e.m. (n= 12-24 animals). * = $P \leq 0.05$, ** = $P \leq 0.01$; *** = $P \leq 0.001$ versus control group (Vehicle) (Dunnett's posthoc test after ANOVA).

Figure 9. The effects of GS39783, and chlordiazepoxide on ethanol induced alterations in rotarod performance in mice. Effects of GS39783 (0, 1, 3, 10, 30 mg/kg; p.o.) and Chlordiazepoxide (10 mg/kg; p.o.) on rotarod endurance in (a) vehicle treated mice and (b) ethanol (20%) treated mice. Values represent mean and s.e.m. n = 10 per treatment group. *** groups that differed significantly to vehicle treated mice (P< 0.001).

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Tables

Table 1:

Drug	Rotarod endurance (Sec)	Traction test % successful
Vehicle	300 + 0	100 + 0
GS39763		
0.1 mg/kg	300 + 0	
1 mg/kg	300 + 0	100 + 0
10 mg/kg	300 + 0	100 + 0
50 mg/kg	300 + 0	100 + 0
100 mg/kg	300 + 0	100 + 0
200 mg/kg	231 + 37	100 + 0
Baclofen		
2.5 mg/kg	214 + 38	50*
5 mg/kg	171 + 43*	30**
Chlordiazepoxide		
5 mg/kg	208 + 34	100 + 0
10 mg/kg	236 + 33	100 + 0

Table 1. Effects of GS39783, baclofen and chlordiazepoxide on motor performance in the rotarod and traction test. Effects of GS39783 (0, 1, 3, 10, 30 mg/kg; p.o.), l-baclofen and chlordiazepoxide (10 mg/kg; p.o.) on rotarod endurance traction and core body temperature in mice Values represent mean and s.e.m. n = 10 per treatment group. * = $P < 0.05$; ** = $P < 0.01$ versus vehicle treated mice

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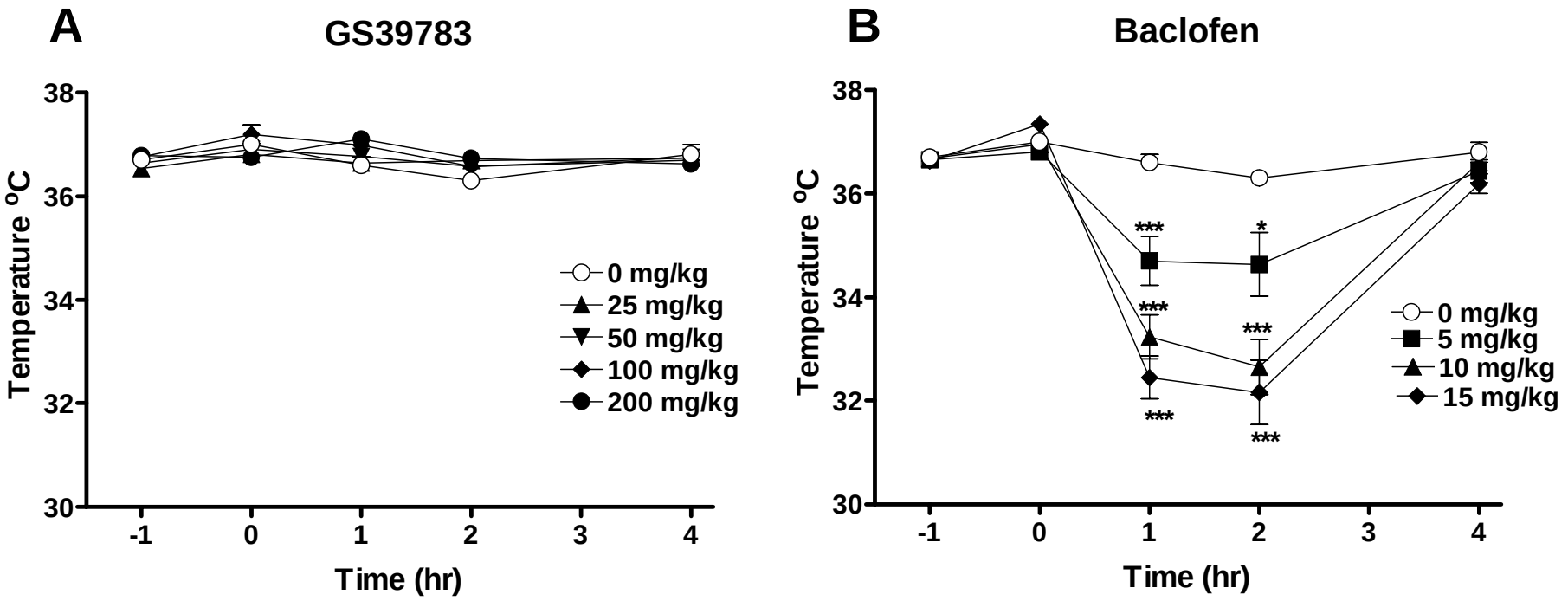
Table 2:

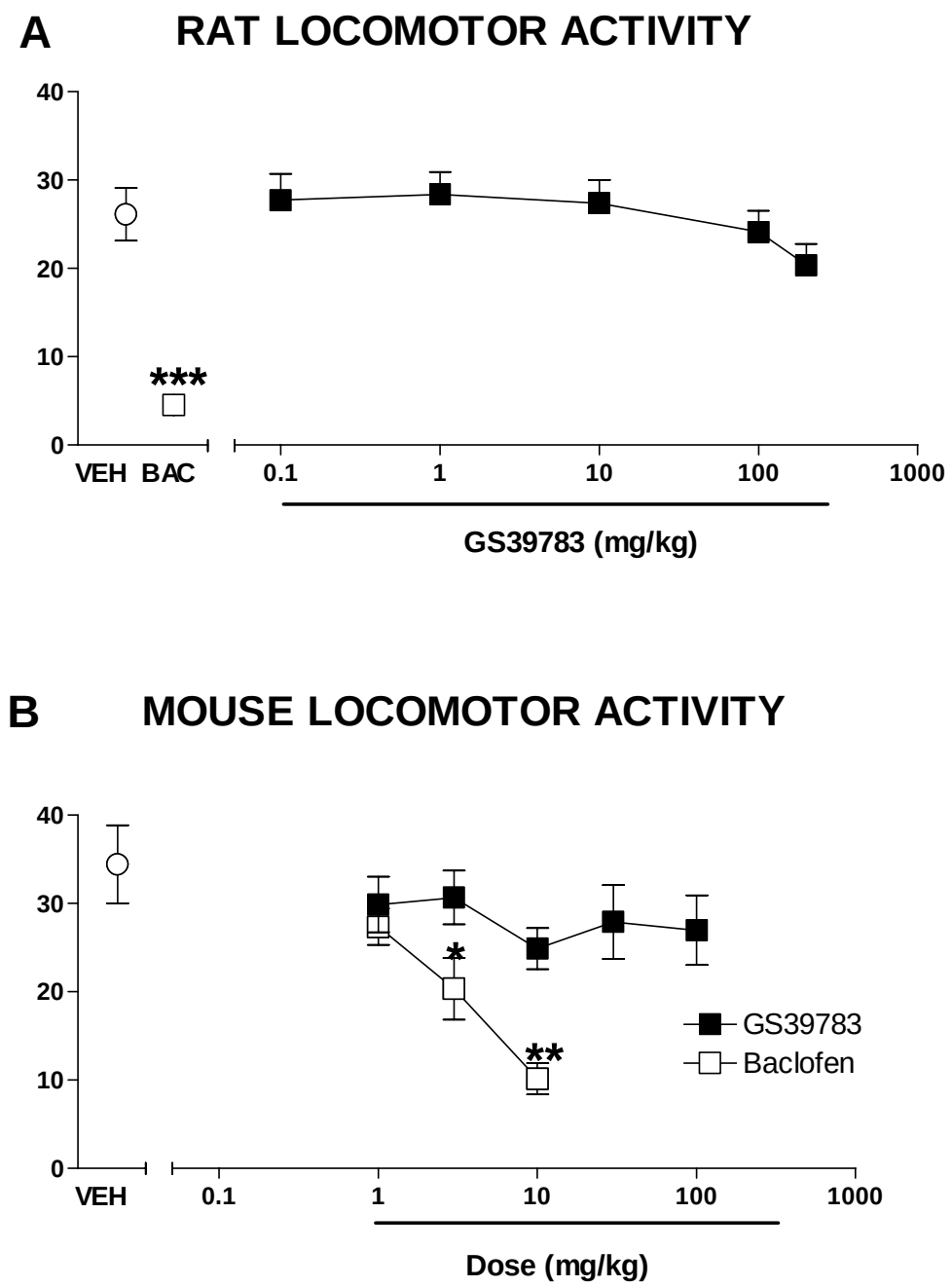
Pretreatment Drug (-60 min)	Experiment. A (50.mg/kg i.p. PB)	Experiment B (50 mg/kg i.p. PB)		Experiment C (4 g/kg i.p. EtOH)
Vehicle	61 ± 3	44 ± 6		18 ± 2
GS39783 1 mg/kg	56 ± 3	----		17 ± 2
GS39783 3 mg/kg	---	58 ± 6		---
GS39783 10 mg/kg	78 ± 8	----		21 ± 2
GS39783 30 mg/kg	----	51 ± 6		----
GS39783 100 mg/kg	59 ± 4	-----		14 ± 2
CDZ 7.5 mg/kg	83 ± 7 *	91 ± 8 *		96 ± 11 *

Table 2. **The effects of GS39783, and chlordiazepoxide (CDZ) on ethanol and pentobarbital (PB)-induced sleeping time in mice** Values are means ± SEM of n=14-15 mice per treatment group; CDZ: chlordiazepoxide; PB: pentobarbital; EtOH: ethanol *: $p < 0.05$ (Dunn's test vs. vehicle-group, following significant ANOVA on ranks (in all three studies).

JPET #66753 Cryan et al., Figure 1.



JPET #66753 Cryan et al., Figure 2.



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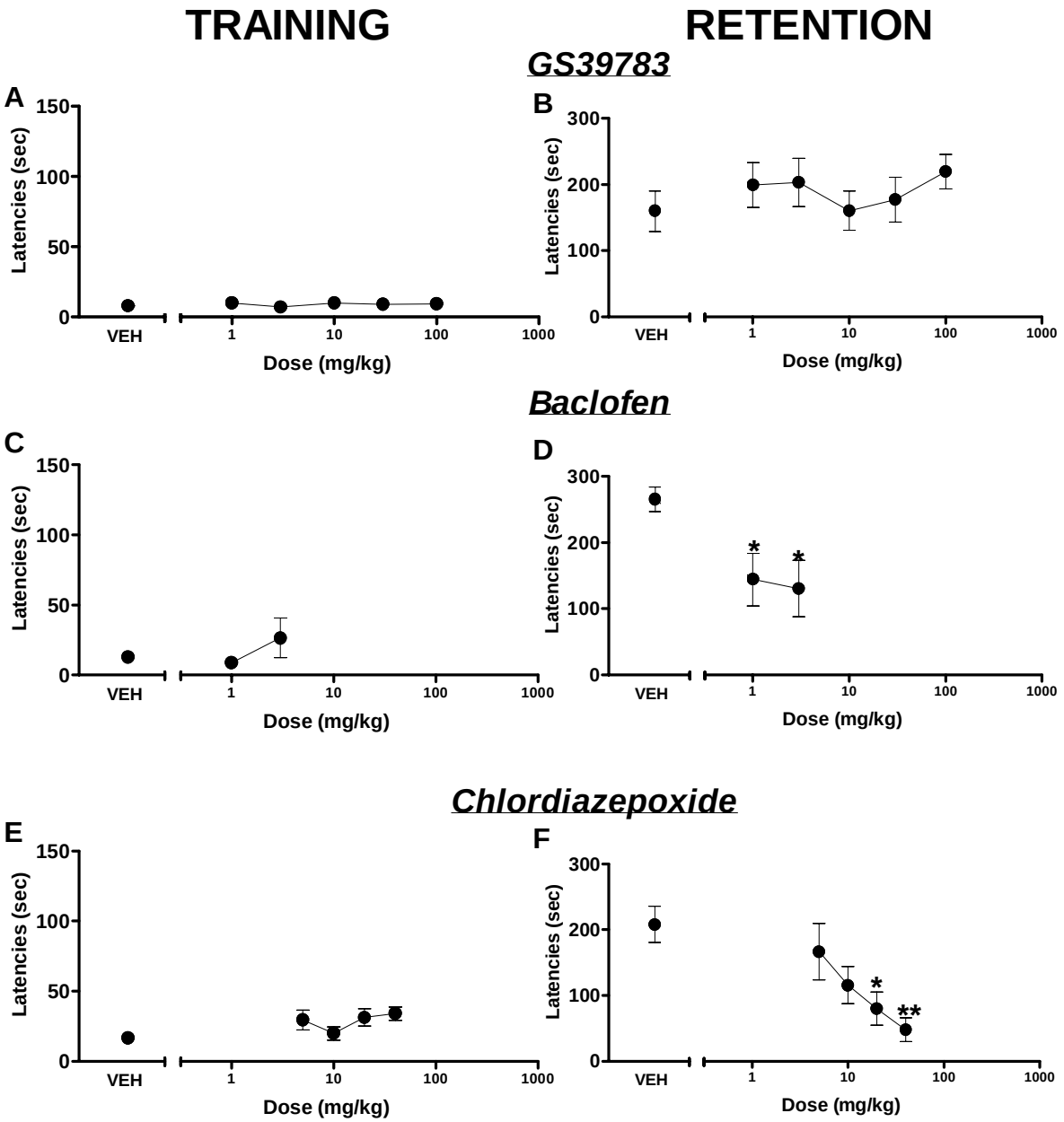

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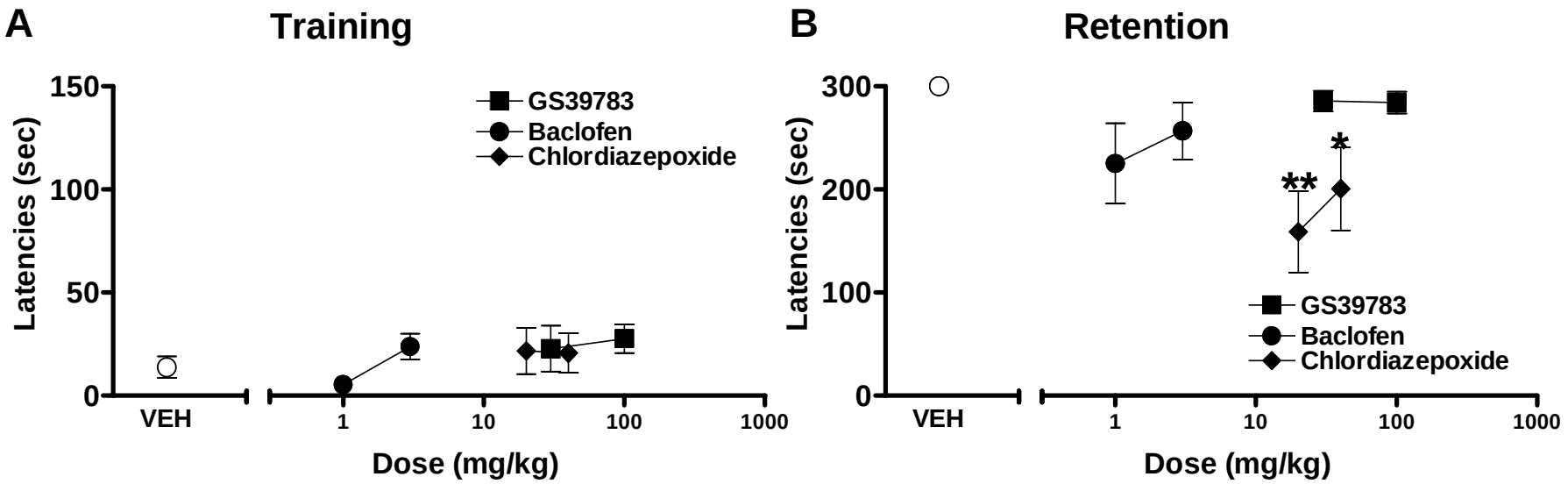

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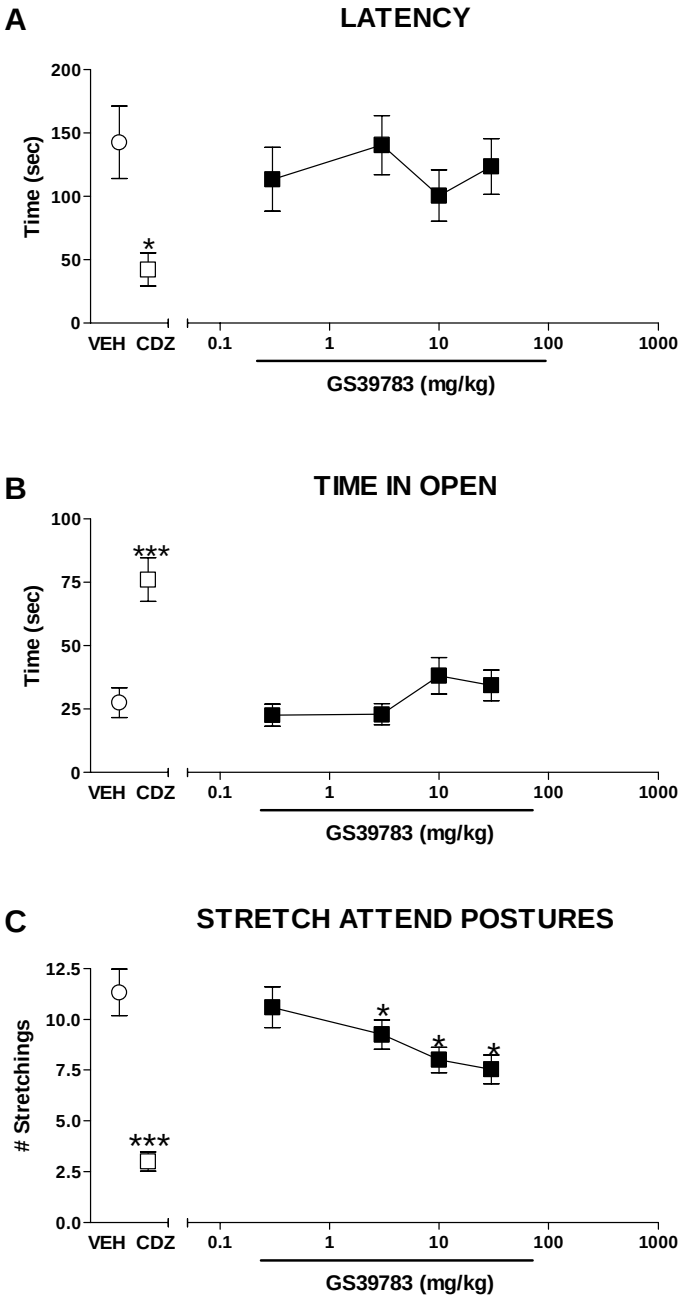

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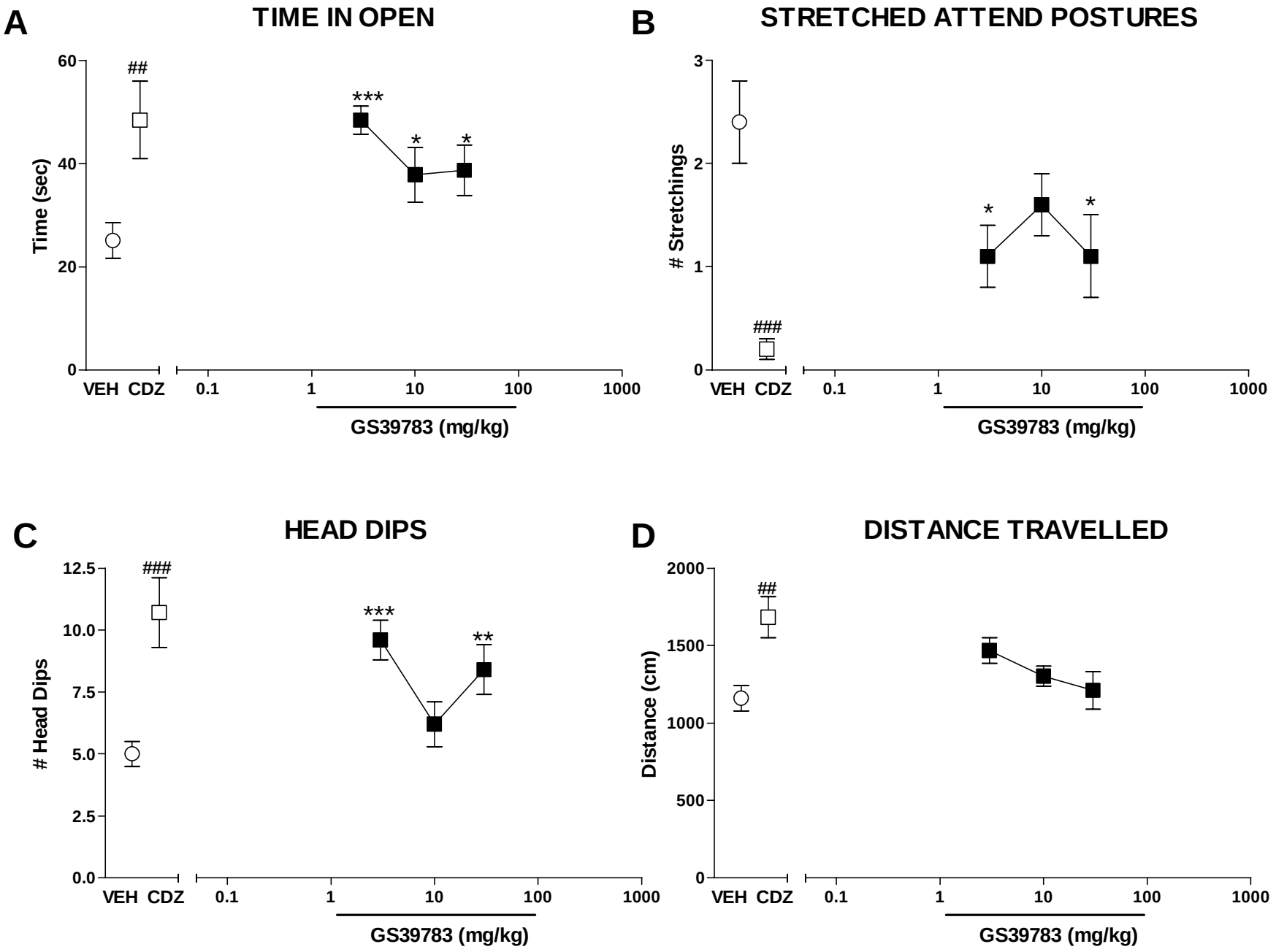

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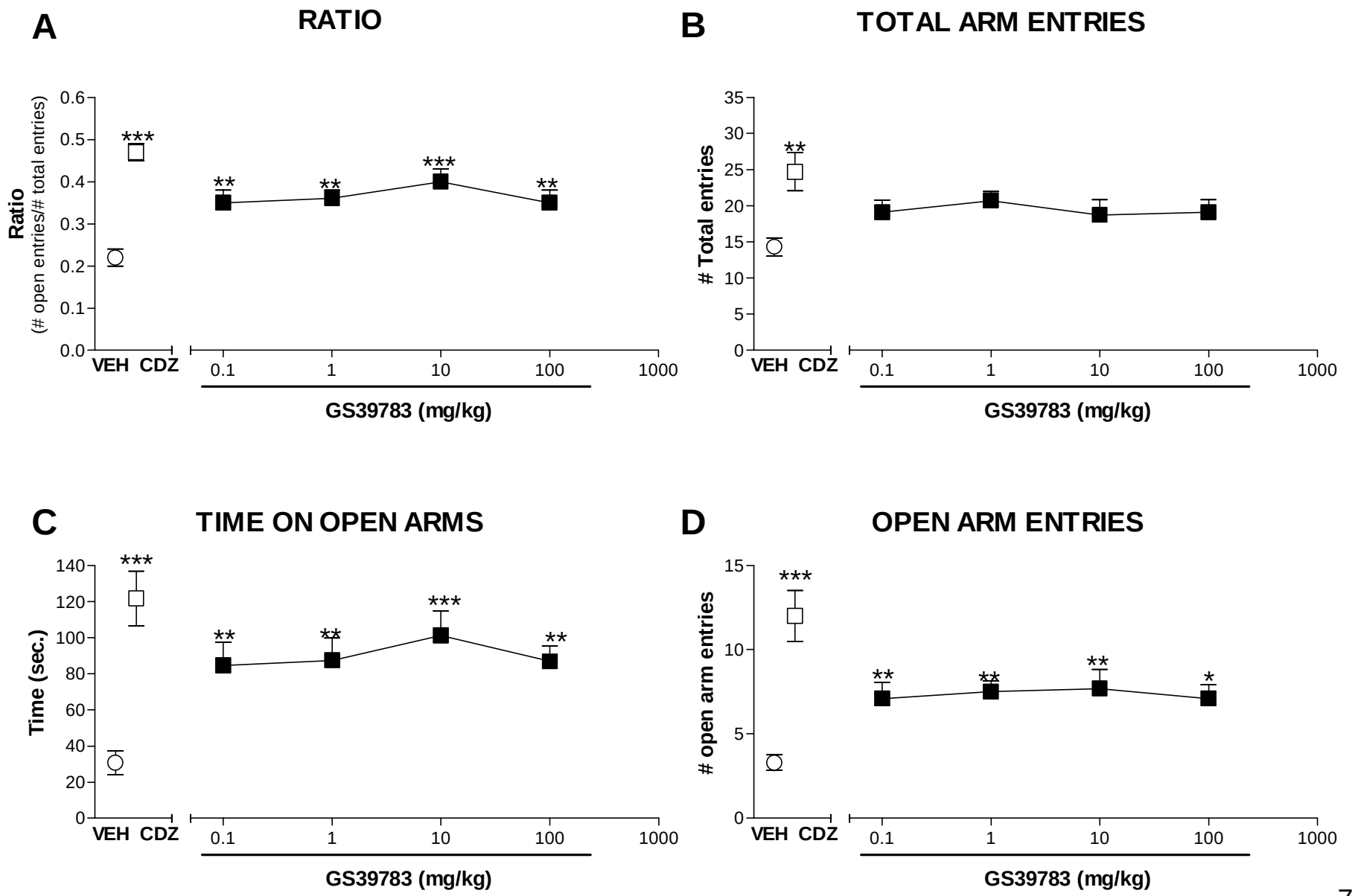

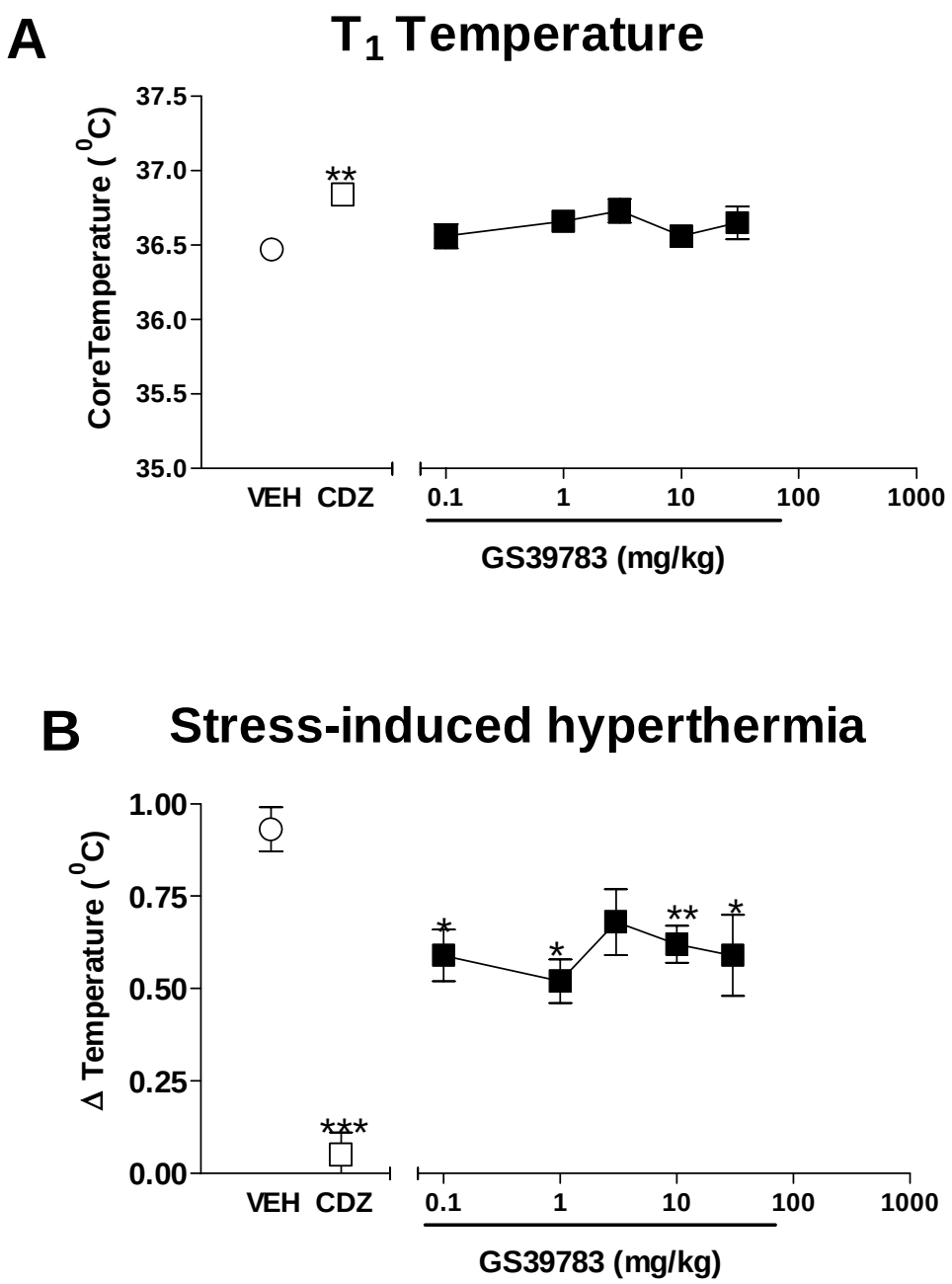

JPET #66753 Cryan et al., Figure 9.