Gene expression profile of sodium channel subunits in the anterior cingulate cortex during experimental paclitaxel-induced neuropathic pain in mice

Willias Masocha Corresp. 1

1 Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Kuwait University, Safat, Kuwait

Corresponding Author: Willias Masocha
Email address: masocha@hsc.edu.kw

Paclitaxel, a chemotherapeutic agent, causes neuropathic pain whose supraspinal pathophysiology is not fully understood. Dysregulation of sodium channel expression, studied mainly in the periphery and spinal cord level, contributes to the pathogenesis of neuropathic pain. We examined gene expression of sodium channel (Nav) subunits by real time PCR in the anterior cingulate cortex (ACC) at day 7 post first administration of paclitaxel, when mice had developed paclitaxel-induced thermal hyperalgesia. The ACC was chosen because increased activity in the ACC has been observed during neuropathic pain. In the ACC of vehicle-treated animals the threshold cycle (Ct) values for Nav1.4, Nav1.5, Nav1.7, Nav1.8 and Nav1.9 were above 30 and/or not detectable in some samples. Thus, comparison in mRNA expression between untreated control, vehicle-treated and paclitaxel treated animals was done for Nav1.1, Nav1.2, Nav1.3, Nav1.6, Nax as well as Navβ1-Navβ4. There were no differences in the transcript levels of Nav1.1-Nav1.3, Nav1.6, Nax, Navβ1-Navβ3 between untreated and vehicle-treated mice, however, vehicle treatment increased Navβ4 expression. Paclitaxel treatment significantly increased the mRNA expression of Nav1.1, Nav1.2, Nav1.6 and Nax, but not Nav1.3, sodium channel alpha subunits compared to vehicle-treated animals. Treatment with paclitaxel significantly increased the expression of Navβ1 and Navβ3, but not Navβ2 and Navβ4, sodium channel beta subunits compared to vehicle-treated animals. These findings suggest that during paclitaxel-induced neuropathic pain there is differential upregulation of sodium channels in the ACC, which might contribute to the increased neuronal activity observed in the area during neuropathic pain.

1   **Gene expression profile of sodium channel subunits in the anterior cingulate cortex during**

2   **experimental paclitaxel-induced neuropathic pain in mice**

4   **Abstract**

5   Paclitaxel, a chemotherapeutic agent, causes neuropathic pain whose supraspinal

6   pathophysiology is not fully understood. Dysregulation of sodium channel expression, studied

7   mainly in the periphery and spinal cord level, contributes to the pathogenesis of neuropathic

8   pain. We examined gene expression of sodium channel (Na_v) subunits by real time PCR in the

9   anterior cingulate cortex (ACC) at day 7 post first administration of paclitaxel, when mice had

10   developed paclitaxel-induced thermal hyperalgesia. The ACC was chosen because increased

11   activity in the ACC has been observed during neuropathic pain. In the ACC of vehicle-treated

12   animals the threshold cycle (Ct) values for $Na_v1.4$, $Na_v1.5$, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ were

13   above 30 and/or not detectable in some samples. Thus, comparison in mRNA expression

14   between untreated control, vehicle-treated and paclitaxel treated animals was done for $Na_v1.1$,

15   $Na_v1.2$, $Na_v1.3$, $Na_v1.6$, Na_x as well as $Na_v\beta1$-$Na_v\beta4$. There were no differences in the transcript

16   levels of $Na_v1.1$-$Na_v1.3$, $Na_v1.6$, Na_x, $Na_v\beta1$-$Na_v\beta3$ between untreated and vehicle-treated mice,

17   however, vehicle treatment increased $Na_v\beta4$ expression. Paclitaxel treatment significantly

18   increased the mRNA expression of $Na_v1.1$, $Na_v1.2$, $Na_v1.6$ and $Na_{x,}$ but not $Na_v1.3$, sodium

19   channel alpha subunits compared to vehicle-treated animals. Treatment with paclitaxel

20   significantly increased the expression of $Na_v\beta1$ and $Na_v\beta3$, but not $Na_v\beta2$ and $Na_v\beta4$, sodium

21   channel beta subunits compared to vehicle-treated animals. These findings suggest that during

22   paclitaxel-induced neuropathic pain there is differential upregulation of sodium channels in the

23   ACC, which might contribute to the increased neuronal activity observed in the area during

24   neuropathic pain.

25   Willias Masocha

26   Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Kuwait University, Safat,

27   Kuwait

28   Phone number: +965 24636078 Email: masocha@hsc.edu.kw

Introduction

30   Voltage-gated sodium channels (Na_v) are responsible for action potential initiation and

31   propagation in neurons and other excitable cells. Sodium channels are composed of a pore-

32   forming α subunit associated with one or more auxiliary β subunits that modulate channel gating,

33   expression and localisation (Catterall et al. 2005; Isom 2001). There are ten sodium channel α

34   subunits $Na_v1.1$-$Na_v1.9$ and Na_x encoded by genes SCN1A-SCN11A, and four β subunits $Na_v\beta1$-

35   $Na_v\beta4$, encoded by genes SCN1B-SCN4B (Brackenbury & Isom 2008; Cummins et al. 2007; Yu

36   & Catterall 2003). These sodium channel subunits are expressed in a wide variety of tissues and

37   the level of expression of each channel varies between tissues.

38   Sodium channels play an important role in the propagation of nociceptive signals. Changes in

39   sodium channel function or expression can result in altered pain sensitivity and perception in

40   various conditions including neuropathic pain (Bagal et al. 2015; Cummins et al. 2007).

41   Dysregulated expression of sodium channels in both the periphery and the central nervous

42   system (CNS), which can result in frequent and ectopic firing in neurons, have been associated

43   with the pathogenesis of neuropathic pain (Craner et al. 2002; Lindia et al. 2005; Pertin et al.

44   2005; Rogers et al. 2006).

45   In the periphery, the expression all sodium channel α subunits was downregulated, except for

46   Na$_v$1.2, in the dorsal root ganglia (DRG) of rats with spared nerve injury (SNI) (Laedermann et

47   al. 2014). Another study observed downregulation of Na$_v$1.8 and Na$_v$1.9 in the DRG of a chronic

48   constriction injury (CCI) model of neuropathic pain (Dib-Hajj et al. 1999). However, other

49   studies have observed upregulation of sodium channel subunits such as Nav1.3, Nav1.6, Nav1.9,

50   Na$_v$β2 and Na$_v$β3 in the DRG of animal models of neuropathic pain (Craner et al. 2002; Lindia et

51   al. 2005; Pertin et al. 2005; Shah et al. 2001; Shah et al. 2000).

52   In the spinal cord Na$_v$1.3 was also found to be upregulated in the dorsal horn neurons of CCI

53   and spinal cord injury (SCI) models of neuropathic pain (Hains et al. 2003; Hains et al. 2004).

54   Sciatic nerve injury (axotomy) resulted in upregulation of Nav1.7 in the spinal cord, which had

55   strong correlation with the level of pain behaviour (Persson et al. 2009). In a model of painful

56   diabetic neuropathy there was upregulation of Na$_v$β3 expression in spinal cord (Shah et al. 2001).

57   Na$_v$β1 expression increased whereas Navβ2 decreased in the spinal cord of neuropathic rats

58   (Blackburn-Munro & Fleetwood-Walker 1999).

59   In the brain dysregulation of sodium channel expression has been observed in different areas

60   during neuropathic pain. In the prefrontal cortex Na$_v$1.1 expression was upregulated in mice with

61   SNI (Alvarado et al. 2013). The expression of Na$_v$1.3 was upregulated in the ventral

62   posterolateral (VPL) nucleus of the thalamus of rats with CCI or spinal cord contusion injury

63   (Hains et al. 2005; Zhao et al. 2006).

64   Recently, we observed increased excitability of the anterior cingulate cortex (ACC) to

65   electrophysiological stimulation in a rat model of paclitaxel-induced neuropathic pain (PINP)

66   (Nashawi et al. 2016). Paclitaxel is a chemotherapeutic drug whose therapeutic use is sometimes

67   limited by the development of dose-dependent painful neuropathy (Scripture et al. 2006; Wolf et

68   al. 2008). The ACC is an area in the brain involved in pain perception and modulation, and has

69   increased activity during neuropathic pain (Hsieh et al. 1995; Vogt 2005; Xie et al. 2009; Zhuo

70   2008). In previous studies, we observed changes in the expression of gamma-aminobutyric acid

71   (GABA)-ergic and glutamatergic molecules in the ACC of a mouse model of PINP (Masocha

72   2015a; Masocha 2015b). However, the expression of sodium channels in the ACC during PINP

73   has not been studied as yet. Studying the expression of sodium channels in the ACC during PINP

74   is important as they might contribute to the increased neuronal excitability, which we observed

75   in the ACC during PINP (Nashawi et al. 2016). Thus, in the current study the gene expression of

76   sodium channel subunits in the ACC was evaluated in mice at a time point when the mice had

77   paclitaxel-induced thermal hyperalgesia (Masocha 2015a; Nieto et al. 2008; Parvathy &

78   Masocha 2013). In previous studies, gene expression changes of other molecules were observed

79   in the ACC of mice with paclitaxel-induced thermal hyperalgesia (Masocha 2015a; Masocha

80   2015b).

Materials and Methods

Animals

83   Female BALB/c mice (8 to 12 weeks old; 20 – 30 g; n = 49) supplied by the Animal Resources

84   Centre (ARC) at the Health Sciences Center (HSC), Kuwait University were used. The animals

85   were kept in temperature controlled (24 ± 1°C) rooms with food and water given *ad libitum*.

86   Animals were handled in compliance with the Kuwait University, HSC, ARC guidelines and in

87   compliance with Directive 2010/63/EU of the European Parliament and of the Council on the

88   protection of animals used for scientific purposes. All animal experiments were approved by the

89 Ethical Committee for the use of Laboratory Animals in Teaching and in Research, HSC, Kuwait

90 University.

Paclitaxel administration

92 Paclitaxel (Cat. No. 1097, Tocris, Bristol, UK) was dissolved in a solution made up of 50%

93 Cremophor EL and 50% absolute ethanol to a concentration of 6 mg/ml and then diluted in

94 normal saline (NaCl 0.9%), to a final concentration of 0.2 mg/ml just before administration.

95 Mice were treated intraperitoneally (i.p.) for 5 consecutive days with paclitaxel 2 mg/kg, the

96 cumulative dose was 10 mg/kg, or its vehicle. This treatment regimen produces painful

97 neuropathy and thermal hyperalgesia in mice on day 7 post first administration (Nieto et al.

98 2008; Parvathy & Masocha 2013). A group of control mice was left untreated.

Tissue preparation and Real time RT-PCR

100 Mice were anesthetized with isoflurane, sacrificed by decapitation on day 7 post first

101 administration of paclitaxel. The ACC was dissected and prepared for RNA extraction as

102 described previously (Masocha 2015b)

103 Gene transcripts of the 10 sodium channel alpha subunits ($Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.4$,

104 $Na_v1.5$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$, $Na_v1.9$ and Na_x) and 4 sodium channel beta subunits ($Na_v\beta1$,

105 $Na_v\beta2$, $Na_v\beta3$ and $Na_v\beta4$) were quantified in the ACC of untreated, vehicle-treated and

106 paclitaxel-treated mice by real time PCR. Total RNA was extracted from the fresh frozen ACC

107 using the RNeasy Kit (Qiagen GmbH), reverse-transcribed, and the mRNA levels were

108 quantified on an ABI Prism® 7500 sequence detection system (Applied Biosystems) as

109 previously described (Masocha 2009; Masocha 2015a). The primer sequences which were used,

110 listed in Table 1, were ordered from Invitrogen (Life Technologies) and/or synthesized at the

111   Research Core Facility (RCF), HSC, Kuwait University. Threshold cycle (Ct) values for all

112   cDNA samples were obtained and the amount of mRNA of individual animal sample (n = 8 to 12

113   per group) was normalized to cyclophilin (housekeeping gene) (ΔCt). The relative amount of

114   target gene transcripts was calculated using the $2^{-\Delta\Delta Ct}$ method as described previously (Livak &

115   Schmittgen 2001). These values were then used to calculate the mean and standard error of the

116   relative expression of the target gene mRNA in the ACC of paclitaxel- and vehicle-treated mice.

117   **Statistical analyses**

118   Statistical analyses were performed using Mann Whitney U test using Graph Pad Prism software

119   (version 5.0). The differences were considered significant at $p < 0.05$. The results in the text and

120   figures are expressed as the means ± S.E.M.

Results

The mRNA expression of sodium channel subunits were analysed in the ACC at day 7, a time when the mice treated with paclitaxel had developed thermal hyperalgesia as we described previously (Masocha 2014; Parvathy & Masocha 2013) i.e. reduction in reaction latency compared to the baseline latency and vehicle-treated mice (5.7 ± 0.3 s compared to 9.6 ± 0.3 s and 9.3 ± 0.3 s, respectively; n = 8 vehicle-treated mice and 10 paclitaxel treated-mice; $p < 0.01$ for both comparisons).

Expression of sodium channel alpha subunits transcripts in the ACC at 7 days after paclitaxel administration

In vehicle-treated animals the Ct values for $Na_v1.4$, $Na_v1.5$, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ were above 30 and not detectable in some samples, whereas the Ct values for $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.6$ and Na_x were below 30. Thus, comparison in mRNA expression between control and paclitaxel treated animals was done for $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.6$ and Na_x.

Treatment with vehicle did not alter the expression of the 5 sodium channel alpha subunits evaluated, $Na_v1.1$ ($p = 1.000$), $Na_v1.2$ ($p = 0.1143$), $Na_v1.3$ ($p = 0.6857$), $Na_v1.6$ ($p = 0.3429$) and Na_x ($p = 0.3429$), compared to untreated control (Figures 1A and 2A). Amongst the 5 sodium channel alpha subunits ($Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.6$ and Na_x) treatment with paclitaxel did not significantly alter the mRNA expression of the $Na_v1.3$ ($p = 0.1379$), but significantly increased the expression of $Na_v1.1$ by 2.1 ± 0.2 fold ($p = 0.0002$), $Na_v1.2$ by 6.2 ± 1.6 fold ($p = 0.0003$), $Na_v1.6$ by 3.8 ± 0.7 fold ($p = 0.0051$), compared to vehicle-treated controls (Figure 1B). Na_x was significantly upregulated by 7.6 ± 2.2 fold ($p = 0.0012$) in the ACC by treatment with paclitaxel compared to treatment with vehicle (Figure 2B). The most upregulated sodium

143 channel alpha subunits were $Na_v1.2$ and Na_x, which were increased by more than sixfold after

144 treatment with paclitaxel.

145 **Expression of sodium channel beta subunits transcripts in the ACC at 7 days after**
146 **paclitaxel administration**

147 Treatment with vehicle did not alter the expression of 3 sodium channel beta subunits, Navβ1 (p

148 = 0.2000), Navβ2 (p = 0.4857), Navβ3 (p = 0.6857), but significantly increased the expression of

149 Navβ4 (p = 0.0286), compared to untreated control (Figure 3A). Amongst the 4 sodium channel

150 beta subunits analysed treatment with paclitaxel significantly increased the expression of Na_vβ1

151 by 2.8 ± 0.5 fold (p = 0.0047) and Na_vβ3 by 4.4 ± 1.1 fold (p = 0.0127), but not Na_vβ2 (p =

152 0.2301) and Na_vβ4 (p = 0.0525), compared to vehicle-treated controls (Figure 3). The most

153 upregulated sodium channel beta subunit was Na_vβ3, which was increased by more than fourfold

154 after treatment with paclitaxel.

155 Discussion

156 This study presents the first comprehensive analysis of the expression of transcripts of sodium

157 channel subunits in the ACC during neuropathic pain, specifically paclitaxel-induced

158 neuropathic pain (PINP). The ACC is an area of the brain associated with pain perception and

159 modulation (Vogt 2005; Xie et al. 2009; Zhuo 2008).

160 No reports about the expression of sodium channels in the ACC specifically were found.

161 However, $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.6$ and also Na_x have been reported to be expressed

162 predominantly (but not exclusively) in the brain with differential expression in different brain

163 areas such as hippocampus, thalamus, cerebellum etc. (Beckh et al. 1989; Catterall 2000;

164 Gautron et al. 1992; Levy-Mozziconacci et al. 1998; Schaller & Caldwell 2003; Westenbroek et

165 al. 1989; Whitaker et al. 2000; Whitaker et al. 2001). On the other hand, $Na_v1.4$ is expressed

166   principally in the skeletal muscle, $Na_v1.5$ is mainly expressed in cardiac muscle, while $Na_v1.7$,

167   $Na_v1.8$ and $Na_v1.9$ are expressed preferentially in peripheral neurons (Cummins et al. 2007; Dib-

168   Hajj et al. 2015). In the current study using real time PCR all the 10 α subunits and 4 β subunits

169   were detected in the ACC with different degrees of expression. $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.6$

170   and Na_x as well as $Na_v\beta1 - Na_v\beta4$ were highly expressed in the ACC. On the other hand,

171   although $Na_v1.4$, $Na_v1.5$, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ were detected in the ACC they were lowly

172   expressed and/or were not detectable in some samples. Thus, the findings of this study are in

173   agreement with studies described above. This suggests that the different sodium channel subunits

174   have different roles in the ACC and the brain in general. $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.6$ and Na_x

175   as well as $Na_v\beta1 - Na_v\beta4$ most likely have more important roles in neuronal activity in the ACC

176   than $Na_v1.4$, $Na_v1.5$, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$. This could be important for drug development

177   of specific sodium channel blockers; for example a specific blocker of $Na_v1.1$ or $Na_v1.2$ would

178   more likely have more effect in the ACC compared to a specific inhibitor of $Na_v1.7$ or $Na_v1.8$

179   based on their expression patterns. Further studies are necessary to understand the specific

180   properties and activities of specific sodium channel subunits in the ACC under normal conditions

181   and during neuropathic pain.

182   Administration of tetrodotoxin (TTX), a voltage-gated sodium channel blocker, was reported to

183   prevent and treat signs of PINP such as thermal hyperalgesia, cold and mechanical allodynia in

184   mice, suggesting that TTX-sensitive voltage-gated sodium channels play a role in the

185   pathophysiology of PINP (Nieto et al. 2008). Mexiletine, a non-selective voltage-gated sodium

186   channel blocker was also found to have antinociceptive effects in rats with paclitaxel-induced

187   mechanical allodynia and hyperalgesia (Xiao et al. 2008). However, we found no studies that

188   investigated the expression of sodium channels in the periphery or CNS during PINP. In the

189   current study, $Na_v1.1$, $Na_v1.2$, $Na_v1.6$ and Na_x as well as Na_vB1 and Na_vB3 were upregulated in

190   the ACC of mice with paclitaxel-induced thermal hyperalgesia. Upregulation of sodium channel

191   expression has been observed in other areas of the brain during neuropathic pain. In the

192   prefrontal cortex $Na_v1.1$ expression was upregulated in mice with SNI (Alvarado et al. 2013).

193   Thus, our data are in agreement with the findings of Alvarado et al. and the suggestion that over-

194   expression of $Na_v1.1$ is involved in increased cortical excitability associated with chronic pain

195   (Alvarado et al. 2013). It is also possible that the increased expression of $Na_v1.2$, $Na_v1.6$, Na_x,

196   Na_vB1 and Na_vB3 in the ACC are involved in the increased excitability of this area observed

197   during PINP (Nashawi et al. 2016). Although $Na_v1.3$ was not significantly altered in the ACC

198   during PINP it was reported to be upregulated in the ventral posterolateral (VPL) nucleus of the

199   thalamus of rats with CCI and spinal cord contusion injury (Hains et al. 2005; Zhao et al. 2006).

200   The findings of the current study suggest that upregulation of specific sodium channel subunits

201   might contribute to hyperexcitability in the ACC. Hyperexcitability has been associated with

202   dysregulation in sodium channels (Devor 2006). A link between upregulation of $Na_v1.3$ and

203   hyperexcitability of neurons in the spinal cord was found in neuropathic pain after spinal cord

204   injury (Hains et al. 2003). Recently, we observed increased excitability of the ACC to

205   electrophysiological stimulation in a rat model PINP (Nashawi et al. 2016), which could be in

206   part be due upregulation of sodium channels amongst other mechanisms such as decreased

207   GABA availability at the synapse because of increased GABA transporter 1 (GAT-1) expression

208   (Masocha 2015b). Changes in the expression of other molecules such as those of the

209   GABAergic, glutamatergic, muscarinic dopaminergic systems have also been observed in the

210   ACC during experimental neuropathic pain (Masocha 2015a; Masocha 2015b; Ortega-Legaspi et

211   al. 2011; Ortega-Legaspi et al. 2010). These findings suggest that the ACC plays an important

212   role in the pathophysiology of PINP in addition to other brain areas, the spinal cord and

213   peripheral nerve damage. Paclitaxel has limited ability to cross the blood-brain barrier (Glantz et

214   al. 1995; Kemper et al. 2003), thus a direct effect of paclitaxel in the ACC is unlikely. In a rat

215   model paclitaxel induced microglial activation in the spinal cord (Peters et al. 2007). They

216   proposed (Peters et al. 2007) that paclitaxel-induced nerve injury possibly induced

217   neurochemical reorganization within the spinal cord leading to central sensitization (Cata et al.

218   2006) and that the microglial reaction they observed occurred as a result of degeneration of

219   central terminals of injured primary afferent fibers or possibly due to the spinal release of factors

220   from injured neurons rather than direct injury of spinal cord neurons by paclitaxel. In the

221   periphery paclitaxel causes nerve damage by direct effects on the neurons (Cavaletti et al. 2000;

222   Scuteri et al. 2006; Theiss & Meller 2000) or via inflammation and the increased infiltration of

223   macrophages into the dorsal root ganglia (Peters et al. 2007; Zhang et al. 2016), which cause

224   further nerve damage. Thus, the changes observed in the ACC could be due to an increased

225   nociceptive input from the peripheral nerves damaged by paclitaxel resulting in central

226   sensitization. However, information on protein expression is critical to subsequently define the

227   meaning of expression changes in the mRNA level observed in the ACC.

228

Conclusions

230   In conclusion, the findings of this study show that sodium channel subunit transcripts are

231   differentially expressed in the ACC; with those known to be preferentially expressed in the CNS

232   being highly expressed in the ACC, whereas those known to be preferentially expressed in the

233   periphery being lowly expressed in the ACC. More importantly, the results show that during

234   experimental paclitaxel-induced neuropathic pain there is increased expression of various sodium

235   channel subunit transcripts in the ACC, which could contribute to the increased excitability and

236   activity observed in this brain region during neuropathic pain.

237

238 **Acknowledgements**

239 I am grateful to Dr Subramanian S Parvathy, Ms. Salini Soman, Ms. Amal Thomas from the

240 Department of Pharmacology and Therapeutics, Faculty of Pharmacy, for their technical

241 assistance and to the staff from the Animal Resources Centre, HSC, Kuwait University for their

242 support.

243 **References**

244 Alvarado S, Tajerian M, Millecamps M, Suderman M, Stone LS, and Szyf M. 2013. Peripheral nerve injury
245   is accompanied by chronic transcriptome-wide changes in the mouse prefrontal cortex. *Mol Pain*
246   9:21.

247 Bagal SK, Marron BE, Owen RM, Storer RI, and Swain NA. 2015. Voltage gated sodium channels as drug
248   discovery targets. *Channels (Austin)* 9:360-366.

249 Beckh S, Noda M, Lubbert H, and Numa S. 1989. Differential regulation of three sodium channel
250   messenger RNAs in the rat central nervous system during development. *EMBO J* 8:3611-3616.

251 Blackburn-Munro G, and Fleetwood-Walker SM. 1999. The sodium channel auxiliary subunits beta1 and
252   beta2 are differentially expressed in the spinal cord of neuropathic rats. *Neuroscience* 90:153-
253   164.

254 Brackenbury WJ, and Isom LL. 2008. Voltage-gated Na+ channels: potential for beta subunits as
255   therapeutic targets. *Expert Opin Ther Targets* 12:1191-1203.

256 Cata JP, Weng HR, Lee BN, Reuben JM, and Dougherty PM. 2006. Clinical and experimental findings in
257   humans and animals with chemotherapy-induced peripheral neuropathy. *Minerva Anestesiol*
258   72:151-169.

259 Catterall WA. 2000. From ionic currents to molecular mechanisms: the structure and function of voltage-
260   gated sodium channels. *Neuron* 26:13-25.

261 Catterall WA, Goldin AL, and Waxman SG. 2005. International Union of Pharmacology. XLVII.
262   Nomenclature and structure-function relationships of voltage-gated sodium channels.
263   *Pharmacol Rev* 57:397-409.

264 Cavaletti G, Cavalletti E, Oggioni N, Sottani C, Minoia C, D'Incalci M, Zucchetti M, Marmiroli P, and
265   Tredici G. 2000. Distribution of paclitaxel within the nervous system of the rat after repeated
266   intravenous administration. *Neurotoxicology* 21:389-393.

267 Craner MJ, Klein JP, Renganathan M, Black JA, and Waxman SG. 2002. Changes of sodium channel
268   expression in experimental painful diabetic neuropathy. *Ann Neurol* 52:786-792.

269 Cummins TR, Sheets PL, and Waxman SG. 2007. The roles of sodium channels in nociception:
270   Implications for mechanisms of pain. *Pain* 131:243-257.

271 Devor M. 2006. Sodium channels and mechanisms of neuropathic pain. *J Pain* 7:S3-S12.

272 Dib-Hajj SD, Black JA, and Waxman SG. 2015. NaV1.9: a sodium channel linked to human pain. *Nat Rev*
273   *Neurosci* 16:511-519.

274 Dib-Hajj SD, Fjell J, Cummins TR, Zheng Z, Fried K, LaMotte R, Black JA, and Waxman SG. 1999. Plasticity
275   of sodium channel expression in DRG neurons in the chronic constriction injury model of
276   neuropathic pain. *Pain* 83:591-600.

277 Gautron S, Dos Santos G, Pinto-Henrique D, Koulakoff A, Gros F, and Berwald-Netter Y. 1992. The glial
278   voltage-gated sodium channel: cell- and tissue-specific mRNA expression. *Proc Natl Acad Sci U S*
279   *A* 89:7272-7276.

280 Glantz MJ, Choy H, Kearns CM, Mills PC, Wahlberg LU, Zuhowski EG, Calabresi P, and Egorin MJ. 1995.
281   Paclitaxel disposition in plasma and central nervous systems of humans and rats with brain
282   tumors. *J Natl Cancer Inst* 87:1077-1081.

283 Hains BC, Klein JP, Saab CY, Craner MJ, Black JA, and Waxman SG. 2003. Upregulation of sodium channel
284   Nav1.3 and functional involvement in neuronal hyperexcitability associated with central
285   neuropathic pain after spinal cord injury. *J Neurosci* 23:8881-8892.

286 Hains BC, Saab CY, Klein JP, Craner MJ, and Waxman SG. 2004. Altered sodium channel expression in
287   second-order spinal sensory neurons contributes to pain after peripheral nerve injury. *J Neurosci*
288   24:4832-4839.

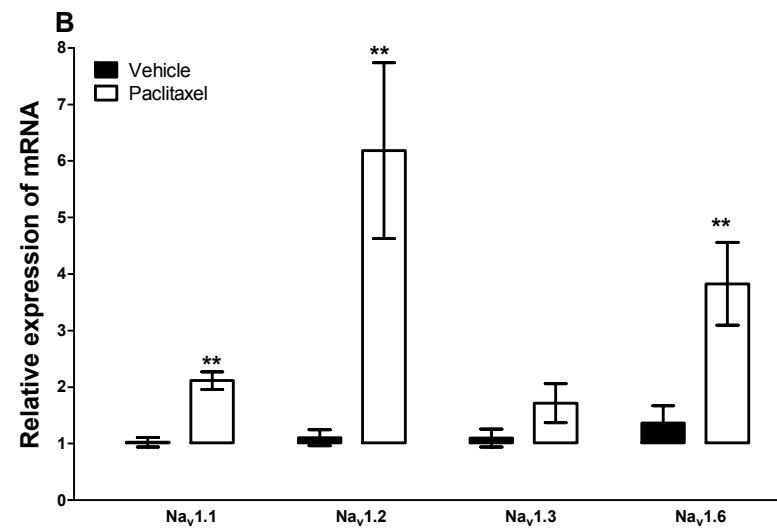
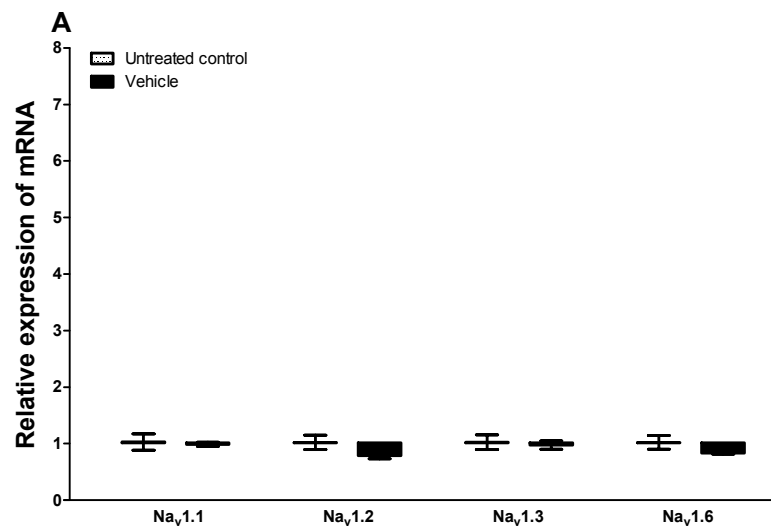
289 Hains BC, Saab CY, and Waxman SG. 2005. Changes in electrophysiological properties and sodium
290   channel Nav1.3 expression in thalamic neurons after spinal cord injury. *Brain* 128:2359-2371.
291 Hsieh JC, Belfrage M, Stone-Elander S, Hansson P, and Ingvar M. 1995. Central representation of chronic
292   ongoing neuropathic pain studied by positron emission tomography. *Pain* 63:225-236.
293 Isom LL. 2001. Sodium channel beta subunits: anything but auxiliary. *Neuroscientist* 7:42-54.
294 Kemper EM, van Zandbergen AE, Cleypool C, Mos HA, Boogerd W, Beijnen JH, and van Tellingen O. 2003.
295   Increased penetration of paclitaxel into the brain by inhibition of P-Glycoprotein. *Clin Cancer Res*
296   9:2849-2855.
297 Laedermann CJ, Pertin M, Suter MR, and Decosterd I. 2014. Voltage-gated sodium channel expression in
298   mouse DRG after SNI leads to re-evaluation of projections of injured fibers. *Mol Pain* 10:19.
299 Levy-Mozziconacci A, Alcaraz G, Giraud P, Boudier JA, Caillol G, Couraud F, and Autillo-Touati A. 1998.
300   Expression of the mRNA for the beta 2 subunit of the voltage-dependent sodium channel in rat
301   CNS. *Eur J Neurosci* 10:2757-2767.
302 Lindia JA, Kohler MG, Martin WJ, and Abbadie C. 2005. Relationship between sodium channel NaV1.3
303   expression and neuropathic pain behavior in rats. *Pain* 117:145-153.
304 Livak KJ, and Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative
305   PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25:402-408.
306 Masocha W. 2009. Systemic lipopolysaccharide (LPS)-induced microglial activation results in different
307   temporal reduction of CD200 and CD200 receptor gene expression in the brain. *J Neuroimmunol*
308   214:78-82.
309 Masocha W. 2014. Paclitaxel-induced hyposensitivity to nociceptive chemical stimulation in mice can be
310   prevented by treatment with minocycline. *Sci Rep* 4:6719.
311 Masocha W. 2015a. Astrocyte activation in the anterior cingulate cortex and altered glutamatergic gene
312   expression during paclitaxel-induced neuropathic pain in mice. *PeerJ* 3:e1350.
313 Masocha W. 2015b. Comprehensive analysis of the GABAergic system gene expression profile in the
314   anterior cingulate cortex of mice with Paclitaxel-induced neuropathic pain. *Gene Expr* 16:145-
315   153.
316 Nashawi H, Masocha W, Edafiogho IO, and Kombian SB. 2016. Paclitaxel Causes Electrophysiological
317   Changes in the Anterior Cingulate Cortex via Modulation of the gamma-Aminobutyric Acid-ergic
318   System. *Med Princ Pract* 25:423-428.
319 Nieto FR, Entrena JM, Cendan CM, Pozo ED, Vela JM, and Baeyens JM. 2008. Tetrodotoxin inhibits the
320   development and expression of neuropathic pain induced by paclitaxel in mice. *Pain* 137:520-
321   531.
322 Ortega-Legaspi JM, de Gortari P, Garduno-Gutierrez R, Amaya MI, Leon-Olea M, Coffeen U, and Pellicer
323   F. 2011. Expression of the dopaminergic D1 and D2 receptors in the anterior cingulate cortex in
324   a model of neuropathic pain. *Mol Pain* 7:97.
325 Ortega-Legaspi JM, Leon-Olea M, de Gortari P, Amaya MI, Coffeen U, Simon-Arceo K, and Pellicer F.
326   2010. Expression of muscarinic M1 and M2 receptors in the anterior cingulate cortex associated
327   with neuropathic pain. *Eur J Pain* 14:901-910.
328 Parvathy SS, and Masocha W. 2013. Matrix metalloproteinase inhibitor COL-3 prevents the development
329   of paclitaxel-induced hyperalgesia in mice. *Med Princ Pract* 22:35-41.
330 Persson AK, Thun J, Xu XJ, Wiesenfeld-Hallin Z, Strom M, Devor M, Lidman O, and Fried K. 2009.
331   Autotomy behavior correlates with the DRG and spinal expression of sodium channels in inbred
332   mouse strains. *Brain Res* 1285:1-13.
333 Pertin M, Ji RR, Berta T, Powell AJ, Karchewski L, Tate SN, Isom LL, Woolf CJ, Gilliard N, Spahn DR, and
334   Decosterd I. 2005. Upregulation of the voltage-gated sodium channel beta2 subunit in
335   neuropathic pain models: characterization of expression in injured and non-injured primary
336   sensory neurons. *J Neurosci* 25:10970-10980.

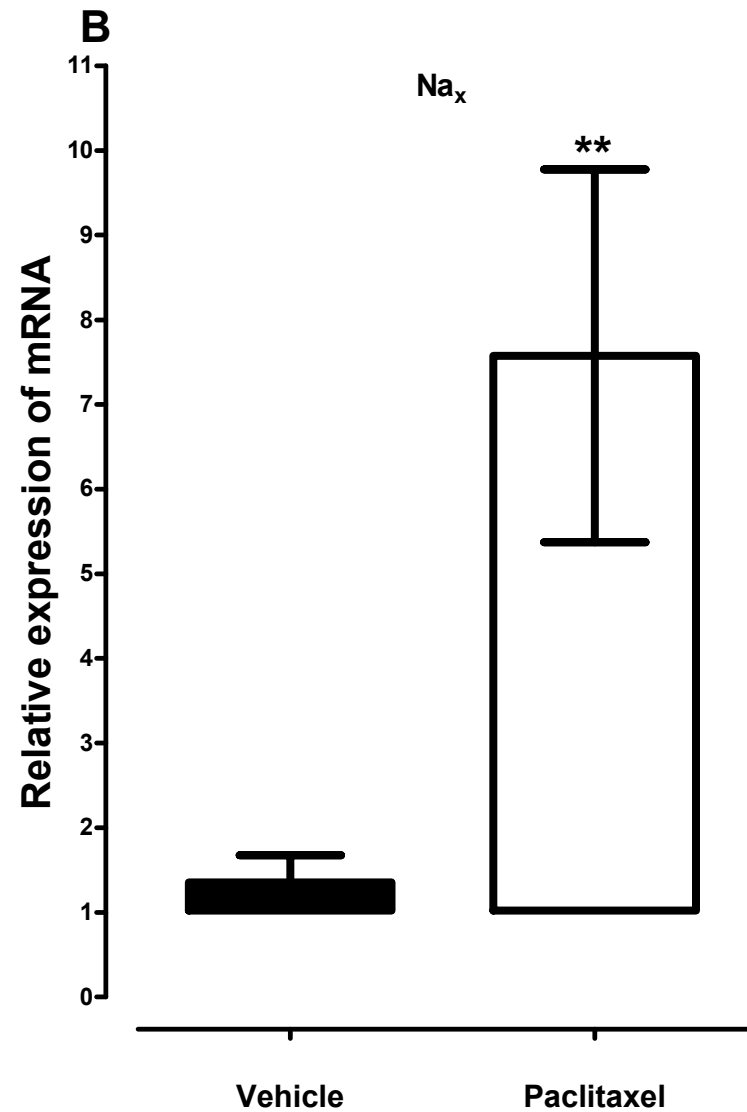
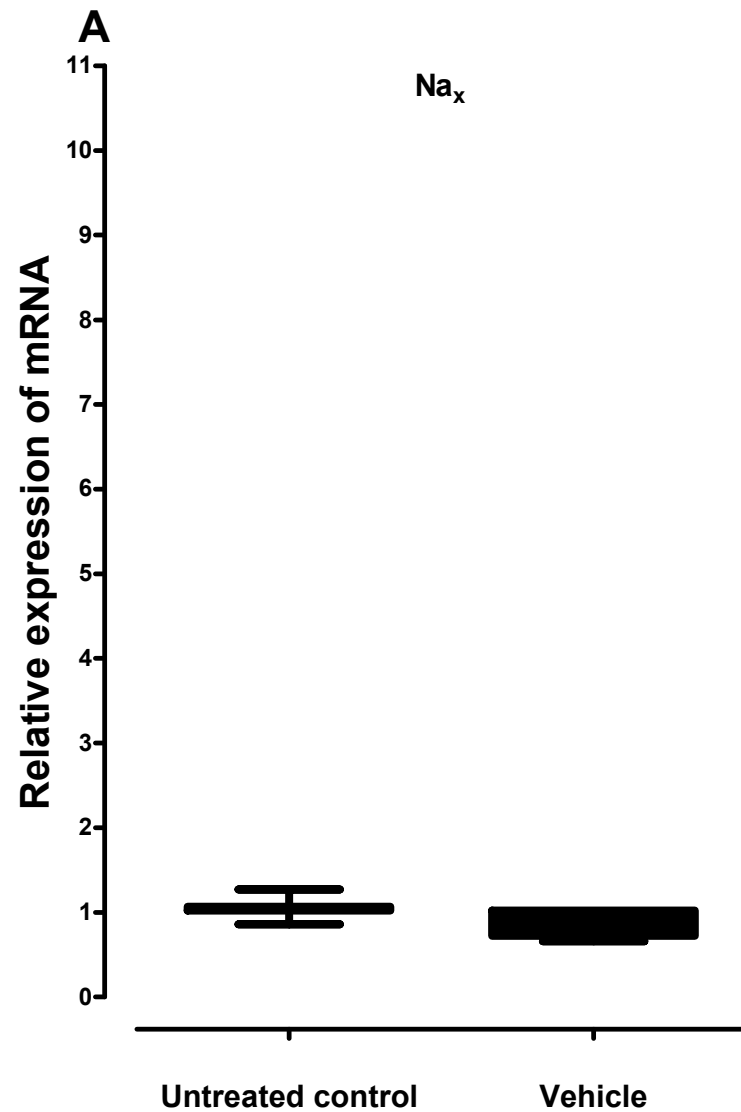
337   Peters CM, Jimenez-Andrade JM, Jonas BM, Sevcik MA, Koewler NJ, Ghilardi JR, Wong GY, and Mantyh
338   PW. 2007. Intravenous paclitaxel administration in the rat induces a peripheral sensory
339   neuropathy characterized by macrophage infiltration and injury to sensory neurons and their
340   supporting cells. *Exp Neurol* 203:42-54.
341   Rogers M, Tang L, Madge DJ, and Stevens EB. 2006. The role of sodium channels in neuropathic pain.
342   *Semin Cell Dev Biol* 17:571-581.
343   Schaller KL, and Caldwell JH. 2003. Expression and distribution of voltage-gated sodium channels in the
344   cerebellum. *Cerebellum* 2:2-9.
345   Scripture CD, Figg WD, and Sparreboom A. 2006. Peripheral neuropathy induced by paclitaxel: recent
346   insights and future perspectives. *Curr Neuropharmacol* 4:165-172.
347   Scuteri A, Nicolini G, Miloso M, Bossi M, Cavaletti G, Windebank AJ, and Tredici G. 2006. Paclitaxel
348   toxicity in post-mitotic dorsal root ganglion (DRG) cells. *Anticancer Res* 26:1065-1070.
349   Shah BS, Gonzalez MI, Bramwell S, Pinnock RD, Lee K, and Dixon AK. 2001. Beta3, a novel auxiliary
350   subunit for the voltage gated sodium channel is upregulated in sensory neurones following
351   streptozocin induced diabetic neuropathy in rat. *Neurosci Lett* 309:1-4.
352   Shah BS, Stevens EB, Gonzalez MI, Bramwell S, Pinnock RD, Lee K, and Dixon AK. 2000. beta3, a novel
353   auxiliary subunit for the voltage-gated sodium channel, is expressed preferentially in sensory
354   neurons and is upregulated in the chronic constriction injury model of neuropathic pain. *Eur J*
355   *Neurosci* 12:3985-3990.
356   Theiss C, and Meller K. 2000. Taxol impairs anterograde axonal transport of microinjected horseradish
357   peroxidase in dorsal root ganglia neurons in vitro. *Cell Tissue Res* 299:213-224.
358   Vogt BA. 2005. Pain and emotion interactions in subregions of the cingulate gyrus. *Nat Rev Neurosci*
359   6:533-544.
360   Westenbroek RE, Merrick DK, and Catterall WA. 1989. Differential subcellular localization of the RI and
361   RII Na+ channel subtypes in central neurons. *Neuron* 3:695-704.
362   Whitaker WR, Clare JJ, Powell AJ, Chen YH, Faull RL, and Emson PC. 2000. Distribution of voltage-gated
363   sodium channel alpha-subunit and beta-subunit mRNAs in human hippocampal formation,
364   cortex, and cerebellum. *J Comp Neurol* 422:123-139.
365   Whitaker WR, Faull RL, Waldvogel HJ, Plumpton CJ, Emson PC, and Clare JJ. 2001. Comparative
366   distribution of voltage-gated sodium channel proteins in human brain. *Brain Res Mol Brain Res*
367   88:37-53.
368   Wolf S, Barton D, Kottschade L, Grothey A, and Loprinzi C. 2008. Chemotherapy-induced peripheral
369   neuropathy: prevention and treatment strategies. *Eur J Cancer* 44:1507-1515.
370   Xiao W, Naso L, and Bennett GJ. 2008. Experimental studies of potential analgesics for the treatment of
371   chemotherapy-evoked painful peripheral neuropathies. *Pain Med* 9:505-517.
372   Xie YF, Huo FQ, and Tang JS. 2009. Cerebral cortex modulation of pain. *Acta Pharmacol Sin* 30:31-41.
373   Yu FH, and Catterall WA. 2003. Overview of the voltage-gated sodium channel family. *Genome Biol*
374   4:207.
375   Zhang H, Li Y, de Carvalho-Barbosa M, Kavelaars A, Heijnen CJ, Albrecht PJ, and Dougherty PM. 2016.
376   Dorsal Root Ganglion Infiltration by Macrophages Contributes to Paclitaxel Chemotherapy-
377   Induced Peripheral Neuropathy. *J Pain*.
378   Zhao P, Waxman SG, and Hains BC. 2006. Sodium channel expression in the ventral posterolateral
379   nucleus of the thalamus after peripheral nerve injury. *Mol Pain* 2:27.
380   Zhuo M. 2008. Cortical excitation and chronic pain. *Trends Neurosci* 31:199-207.

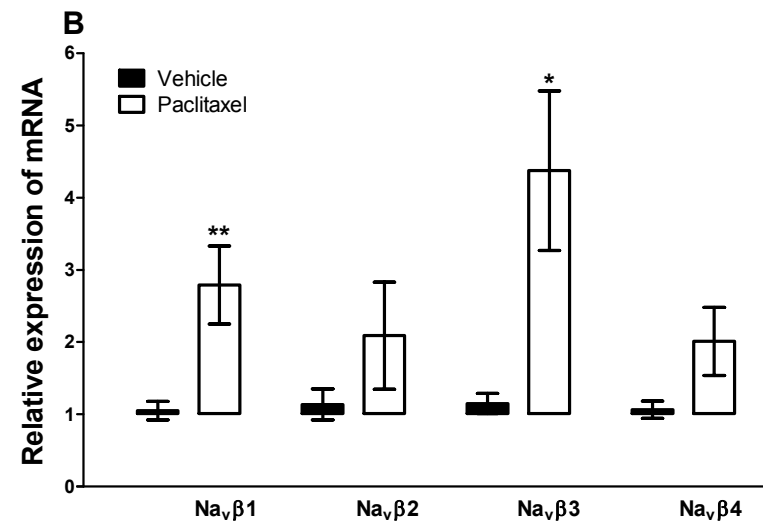
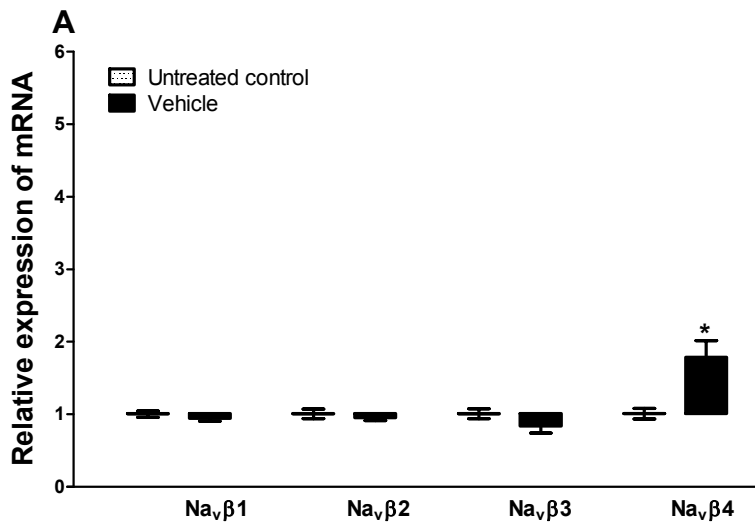
381

382

Effects of paclitaxel on sodium channel alpha subunits transcript levels in the anterior cingulate cortex (ACC)

Relative mRNA expression of sodium channel alpha subunits $Na_v1.1$, $Na_v1.2$, $Na_v1.3$ and $Na_v1.6$ in the ACC of BALB/c mice (A) vehicle-treated mice versus untreated mice. Each bar represents the mean ± S.E.M of the values obtained from 4 untreated mice and 4 vehicle-treated mice. (B) Relative mRNA expression of sodium channel alpha subunits on day 7 after first administration of the drug or its vehicle. Each bar represents the mean ± S.E.M of the values obtained from 9-11 vehicle-treated mice and 12 paclitaxel-treated mice. ** $p < 0.01$ compared to vehicle-treated mice.

Effects of paclitaxel on the sodium channel alpha subunit Na_x transcript levels in the anterior cingulate cortex (ACC)

Relative mRNA expression of Na_x in the ACC of BALB/c mice (A) vehicle-treated mice versus untreated mice. Each bar represents the mean ± S.E.M of the values obtained from 4 untreated mice and 4 vehicle-treated mice. (B) Relative mRNA expression of sodium channel alpha subunits on day 7 after first administration of the drug or its vehicle. Each bar represents the mean ± S.E.M of the values obtained from 11 vehicle-treated mice and 12 paclitaxel-treated mice . * * p < 0.01 compared to vehicle-treated control mice.

Effects of paclitaxel on sodium channel beta subunits transcript levels in the anterior cingulate cortex (ACC)

Relative mRNA expression of sodium channel beta subunits $Na_v\beta1$ to 4 in the ACC of BALB/c mice (A) vehicle-treated mice versus untreated mice. Each bar represents the mean ± S.E.M of the values obtained from 4 untreated mice and 4 vehicle-treated mice. * $p < 0.05$ compared to untreated mice. (B) Relative mRNA expression of sodium channel alpha subunits on day 7 after first administration of the drug or its vehicle. Each bar represents the mean ± S.E.M of the values obtained from 8-11 vehicle-treated control mice and 8-12 paclitaxel-treated mice . * $p < 0.05$ and ** $p < 0.01$ compared to vehicle-treated mice.

PCR primer sequences of cyclophilin, and sodium channel subunits

1   **Table 1. PCR primer sequences of cyclophilin, and sodium channel subunits**

Gene	Polarity	
	Sense **Sequence 5´to 3´**	Anti-sense **Sequence 5´to 3´**
Cyclophilin	GCTTTTCGCCGCTTGCT	CTCGTCATCGGCCGTGAT
$Na_v1.1$	AACAAGCTTCATTCACATACAATAAG	AGGAGGGCGGACAAGCTG
$Na_v1.2$	GGGAACGCCCATCAAAGAAG	ACGCTATCGTAGGAAGGTGG
$Na_v1.3$	GGGTGTTGGGTGAGAGTGGAG	AATGTAGTAGTGATGGGCTGATAAGAG
$Na_v1.4$	CGCGCTGTTCAGCATGTT	CTCCACGTCCTTGGACCAAG
$Na_v1.5$	AGACTTCCCTCCATCTCCAGATA	TGTCACCTCCAGAGCTAGGAAG
$Na_v1.6$	AGCAAAGACAAACTGGACGATACC	CACTTGAACCTCTGGACACAACC
$Na_v1.7$	TCCTTTATTCATAATCCCAGCCTCAC	GATCGGTTCCGTCTCTCTTTGC
$Na_v1.8$	ACCGACAATCAGAGCGAGGAG	ACAGACTAGAAATGGACAGAATCACC
Nav1.9	TGAGGCAACACTACTTCACCAATG	AGCCAGAAACCAAGGTACTAATGATG
Na_x	TGTCTCCTCTAAACTCCCTCAG	TGCGTAAATCCCAAGCAAAGT
$Na_v\beta1$	GTGTATCTCCTGTAAGCGTCGTAG	ATTCTCATAGCGTAGGATCTTGACAA
$Na_v\beta2$	GGCCACGGCAAGATTTACCT	CACCAAGATGACCACAGCCA
$Na_v\beta3$	ACTGAAGAGGCGGGAGAAGAC	GGTGAGGAAGACCAGGAGGATG
$Na_v\beta4$	CCCTTGGTGTAGAAACTAAGCAGAG	CAGAAGCGAGTCAGTCAGATACG