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RESEARCH PAPER

Role of 5-HT$_1$ receptor subtypes in the modulation of pain and synaptic transmission in rat spinal superficial dorsal horn

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BACKGROUND AND PURPOSE

5-HT receptor agonists have variable nociceptive effects within the spinal cord. While there is some evidence for 5-HT$_{1A}$ spinally-mediated analgesia, the role of other 5-HT$_1$ receptor subtypes remains unclear. In the present study, we examined the spinal actions of a range of 5-HT$_1$ agonists, including sumatriptan, on acute pain, plus their effect on afferent-evoked synaptic transmission onto superficial dorsal horn neurons.

EXPERIMENTAL APPROACH

For *in vivo* experiments, 5-HT agonists were injected via chronically implanted spinal catheters to examine their effects in acute mechanical and thermal pain assays using a paw pressure analgesymeter and a Hargreave's device. For *in vitro* experiments, whole-cell patch-clamp recordings of primary afferent-evoked glutamatergic EPSC were made from lamina II neurons in rat lumbar spinal slices.

KEY RESULTS

Intrathecal (i.t.) delivery of the 5-HT$_{1A}$ agonist R ± 8-OH-DPAT (30–300 nmol) produced a dose-dependent thermal, but not mechanical, analgesia. Sumatriptan and the 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1F}$ agonists CP93129, PNU109291 and LY344864 (100 nmol) had no effect on either acute pain assay. R ± 8-OH-DPAT (1 μM) and sumatriptan (3 μM) both reduced the amplitude of the evoked EPSC. In contrast, CP93129, PNU109291 and LY344864 (0.3–3 μM) had no effect on the evoked EPSC. The actions of both R ± 8-OH-DPAT and sumatriptan were abolished by the 5-HT$_{1A}$ antagonist WAY100635 (3 μM).

CONCLUSIONS AND IMPLICATIONS

These findings indicate that the 5-HT$_{1A}$ receptor subtype predominantly mediates the acute antinociceptive and cellular actions of 5-HT$_1$ ligands within the rat superficial dorsal horn.

Abbreviations

ACSF, artificial cerebrospinal fluid; CNQX, 6-cyano-2,3-dihdroxy-7-nitro-quinoxaline; sumatriptan, 3-[2-(dimethylamino)ethyl]-*N*-methyl-1*H*-indole-5-methanesulphonamide succinate

Introduction

Brain structures such as the rostroventral medial medulla provide the major 5-hydroxytryptaminergic descending pathway to the spinal superficial dorsal horn, which is the initial relay point for nociceptive inputs to the CNS (Millan, 2002). The neurotransmitter 5-HT (serotonin) modulates spinal nociceptive transmission in a complex manner because of the involvement of multiple 5-HT receptor subtypes and their specific localisation. One group of the 5-HT receptor superfamily, the 5-HT$_1$ receptors, are inhibitory G$_{i/o}$-coupled receptors and include 5-HT$_{1A, B, D, E \text{ and } F}$ receptor subtypes (Barnes and Sharp, 1999; Alexander *et al.*, 2011). Both 5-HT$_{1A}$ and 5-HT$_{1B}$ agonists have been reported to produce

antinociception, although there is some variability between studies, which may be related to differences in the pain assays (Schmauss *et al.*, 1983; Mjellem *et al.*, 1992; Xu *et al.*, 1994; Oyama *et al.*, 1996; Bardin *et al.*, 2000; Sasaki *et al.*, 2001; Nadeson and Goodchild, 2002; Jeong *et al.*, 2004). The role of 5-HT$_{1D}$ and 5-HT$_{1F}$ receptors in spinal nociceptive modulation is poorly understood (e.g. Jeong *et al.*, 2004).

Sumatriptan and other triptans have efficacy in the treatment of migraine via their actions at 5-HT$_{1B,\ D\ and\ F}$ receptors (Humphrey *et al.*, 1990; Monteith and Goadsby, 2011). The anti-migraine actions of triptans are partly mediated by inhibition of nociceptive transmission from pain sensitive intracranial structures within the trigeminal dorsal horn induced by 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Nozaki *et al.*, 1992; Goadsby and Hoskin, 1996; Storer and Goadsby, 1997; Cumberbatch *et al.*, 1998; Donaldson *et al.*, 2002). In addition to trigeminal sensory neurons, it has been demonstrated that 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors are located in lumbar dorsal root ganglion neurons (Pierce *et al.*, 1996; Wotherspoon and Priestley, 2000; Potrebic *et al.*, 2003). Thus, triptans also have the potential to modulate nociceptive transmission at the spinal level. While some studies have shown that triptans lack antinociceptive effects in spinally-mediated models of pain (Skingle *et al.*, 1990; Connor *et al.*, 1997), others have shown that they do have some efficacy (Ghelardini *et al.*, 1996; Bingham *et al.*, 2001; Nikai *et al.*, 2008). This analgesia, however, may be restricted to specific chronic pain states (Bingham *et al.*, 2001; Kayser *et al.*, 2002; Nikai *et al.*, 2008).

At the cellular level, 5-HT produces both pre- and postsynaptic inhibition and excitation within the spinal and trigeminal superficial dorsal horn (Grudt *et al.*, 1995; Hori *et al.*, 1996; Travagli and Williams, 1996; Ito *et al.*, 2000; Lu and Perl, 2007). In the spinal dorsal horn, presynaptic 5-HT$_{1A}$ ligands inhibit primary afferent Aδ- and C-fibre-evoked EPSC (Hori *et al.*, 1996; Li and Zhuo, 1998; Ito *et al.*, 2000; Garraway and Hochman, 2001; Lu and Perl, 2007). It has also been shown that the anti-migraine drug sumatriptan inhibits afferent-evoked EPSC in the trigeminal dorsal horn via a presynaptic mechanism (Travagli and Williams, 1996; Jennings *et al.*, 2004) and inhibits afferent-evoked neuropeptide release within the spinal cord (Arvieu *et al.*, 1996). In the trigeminal system, there is pharmacological evidence that the sumatriptan-induced inhibition is mediated by 5-HT$_{1D}$ receptors (Jennings *et al.*, 2004). The role of 5-HT$_1$ receptor subtypes, other than 5-HT$_{1A}$ receptors, within the superficial dorsal horn of the spinal cord remains poorly understood. In the present study, we examined the effect of i.t. injections of a range of 5-HT$_1$ agonists, including sumatriptan, on assays of acute pain, and the effect of these agonists on primary afferent-evoked neurotransmission in order to determine the relative contribution of 5-HT$_{1A,\ B,\ D\ and\ F}$ receptors.

Methods

Experiments were carried out on 2–3 week old (electrophysiology) and 6–8 week old (behaviour) Sprague–Dawley rats obtained from Animal Resources Centre (Canning Vale, Australia). All animal care and experimental procedures complied with the guidelines of the 'NH&MRC Code of Practice for the Care and Use of Animals in Research in Australia' and were approved by the Royal North Shore Hospital Animal Care and Ethics Committee. Animals were housed in individually-ventilated cages under a 12:12 h light/dark cycle, with environmental enrichment and free access to water and standard rat chow. 6 to 8 week old animals were housed in groups of three; 2–3 week old animals were housed with their littermates and mother.

Reagents

1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrol[3,2-*b*]pyridin-5-one dihydrochloride (CP93129), *N*-[(3*R*)-3-(dimethylamino)-2,3,4,9-tetrahydro-1*H*-carbazol-6-yl]-4-fluorobenzamide hydrochloride (LY344864), (*S*)-3,4-Dihydro-1-[2-[4-(4-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-methyl-1*H*-2-benzopyran-6-carboxamide (PNU109291) and (2*R*)-(+)-8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (*R*(+)-8-OH-DPAT) were obtained from Tocris Cookson (Bristol, UK); 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), DL-(–)-2-amino-5-phosphonopentanoic acid (AP5) and *n*-ethyllidocaine bromide (QX314) were from Ascent Scientific (Bristol, UK); DAMGO, lignocaine hydrochloride, methionine enkephalin, picrotoxin, strychnine hydrochloride, 3-[2-(dimethylamino)ethyl]-*N*-methyl-1*H*-indole-5-methanesulphonamide succinate (sumatriptan), *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate (WAY100635) and all other reagents were from Sigma (Sydney, Australia).

Behavioural experiments

All animals were allowed to acclimatize to their holding cages for 2–3 days before any procedures were carried out. For the behavioural experiments, chronic polyethylene lumbar intrathecal catheters were inserted between vertebrae L5–6, advanced 3 cm rostrally and exteriorized via the occipital region under isoflurane (2–3% in O_2) anaesthesia, as described previously (Vuong *et al.*, 2008). Animals were then allowed to recover for 3–4 days. Solutions of all agents were made up just before their administration. All agents were made up in a vehicle solution comprising either 100% normal saline, or 50:50% DMSO/normal saline, and injected i.t. in a total volume of 20 μL, followed by a 15 μL flush of normal saline.

To measure mechanical pain, animals were gently restrained in a loose sock, and the left hind paw was placed in a paw pressure analgesymeter (Randall-Sellito device, Ugo Basile, Comeria, Italy). A steadily increasing force (rate = 17 g·s^{-1}) was applied to the hind paw until the animal attempted to withdraw its paw, or a cut-off threshold of 500 g was reached. This gave a measure of the mechanical paw withdrawal threshold (PWT). A plantar tester (Ugo Basile) was used to measure thermal pain. Rats were placed in Perspex enclosures (15 × 15 × 18 cm) and given 10–15 min to acclimatize. Focal infrared heat was applied through the glass-bottomed of the cage to the left hind paw, and the time for the rat to respond by moving its paw away from the noxious heat source was recorded (thermal paw withdrawal latency, PWL). To assess motor performance, the duration for which the animal could maintain balance on the rotating drum of a rotarod device (Ugo Basile) was measured as the rotarod latency, with a maximal cut-off time of 300 s. Animals were

habituated to each testing apparatus three to four times before experiments were performed.

All procedures were carried out in the light cycle, commencing 09:00 h. Each animal underwent an i.t. drug injection experiment on two consecutive days. The two drugs injected i.t. in each animal were delivered in a randomized order, and the experimenter was unaware of (blind to) the drugs being tested. On the experiment day, testing on each device was carried out 30 min before and immediately before, then at set time points over a 3 h period following the i.t. drug injection. At each time point, testing on each device was carried out once. At the completion of the experiment, catheter placement was confirmed by the occurrence of rapid, bilateral hind limb paralysis following an i.t. injection of lignocaine (2%). *R*(+)-8-OH-DPAT was tested over a range of doses (30–300 nmol). The other 5-HT ligands were tested at 100 nmol, the dose at which *R*(+)-8-OH-DPAT produced a maximal effect; higher doses were not tested as side effects (such as hypersensitivity and vocalization with sumatriptan) were observed in preliminary studies.

Electrophysiological experiments

For the electrophysiological experiments, animals were deeply anaesthetized with isoflurane and decapitated. The spinal cord was exposed, the dura was incised and the spinal column was quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) of composition (mM): NaCl, 126; KCl, 2.5; NaH_2PO_4, 1.4; $MgCl_2$, 1.2; $CaCl_2$, 2.4; glucose, 11; $NaHCO_3$, 25. Transverse (300 μm) slices of the lumbar spinal cord (L4–6) were obtained using a vibratome (VT1000S: Leica, Nussloch, Germany) and maintained at 34°C in a submerged chamber containing ACSF equilibrated with 95% O_2 and 5% CO_2. Individual slices were transferred to a slice chamber (volume 0.5 mL) and superfused continuously (1.8 mL·min^{-1}) with ACSF at 34°C using an inline temperature controller.

Superficial dorsal horn neurons located throughout lamina II were visualized using infra-red Dodt-tube optics on an upright microscope (Olympus BX50, Olympus, Sydney, Australia). Whole-cell voltage clamp recordings (holding potential −65 mV, liquid junction potential corrected) were made using an Axopatch 200B (Molecular Devices, Sunnyvale, CA). The internal solution contained (mM): CsCl 140, HEPES 10, EGTA 0.2, $MgCl_2$ 1, QX-314 3, MgATP 2 and NaGTP 0.3 (pH 7.3 and osmolality 280–285 mosmol·L^{-1}). Series resistance (<25 MΩ) was compensated by 80% and continuously monitored during experiments. Electrically evoked EPSC were elicited in neurons via a glass unipolar stimulating electrode placed on dorsal rootlets adjacent to the dorsal root entry zone (once every 12 s, intensity 2–20 V, 0.05–0.3 ms) in the presence of the $GABA_A$ channel blocker picrotoxin (100 μM), the glycine receptor antagonist strychnine (3 μM) and the NMDA receptor antagonist AP5 (25 μM). Stock solutions of all 5-HT ligands were made in H_2O, or DMSO, and then diluted (1:1000–10 000) to working concentrations in ACSF immediately before use and applied by superfusion to the slice chamber. 5-HT agents were used at maximal concentrations, as in our previous studies (Marinelli *et al.*, 2004; Jeong *et al.*, 2008).

Analysis

For behavioural experiments, comparisons of drug effects over time were made using two-way repeated measures ANOVAs, with time and drug treatment as a within- and between-subjects factors respectively (Prism, GraphPad Software, La Jolla, CA). When two-way ANOVAs were significant, *post hoc* comparisons between drug treatment groups and vehicle at individual time points were made using the Bonferroni adjustment for multiple comparisons. In addition, comparisons of drug effects at a set time (30 min post-drug for thermal PWL and mechanical PWT, 5 min post-drug for rotarod latency) were made using one-way ANOVAs, and, when significant, *post hoc* comparisons between drug treatment groups and vehicle were made using Dunnett's adjustment for multiple comparisons.

For electrophysiological experiments, recordings were filtered (2 kHz low-pass filter) and sampled (10 kHz) for online and offline analysis using AxographX (Axograph Scientific, Sydney Australia). Evoked EPSC amplitude was measured as the difference between the peak of the EPSC and a 2 ms baseline period preceding the stimulus artefact. Neurons were considered to be drug responders if there was a decrease in evoked EPSC amplitude, which was greater than 15% (approximately two times the standard deviation of baseline evoked EPSC amplitude). Statistical assessment of individual drug effects were made using paired *t*-tests (pre vs. drug), and comparisons of individual drug effects between lamina II-outer and II-inner neurons were made using unpaired *t*-tests. Differences were considered significant when $P < 0.05$, and all numerical data are expressed as mean ± SEM.

Results

5-HT$_{1A}$ agonist, but not 5-HT$_{1B/D/F}$ agonists, produces antinociception

We first compared the actions of the 5-HT_{1A} receptor subtype agonist *R*(+)-8-OH-DPAT, sumatriptan and the μ-opioid receptor agonist DAMGO in assays of acute thermal and mechanical pain. I.t. injection of the 5-HT_{1A} agonist *R*(+)-8-OH-DPAT (100 nmol) produced an increase in thermal PWL, which was significantly greater than that produced by vehicle at 30–120 min, then declined to vehicle levels at 180 min (Figure 1A, $n = 6$). By contrast, sumatriptan (100 nmol) did not produce a significant change in thermal PWL compared with vehicle (Figure 1A, $n = 6$). DAMGO (1 nmol) produced an increase in thermal PWL, which was significantly greater than that produced by vehicle at 30–60 min, and returned to vehicle levels at 120 min (Figure 1A, $n = 5$).

R(+)-8-OH-DPAT and sumatriptan (100 nmol) did not produce a significant change in mechanical PWT compared with vehicle (Figure 1B, $n = 6$). By contrast, DAMGO (1 nmol) produced an increase in mechanical PWT, which was significantly greater than that produced by vehicle at 30 min, and returned to vehicle levels at 60 min (Figure 1B, $n = 5$). *R*(+)-8-OH-DPAT (100 nmol) and DAMGO (1 nmol) produced a significant decrease in rotarod latency, compared with vehicle at 5 and 5–30 min, respectively, and returned to vehicle levels at 30–60 min (Figure 1C, $n = 6, 5$). By contrast, sumatriptan (100 nmol) did not produce a change in rotarod latency significantly different to vehicle (Figure 1C, $n = 6$).

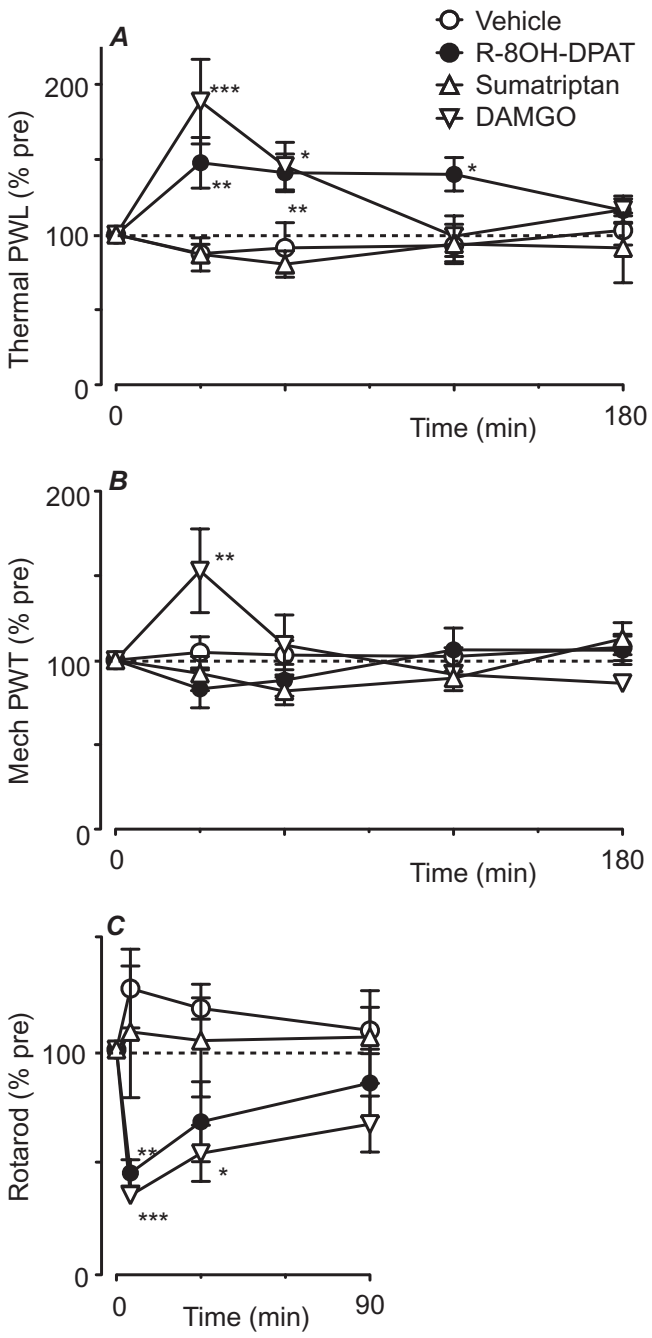

Figure 1

R(+)-8-OH-DPAT, but not sumatriptan, produces analgesia. Change in (A) thermal PWL, (B) mechanical PWT and (C) rotarod latency produced by i.t. injection of the 5-HT$_{1A}$ agonist *R*(+)-8-OH-DPAT (R-8OH-DPAT, 100 nmol), sumatriptan (100 nmol), the μ-opioid agonist DAMGO (1 nmol) and matched vehicle. Data are expressed as a percentage of the pre-injection baseline levels. *P < 0.05, **P < 0.01 and ***P < 0.001, when compared with vehicle at corresponding time points.

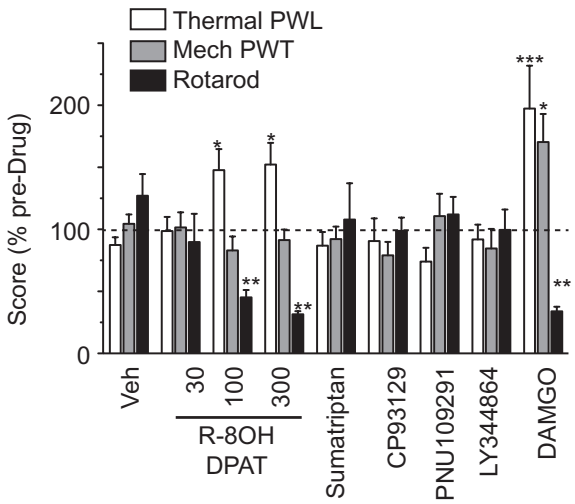

Figure 2

A 5-HT$_{1A}$ agonist, but not sumatriptan or 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-HT$_{1F}$ agonists, produce dose-dependent analgesia. Change in thermal PWL, mechanical PWT and rotarod latency produced by i.t. injection of vehicle; sumatriptan (100 nmol); the 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-HT$_{1F}$ agonists *R*(+)-8-OH-DPAT (R-8OH-DPAT, 30–300 nmol); CP93129 (100 nmol); PNU109291 (100 nmol) and LY344864 (100 nmol); and the μ-opioid agonist DAMGO (1 nmol) and matched vehicle. Thermal PWL and mechanical PWT at 30 min post-injection and rotarod latency at 5 min post-injection are expressed as a percentage of the pre-injection baseline levels. *P < 0.05, **P < 0.01 and ***P < 0.001, when compared with vehicle.

The increase in thermal PWL at 30 min post-injection and the decrease in rotarod latency at 5 min post-injection produced by *R*(+)-8-OH-DPAT (30–300 nmol) were both dose-dependent (Figure 2). We also examined the effect of the 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-HT$_{1F}$ subtype-selective agonists CP93129, PNU109291 and LY344864. Like sumatriptan, CP93129, PNU109291 and LY344864 (100 nmol) had no effect on mechanical PWT, thermal PWL and rotarod latency (Figure 2, P > 0.05, n = 6 each).

5-HT$_{1A}$ mediated-presynaptic inhibition of evoked EPSC predominates within lamina II

In the presence of picrotoxin (100 μM), strychnine (3 μM) and AP5 (25 μM), stimulation of the dorsal rootlets evoked EPSC in lamina II neurons, which had stable latencies and were abolished by TTX (500 nM, n = 4), or by CNQX (5 μM) (Figure 3C,E). Superfusion of *R*(+)-8-OH-DPAT (1 μM) produced a decrease in the amplitude of evoked EPSC in most lamina II neurons (81%, n = 13/16) (Figure 3A,B). Sumatriptan (3 μM) produced a decrease in the amplitude of evoked EPSC in a subpopulation of lamina II neurons (47%, n = 7/15), which did not always reverse following washout (Figure 3C,D). When averaged across all neurons tested, *R*(+)-8-OH-DPAT (P < 0.0001, n = 16) and sumatriptan (P = 0.04, n = 15) produced a significant decrease in evoked EPSC amplitude (Figure 4A).

In most lamina II neurons, CP93129 (3 μM, 85% n = 11/13), PNU109291 (300 nM, 100%, n = 9) and LY344864 (1 μM, 86%, n = 6/7) had no effect on the amplitude of evoked EPSC (Figure 3E,F). When averaged across all neurons tested, CP93129, PNU109291 and LY344864 had no significant effect on the amplitude of evoked EPSC (Figure 4A,

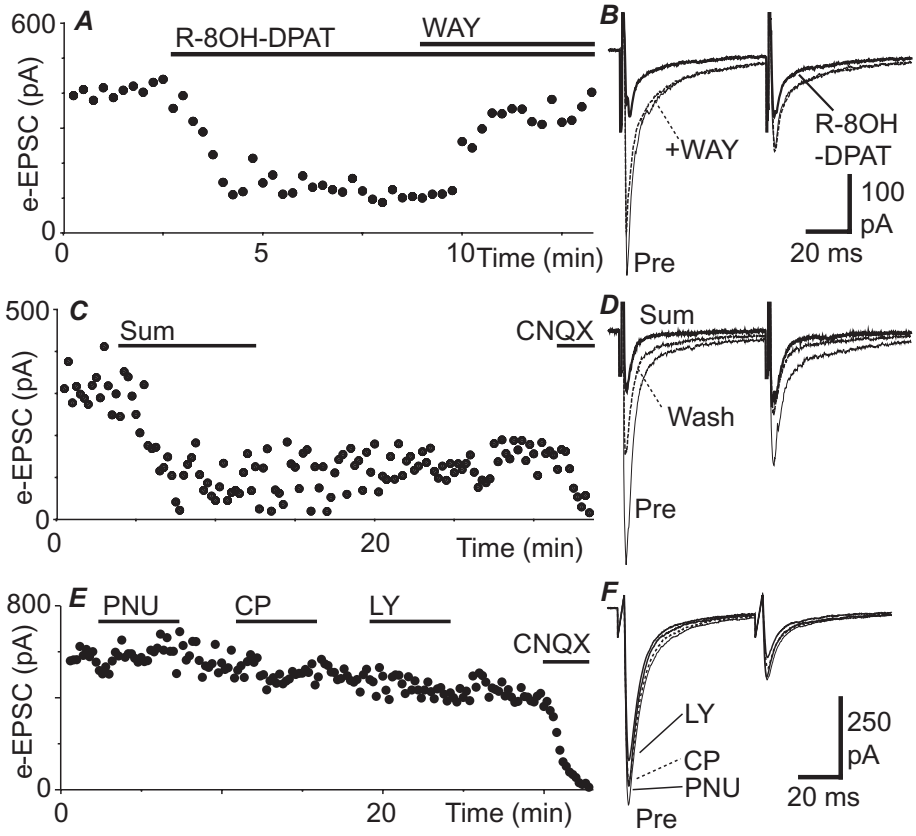

Figure 3

R(+)-8-OH-DPAT and sumatriptan inhibit evoked EPSC in lamina II. Time plots of the amplitude of dorsal rootlet-evoked EPSC during superfusion of (A) *R*(+)-8-OH-DPAT (R-8OH-DPAT, 1 μM) and addition of WAY100635 (WAY, 3 μM) (C) sumatriptan (Sum, 3 μM) and CNQX (3 μM) (E) PNU109291 (PNU, 300 nM), CP93129 (CP, 3 μM), LY344864 (LY, 1 μM) and CNQX (3 μM). (B) (D) and (F) Averaged traces of evoked EPSC taken from epochs in corresponding time plots (A) (C) and (E), before (Pre) and during application each agonist/antagonist. (A, B), (C, D) and (E, F) are taken from three different neurons.

P = 0.9, 0.2, 0.9). Subsequent superfusion of either DAMGO (3 μM, n = 5), or met-enkephalin (10 μM, n = 13) produced a reduction in evoked EPSC amplitude in all neurons tested (Figure 4A and 44 ± 9 and 45 ± 6% of pre-drug, n = 6 and 14, for DAMGO and met-enkephalin, respectively, P < 0.0001 each).

We next examined whether these responses differed between lamina II-outer and II-inner neurons. *R*(+)-8-OH-DPAT produced a reduction in evoked EPSC amplitude, which was similar in lamina II-outer and II-inner neurons (Figure 4C, P = 0.3, n = 9 and 5). The reduction in evoked EPSC amplitude produced by sumatriptan was similar for lamina II-outer and II-inner neurons (Figure 4C, P = 0.9, n = 7 and 5). The reduction in evoked EPSC amplitude produced by DAMGO (3 μM) and met-enkephalin (10 μM) was greater for lamina II-outer neurons than lamina II-inner neurons (Figure 4C, P = 0.02, n = 11 and 9). In addition, the effect of CP93129, PNU109291 and LY344864 on evoked EPSC amplitude did not differ between lamina II-outer and II-inner neurons (P = 0.7, 0.2, 0.8).

In some of these neurons, paired evoked EPSC were elicited by two stimuli of identical strength in close succession (inter-stimulus interval = 70 ms) to determine whether inhibition of the first evoked EPSC was associated with relative facilitation of the second evoked EPSC (evoked $EPSC_2$/evoked $EPSC_1$). Before drug application, the ratio of evoked $EPSC_2$/evoked $EPSC_1$ was 0.56 ± 0.04 (evoked $EPSC_2$/evoked $EPSC_1$ range = 0.17–2.45), with both paired pulse inhibition and facilitation being observed (Figure 3B,D,F, n = 83). In neurons that displayed *R*(+)-8-OH-DPAT-induced inhibition of evoked $EPSC_1$, the ratio of evoked $EPSC_2$/evoked $EPSC_1$ increased to 138 ± 10% of the pre-*R*(+)-8-OH-DPAT value (P = 0.004, n = 12). In neurons that displayed sumatriptan-induced inhibition of evoked $EPSC_1$, the ratio of evoked $EPSC_2$/evoked $EPSC_1$ was 134 ± 22% of the pre-sumatriptan value (P = 0.15, n = 7). DAMGO and met-enkephalin increased the ratio of evoked $EPSC_2$/evoked $EPSC_1$ to 164 ± 13% of the pre-DAMGO/met-enkephalin value (P < 0.0001, n = 20). There was no relationship between the initial ratio of evoked $EPSC_2$/evoked $EPSC_1$ and the subsequent inhibition of evoked $EPSC_1$ in the responding neurons (r^2 = 0.01, P = 0.5, linear regression).

We finally examined the receptor subtypes that mediate the actions of *R*(+)-8-OH-DPAT and sumatriptan. The reduc-

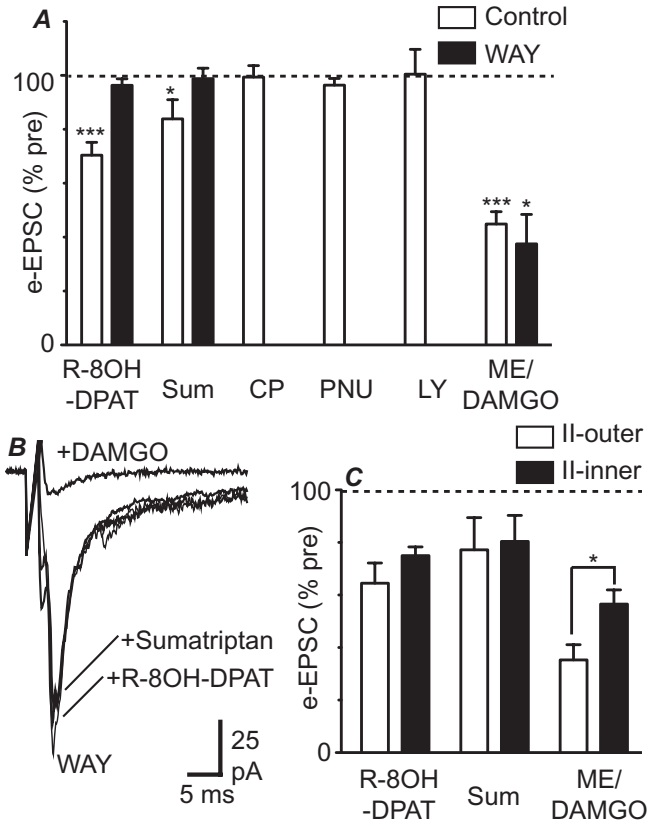

Figure 4

The effects of *R*(+)-8-OH-DPAT and sumatriptan on evoked EPSCs are both mediated by 5-HT$_{1A}$ receptors. (A) Histogram of evoked EPSC amplitude during application of *R*(+)-8-OH-DPAT (R-8OH-DPAT, 1 μM), sumatriptan (Sum, 3 μM), CP93129 (CP, 3 μM), PNU109291 (PNU, 300 nM), LY844864 (LY, 1 μM) and DAMGO (3 μM) or met-enkephalin (10 μM) (DAMGO/ME). Data are expressed as a percentage of the pre-drug application value and are shown for neurons from untreated slices (Control) and slices pre-incubated with WAY100635 (WAY, 3 μM). (B) Averaged traces of evoked EPSC before and during addition of *R*(+)-8-OH-DPAT (R-8OH-DPAT, 1 μM), sumatriptan (3 μM), then DAMGO (3 μM) in a slice pre-incubated with WAY100635 (WAY, 3 μM). (C) Histogram of evoked EPSC amplitude during application of *R*(+)-8-OH-DPAT (R-8OH-DPAT, 1 μM), sumatriptan (Sum, 3 μM) and DAMGO (3 μM) or met-enkephalin (10 μM) (DAMGO/ME) for neurons in lamina II-outer and II-inner; data are expressed as a percentage of the pre-drug application value. **P* < 0.05 and ****P* < 0.001.

tion in evoked EPSC amplitude produced by *R*(+)-8-OH-DPAT was reversed by addition of the 5-HT$_{1A}$ antagonist WAY100635 (3 μM) (Figure 3A and 97 ± 3% of the pre-*R*(+)-8-OH-DPAT level, n = 13). In slices pre-incubated in WAY100635 (3 μM), addition of *R*(+)-8-OH-DPAT (1 μM) did not produce a significant change in evoked EPSC amplitude (Figure 4B, P = 0.2, n = 6). Similarly, in slices pre-incubated in WAY100635 (3 μM) addition of sumatriptan (3 μM) did not produce a significant change in evoked EPSC amplitude (Figure 4A,B, P = 0.8, n = 6). In the presence of WAY100635, DAMGO (3 μM) produced a reduction in evoked EPSC amplitude (Figure 4B, P = 0.01, n = 4).

Discussion

In the present study, it has been demonstrated that spinal administration of the 5-HT$_{1A}$ agonist *R*(+)-8-OH-DPAT produces thermal, but not mechanical antinociception in the rat. In addition, 5-HT$_{1A}$ receptor activation presynaptically inhibited glutamatergic primary afferent-evoked synaptic transmission onto a subpopulation of lamina II lumbar dorsal horn neurons. By contrast, sumatriptan and 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-HT$_{1F}$ ligands had no spinal antinociceptive effects, and sumatriptan produced 5-HT$_{1A}$-mediated presynaptic inhibition. These findings indicate that 5-HT$_{1A}$, but not 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-HT$_{1F}$, receptor activation has an antinociceptive effect in acute thermal pain assays and inhibits nociceptive transmission into the lumbar superficial dorsal horn of the rat.

5-HT$_{1A}$-mediated spinal analgesia

The 5-HT$_{1A}$ receptor agonist *R*(+)-8-OH-DPAT produced dose-dependent antinociception in an acute noxious thermal paw withdrawal assay, but not in a noxious mechanical paw withdrawal assay. This is consistent with results from previous studies that have shown 5-HT$_{1A}$-mediated analgesia in acute thermal, but not mechanical pain assays (Mjellem *et al.*, 1992; Xu *et al.*, 1994; Bardin *et al.*, 2000). It might also be noted that another study has not observed 5-HT$_{1A}$-mediated thermal analgesia (Nadeson and Goodchild, 2002), and the effect of spinal 5-HT$_{1A}$ receptor activation in the formalin test is variable (Xu *et al.*, 1994; Oyama *et al.*, 1996; Sasaki *et al.*, 2001; Jeong *et al.*, 2004). While *R*(+)-8-OH-DPAT also produced a dose-dependent reduction in motor performance in the rotarod assay, the thermal antinociception was unlikely to be due to a motor side effect because of the lack of effect on paw withdrawal in response to a noxious mechanical stimulus. Like *R*(+)-8-OH-DPAT, DAMGO produced greater antinociception in the thermal pain assay than in the mechanical pain assay. It has recently been shown that spinal μ-opioid receptor agonists target thermal, as opposed to mechanical pain in the mouse (Scherrer *et al.*, 2009 and see below). The lesser selectivity of DAMGO in the present study may have been due to the use of higher doses, or species differences. These findings suggest that, like μ-opioids, spinally delivered 5-HT$_{1A}$ agonists are at least partially selective for thermal nociceptive modalities in the rat.

Unlike *R*(+)-8-OH-DPAT, the 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-HT$_{1F}$ agonists CP93129, PNU109291 and LY344864 had no effect in either thermal, or mechanical pain assays, or on motor performance in the rotarod assay. The lack of effect of these agonists is consistent with some studies on 5-HT$_{1B}$ agonists such as CP93129 and RU24969 (Mjellem *et al.*, 1992; Sasaki *et al.*, 2001), but not others using CGS12066 and TFMPP (Alhaider and Wilcox, 1993; Xu *et al.*, 1994; Jeong *et al.*, 2004). Compared with CP93129, CGS12066 and TFMPP have relatively low selectivity for 5-HT$_{1B}$ receptors over 5-HT$_{1A}$ receptors (e.g. Neale *et al.*, 1987), suggesting that the analgesic effects of 5-HT$_{1B}$ agonists in some studies are mediated by 5-HT$_{1A}$ receptors. While there are no reported studies on the spinal analgesic effects of 5-HT$_{1D}$ and 5-HT$_{1F}$ agonists, it has been shown that the 5-HT$_{1D}$ antagonist BRL15572 has no effect on 5-HT-induced analgesia in the formalin test (Jeong

et al., 2004). Together, these findings suggest that 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} receptor activation in the spinal cord has little effect on acute thermal and mechanical nociception.

5-HT_{1A} receptor activation presynaptically inhibits primary afferent neurotransmission

In the present study, it was found that *R*(+)-8-OH-DPAT inhibited dorsal rootlet-evoked EPSC in neurons throughout lamina II of the spinal dorsal horn. This is similar to that previously observed for the enantiomer 8-OH-DPAT in both spinal and trigeminal superficial dorsal horn neurons (Hori *et al.*, 1996; Travagli and Williams, 1996; Ito *et al.*, 2000). This was probably due to presynaptic inhibition of glutamate release from nociceptive primary afferent inputs because it was associated with an increase in the paired pulse ratio of closely spaced EPSC, as observed previously for 5-HT in the trigeminal dorsal horn (Travagli and Williams, 1996). A presynaptic locus of action is supported by other studies that have shown that 5-HT and 8-OH-DPAT reduce the frequency but have no effect on the amplitude of spontaneous, tetrodotoxin-resistant miniature EPSC in lumbar and trigeminal dorsal horn (Hori *et al.*, 1996; Travagli and Williams, 1996). While the specific afferent fibre types that convey 5-HT_{1A} sensitive inputs were not examined in the present study, it has previously been reported that 5-HT inhibits both Aδ- and C-fibre-evoked EPSC in lumbar dorsal horn, and 8-OH-DPAT inhibits only C-fibre-evoked EPSC (Ito *et al.*, 2000; Lu and Perl, 2007).

While there is good evidence that 5-HT_{1A} receptors mediate the postsynaptic action of 8-OH-DPAT in the dorsal horn (Ito *et al.*, 2000; Abe *et al.*, 2009), the role of 5-HT_{1A} receptors in the presynaptic actions of 8-OH-DPAT are less clear. *R*(+)-8-OH-DPAT, like 8-OH-DPAT, is an agonist at both 5-HT_{1A} and 5-HT_7 receptors, and some *in vitro* studies on synaptic transmission in the dorsal horn have used less selective agents, such as the 5-$HT_{1A/7}$ antagonist NAN-190 (Hori *et al.*, 1996; Travagli and Williams, 1996). In addition, it has been shown that the 8-OH-DPAT (10 μM)-induced inhibition of evoked EPSC is unaffected by the 5-HT_{1A} antagonist WAY100635 (10 μM) (Ito *et al.*, 2000). These studies suggest that 5-HT_{1A}, 5-HT_7 and other unidentified 5-HT receptors may have a role in the presynaptic actions of 8-OH-DPAT. In the present study, however, the *R*(+)-8-OH-DPAT-induced inhibition of evoked EPSC was abolished by WAY100635 (3 μM), suggesting that 5-HT_{1A} receptors mediate its presynaptic effects. The difference between these studies might be due to the use of lower concentrations of a more efficacious enantiomer (*R*(+)-8-OH-DPAT vs. 8-OH-DPAT), or younger animals (2–4 vs. 7 weeks) in the present study.

The present findings indicate that 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} receptor activation has little effect on primary afferent transmission in lumbar dorsal horn because CP93129, PNU109291 and LY344864 did not produce a significant inhibition of evoked EPSC. While this is the first study to examine the cellular actions of 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} agonists in the lumbar dorsal horn, it has been reported that 5-HT_{1D} activation inhibits evoked EPSC in the trigeminal dorsal horn (see below). Our *in vitro* findings differ from *in vivo* evidence that 8-OH-DPAT and the 5-HT_{1B} agonist CP94253 inhibit C-fibre-evoked field potentials in lamina II (Aira *et al.*, 2010), and that CP93129 inhibits Aδ-evoked firing in deep dorsal horn neurons (Gjerstad *et al.*, 1997).

Sumatriptan acts via 5-HT_{1A} receptors in the lumbar dorsal horn

In the present study, the anti-migraine drug sumatriptan had no effect on acute mechanical, or thermal pain assays when delivered i.t., as observed previously (Skingle *et al.*, 1990; Bingham *et al.*, 2001; Nikai *et al.*, 2008). This was consistent with the lack of effect of selective 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} agonists and supports the notion that sumatriptan is selective for specific forms of cranial pain (Nozaki *et al.*, 1992; Donaldson *et al.*, 2002). There is some evidence, however, that this may differ in somatic and visceral inflammatory pain models in mice where i.t. delivered sumatriptan has analgesic effects at doses below those used in the present study (Nikai *et al.*, 2008).

In contrast to the *in vivo* findings, sumatriptan produced a reduction in evoked EPSC, as observed previously in the trigeminal dorsal horn (Travagli and Williams, 1996; Jennings *et al.*, 2004). This is consistent with the finding that sumatriptan inhibits afferent evoked release of CGRP and substance P in spinal cord slices (Arvieu *et al.*, 1996) In the trigeminal dorsal horn, sumatriptan-induced inhibition of primary afferent-evoked EPSC is thought be mediated by 5-HT_{1D} receptors because it is abolished by the 5-$HT_{1B/D}$ antagonist BRL15572 and not mimicked by CP93129 (Jennings *et al.*, 2004). The sumatriptan-induced inhibition observed in the present study was unlikely to be mediated by 5-HT_{1B}, 5-HT_{1D} or 5-HT_{1F} receptors because of the overall lack of effect of selective 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} agonists (see above). The sumatriptan-induced inhibition of evoked EPSC was, however, abolished by WAY100635, suggesting that it is likely to be mediated by 5-HT_{1A} receptors. In this regard, sumatriptan has significant affinity for human and rat 5-HT_{1A} receptors (Schoeffter and Hoyer, 1989;, e.g. Newman-Tancredi *et al.*, 1997). We cannot, however, exclude a role for 5-HT_{1B} receptors because CP93129 inhibited primary afferent-evoked EPSC in a minor subpopulation of lamina II neurons.

Like trigeminal sensory neurons, 5-HT_{1B} and 5-HT_{1D} receptors are located in lumbar dorsal root ganglion neurons (Pierce *et al.*, 1996; Wotherspoon and Priestley, 2000; Potrebic *et al.*, 2003). The lack of 5-HT_{1B} or 5-HT_{1D} presynaptic inhibition observed in the present study might have been due to a lack of surface receptors because 5-HT_{1D} receptors are localized exclusively within dense core vesicles of primary afferent presynaptic terminals, in both the lumbar and trigeminal dorsal horn (Potrebic *et al.*, 2003). It might be noted, however, that sumatriptan acts via 5-HT_{1D} receptors to inhibit primary afferent-evoked EPSC in the trigeminal dorsal horn (Jennings *et al.*, 2004), under conditions identical to those used in the present lumbar spinal cord preparation. Thus, the difference in 5-HT_{1B} and 5-HT_{1D} presynaptic inhibition between trigeminal and lumbar dorsal horn *in vitro* preparations is unlikely to be due to a lack of receptors on the membrane of presynaptic terminals.

Functional implications

Generally, the selective 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} agonists had consistent profiles of action in the *in vitro* and *in*

vivo assays used in the present study. By contrast, while both *R*(+)-8-OH-DPAT and sumatriptan produced 5-HT$_{1A}$-mediated inhibition of primary afferent transmission, only *R*(+)-8-OH-DPAT had a spinal analgesic action. The disparity between the *in vivo* and *in vitro* findings for sumatriptan may have been due to a number of factors. Firstly, spinal pain circuits receive descending 5-hydroxytryptaminergic inputs from brainstem structures such as the RVM (Millan, 2002). There was no 5-hydroxytryptaminergic 'tone' in our *in vitro* preparation because a 5-HT$_{1A}$ antagonist had no effect on basal synaptic transmission, which is consistent with the loss of supraspinal inputs in isolated spinal cord slices. By contrast, tonically active descending 5-hydroxytryptaminergic pathways could partly occlude the *in vivo* actions of exogenously applied agonists, particularly given that *R*(+)-8-OH-DPAT displayed greater *in vitro* efficacy than sumatriptan (30 vs. 16% 5-HT$_{1A}$-mediated inhibition of evoked EPSC). The descending 5-hydroxytryptaminergic pathway from the RVM, however, displays either a low level of activity, or is quiescent under basal conditions in unstressed rats (Heinricher and Kaplan, 1991; Mitchell *et al.*, 1998). Thus, the *in vitro* approach might provide a more sensitive measure of 5-HT$_{1A}$ actions in the spinal cord. Secondly, the difference between preparations may have been due to the use of neonatal animals for *in vitro* experiments because cellular spinal processing and descending 5-hydroxytryptaminergic control of spinal analgesia varies with age (e.g. Nakatsuka *et al.*, 2000; Hathway *et al.*, 2009). It might be noted, however, that 5-HT-induced inhibition of primary afferent transmission has been observed in adult rats (Ito *et al.*, 2000).

It has been suggested that thermal and mechanical pain are conveyed via distinct pain pathways through lamina II outer and inner, respectively, and that μ-opioids specifically target the former (Scherrer *et al.*, 2009). The present findings suggest that 5-HT$_{1A}$ receptor activation also targets thermal pain, rather than mechanical pain. While μ-opioid inhibition of evoked EPSC was greater in lamina II outer, we found no corresponding difference in 5-HT$_{1A}$-mediated presynaptic inhibition. Nonetheless, 5-HT$_{1A}$ pre-synaptic inhibition of primary afferent-evoked synaptic transmission was restricted to subpopulation of lamina II neurons, as observed previously (Hori *et al.*, 1996; Ito *et al.*, 2000). Similarly, previous studies have shown that 5-HT-induced postsynaptic inhibition is restricted to subpopulations of morphologically distinct lamina I–II neurons (Grudt *et al.*, 1995; Ito *et al.*, 2000; Lu and Perl, 2007; Yasaka *et al.*, 2010). Together these findings suggest that the analgesic actions of 5-HT$_{1A}$ agonists are mediated by distinct pre- and postsynaptic mechanisms within spinal pain pathways. However, the precise cellular elements that mediate thermal and mechanical pain within the spinal cord remain to be determined.

Conclusion

The present findings suggest that, at the spinal level, 5-HT$_{1A}$ agonists might be more effective in specific pain modalities. At the cellular level, 5-HT$_{1A}$, but not 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-HT$_{1F}$ receptor activation presynaptically inhibits primary afferent-evoked synaptic transmission onto a subpopulation of lamina II superficial dorsal horn neurons. In addition, the presynaptic cellular actions of the anti-migraine drug sumatriptan within the rat lumbar dorsal horn are mediated by 5-HT$_{1A}$ receptors. This suggests that the pharmacological profile of sumatriptan needs to be evaluated pharmacologically or by using knockout strategies (e.g. Kayser *et al.*, 2007).

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Conflicts of interest

The authors have no conflicting financial interests.

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