Available online at www.sciencedirect.com
SCIENCE DIRECT
Brain Research 969 (2003) 14–26
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

Effects of m-CPP in altering neuronal function: blocking depolarization in invertebrate motor and sensory neurons but exciting rat dorsal horn neurons

Garrett M. Sparks[a], Eugen Brailoiu[b], G. Cristina Brailoiu[b], Nae J. Dun[b], Jami Tabor[a], Robin L. Cooper[a,*]

[a]*Department of Biology, University of Kentucky, Lexington, KY 40506-0225, USA*
[b]*Department of Pharmacology, James H. Quillen College of Medicine, East Tennessee State University, P.O. Box 70577, Johnson City, TN 37614, USA*

Accepted 2 January 2003

Abstract

The compound *m*-chlorophenylpiperazine (m-CPP) is used clinically to manipulate serotonergic function, though its precise mechanisms of actions are not well understood. m-CPP alters synaptic transmission and neuronal function in vertebrates by non-selective agonistic actions on 5-HT_1 and 5-HT_2 receptors. In this study, we demonstrated that m-CPP did not appear to act through a 5-HT receptor in depressing neuronal function in the invertebrates (crayfish and *Drosophila*). Instead, m-CPP likely decreased sodium influx through voltage-gated sodium channels present in motor and primary sensory neurons. Intracellular axonal recordings showed that m-CPP reduced the amplitude of the action potentials in crayfish motor neurons. Quantal analysis of excitatory postsynaptic currents, recorded at neuromuscular junctions (NMJ) of crayfish and *Drosophila,* indicated a reduction in the number of presynaptic vesicular events, which produced a decrease in mean quantal content. m-CPP also decreased activity in primary sensory neurons in the crayfish. In contrast, serotonin produces an increase in synaptic strength at the crayfish NMJ and an increase in activity of sensory neurons; it produces no effect at the *Drosophila* NMJ. In the rat spinal cord, m-CPP enhances the occurrence of spontaneous excitatory postsynaptic potentials with no alteration in evoked currents.

Keywords: Serotonin; Sodium; Neuromodulation; Synapse; Crayfish; Presynaptic; Rat

1. Introduction

Past research investigating the mechanism by which *m*-chlorophenylpiperazine (m-CPP) alters synaptic transmission and neuronal function has focused on its ability to act as a non-selective agonist to vertebrate 5-HT_1 and 5-HT_2-family receptors. The latter activate phospholipase C and increase inositol phosphates and intracellular Ca^{2+} [6]. However, m-CPP antagonizes 5-HT_{2B} receptors in some models (Ref. [84] see also reviews [10,74]), and also has a high affinity for 5-HT_{2C} receptors [45,62]. The serotonergic effects may be related to the ability of the compound to reverse the 5-HT transporter [7,8], which increases extracellular serotonin concentrations in the rat central nervous system (CNS). In addition, m-CPP also affects endocrine responses [11,71], which in some cases are related to an increase in release of prolactin and corticosteroids [17,60]. This compound has also been shown to effect alpha$_2$-adrenergic receptors, as well as, to a lesser degree, alpha$_1$-adrenergic, beta-adrenergic [44], and dopamine sites [39].

The variety of this compound's actions may explain some of the varied responses induced by psychiatric treatments with the antidepressant drug trazodone. m-CPP, a metabolite of trazodone, elicits or aggravates anxiety attacks in patients with anxiety disorders, obsessions in patients with obsessive compulsive disorder, elation in alcoholics and cocaine addicts, and positive symptoms in schizophrenic patients [1,13,45,47,48,63]. It also causes

*Corresponding author. Tel.: +1-859-257-5950; fax: +1-859-257-1717.
E-mail address: RLCOOP1@pop.uky.edu (R.L. Cooper).

0006-8993/03/$ – see front matter
doi:10.1016/S0006-8993(03)02271-6

anorexia in rats [73], causes a reduction in aggressive behavior [40], and possesses analgesic properties [68].

The reported 5-HT_1 and 5-HT_2 receptor agonist activity of m-CPP in vertebrates has made m-CPP an attractive choice for use in invertebrate models that are known to have 5-HT_2-like receptor function [19,82]. m-CPP was used in an earlier study with crayfish to examine responsiveness of 5-HT in excised ventral nerve cord preparations of aggressive and submissive crayfish [89,90]. Whole animal studies, in crayfish, have employed systemic injections of m-CPP, as well as other 5-HT receptor agonists, to determine which compounds most closely mimic 5-HT injections with behavioral assays [85].

In a previous study in which pharmacological identification of the 5-HT receptor subtypes at the crayfish neuromuscular junction (NMJ) was investigated, m-CPP depressed synaptic transmission [82]. This was surprising, since other selective 5-HT_2 agonists enhance synaptic transmission. Thus, this current study was undertaken to investigate the potential mechanisms of synaptic depression caused by m-CPP at the crayfish NMJ, and also to extend the investigation to NMJs of the larval *Drosophila melanogaster*, which are not influenced by exogenous application of 5-HT, but like the crayfish are glutamatergic.

5-HT effects synaptic transmission at the crayfish NMJ by increasing the probability of presynaptic vesicular release [24,79,81]. At the crayfish NMJ, 5-HT acts through an IP_3 cascade, suggesting that the invertebrate 5-HT receptors present at the crayfish NMJ are analogous to 5-HT_2 vertebrate receptors. Pharmacological studies that have examined the use of a number of 5-HT_2 agonists and antagonists have suggested that selective 5-HT2 subfamily antagonists [82] can block approximately 80% of the 5-HT enhancement in synaptic transmission.

In crayfish and lobsters, neurosecretory cells in the ventral nerve cord release 5-HT. A large number of varicose endings of neurons are located within the 2nd nerve roots of the ventral nerve cord ganglia, suggesting release sites are present directly in the hemolymph [9,78]. It is well established in crayfish that 5-HT enhances synaptic strength at NMJs [31,79], increases heart rate [54], and enhances sensory neuronal activity [26,81].

A variety of preparations were used to illuminate the mechanism of m-CPP's action in the crayfish. First, we examined pre- and post-synaptic actions of m-CPP at the NMJ of the crayfish opener muscle. Secondly, we addressed the action of m-CPP directly on action potentials within a motor neuron. Thirdly, we assessed the actions of m-CPP on primary sensory neuron function for similar mechanisms of action on motor neuronal function. Fourthly, we utilized a specific larval *Drosophila* NMJ, since it is insensitive to exogenous 5-HT application, to assess direct effects of m-CPP at another glutamatergic NMJ. Lastly, a vertebrate model, rat dorsal horn neurons, were studied to substantiate if the actions of m-CPP observed in the invertebrate models were similar to those in a vertebrate system over the same concentration range.

Using these various approaches to investigating the action of m-CPP allowed us to assess mechanisms of action in systems in which synaptic transmission could be quantified both pre- and postsynaptically at discrete synaptic sites.

2. Methods

The methods were similar to those described earlier for the fly [53], crayfish [23], and rat [2,88]. In brief, the following procedures were followed.

2.1. Animals

Mid-sized crayfish (*Procambarus clarkii*), measuring 8–10 cm in body length and weighing 20–36 g, were obtained from Atchafalaya Biological Supply Co. (Raceland, LA). Animals were housed in an aquatic facility within the laboratory in individual tanks, and were fed fish food pellets every 3 days. Only male crayfish in their intermolt stage were used.

The 'wild-type' laboratory strain of *Drosophila melanogaster*, Canton S, was used in these studies. The eggs were allowed to hatch and develop at 25 °C with a 12:12 dark–light cycle. The methods used to stage fly larvae have been described previously [14,53]. All animals were maintained in vials partially filled with a cornmeal–agar–dextrose–yeast medium. Larvae at the beginning of the 'wandering' phase of the third instar were used in these experiments.

A breeding colony of Sprague–Dawley rats was established at the Division of Laboratory Animal Resources, East Tennessee State University. Immature, 10- to 15-day-old rats of either sex were used for the electrophysiological study. Animal protocols were reviewed and approved by the University Animal Care and Use Committee.

2.2. Pharmacology

In the crayfish and *Drosophila* studies, exogenous application of 5-HT (Sigma, St. Louis, MO) or m-CPP (Sigma), as well as combinations were applied by fully exchanging the bathing medium of the preparation three times. The concentrations used are reported in the Results for each experimental paradigm.

In the electrophysiological studies utilizing rat spinal cord, m-CPP (1 mM) dissolved in oxygenated Krebs solution, was applied by superfusion.

2.3. Dissection and physiology

2.3.1. Crayfish neuromuscular junctions

These procedures have previously been described in

detail [28,29,82]. In brief, the opener muscle in either the first or second walking legs, which is innervated by a single, purely-tonic excitatory motor neuron, was used throughout these studies. The opener muscle was prepared by the standard dissection. The tissue was pinned out in a Slygard dish for viewing with a Nikon Optiphot-2 upright fluorescent microscope using a 40× (0.55 NA) Nikon water-immersion objective. All dissected preparations were maintained in crayfish saline, a modified Van Harreveld's solution (in mM: 205 NaCl; 5.3 KCl; 13.5 $CaCl_2$ $2H_2O$; 2.45 $MgCl_2 6H_2O$; 0.5 HEPES) adjusted to pH 7.4.

2.3.2. Larval Drosophila neuromuscular junctions

The dissections were performed as described earlier [25]. In brief, a longitudinal mid-dorsal incision was made, and the edges pinned, so that the preparation was spread out on a glass slide in the preparation dish as originally described for studies of the leech nervous system [61]. Internal organs were carefully removed to expose the body wall muscles, particularly the ventral longitudinal muscles of segment 4. The electrical recordings were obtained from the prominent longitudinal m6 muscle.

The physiological solution used is the same as previously described [80]. The physiological saline contains (in mM): 1.0 $CaCl_2 \cdot 2H_2O$, 70 NaCl, 5 KCl, 10 $NaHCO_3$, 5 trehalose, 115 sucrose, 5 BES (*N*,*N*-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid). All experiments were performed at room temperature (19–21 °C).

2.4. Excitatory postsynaptic potentials (EPSPs) in crayfish and Drosophila

EPSPs at the crayfish NMJ were recorded by intracellular electrodes, with 30–60 MΩ resistance microelectrodes, filled with 3 M KCl. Responses were recorded with a standard intracellular electrode amplifier (AxoClamp 2A, Axon Instruments). Electrical signals were recorded onto VHS tape and on-line to a Power Mac 9500 via a MacLab/4s interface. EPSPs were recorded at 10 kHz. All events were appropriately scaled to known values measured on an oscilloscope. The corrected scale was then adjusted with MacLab Scope software (version 3.5.4). A Grass S-88 stimulator and stimulus isolation unit (Grass), with leads to a standard suction electrode set-up, were used to stimulate the excitatory nerves in each preparation. The opener muscle preparations were stimulated to induce a short-term facilitation (STF) by giving a 40 Hz train of ten pulses at intervals of 5 or 10 s.

Electrophysiological recordings made from the larval *Drosophila* preparations were performed as previously described [64,80]. Intracellular recordings were made with 30–60 MΩ resistance, 3 M KCl-filled microelectrodes. Electrophysiological parameters of interest were the resting membrane potential (Rp) and the EPSP amplitudes for Is and Ib motor nerve terminals in segment 4 of muscle m6. Single stimuli at 0.5 Hz were given to determine whether the EJP amplitudes were altered due to the presence of m-CPP or 5-HT in the bathing media. Responses were recorded with a 1×LU head stage and an Axoclamp 2A amplifier. Electrical signals were recorded to VHS tape (Vetter, 400) as well as on-line to a PowerMac 9500 via a MacLab/4s interface. All events were measured and calibrated with the MacLab Scope software 3.5.4 version. All experiments were performed at room temperature (19–22 °C).

2.5. Excitatory postsynaptic currents (EPSCs) in crayfish

At the crayfish NMJ, synaptic currents were measured with focal macropatch electrodes, allowing for a determination of the effect of agents on presynaptic vesicular events as well as receptivity of the postsynaptic glutamate receptors. Living terminals were visualized by exposure to vital fluorescent dye 4-Di-2-ASP (Molecular Probes) [17,56]. Synaptic currents were obtained using the loose patch technique by lightly placing a 10–20 μm fire polished glass electrode directly over a spatially isolated varicosity along the nerve terminal [22]. The macropatch electrode is specific for current recording within the region of the electrode lumen. The seal resistance was in the range of 100 KΩ to 1 MΩ. Both the evoked excitatory postsynaptic currents (EPSCs) and miniature excitatory postsynaptic currents (mEPSCs) were recorded [20,25,86]. By directly counting evoked quantal events, alterations in the number of vesicles fusing within the presynaptic terminal during exposure to pharmacological agents may be observed.

To monitor quanta released over time, direct counts were obtained and, in addition, the area of the evoked current was measured for each event throughout the experiment. The tonic excitatory motor nerve was stimulated at a rate of 1 Hz in order not to facilitate the responses between trials. Exposure of 5-HT to this preparation has a gradual effect on all of the quantal parameters over several minutes, so recordings were maintained for at least 20 min or longer after exposure to the various compounds [79,81].

2.6. Intracellular recordings within the opener motor neuron

Intracellular axonal recordings were made by placing a microelectrode into the excitatory axon of the opener muscle close to where the axon bifurcates. One can easily determine if the excitatory or the inhibitory axon was penetrated with the microelectrode by the occurrence of an evoked action potential, since the excitatory axon is selectively stimulated in the meropodite of the leg [32]. The characteristics of the action potential (rise time, amplitude and decay rate) were monitored before and during exposure to m-CPP.

2.7. Crayfish sensory neurons

The muscle receptor organs (MRO) of the crayfish were exposed in the same manner as detailed earlier [26]. In brief, the shell along the lower lateral border of the abdomen on each side, along the series of small indentations, was cut. The shell was separated into two parts—a dorsal section and a ventral section. The ventral half was discarded. The preparation was anchored to a Sylgard-coated dish with the ventral view, or muscle-side-up. Each abdominal segment has two sets of the rapidly- and slowly-adapting MROs on the right and left hemi-segments. Only the slowly-adapting neurons were used.

For the sensory responses of the MRO, the firing frequency of the static response was monitored over time before and during exposure to m-CPP.

2.8. Rat spinal dorsal horn neurons

2.8.1. Spinal cord slice preparation

Transverse thoracolumbar spinal cord slices were prepared from immature (10- to 15-day-old) rats using a procedure similar to that described earlier [2,88]. Rats were anaesthetized with urethane (1.2 g kg^{-1}, i.p.) and decapitated immediately. After a laminectomy, a segment of thoracolombar spinal cord was removed and placed in a Petri dish containing ice-cold Krebs solution of the following composition (in mM): 127 NaCl, 1.9 KCl, 1.2 KH_2PO_4, 2.4 $CaCl_2$, 1.3 $MgCl_2$, 26 $NaHCO_3$, and 10 glucose, saturated with 95% O_2 and 5% CO_2. Transverse 400 μm sections were prepared with the use of a vibrotome. Slices were incubated in oxygenated Krebs solution at room temperature (21±1 °C) for at least 1 h before the start of the experiments.

2.8.2. Whole cell patch recording

Slices were transferred to the recording chamber (model RC-22C, Warner Instrument Inc., Hamden, CT) and superfused with oxygenated Krebs solution at a rate of 1–2 ml/min using a valve control perfusion system (model BPS-4; ALA Scientific Instruments Inc., Westbury, NY). The whole cell patch recording technique was similar to that described earlier [2,88]. Patch electrodes were pulled from thin-walled borosilicate glass capillaries, filled with a solution containing (in mM) 130 K-gluconate, 1 $MgCl_2$, 2 $CaCl_2$, 4 ATP, 0.3 GTP, 10 EGTA, and 10 HEPES, had a resistance of 3–5 MΩ. Signals were recorded using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA) in current or voltage clamp mode, filtered at 2 kHz, displayed on a Tektronix 5110 oscilloscope (Tektronix Inc., Beaverton, OR) and on a two-channel Gould chart recorder RS 3200 (Gould Instruments Systems Inc., Valley View, OH). Experimental protocols were controlled and data acquired by a personal computer using Clampex 8.0 software (Axon Instruments, Foster City, CA).

3. Results

3.1. Excitatory postsynaptic potentials (EPSPs) at the crayfish NMJ

The ventral opener neuromuscular preparation, as depicted in Fig. 1, has routinely been used to address the mechanisms by which 5-HT enhances synaptic transmission. Exposure to 5-HT (100 nM) shows a marked increase in all the EPSP responses throughout the pulse train while m-CPP (1 mM) depresses the EPSPs throughout the stimulus response (Fig. 1A). Previous pharmacological investigations have suggested that 5-HT_2-like receptor subtypes are present at these junctions [82]. However, the responses to exposure of m-CPP contradicted the notion that the compound behaves as a 5-HT_2 family agonist, since it depresses synaptic transmission (Fig. 1B, C). Extensive washing of the preparations with saline was performed to examine if the depressed responses were able to recover from exposure to m-CPP. The responses did partially recover after 20 min, but the recovery was slow in comparison to the initial depression. The general depression and slow recovery occurred in all five preparations examined (Fig. 1D).

3.2. Excitatory postsynaptic currents (EPSCs) at the crayfish NMJ

To ascertain if the reduction in the EPSP amplitudes was due to a presynaptic effect on transmitter release and/or a reduction in the sensitivity of the postsynaptic glutamate receptors on the muscle fiber, recording excitatory postsynaptic currents (EPSCs) and counting the number of evoked quanta over time was performed before and during exposure to m-CPP (Fig. 2). If the effect were presynaptic, then the number of quanta released per evoked event should be reduced upon exposure to m-CPP. The exposure of the crayfish NMJ resulted in a reduction of the number of evoked quantal events over time (Table 1). Primary varicosities along the terminals were used to monitor EPSCs since they are known to be higher in synaptic efficacy in comparison to the more distal varicosities along the terminals [4,20]. The results indicated that the number of evoked events was decreased by exposure to m-CPP without altering the size or shape of single postsynaptic evoked events (Table 1). Since the mean quantal content (*m*) decreased with exposure to m-CPP, a more thorough analysis of the probability of vesicular release was performed by counting the number of single, double, etc. quantal events during evoked stimulation. This approach allowed us to determine the site of m-CPP's action in reducing transmitter release to the presynaptic terminals. To obtain an index of evoked release, 500 trial subsets were examined, and *m* was calculated (Table 1) from these distributions. There was a significant decrease in the number of single and multiple events occurring in the

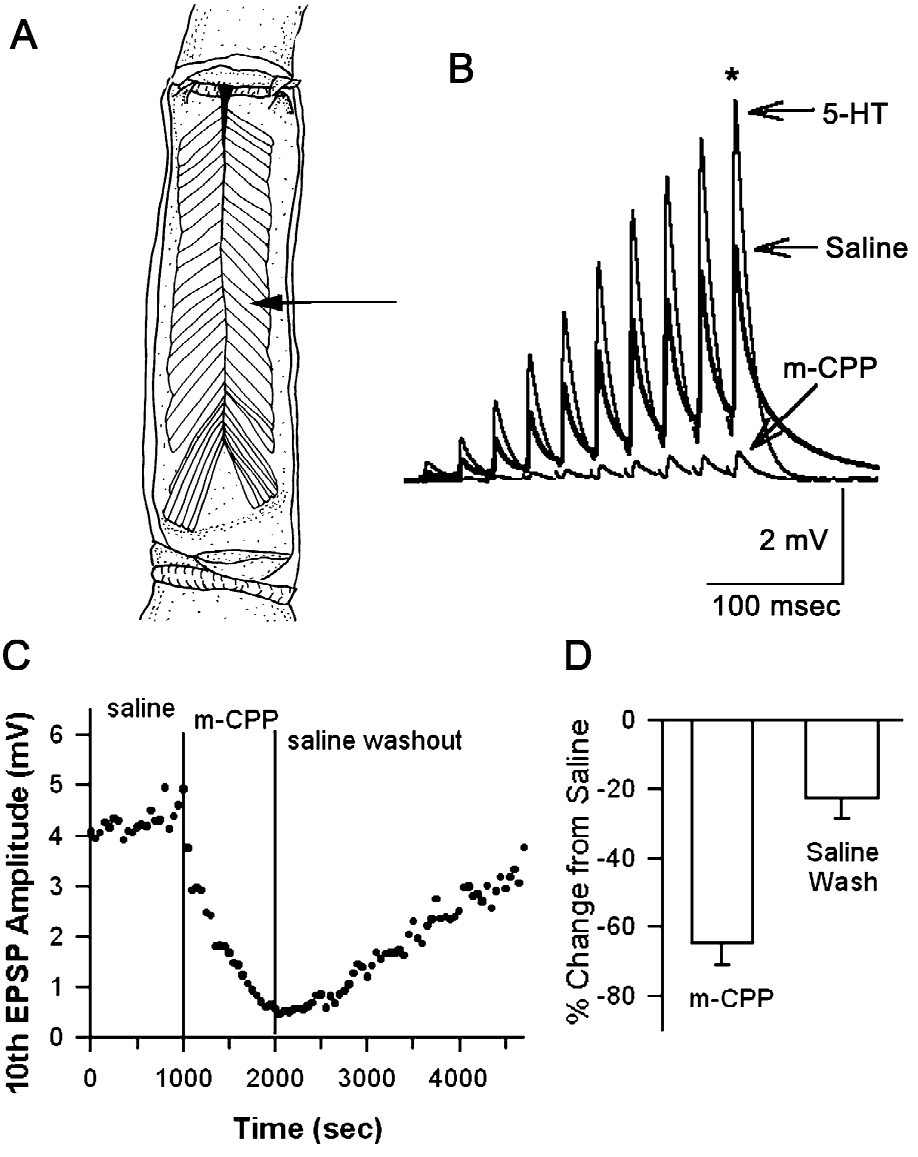

Fig. 1. (A) schematic of the opener muscle in the crayfish walking leg (A). Representative EPSPs responses to a train of 10 stimulation pulses given at 40 Hz before and during exposure to m-CPP (1 mM) (B). After the responses recovered to baseline values, 5-HT (100 nM) produced a normal enhancement of the EPSP amplitude (B). The effects in reducing the amplitude of the 10th EPSP within the train by m-CPP are gradual and reversible (C). The 10th event (marked by a star) within the train of EPSPs were used as measures of responsiveness to the various pharmacological agents. The responses did partially recover after 20 min, but it was slow in comparison to the initial depression. The general depression after 1000 s is shown for the five preparations examined (D).

presence of m-CPP. Thus, the probability of a vesicle fusing during evoked stimulation was decreased by the presence of m-CPP.

The area of the evoked current trace (a measure of charge) over time also indicated a decrease in the number of occurrences without a loss of the amount of charge for single evoked events (Fig. 2B). In addition, the magnitude of spontaneous or miniature excitatory postsynaptic currents (mEPSCs) before and during exposures to m-CPP was compared over time (Fig. 2C). This measure indicated that receptivity to glutamate was not compromised postsynaptically. No differences were noted in three out of three experiments. In two other preparations the occurrences of mEPSCs were too sparse throughout the recordings for comparison, however measures of the evoked charge showed a decrease in the occurrence without an alteration in the unitary size of single events, which was also the case for the three other preparations. The results of quantal measures led us to investigate the possibility that m-CPP might alter the evoked depolarization of the nerve terminal.

3.3. Actions of m-CPP on the action potential

Intracellular recordings of the evoked action potentials within the axon of the motor neuron revealed that m-CPP

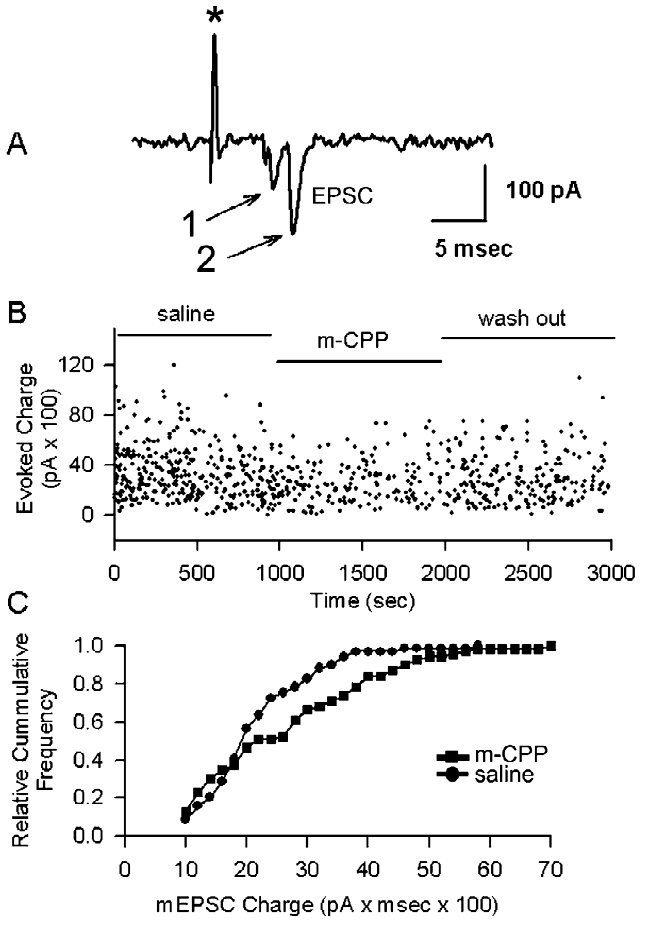

Fig. 2. Evoked excitatory postsynaptic currents (EPSCs) are obtained by a focal macropatch electrode being placed directly over a visualized varicosity (A). Two discrete evoked quantal currents are shown (1 and 2) in the recording. As shown in a representative trail, the area (or charge) of the evoked EPSCs (B) measured over time before and during exposure to m-CPP (1 mM) indicate that fewer evoked responses are occurring without a change in the individual quantal current size. A plot of relative cumulative number of occurrences for the charge of mEPSCs shows no significant shift between saline and m-CPP exposure (C).

Table 1
Direct counts of the quantal events recorded from tonic terminals of the crayfish NMJ before, during exposure to m-CPP and after washing of the preparation

	Saline		m-CPP		Wash	
	A	B	C	D	E	F
Prep 1	Obs	Obs	Obs	Obs	Obs	Obs
0	317	353	415	416	380	394
1	164	145	91	84	115	104
2	9	2	3	0	5	2
m	0.37	0.29	0.19	0.168	0.25	0.22
Prep 2	Obs	Obs	Obs	Obs	Obs	Obs
0	87	113	145	205	176	120
1	392	368	329	273	298	352
2	21	19	25	22	24	27
3	0	0	1	0	2	1
m	0.87	0.81	0.76	0.63	0.7	0.82
Prep 3	Obs	Obs	Obs	Obs	Obs	Obs
0	75	86	74	228	188	199
1	329	333	320	238	285	280
2	76	73	89	28	27	21
3	14	8	16	4	0	0
4	4	0	1	0	0	0
m	1.09	1.01	1.1	0.62	0.68	0.64
Prep 4	Obs	Obs	Obs	Obs	Obs	Obs
0	400	401	434	422	435	426
1	88	70	63	64	64	68
2	2	0	3	2	1	6
m	0.19	0.15	0.14	0.12	0.13	0.16
Prep 5	Obs	Obs	Obs	Obs	Obs	Obs
0	457	431	466	470	452	474
1	43	68	26	18	45	25
2	0	1	3	3	3	1
3	0	0	0	1	0	0
m	0.09	0.14	0.07	0.06	0.1	0.05

The first column states the number of discrete events (0, failures; 1, single events; 2 double events, etc.) that occurred per stimulus trial. The second column states the observed number of occurrences during each of the two sets of 500 trials before and during exposure to m-CPP, respectively. The mean quantal content (*m*) for each condition is shown for each of the 500 trials.

(1 mM) reduced the amplitude and broadened the duration, resulting in an increase in the time to peak response (Fig. 3A). In addition, the conduction velocity was decreased, along with a slight decrease (less negative) in the resting membrane potential by 2–3 mV. A representative preparation to illustrate the onset and extent of the effects over time is shown in Fig. 3B and C. A reduction in the amplitude and conduction velocity and increased time to peak response were observed in three out of three experiments, with slight differences in the onset of the effect and rate of recovery upon washing out m-CPP. The mean rate in the decline of the amplitude was 0.03 mV/s (S.E.M.$\pm$0.0051 mV/s, n=3). The reduced amplitude of the action potential is likely related to the reduction in the EPSP amplitudes since fewer voltage-gated calcium channels would be activated within the terminals. This could also account for the abrupt failure in the ability to induce an EPSP in some preparations during prolonged exposure to m-CPP. The recovery time after washing the preparations was quite variable. It ranged from 3000 s to more then 5000 s in the three preparations.

3.4. Effects of m-CPP on primary sensory neurons within the crayfish

Since m-CPP had direct effects on the motor neurons, we examined if similar alterations occurred for primary sensory neurons within the crayfish. For this study, we used the well-established preparation of the proprioceptive sensory neuron associated with the slowly-adapting muscle receptive organ (MRO) in the abdomen of the crayfish [36,49]. The cell maintains a relatively constant firing frequency upon static sensory stimulation.

A representative activity profile of a slowly-adapting MRO while it is bathed in saline is shown in Fig. 4A. Upon exchanging the bathing medium with saline con-

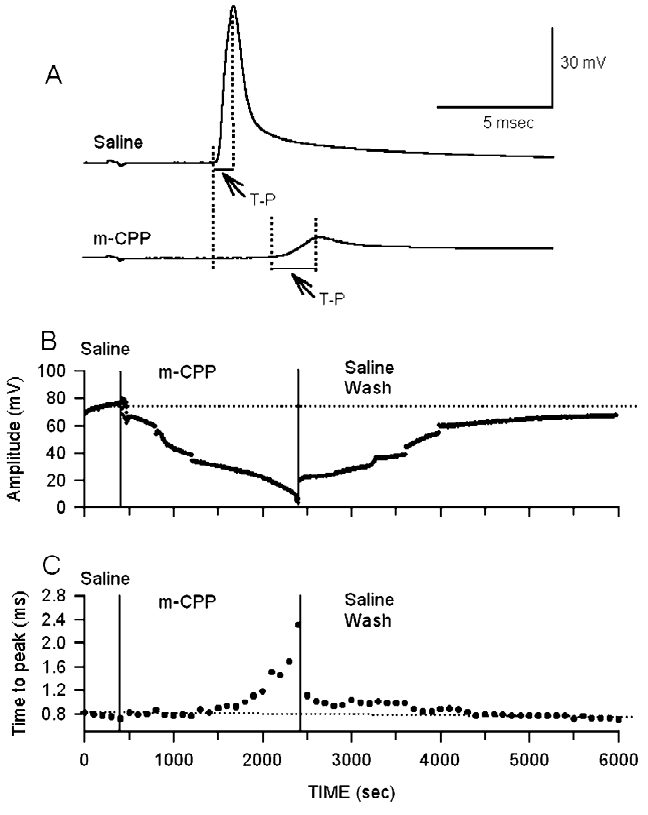

Fig. 3. The conduction velocity and the amplitude of the motor neuron action potential gradually decreases while the width increase during exposure to m-CPP (A). The decrease in the amplitude continues to decrease while exposed to m-CPP, however it is reversible upon extensive washing of the preparation albeit at a slower rate then the decline (B). The time to peak of the action potential gradually increases during exposure to m-CPP which parallels the reduction in the peak amplitude (C).

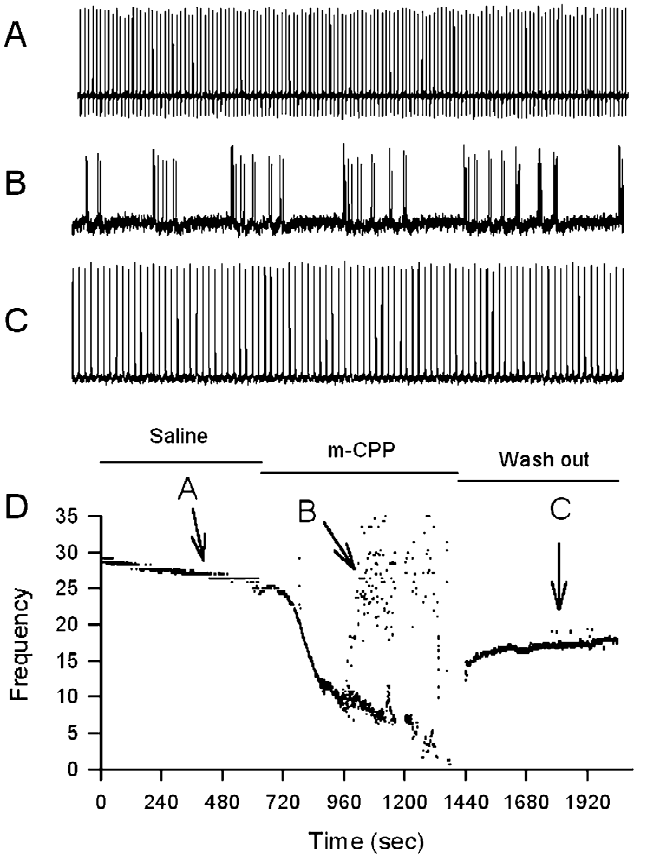

Fig. 4. The actions of m-CPP depressed the electrical activity of the primary sensory neuron associated with proprioception of the abdomen (i.e. the slowly-adapting muscle receptor). The steady firing frequency of the neuron (A) gradually decreases upon exposure to m-CPP (100 μM) (B) and partially recovers upon wash out (C). In five out of five preparations m-CPP resulted in the neuron to show short burst of activity ranging from two spikes to a series of 10 or 15 spikes at a high frequency which eventually decreases together along with the overall firing frequency (D). The traces shown in A–C are snap shots at various 5 s periods as indicated in D.

taining 100 μM or 1 mM m-CPP, the firing frequency decreases (Fig. 4B). Plots of instantaneous frequency profiles revealed bursts in the activity after several minutes during exposure to m-CPP, while the overall firing frequency showed a marked decrease (Fig. 4D). Windows of 5 s are shown in Fig. 4A–C for the responses during saline, m-CPP exposure, and upon wash out, respectively. In five out of five preparations exposed to 100 μM m-CPP, the firing frequency decreased within a few minutes. In three out of the five preparations, the majority of the depression was recovered with wash out within 20 min. As with the EPSPs recorded at the crayfish NMJ, recovery is slower than the onset of the initial depression. The decrease in firing frequency was markedly pronounced at 1 mM and wash out was not successful even after 20 min of extensive washing of the preparation with saline. This was observed in five out of five experiments conducted with 1 mM.

For controls in mechanical disturbance induced by switching the bathing medium, sham experiments were conducted in an earlier investigation [26] in which the original bathing saline was replaced by fresh saline. Completely exchanging the bathing medium took less than 30 s. Five sham experiments were performed. In three out of the five cases, the neuron decreased in firing frequency during the initial solution transition followed by another period of reduced activity and then small bursts of activity, each lasting only a second or two. The average firing frequency, measured for each sequential 10 s, throughout the entire recording period reveal no prolonged effects due to exchanging of the bathing medium in sham controls.

The time course for the activity depression induced by m-CPP at a given concentration did vary among preparations but the overall trends were identical. During 100 μM exposure, the firing frequency would decrease and, shortly afterwards, two spikes would appear as a pair, followed by a delay before the next pair of spikes appeared. After a few minutes, the paired spikes would transform to short trains of bursts with a high-intraburst frequency (Fig. 4B and D). At the high dose (1 mM), three of the five preparations

shut down completely in less that 1 min without showing bursts.

3.5. Effects of m-CPP on EPSPs at the larval fly NMJ

Since the actions of m-CPP at the crayfish NMJ appeared to be independent of 5-HT receptor mediated responses, a similar type of NMJ was examined in another invertebrate skeletal muscle preparation, but this time one that was known to be insensitive to exogenous application to 5-HT. This preparation was the ventral longitudinal muscle m6 of segment 4 in the wandering 3rd instar.

The muscle, m6, is innervated by both Ib and Is motor neurons [3,50]. Both Ib and Is neurons can be recruited individually or together to produce a composite EPSP as shown in Fig. 5A. The Ib axon has large terminal varicosities on the muscle fibers but gives rise to small EPSPs, whereas the Is has small terminal varicosities and produces large EPSPs [50]. The individual responses as well as the composite EPSP were rapidly reduced in amplitude upon exposure to 100 μM m-CPP (Fig. 5B). This was consistent in five out of five preparations with about the same rate as indicated by the percent change in EPSP amplitudes over time (Fig. 5C). Exposure of a preparation to 1 mM m-CPP attenuated the EPSPs within seconds, and recovery was difficult to obtain (data not shown).

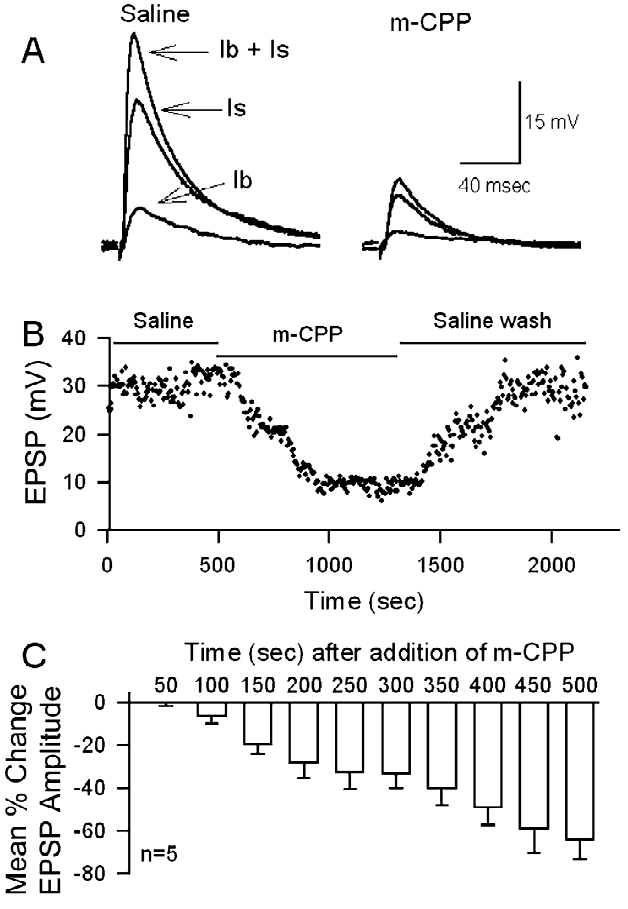

Fig. 5. In the 3rd instar of larval *Drosophila*, the abdominal muscle 6 is innervated by two axons, Ib and Is. The Ib axon can usually be recruited at a lower threshold and it produces a smaller EPSPs than the Is axon. Representative examples are shown before (A) and after 2 min of exposure to m-CPP (1 mM) (B). Note the amplitudes of the Ib and the Is EPSPs both decrease as a result of m-CPP exposure. A representative example in the rate of depression in the amplitude of the combined Ib + Is EPSPs is shown (C). Note the responses partially recover from extensive washing.

3.6. Effects of m-CPP on rat dorsal horn neurons

The effects of m-CPP (1 mM) were investigated in six spinal cord neurons, in which four of the neurons were from the superficial dorsal horn (lamina II, substantia gelatinosa) and two neurons were from lamina V. The resting membrane potential and input resistance were -57 ± 2.3 mV and 737 ± 67 MΩ, respectively. Application of m-CPP (1 mM) by superfusion did not produce a significant change in *I–V* relationship in five neurons and induced an inward current in one neuron. The amplitude of the evoked action potentials (Fig. 6A) as well as the amplitude of the fast inward current were not affected by m-CPP. However, the compound at 1 mM did produce, in five out of six neurons, an enhancement in the occurrences of the excitatory spontaneous synaptic activity (mEPSCs) (Fig. 6B). The general effect lasted approximately 8–10 min in all the preparations and was reversible after washing the preparation with Krebs solution. Two cells were examined for repetitive exposure of m-CPP at the same concentration, but this time, TTX (500 nM), bicuculline (20 mM) and strychnine (1 mM) were applied by superfusion to exclude any indirect effect and possible contamination from inhibitory synaptic responses before the application of m-CPP. No differences in the activity profiles were observed between the first trial and the second trial for these two preparations.

4. Discussion

In this study, we demonstrated that m-CPP, commonly used as a 5-HT-2C receptor agonist in human clinical studies [6,71] and various vertebrate studies [1,16], did not appear to act through a 5-HT receptor in depressing neuronal function in the invertebrates (crayfish and *Drosophila*). Instead, m-CPP likely decreased sodium influx through voltage-gated sodium channels present in motor and primary sensory neurons. This reduction in the amplitude of action potentials presynaptically led to depression in glutamatergic synaptic transmission at the NMJs in both crayfish and *Drosophila*. However, when the same concentration was used on the rat spinal cord, an enhancement in the occurrence of mEPSPs was observed.

The precise mechanism of action of m-CPP in vertebrate systems does not appear to be resolved; a wide range of

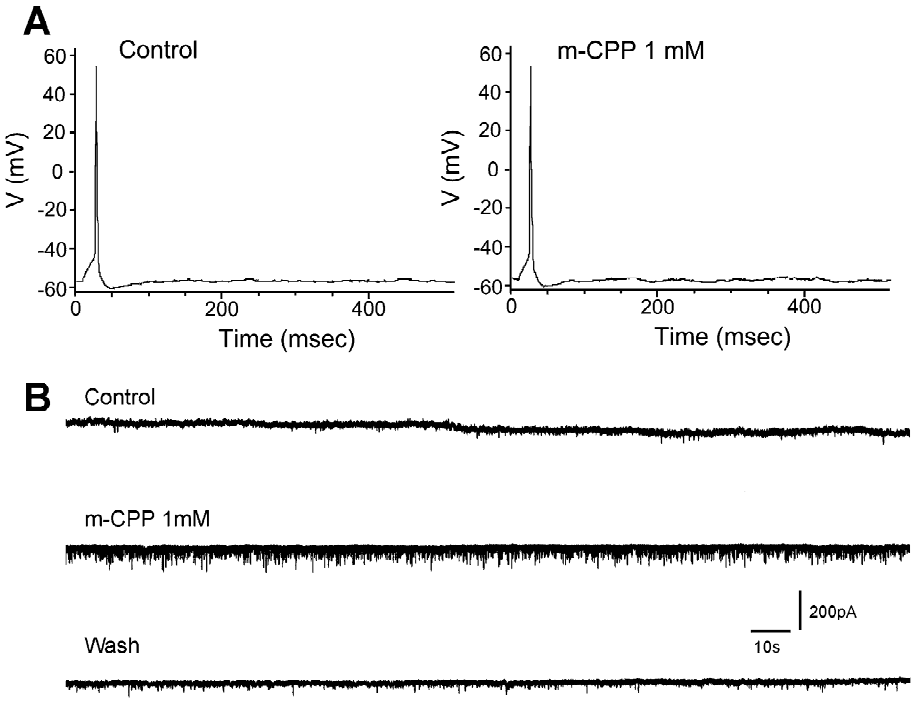

Fig. 6. Application of m-CPP (1 mM) by superfusion of thoracolumbar spinal cord slices did not produce a significant change in the amplitude of the action potentials when exposed to m-CPP. The cell was maintained in current-clamp (A). In five out of six neurons, there was an enhancement in the frequency of excitatory spontaneous synaptic activity as measured by the number of mEPSCs while in voltage-clamp. This enhanced effect lasted 8–10 min and was reversible after wash (B).

behavioral and physiological actions have been observed and various postulations made as to its mechanism of action. It has been suggested that m-CPP blocks the 5-HT transporter, resulting in an acute increase in levels of 5-HT within the extracellular fluid in the CNS of rats. It has also been reported that m-CPP can act as both 5-HT_1 and 5-HT_2 receptor agonist and down-regulate the number of 5-HT receptors in rats [37]. The reduction in food intake in rats induced by m-CPP is assumed to work via m-CPP's action as a 5-HT(1B/2C) receptor agonist [42,67,77]. In addition, recent evidence supports the notion that mCPP releases neuronal 5-HT by a common non-exocytotic diffusion-exchange mechanism involving the 5-HT transporter [70]. It is also noted that m-CPP can increase the extracellular concentrations of dopamine [35]. Human experimentation has indicated that m-CPP possesses 5-HT receptor agonist/antagonist actions and that such broad actions cause the 'serotonin syndrome' that can be induced from a single oral dose of m-CPP [46].

As for the invertebrates, m-CPP has been used as a vertebrate analog to 5-HT_1 receptor agonist to demonstrate altered sensory drive into the animals ventral nerve cord based on social status [89,90]. Yeh et al. [89,90] demonstrated that a difference in the response to m-CPP (50 μM) occurred after crayfish were paired for a few days. The implications of these studies are hard to interpret based on our recent findings that m-CPP (100 μM m-CPP or 1 mM) depressed the amplitude of action potentials in motor and sensory neurons in crayfish. In addition, we reported that m-CPP blocked glutamatergic synaptic transmission by an unknown mechanism [19]. In this past study, we also reported a depression to acutely applied 5-HT at the NMJ of crayfish that were chronically exposed to m-CPP, suggesting a 5-HT receptor down-regulation by chronic exposure to m-CPP. In light of current evidence we now report, the past responses in which m-CPP was used to asses 5-HT responsiveness should be re-evaluated, since neuronal function was compromised.

We are not aware of any study that has directly examined the role of m-CPP as a potential blocker of voltage-gated ion channels in neurons, but this would resolve a likely mechanism of action for m-CPP in the crayfish and *Drosophila* preparations. The decrease in the amplitude and widening of the action potentials during exposure to m-CPP very closely mimics the responses noted, in the squid giant axons, when extracellular sodium chloride was replaced with choline chloride [43]. Since we observed similar responses in alterations of the action potential shape in the axons of motor neurons to m-CPP, which does not occur for exposure to 5-HT [26,30], it is unlikely m-CPP has actions similar to vertebrates in these species. However, m-CPP may show promise in these preparations as a blocker of sodium channels in motor nerve terminals when depolarizations are induced to activate voltage-gated calcium channels to assess calcium's role, independent of sodium influx, in properties of synap-

tic transmission such as: short-term facilitation [29,33], long-term facilitation [76] or in computational modeling of sodium and calcium domains at synapses [27,87].

The ability of m-CPP to increase the frequency of mEPSCs of the rat dorsal horn neurons in our study suggests that m-CPP caused nerve terminals, which were in contact with the cell being monitored, to spontaneously release transmitter. This occurred in the absence of nerve stimulation. In addition, since evoked responses and the shape of the induced currents were not altered by m-CPP, the actions of m-CPP are likely to be selective on the presynaptic nerve terminals. These actions do not mimic the actions of 5-HT application, which may in part be due to the varied receptor subtypes present. The 5-HT receptors subtypes within spinal cord of rat have been intensively investigated.

It is known by autoradiographic mapping that 5-HT_{1A}, 5-HT_{1B}, and 5-HT_2 receptors are present in the rat spinal cord. 5-HT_{1A} receptors are mainly present in the dorsal horn, while 5-HT_{1B} receptors occur throughout the spinal cord; fewer 5-HT_2 receptors are noted in the dorsal horn [57]. Binding and immunohistochemical studies showed the presence of 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors in the dorsal horn [18,41]. The source of spinal 5-HT_{2C} might be the intrinsic dorsal horn neurons or the primary afferent fibers [66]. Serotonin is one of the compounds responsible for the modulation of sensory transmission in the dorsal horn. Although the descending central projections involved in anti-nociception contain serotonin, there is growing evidence that supports a role for the serotonergic pathways in descending inhibition as well as in descending facilitation within the dorsal horn [58]. The opposing actions of 5-HT could be explained by the presence of different subtypes of 5-HT receptors, their different localization on dorsal horn structures (primary afferents fibers, excitatory/inhibitory interneurons, projection neurons), or by 5-HT interacting with other transmitters involved in nociception. The excitatory effect of 5-HT_{2C} receptor activation on neuronal activity and their distribution in the dorsal horn are compatible with a pronociceptive role [58,63].

The effects of 5-HT on synaptic transmission are also dependent on cell types. For example, 5-HT depresses synaptic transmission, which is likely mediated by 5-HT_{1A} receptors, in the deep dorsal horn neurons [38,55]. There are several proposed models as to how 5-HT may exert its action on spinal cord neurons. For example, indirect effects may be a result of 5-HT causing the release of noradrenaline and/or adenosine from impinging nerve terminals in the region [75]. In addition, 5-HT may modulate the behavioral response to substance P on the neurons [34]. These actions are thought to be mediated by 5-HT_1 receptors, since selective agonists, including m-CPP, can mimic this response. Since there is a potentiation of GABA effects mediated by 5-HT2 receptors through a protein kinase-dependent transduction pathway [52], we blocked GABA receptors with the use of bicuculline (20 mM) during m-CPP exposure experiments in the rats. Since no differences were observed in the responses for m-CPP application before or after GABA receptor blockade, there is likely no GABAergic intrinsic activity influencing the responses measured in the paradigm used in the study.

Use of the *Drosophila* NMJ shares many of the advantages of the crayfish model, as there are few motor neurons to consider, and it is possible to perform direct quantal counts of vesicular release over individual nerve terminal varicosities. It has been shown that 5-HT has no direct effect on modulating synaptic transmission at the *Drosophila* skeletal neuromuscular junction as used in this current study [21], thus any effects caused by m-CPP in this system can be assumed to be acting independent of 5-HT effects.

Since the cDNA and protein sequence of the voltage-gated sodium channel and 5-HT receptors of crayfish are not known, we can not compare to the vertebrate systems for a common molecular signature that binds to m-CPP. However, since we observed the same physiological phenomena to be present in the *Drosophila* model for m-CPP's actions, we compared known sequences for the *Drosophila* and vertebrates. In examining for similarities in cDNA sequence and the protein sequence between Human 5-HT2A receptor (accession # NM 000621; NP 000612.1) and the *Drosophila malanogaster* voltage gated sodium channel (accession # NM 079134; NP 523858.4) no significant similarity appears. However, there are a number of differences in sodium channel function in insects suggesting alternative splicing in various tissues. Tan et al. [83] have noted, 'Similarly, early electrophysiological studies show that pyrethroid insecticides affect the insect PNS, e.g. sensory neurons, more effectively than the CNS [12,59,65,69,72], suggesting the existence of distinct types of sodium channels. The molecular basis of this diversity, however, is not understood.' which supports the notion of selective alternative splicing.

There are several reports that antagonists to vertebrate 5-HT3 subtype receptors also alter the function of ion channels. For example, the 5-HT3 receptor antagonists granisetron (Kytril), ondansetron (Zofran), and dolasetron (Anzemet) also appear to block the human cardiac sodium channel [51]. Even high concentrations (micromolar range) of steroids are known to inhibit both the 5-HT3 receptor channel and the voltage-gated sodium channel expressed in neuroblastoma cells [5]. It appears that there is some non-selective actions among the compounds that effect 5-HT3 receptors and voltage-gated sodium channels. Such altered actions of compounds for the 5-HT2 subfamily and voltage-gated sodium channels are not common. However, one recent report [15] presents evidence that 5-HT, working via a 5-HT2a or 5-HT2c receptors, inhibits voltage-gated sodium channels through a protein kinase-C mechanism in the apical dendrites of pyramidal neurons in mice. The general pharmacology for vertebrate 5-HT2 family of

vertebrate receptors, except for the actions of m-CPP, appear to imply the actions of 5-HT at the crayfish NMJ are working in part through a similar 5-HT2 family of receptors which results in enhancement of synaptic transmission [23,82].

In summary, it seems that while much of the activity of m-CPP in altering synaptic transmission may be attributed to serotonergic effects, some of the discrepancies in the activity of the compound may be due to sodium channel binding.

Acknowledgements

Funding was provided by NSF grants IBN-9808631 and IBN-0131459 (R.L. Cooper), NSF-ILI-DUE 9850907 (R.L. Cooper) and a G. Ribble Fellowship for undergraduate studies in the School of Biological Sciences at the University of Kentucky (J. Tabor and G.M. Sparks) and a Undergraduate Research Scholarship awarded by the Arnold and Mabel Beckman Foundation (G.M. Sparks), NIH NS18710 (N.J. Dun)

References

[1] W. Abi-Saab, J.P. Seibyl, D.C. D'Souza, L.P. Karper, R. Gueorgueva, A. Abi-Dargham, M.L. Wong, S. Rajhans, J.P. Erdos, G.R. Heninger, D.S. Charney, J.H. Krystal, Ritanserin antagonism of m-chlorophenylpiperazine effects in neuroleptic-free schizophrenics patients: support for serotonin-2 receptor modulation of schizophrenia symptoms, Psychopharmacology 162 (2002) 55–62.

[2] V.R. Antunes, G.C. Brailoiu, E.H. Kwok, P. Scruggs, N.J. Dun, Orexins/hypocretins excite rat sympathetic preganglionic neurons in vivo and in vitro, Am. J. Physiol. Regul. Integr. Comp. Physiol. 281 (2001) R1801–R1807.

[3] H.L. Atwood, R.L. Cooper, Functional and structural parallels in crustaceans and *Drosophila* neuromuscular systems, Am. Zool. 35 (1995) 556–565.

[4] H.L. Atwood, R.L. Cooper, Synaptic diversity and differentiation: Crustacean neuromuscular junctions, Invert. Neurosci. 1 (1996) 291–307.

[5] M. Barann, M. Göthert, M. Brüss, H. Bönisch, Inhibition by steroids of [^{14}C]-guanidinium flux through the voltage-gated sodium channel and the cation channel of the 5-HT3 receptor of N1E-115 neuroblastoma cells, Naunyn-Schmiedeberg's Arch. Pharmacol. 360 (1999) 234–241.

[6] N.M. Barnes, T. Sharp, A review of central 5-HT receptors and their function, Neuropharmacology 58 (1999) 1083–1152.

[7] M.H. Baumann, D.C. Mash, J.K. Staley, The serotonin agonist *m*-chlorophenylpiperazine (mCPP) binds to serotonin transporter sites in human brain, Neuroreport 6 (1995) 2150–2152.

[8] M.H. Baumann, J.J. Rutter, S.B. Auerbach, Intravenous administration of the serotonin agonist *m*-chlorophenylpiperazine (mCPP) increases extracellular serotonin in the diencephalons of awake rats, Neuropharmacology 32 (1993) 1381–1386.

[9] B.S. Beltz, E.A. Kravitz, Mapping of serotonin-like immunoreactivity in the lobster nervous system, J. Neurosci. 3 (1983) 585–602.

[10] F.G. Boess, I.L. Martin, Molecular biology of 5-HT receptors, Neuropharmacology 33 (1994) 275–317.

[11] A. Broocks, T. Meyer, A. George, U. Hillmer-Vogel, D. Meyer, B. Bandelow, G. Hajak, U. Bartmann, C.H. Gleiter, E. Ruther, Decreased neuroendocrine responses to meta-chlorophenylpiperazine (m-CPP) but normal responses to ipsapirone in marathon runners, Can. J. Physiol. Pharmacol. 76 (1998) 278–283.

[12] P.E. Burt, R.E. Goodchild, The site of action of pyrethrin I in the nervous system of the cockroach *Periplaneta americana,* Ent, Exp. Appl. 14 (1971) 179–189.

[13] L. Buydens-Branchey, M. Branchey, P. Fergeson, J. Hudson, C. McKernin, Euphorogenic properties of the serotonergic partial agonist *m*-chlorophenylpiperazine in cocaine addicts, Arch. Gen. Psychiatr. 50 (1993) 1001.

[14] J.A. Campos-Ortega, V. Hartenstein, The Embryonic Development of *Drosophila melanogaster*, Spring-Verlag, Berlin, 1985.

[15] D.B. Carr, D.C. Cooper, S.L. Ulrich, N. Spruston, D.J. Surmeier, Serotonin receptor activation inhibits sodium current and dendritic excitability in prefrontal cortex via a protein kinase C-dependent mechanism, J. Neurosci. 22 (2002) 6846–6855.

[16] L. Castro, I. Maldonado, I. Campos, B. Varjao, A.L. Angelo, R.A. Athanazio, M.C. Barbetta, A.C. Ramos, J.B. Fregoneze, E. De Castro e Silva, Central administration of mCPP, a serotonin 5-HT(2B/2C) agonist, decreases water intake in rats, Pharmacol. Biochem. Behav. 72 (2002) 891–898.

[17] D.S. Charney, S.W. Woods, W.K. Goodman, G.R. Heninger, Serotonin function in anxiety. Effects of the serotonin agonist, mCPP, in panic disorder patients and healthy subjects, Psychopharmacology 92 (1987) 14–24.

[18] R.E. Coggeshall, S.M. Carlton, Receptor localization in the mammalian dorsal horn and primary afferent neurons, Brain Res. 24 (1997) 28–66.

[19] R.L. Cooper, C.C. Harrington, L. Marin, H.L. Atwood, Quantal release at visualized terminals of a crayfish motor axon: Intraterminal and regional differences, J. Comp. Neurol. 375 (1996) 583–600.

[20] R.L. Cooper, L. Marin, H.L. Atwood, Synaptic differentiation of a single motor neuron: conjoint definition of transmitter release, presynaptic calcium signals, and ultrastructure, J. Neurosci. 15 (1995) 4209–4222.

[21] R.L. Cooper, W.S. Neckameyer, Dopaminergic neuromodulation of motor neuron activity and neuromuscular function in *Drosophila melanogaster*, Comp. Biochem. Physiol. 122 (1999) 199–210.

[22] R.L. Cooper, M.E. Ruffner, Depression of synaptic efficacy at intermolt in crayfish neuromuscular junctions by 20-hydroxyecdysone, a molting hormone, J. Neurophysiol. 79 (1998) 1931–1941.

[23] R.L. Cooper, R.J. Chase, J. Tabor, Altered responsiveness to 5-HT at the crayfish neuromuscular junction due to chronic p-CPA and m-CPP treatment, Brain Res. 916 (2001) 143–151.

[24] R.L. Cooper, A. Dönmezer, J. Shearer, Intrinsic differences in sensitivity to 5-HT between high- and low-output terminals innervating the same target, Neurosci. Res. 45 (2003) 163–172.

[25] R.L. Cooper, B.A. Stewart, J.M. Wojtowicz, S. Wang, H.L. Atwood, Quantal measurement and analysis methods compared for crayfish and *Drosophila* neuromuscular junctions and rat hippocampus, J. Neurosci. Methods 61 (1995) 67–78.

[26] R.L. Cooper, E. Ward, R. Braxton, H. Li, W.M. Warren, The effects of serotonin and ecdysone on primary sensory neurons in crayfish, Microsc. Res. Tech. 60 (2003) 336–345.

[27] R.L. Cooper, J. Winslow, C.K. Govind, H.L. Atwood, Synaptic structural complexity as a factor enhancing probability of calcium-mediated transmitter release, J. Neurophysiol. 75 (1996) 2451–2466.

[28] M.E. Crider, R.L. Cooper, The importance of the stimulation paradigm in determining facilitation and effects of neuromodulation, Brain Res. 842 (1999) 324–331.

[29] M.E. Crider, R.L. Cooper, Differentially facilitation of high- and low-output nerve terminals from a single motor neuron, J. Appl. Physiol. 88 (2000) 987–996.

[30] D. Dixon, H.L. Atwood, Conjoint action of phosphoinositol and

adenylate cyclase systems in serotonin-induced facilitation at the crayfish neuromuscular junction, J. Neurophysiol. 62 (1989) 1251–1259.
[31] J. Dudel, Facilitatory effects of 5-hydroxy-tryptamine on the crayfish neuromuscular junction, Naunyn-Schmiedebergs Arch. Exp. Path.u. Pharmak. 249 (1965) 515–528.
[32] J. Dudel, S.W. Kuffler, The quantal nature of transmission and spontaneous miniature potentials at the crayfish neuromuscular junction, J. Physiol. 155 (1961) 514–529.
[33] J. Dudel, I. Parnas, H. Parnas, Neurotransmitter release and its facilitation in crayfish muscle. Release determined by both, intracellular calcium concentration and depolarization of the nerve terminal, Pflugers Arch. 399 (1983) 1–10.
[34] P.K. Eide, Stimulation of 5-HT1 receptors in the spinal cord changes substance P-induced behaviour, Neuropharmacology 31 (1992) 541–545.
[35] E. Eriksson, G. Engberg, O. Bing, H. Nissbrandt, Effects of mCPP on the extracellular concentrations of serotonin and dopamine in rat brain, Neuropsychopharmacology 20 (1999) 287–296.
[36] H.L. Fields, Crustacean abdominal and thoracic muscle receptor organs, in: P.J. Mill (Ed.), Structure and Function of Proprioceptors in the Invertebrate, Chapman and Hall, New York, 1976, pp. 65–114.
[37] K.C. Fone, R.H. Austin, I.A. Topham, G.A. Kennett, T. Punhani, Effect of chronic m-CPP on locomotion, hypophagia, plasma corticosterone and 5-HT2C receptor levels in the rat, Br. J. Pharmacol. 123 (1998) 1707–1715.
[38] S.M. Garraway, S. Hochman, Pharmacological characterization of serotonin receptor subtypes modulating primary afferent input to deep dorsal horn neurons in the neonatal rat, Br. J. Pharmacol. 132 (2001) 1789–1798.
[39] A. Hamik, S.J. Peroutka, 1-(*m*-chlorophenyl)piperazine (mCPP) Interactions with neurotransmitter receptors in the human brain, Biol. Psych. 25 (1989) 569–575.
[40] R.A. Hahn, M.D. Hynes, R.W. Fuller, Apomorphine-induced aggression in rats chronically treated with oral clonidine: Modulation by central serotonergic mechanisms, J. Pharmacol. Exp. Ther. 220 (1982) 389–393.
[41] L.A. Helton, K.B. Thor, M. Baez, 5-hydroxytryptamine2A, 5-hydroxytryptamine2B, and 5-hydroxytryptamine2C receptor mRNA expression in the spinal cord of rat, cat, monkey and human, Neuroreport 5 (1994) 2617–2620.
[42] K.N. Hewitt, M.D. Lee, C.T. Dourish, P.G. Clifton, Serotonin 2C receptor agonists and the behavioural satiety sequence in mice, Pharmacol. Biochem. Behav. 71 (2002) 691–700.
[43] A.L. Hodgkin, B. Katz, The effect of sodium ions on the electrical activity of the giant axon of the squid, J. Physiol. 108 (1949) 37–77.
[44] R. Invernizzi, C. Fracasso, S. Caccia, S. Garattini, R. Samanin, Effects of intracerebroventricular administration of D-fenfluramine and D-norfenfluramine, as a single injection or 2-h infusion, on serotonin in brain: Relationship to concentrations of drugs in brain, Neuropharmacology 30 (1991) 119–123.
[45] R.S. Kahn, S. Wetzler, *m*-Chlorophenylpiperazine as a probe of serotonergic function, Biol. Psychiatr. 30 (1991) 1139–1166.
[46] T. Klaassen, K.L. Ho Pian, H.G. Westenberg, J.A. den Boer, H.M. van Praag, Serotonin syndrome after challenge with the 5-HT agonist meta-chlorophenylpiperazine, Psychiatry Res. 79 (1998) 207–212.
[47] J.H. Krystal, J.P. Seibyl, L.H. Price, S.W. Woods, G.R. Heninger, G.K. Aghajanian, D.S. Charney, *m*-Chlorophenylpiperazine effects in neuroleptic-free schizophrenic patients. Evidence implicating serotonergic systems in the positive symptoms of schizophrenia, Arch. Gen. Psychiatr. 50 (1993) 624–635.
[48] J.J. Krystal, E. Webb, N. Cooney, H.R. Krazler, D.S. Charney, Specificity of ethanol-like effects elicited by serotonergic and noradrenergic mechanisms, Arch. Gen. Psychiatr. 51 (1994) 898–911.
[49] S.W. Kuffler, Mechanisms of activation and motor control of stretch receptors in lobster and crayfish, J. Neurophysiol. 17 (1954) 558–574.
[50] P. Kurdyak, H.L. Atwood, B.A. Stewart, C.F. Wu, Differential physiology and morphology of motor axons to ventral longitudinal muscle in larval *Drosophila*, J. Comp. Neurol. 350 (1994) 463–472.
[51] Y.A. Kuryshev, A.M. Brown, L. Wang, C.R. Benedict, D. Rampe, Interactions of the 5-Hydroxytryptamine 3 antagonist class of antiemetic drugs with human cardiac ion channels, J. Pharmacol. Exp. Ther. 295 (2000) 614–620.
[52] H. Li, B. Lang, J.F. Kang, Y.Q. Li, Serotonin potentiates the response of neurons of the superficial laminae of the rat spinal dorsal horn to gamma-aminobutyric acid, Brain Res. Bull. 52 (2000) 559–565.
[53] H. Li, X. Peng, R.L. Cooper, Development of *Drosophila* larval neuromuscular junctions: Maintaining synaptic strength, Neuroscience 115 (2002) 505–513.
[54] L. Listerman, J. Deskins, H. Bradacs, R.L. Cooper, Measures of heart rate during social interactions in crayfish and effects of 5-HT, Comp. Biochem. Physiol. A. 125 (2000) 251–264.
[55] J.A. Lopez-Garcia, A.E. King, Pre- and post-synaptic actions of 5-hydroxytryptamine in the rat lumbar dorsal horn in vitro: implications for somatosensory transmission, Eur. J. Neurosci. 8 (1996) 2188–2197.
[56] L. Magrassi, D. Purves, J.W. Lichtman, Fluorescent probes that stain living nerve terminals, J. Neurosci. 7 (1987) 1207–1214.
[57] L. Marlier, J.R. Teilhac, C. Cerruti, A. Privat, Autoradiographic mapping of 5-HT1, 5-HT1A, 5-HT1B and 5-HT2 receptors in the rat spinal cord, Brain Res. 31 (1991) 15–23.
[58] M.J. Millan, Descending control of pain, Prog. Neurobiol. 66 (2002) 355–474.
[59] T.A. Miller, M.E. Adams, Central vs. peripheral action of pyrethroids on the house fly nervous system, in: Synthetic pyrethroids ACS Symposium Series, Vol. 42, American Chemical Society, Washington, DC, 1977, pp. 98–115.
[60] E.A. Mueller, D.L. Murphy, T. Sunderland, Neuroendocrine effects of *m*-chlorophenylpiperazine, a serotonin agonist, in humans, J. Clin. Endocrinol. Metab. 61 (1985) 1179–1184.
[61] K.J. Muller, J.G. Nicholls, G.S. Stent, Neurobiology of the Leech, NY, Cold Spring Harbor Laboratory, New York, 1981, p. 254.
[62] D.L. Murphy, K.P. Lesch, C.S. Aulakh, T.A. Pigott, Serotonin-selective arylpiperazines with neuroendocrine, behavioral, temperature, and cardiovascular effects in humans, Pharmacol. Rev. 43 (1991) 527–552.
[63] A.Z. Murphy, R.M. Murphy, F.P. Zemlan, Role of spinal serotonin1 receptor subtypes in thermally and mechanically elicited nociceptive reflexes, Psychopharmacology 108 (1992) 123–130.
[64] W.S. Neckameyer, R.L. Cooper, GABA transporters in *Drosophila melanogaster*: developmental expression, behavior, and physiology, Invert. Neurosci. 3 (1998) 279–294.
[65] M.P. Osborne, R.J. Hart, Neurophysiological studies of the effects of permethrin upon pyrethroid resistant (*kdr*) and susceptible strains of dipteran larvae, Pestic. Sci. 20 (1979) 100–114.
[66] M. Pompeiano, J.M. Palacios, G. Mengod, Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors, Brain Res. Mol. Brain Res. 23 (1994) 163–178.
[67] H.B. Rice, R.L. Corwin, mCPP-induced hypophagia in rats is unaffected by the profile of dietary unsaturated fatty acids, Pharmacol. Biochem. Behav. 73 (2002) 545–550.
[68] C. Rochat, L. Cervo, S. Romandini, R. Samanin, Evidence that *m*-chlorophenylpiperazine inhibits some nociceptive responses of rats by activating 5-hydroxytryptamine mechanisms, J. Pharm. Pharmacol. 34 (1982) 325–327.
[69] M. Roche, J.C. Guillet, Effects of the pyrethroids bioallethrin, deltamethrin and RU-15525 on the electrical activity of a cuticular mechanoreceptor of the cockroach *Periplaneta americana*, Pestic. Sci. 16 (1985) 511–519.

[70] R. Rothman, M. Baumann, Therapeutic and adverse actions of serotonin transporter substrates, Pharmacol. Ther. 95 (2002) 73.
[71] B. Sabbe, W. Hulstijn, M. Maes, M. Pier, S. Scharpe, F. Zitman, Psychomotor slowing, neuroendocrine responses, and behavioral changes after oral administration of meta-chlorophenylpiperazine in normal volunteers, Psychiatry Res. 105 (2001) 151–163.
[72] V.L. Salgado, S.N. Irving, T.A. Miller, The importance of nerve terminal depolarization in pyrethroid poisoning of insects, Pestic. Biochem. Physiol. 20 (1983) 169–182.
[73] R. Samanin, T. Mennini, A. Ferraris, C. Bendotti, F. Borsini, S. Garattini, *m*-Chlorophenylpiperazine: A central serotonin agonist causing powerful anorexia in rats, Naunyn Schmiedeberg Arch. Pharmacol. 308 (1979) 159–163.
[74] E. Sanders-Bush, H. Canton, Serotonin receptors: signal transduction pathways, in: F.E. Bloom, D.J. Kupfer (Eds.), Psychopharmacology, The Fourth Generation of Progress, Raven, New York, 1995, pp. 431–442.
[75] J. Sawynok, A. Reid, Interactions of descending serotonergic systems with other neurotransmitters in the modulation of nociception, Behav. Brain Res. 73 (1996) 63–68.
[76] R.G. Sherman, H.L. Atwood, Synaptic facilitation: long-term neuromuscular facilitation in crustaceans, Science 171 (1971) 1248–1250.
[77] Y. Sugimoto, T. Yoshikawa, T. Noma, J. Yamada, The 5-HT2C/2B receptor agonist *m*-chlorophenylpiperazine (mCPP) inhibits 2-deoxy-D-glucose (2-DG)-induced hyperphagia in rats, Biol. Pharm. Bull. 24 (2001) 1431–1433.
[78] H. Schneider, B.A. Trimmer, J. Rapus, M. Eckert, D.E. Valentine, E.A. Kravitz, Mapping of octopamine-immunoreactive neurons in the central nervous system of the lobster, J. Comp. Neurol. 329 (1993) 129–142.
[79] R.C. Southard, J. Haggard, M.E. Crider, S.W. Whiteheart, R.L. Cooper, Influence of serotonin on the kinetics of vesicular release, Brain Res. 871 (2000) 16–28.
[80] B.A. Stewart, H.L. Atwood, J.J. Renger, J. Wang, C.F. Wu, Improved stability of *Drosophila* larval neuromuscular preparation in haemolymph-like physiological solutions, J. Comp. Physiol. A. 175 (1994) 179–191.
[81] J.R. Strawn, W.S. Neckameyer, R.L. Cooper, The effects of 5-HT on sensory, central and motor neurons driving the abdominal superficial flexor muscles in the crayfish, Comp. Biochem. Physiol. 127B (2000) 533–550.
[82] J. Tabor, R.L. Cooper, Physiologically identified 5-HT2 -like receptors at the crayfish neuromuscular junction, Brain Res. 932 (2002) 91–98.
[83] J. Tan, Z. Liu, Y. Nomura, A.L. Goldin, K. Dong, Alternative splicing of an insect sodium channel gene generates pharmacologically distinct sodium channels, J. Neurosci. 22 (2002) 5300–5309.
[84] D.R. Thomas, T.L. Gager, V. Holland, A.M. Brown, M.D. Wood, *m*-Chlorophenylpiperazine (mCPP) is an antagonist at the cloned human 5-HT2B receptor, NeuroReport 7 (1996) 1457–1460.
[85] A.J. Tierney, L.A. Mangiamele, Effects of serotonin and serotonin analogs on posture and agonistic behavior in crayfish, J. Comp. Physiol. A 187 (2001) 757–767.
[86] K. Viele, A. Stromberg, R.L. Cooper, Estimating the number of release sites within the nerve terminal by statistical analysis of synaptic charge, Synapse 47 (2003) 15–25.
[87] J.L. Winslow, R.L. Cooper, H.L. Atwood, Intracellular ionic concentration by calibration from fluorescence indicator emission spectra, its relationship to the K_d, F_{min}, F_{max} formula, and use Na-Green for presynaptic sodium, J. Neurosci. Methods 118 (2002) 163–175.
[88] S.Y. Wu, S.L. Dun, M.T. Wright, J.K. Chang, N.J. Dun, Endomorphin-like immunoreactivity in the rat dorsal horn and inhibition of substantia gelatinosa neurons in vitro, Neuroscience 89 (1999) 317–321.
[89] S.R. Yeh, R.A. Fricke, D.H. Edwards, The effect of social experience on serotonergic modulation of the escape circuit of crayfish, Science 271 (1996) 366–369.
[90] S.R. Yeh, B.E. Musolf, D.H. Edwards, Neuronal adaptations to changes in the social dominance status of crayfish, J. Neurosci. 17 (1997) 697–708.