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Alterations in the Intrinsic Burst Activity of Purkinje Neurons in Offspring Maternally Exposed to the CB1 Cannabinoid Agonist WIN 55212-2

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Abstract Burst firing plays an important role in normal neuronal function and dysfunction. In Purkinje neurons, where the firing rate and discharge pattern encode the timing signals necessary for motor function, any alteration in firing properties, including burst activity, may affect the motor output. Therefore, we examined whether maternal exposure to the cannabinoid receptor agonist WIN 55212-2 (WIN) may affect the burst firing properties of cerebellar Purkinje cells in offspring. Whole-cell somatic patch-clamp recordings were made from cerebellar slices of adult male rats that were exposed to WIN prenatally. WIN exposure during pregnancy induced long-term alterations in the burst firing behavior of Purkinje neurons in rat offspring as evidenced by a significant increase in the mean number of spikes per burst ($p < 0.05$) and the prolongation of burst firing activity ($p < 0.01$). The postburst afterhyperpolarization potential ($p < 0.001$), the mean intraburst interspike intervals ($p < 0.001$) and the mean intraburst firing frequency ($p < 0.001$) were also significantly increased in the WIN-treated group. Prenatal exposure to WIN enhanced the firing irregularity as reflected by a significant decrease in the coefficient of variation of the intraburst interspike interval ($p < 0.05$). Furthermore, whole-cell voltage-clamp recordings revealed that prenatal WIN exposure significantly enhanced Ca^{2+} channel current amplitude in offspring Purkinje neurons compared to control cells. Overall, the data presented here strongly suggest that maternal exposure to cannabinoids can induce long-term changes in complex spike burst activity, which in turn may lead to alterations in neuronal output.

Keywords Cannabinoid · Intrinsic burst firing · Prenatal exposure · Purkinje neuron · Whole-cell patch clamp · WIN 55212-2

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Introduction

The cerebellum, which is involved in movement regulation and motor learning, could be a possible target for both acute and chronic effects of cannabis during early development and/or adulthood. Neurons in the central nervous system (CNS) undergo developmental changes, including proliferation, migration, synaptogenesis and cellular apoptosis in the fetal and neonatal periods (Nguon et al. 2006). Developmental disturbances in any of these processes can affect either CNS structure and/or function. Accumulating evidence indicates that a number of chemicals (Nguon et al. 2005) during intrauterine and neonatal exposures may affect the developing nervous system. Findings of animal and human studies suggest that cannabinoids, if abused by pregnant women, can be transferred from mother to fetus through the placenta (Hutchings et al. 1989). The cannabinoid CB1 receptor is one of the most abundantly expressed and widely distributed receptors in the brain regions involved in motor control and learning

(Herkenham et al. 1990; Mailleux and Vanderhaeghen 1992; Tsou et al. 1998). Prenatal exposure to cannabinoids can severely affect both fetal and postnatal development (Fernandez-Ruiz et al. 2000; Maccarrone and Wenger 2005; Wang et al. 2006).

In this context, the cerebellum could be one of the brain regions which are most vulnerable to prenatal exposure to cannabinoids (Ma et al. 2008; Shabani et al. 2011). We have previously reported that maternal exposure to WIN 55212-2 (WIN) profoundly affected the intrinsic regular single spike firing properties of Purkinje neurons (Shabani et al. 2011); however, to our knowledge, there is no information about the effect of prenatal exposure to cannabinoids on offspring neuronal burst firing activity. Thus, in the present study, it was hypothesized that exposure to cannabinoids during the prenatal period may alter the electrophysiological properties of Ca^{2+} channels, which in turn influence the burst discharge properties of Purkinje cells (PCs). Therefore, the firing characteristics of Purkinje neurons in young adult offspring prenatally exposed to a CB1 agonist were assessed, using whole-cell patch-clamp recording under both current- and voltage-clamp modes. Cerebellar PCs integrate motor information, at least in part by generating action potentials organized in regular discharges and/or bursts. These cells receive inputs from a single climbing fiber, and its activation produces a distinctive high-frequency burst of spikes. Many neurons including PCs alternate between high-frequency discharges and quiescent intervals, a phenomenon which is called "burst firing." The functional importance of such bursts, instead of single spikes, is that bursts provide effective mechanisms for selective and more reliable neuronal communication (Izhikevich et al. 2003). Burst firing in the cell body could play a particularly important role in synaptic plasticity by action potential-dependent regulation of gene expression (Fields et al. 1997). The burst firing likely reflects the intrinsic membrane properties of Purkinje neurons (Swensen and Bean 2003). Thus, changes in spontaneous bursting discharge characteristics of PCs may affect motor information processing. Postnatal CB1 cannabinoid receptor activation was previously found to alter the synchronized population burst firing induced by kainite in rat hippocampus (Mason and Cheer 2009; Lupica et al. 2004), It is, therefore, of particular interest to determine whether prenatal exposure to WIN, a potent cannabinoid receptor agonist, alters the burst firing properties of nerve cells in offspring. Using the whole-cell configuration of the patch clamp, the present study was designed to test this hypothesis by analyzing somatic burst firing behavior of Purkinje neurons in offspring maternally exposed to WIN. The contribution of voltage-gated Ca^{2+} channels to prenatal WIN treatment–induced changes in burst firing was also assessed.

Materials and Methods

Animals and Exposure

Primiparous Wistar female rats (Pasteur Institute, Karaj, Iran), weighing 200–250 g, were housed for 2 weeks before mating at constant room temperature (22–24 °C) with a light cycle of 12/12 h (8:00 a.m. to 20:00 p.m.) and free access to food and water. Pairs of females were then placed with single male rats in the late afternoon. Vaginal smears or plugs were examined the following day at 9:00 a.m. The day on which sperm was found was designated as gestation day 0 (GD0). Then, pregnant rats were randomly assigned to either a control group or a treatment group that received daily 1-ml/kg subcutaneous injections of 0.5 mg/kg WIN suspended in 1 % Tween 80 and physiological saline from GD5 to GD20 (Mereu et al. 2003). Pups were weaned at day 21 of age. To evaluate the effects of WIN on the burst activity of Purkinje neurons, the whole-cell patch-clamp technique in the cerebellar slice preparation of offspring at postnatal day 50 was used. All animal procedures were conducted in accordance with guidelines set by the Institutional Animal Care Committee at the Shahid Beheshti University of Medical Sciences.

Slice Preparation

Male offspring (50 days old) were divided into three groups of 17 rats in each group: control, vehicle-treated (1 % Tween 80) and maternally WIN-exposed.

Animals were decapitated after anesthesia by inhalation of ether, the brain was then rapidly removed from the skull and rinsed with cold artificial cerebrospinal fluid (ACSF) and the cerebellum was carefully dissected out. Parasagittal slices from the cerebellar vermis (250–300 μm thick) using a vibrating microtome (752 M; Campden Instruments, Loughborough, UK) were obtained as previously described (Janahmadi et al. 2009; Shabani et al. 2011). The ACSF solution used for both slicing and recording contained (in mM) 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 26 $NaHCO_3$ and 10 glucose and was oxygenated (95 % oxygen and 5 % carbon dioxide) to adjust the pH to 7.4. After a period of recovery at 36 °C for 1 h, slices were maintained at room temperature (22–24 °C) in ACSF equilibrated with 95 % O_2–5 % CO_2 until they were used for recording.

Whole-Cell Patch-Clamp Recording

Whole-cell patch-clamp recordings in current clamp mode from Purkinje neurons were made under direct visual control using differential interference contrast optics (BX51WI; Olympus, Tokyo, Japan). Purkinje neurons were

visualized by infrared video imaging (ORCA; Hamamatsu, Hamamatsu City, Japan) with a 60× water immersion objective and approached by applying positive pressure.

For recording, a single slice was placed in a recording chamber, continuously perfused at the rate of 1–2 ml/min with normal bubbled ACSF at room temperature (22–24 °C) and held at the bottom of the recording chamber with a *U*-shaped platinum-frame nylon net. Recordings were made using Multiclamp 700B amplifiers (Axon Instruments, Foster City, CA, USA) and digitized with a Digidata computer interface (Axon Instruments). Patch electrodes were fabricated, using with a PC-10 puller (Narishige, Tokyo, Japan) from thick-walled filament borosilicate glass. The tip resistance of the electrodes was 4–6 MΩ when filled with internal solution containing (in mM) 135 potassium methylsulfate ($KMeSO_4$), 10 KCl, 10 HEPES, 1 $MgCl_2$, 2 Na_2ATP and 0.4 Na_2GTP; osmolarity was adjusted to 290 mOsm. After establishment of a gigaohm seal, the whole-cell configuration was achieved simply by applying a brief suction. Before rupturing the membrane, cells with a seal <1 GΩ were discarded and the test seal function was constantly monitored throughout the recording to ensure that the seal was stable. In addition, series resistance (typically <15 MΩ) was checked for stability during the experiments. Data were collected using pClamp 9 software (Axon Instruments) and stored on computer hard disk for offline analysis. Since there were no statistically significant differences between control and vehicle-treated groups, the results were pooled and presented as a control.

Several burst parameters including mean number of spikes per burst, mean interburst interval, burst duration, intraburst interspike interval and mean amplitude of postburst afterhyperpolarization (AHP) were analyzed.

To record the postburst AHP, cell membrane voltage was held at −60 mV and the peak amplitude of AHP following spontaneous bursting firing was measured from the preburst level to the peak of the postburst AHP. Burst duration was calculated as the time between the first spike of a burst and the last spike of that burst. Interburst interval was measured as the point from the start of one burst to the start of the next burst (Bains et al. 1999; Staley et al. 1998). The intraburst regularity of firing was assessed using the coefficient variation (CV) of the intraburst interspike intervals (the intervals between spikes within a burst), which was calculated as the ratio of the standard deviation to the mean.

Calcium channel currents were recorded under whole-cell voltage-clamp recording in the presence of synaptic blockers (100 μM picrotoxin and 1 mM kynurenic acid) using a patch pipette containing (in mM) CsCl 130, NaCl 4, HEPES 10, EGTA 0.9, Na_2ATP 4, Na_2GTP 0.5, 5 QX314 and 20 Cs_2MeSo_4 and pH 7.4 was adjusted to 7.4 with CsOH. The extracellular ACSF contained NaCl 106, $MgCl_2$ 1.5, $CaCl_2$ 2, $NaHCO_3$ 26, KH_2PO_4 1.25, glucose 10 and 4-aminopyridine (4-AP) 5. To compare the pharmacological properties of Ca^{2+} currents recorded from PCs in offspring prenatally exposed to WIN with those recorded in control conditions, the following antagonists were used: diltiazem (Dilt, 5 μM), mibefradil (Mib, 4 μM), ZD7288 (ZD, 50 μM) and $CdCl_2$ (Cd, 50 μM). All drugs were purchased from Sigma (St. Louis, MO, USA), except ZD7288 (Tocris, Bristol, UK). Doses were chosen based on previous experience and/or literature review.

Data Analysis

Differences between groups were tested for statistical significance using a two-tailed unpaired Student's *t* test or one-way ANOVA followed by Tukey's test on normally distributed data. $p \leq 0.05$ was considered statistically significant, and the results were expressed as mean ± SEM.

Results

The burst firing behavior of Purkinje neurons of offspring prenatally exposed to WIN at postnatal day 50 (PD50) was compared to that in the control group to test whether maternal exposure produces any alterations in neuronal bursting discharge properties. Recordings were obtained after pharmacological blockade of fast glutamatergic and GABAergic synaptic transmissions with a mixture of kynurenic acid (1 mM) and picrotoxin (100 μM). Under this condition, Purkinje neurons exhibited intrinsic bursting activity (Fig. 1). Our findings showed that prenatal exposure to WIN did not significantly affect the mean resting membrane potential of Purkinje neurons in rat offspring (control, −62.04 ± 1.25 mV; WIN-treated, −60.93 ± 1.69 mV).

Induction of Long-Term Changes in the Burst Firing Properties of Offspring Purkinje Neurons Maternally Exposed to WIN

The membrane properties of cerebellar Purkinje neurons are favored for generation of burst firing, which can be seen either during complex spikes elicited by climbing fiber activation or by direct electrical stimulation of somata. To examine the possible effect of prenatal WIN exposure on burst firing properties, several parameters of bursts were measured, as mentioned in "Materials and Methods." In prenatal WIN-treated offspring, the average number of spikes per complex burst was significantly increased (from 2.7 ± 0.24 in control to 3.42 ± 0.16 in the WIN-treated group, $p < 0.05$, $n = 31$ in each group; Fig. 2a). Maternal

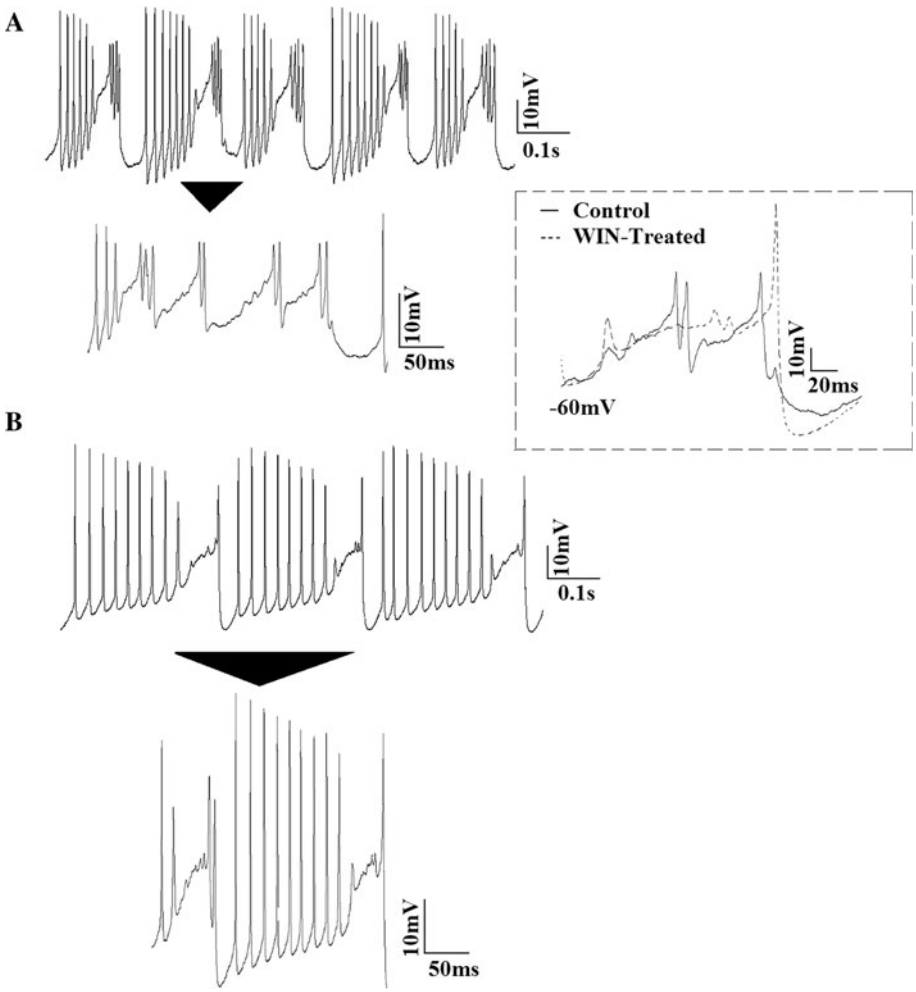

Fig. 1 Effect of maternal WIN treatment on complex spike bursts of Purkinje neurons. Representative complex spike bursts from Purkinje neurons recorded under current clamp in control condition (**a**) and in a PC neuron exposed prenatally to WIN (**b**). Expanded traces show complex spike waveforms. *Inset* shows two superimposed last Ca^{2+} spikes in a burst recorded from a control (*solid line*) and a WIN-treated (*dashed line*) cell, indicating a larger postburst AHP potential in Purkinje neurons of offspring maternally exposed to WIN

WIN treatment resulted also in a significant prolongation of spontaneous complex spike bursts compared to the control condition (Fig. 2b). The amplitude of postburst AHP following a spontaneous complex spike burst was significantly increased in Purkinje neurons of offspring prenatally exposed to WIN when compared with control neurons (Fig. 2c).

In addition, Purkinje neurons of offspring from WIN-treated mothers showed a significant increase in their mean intraburst interspike interval (0.0239 ± 0.0006 s, $p < 0.001$; Fig. 2d), corresponding to the mean intraburst frequency of 43.23 ± 1.38 Hz, compared with the control intraburst interspike interval (0.0131 ± 0.0004 s) and an intraburst firing frequency of 80.21 ± 2.38 Hz. Prenatal exposure to WIN also led to a significant enhancement of the intraburst firing regularity as evidenced by a significant decrease in the CV of the intraburst interspike interval from 0.237 ± 0.01 in the control group to 0.141 ± 0.004 ($p < 0.05$) in WIN-treated rats. Moreover, the interburst interval was significantly increased in Purkinje neurons of prenatally WIN-treated offspring compared with control cells (0.65 ± 0.04 s in control vs. 1.05 ± 0.2 s in WIN-treated rats, $p < 0.01$; Fig. 2e). The peak amplitude of the Ca^{2+} spike was also significantly enhanced by prenatal WIN treatment (from 19.83 ± 0.72 mV in control to 35.38 ± 2.01 mV in WIN-treated offspring, Fig. 2f).

Prenatal Exposure to WIN Altered the Pharmacological Sensitivity of Ca^{2+} Channel Current

To further evaluate whether voltage-dependent Ca^{2+} channel currents contribute to the maternal WIN treatment–induced changes in burst firing activity, whole-cell voltage-clamp recordings were performed in the presence of the T-type Ca^{2+} channel blocker Mib (4 μM) and the L-type Ca^{2+} channel blockers Dilt (5 μM) and Cd (50 μM). There are also several lines of evidence that support the inhibitory effect of ZD, a blocker of hyperpolarization-activated channels, on T-type Ca^{2+} channels (Felix et al. 2003; Sánchez-Alonso et al. 2008). Therefore, ZD (50 μM) was also added to the ACSF containing synaptic blockers and Ca^{2+} channel blockers. With intracellular QX-314 and CsCl to block Na^+ and K^+ channel

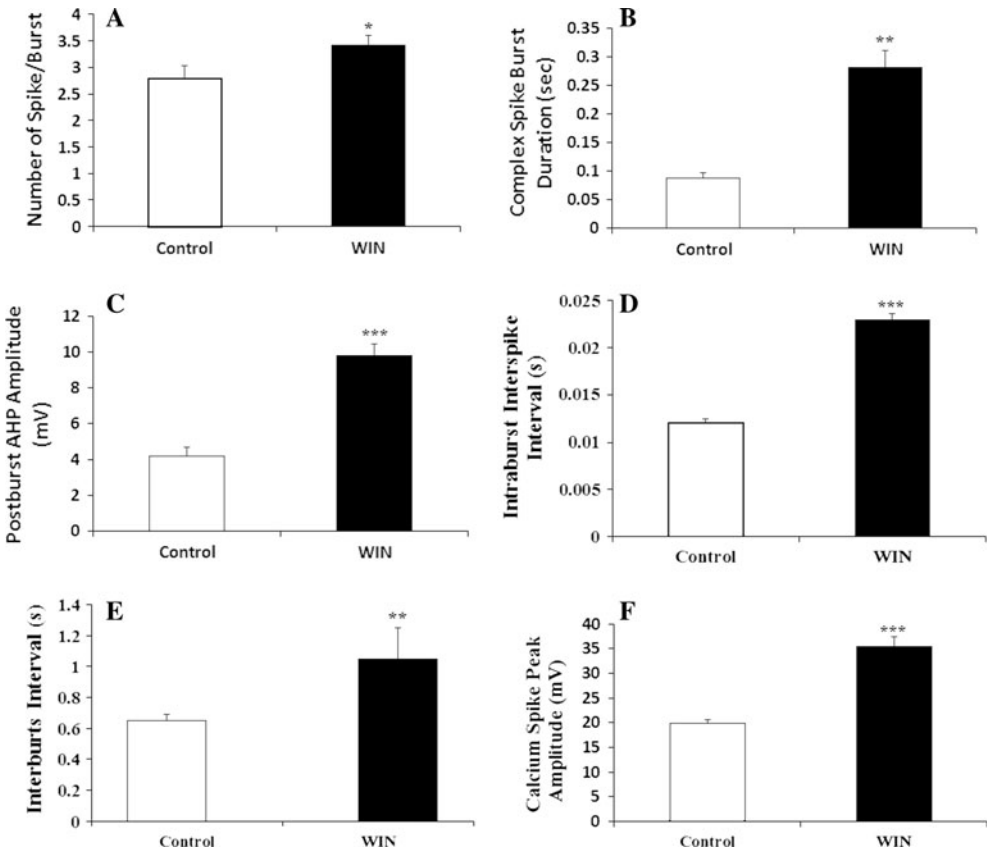

Fig. 2 Prenatal WIN treatment causes profound alterations in Purkinje neuronal bursting parameters. **a** Mean number of Ca^{2+} spikes per burst, **b** complex spike burst duration, **c** mean amplitude of postburst AHP potential following a complex spike burst, **d** average intraburst interspike intervals, **e** average interburst intervals and **f** mean amplitude of Ca^{2+} spikes of Purkinje neurons of control and prenatally WIN-treated offspring. Significant differences from control: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

currents and in the presence of kynurenic acid and picrotoxin, intrinsic Ca^{2+} spikes were somatically recorded from PCs under current clamp condition (Fig. 3a). Then, to assess whether these changes in neuronal firing pattern following prenatal WIN treatment are accompanied by an alteration in Ca^{2+} channel currents, a ramped voltage-clamp pulse from −80 to +20 mV in 1,250 ms at a slope of 80 mV/s was applied to elicit the Ca^{2+} current (Fig. 3b). The maximum peak amplitude of whole-cell Ca^{2+} current in control cells recorded during a ramp voltage command reached a maximum of $-1{,}906.79 \pm 190.79$ (Fig. 3c) at -29.6 ± 2.06 mV, whereas in Purkinje neurons obtained from maternally WIN-treated offspring the mean amplitude of the maximum current was $-2{,}564.67 \pm 177.34$ (Fig. 3c, $p < 0.05$) at -34.37 ± 1.46 mV. Application of Ca^{2+} channel blockers and ZD significantly reduced the peak amplitude of the inward Ca^{2+} current to -938.217 ± 243.562 pA in control cells ($p < 0.01$) and to -495.57 ± 109 in PCs obtained from prenatally WIN-treated offsprings ($p < 0.001$, Fig. 3c). Furthermore, when responses of PCs from the WIN-treated group were recorded upon depolarizing voltage-clamp steps delivered at 10-mV increments from a holding potential of −60 mV, the peak amplitude of Ca^{2+} currents was significantly larger compared to control (Fig. 3d, e), as shown in current–voltage relationship curves (Fig. 3d). Prenatal WIN treatment resulted in significantly increased Ca^{2+} current peak amplitude ($-3{,}488.97 \pm 453.398$ pA, $p < 0.0001$; Fig. 3e) compared with control cells ($-2{,}710.51 \pm 317.13$ pA). Blockage of Ca^{2+} channels produced significant inhibition of the Ca^{2+} current in both the control and WIN-treated groups ($p < 0.001$), but the magnitude of inhibition in WIN-treated rats was less (peak amplitude of inward current $-1{,}906.57 \pm 415.94$ pA in WIN-treated versus $-1{,}288.9 \pm 299.2$ pA in control, $p < 0.05$; Fig. 3d).

Discussion

The findings of the current study indicate that prenatal exposure to WIN, a cannabinoid receptor agonist, alters characteristics of the burst firing patterns of Purkinje neurons in offspring. The results of the present work do not

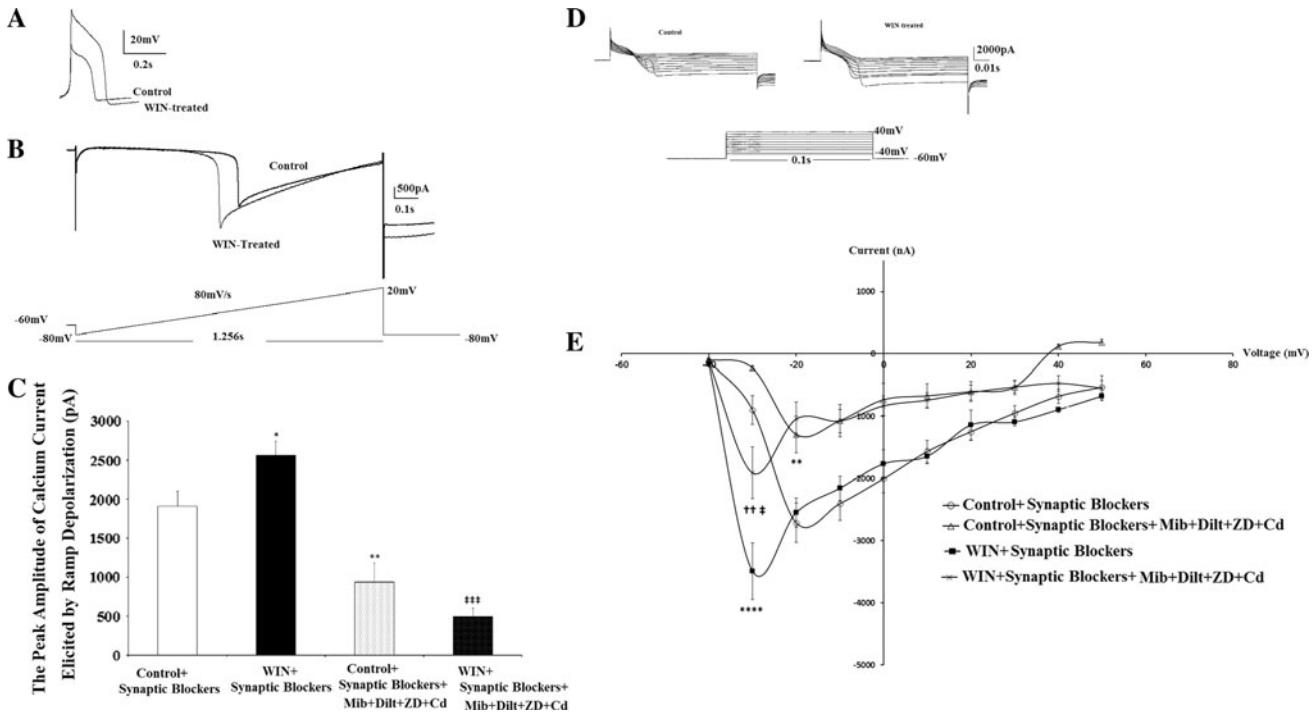

Fig. 3 Pharmacological properties of inward Ca^{2+} current in Purkinje neurons of offspring maternally exposed to cannabinoid agonist WIN 55212-2. **a** Two superimposed Ca^{2+} spikes recorded following blockade of Na^{+}- and voltage-dependent K^{+} channels in control and prenatally WIN-treated rat offspring. **b** Superimposed voltage ramp-evoked current traces recorded from Purkinje cells of control and maternally WIN-treated rats. Ramp voltage-clamp protocol to elicit inward Ca^{2+} current is shown at the *bottom* of the traces. **c** Histogram summarizing the effects of maternal WIN exposure and Ca^{2+} channel blockers on the peak amplitude of inward Ca^{2+} channel current recorded using depolarizing ramp voltage command. *Asterisks* show significant difference from control current (i.e., current recorded after application of synaptic blockers and 4-AP). **d** Representative whole-cell Ca^{2+} current traces recorded from a control Purkinje cell and a cell from prenatal WIN-treated offspring. Voltage-clamp protocol is shown beneath current traces. **e** Current–voltage relationships of maximal inward currents obtained in control and maternally WIN-treated rats and following Ca^{2+} channel blockade. *Asterisks* represent statistical differences from control + synaptic blockers (*open circles*). *Dagger* indicates statistical difference between control + synaptic blockers + Mib + Dilt + ZD + Cd (*triangle*) and WIN + synaptic blockers + Mib + Dilt + ZD + Cd. *Double dagger* shows Statistical difference between WIN + synaptic blockers (*filled square*) and WIN + synaptic blockers + Mib + Dilt + ZD + Cd (*X*). One-way ANOVA was used, followed by Tukey's multiple comparison test, to analyze the statistical significance of the data (*,$^{\ddagger}p < 0.05$; **,$^{\dagger\dagger}p < 0.01$, ***,$^{\ddagger\ddagger\ddagger}p < 0.001$; ****$p < 0.0001$)

provide direct evidence of the cellular mechanisms underlying alterations in burst activity in Purkinje neurons of rat offspring. However, as PCs fired spontaneous rhythmic bursts of simple and/or complex spikes in slice preparations in which their synaptic inputs were blocked, the electrophysiological characteristics of Purkinje neurons may likely contribute to their intrinsic burst firing. In many central neurons including cerebellar PCs, electrophysiological properties play a key role in establishing the cells' physiological function (Gruel and Franklin 1987; McKay et al. 2007) and shaping the cells' firing pattern (Smith and Perrier 2006). Therefore, any alterations in the electrophysiological properties of the firing pattern may affect the most neural mechanisms such as information processing, encoding or generating motor input (Kaczmareck and Levitan 1987). In our previous work we found that tonic firing properties of PCs from offspring prenatally exposed to WIN were profoundly altered (Shabani et al. 2011). In the present account, we show that maternal exposure to a cannabinoid agonist alters significantly several properties of the burst firing of Purkinje neurons from the offspring of prenatally WIN-treated rats. There were significant differences in the number of calcium spikes per burst, Ca^{2+} spike amplitude, burst duration, postburst AHP amplitude and intra- and interburst intervals of PCs from control and prenatally WIN-treated offspring. Burst firing is believed to be important in neural communication and can play a crucial role in neuronal information processing, particularly for motor pattern generation and synchronization in bursting neurons (Llinás 1988). Therefore, persistent changes in burst firing may affect synaptic and/or intrinsic excitability and thereby neuronal function, subsequently altering behavior. It has been reported that cerebellar Purkinje neurons have membrane properties that favor burst firing (Swensen and Bean 2003) and fire spontaneous activity in the form of trains of spikes that are both

Na^+-dependent (high-frequency simple spike) as well as Ca^{2+}-dependent (low-frequency complex spike) (Llinás and Hess 1976; Llinás and Sugimori 1980). There are also several lines of evidence indicating that alterations in the intrinsic electrical properties of PCs may contribute to the underlying cellular changes in physiological and behavioral function (Goudarzi et al. 2010; Chiesa et al. 2000; Ovsepian and Friel 2008).

Endocannabinoids serve as retrograde signals to enable postsynaptic neurons to regulate the strength of their synaptic inputs. Cerebellar Purkinje neurons release endocannabinoids from their dendrites, which then bind to presynaptic CB1 cannabinoid receptors, leading to transient downregulation of both excitatory and inhibitory synapses (Kreitzer and Regehr 2001). Several experimental and human seizure studies indicate that cannabinoid compounds have antiepileptic actions (Cunha et al. 1980; Wallace et al. 2002; Mason and Cheer 2009). We found that PCs of offspring from rats maternally treated with WIN exhibited significantly larger postburst AHP amplitudes, which were associated with a significant increase in the amplitude of either Na^+ or Ca^{2+} spikes. Therefore, an increase in Ca^{2+} and/or Na^+ influx during the bursts may be responsible for the enhancement of postburst AHP observed in the WIN-treated group. It has been reported that both Ca^{2+}- and Na^+-dependent K^+ conductances contribute to the generation of postburst AHP (Franceschetti et al. 2003; Zhang et al. 2010). The postburst AHP plays a major role in determining the neuronal intrinsic excitability and firing pattern and thereby is implicated in several neurological diseases, including Alzheimer disease (Oh et al. 2009). Here, significant increases in the duration of bursts and in the interburst interspike intervals were also documented in the WIN-treated group compared to controls, indicating the suppressive effects of cannabinoid receptor activation on burst firing. This is consistent with similar results reported by Goonawardena et al. (2010) in the hippocampus. A striking property of some neurons including PCs is burst firing, which plays an important role in their proper physiological function. It may also contribute to the synchronization of electrical activity in cells within a network. Furthermore, in the present study, it was shown that prenatal treatment with WIN caused a significant increase in the peak amplitude of Ca^{2+} spikes, which is consistent with our previous finding that offspring from mother rats treated with WIN had a significantly augmented Na^+-dependent action potential (Shabani et al. 2011). One possible reason for this enhancement could be the inhibition of K^+ channels by cannabinoid treatment. Several reports have indicated the inhibitory effects of cannabinoids on K^+ channel currents (Schweitzer 2000; Van den Bossche and Vanheel 2000; Wacnik et al. 2008; Khairy and Houssen 2010). In our previous work, we also reported that PCs from offspring of mothers exposed to WIN during pregnancy exhibited a significant hyperexcitability in their neuronal intrinsic activity, as evidenced by an increase in the population spike amplitude, which is believed to be a function of cellular action potential amplitude, cell synchronization and number of cells firing (Schweitzer et al. 2000). In addition, we previously showed that Ca^{2+}-activated K^+ channels play a major role in the regulation of neuronal hyperexcitability and burst activity in PCs (Haghdoost-Yazdi et al. 2008).

Another observation of the present study was the enhancement of calcium current in offspring from mother rats treated with WIN during pregnancy. This is in contrast to the results from the literature that investigated the inhibitory effects of cannabinoids on presynaptic voltage-dependent Ca^{2+} channels.

Although substantial efforts have been focused on understanding the acute electrophysiological effects of cannabinoid receptor activation and/or inhibition, very few studies have been conducted on the long-term electrophysiological consequences of cannabinoid receptor function. Several studies have addressed the acute modulatory effects of CB1 receptor activation on synaptic transmission, which have been attributed to the inhibition of presynaptic voltage-gated Ca^{2+} channels, including N-, P/Q- and R-type channels (Daniel et al. 2004; Rusakov et al. 2005; Yamasaki et al. 2006). However, a direct acute inhibitory effect of cannabinoids on excitability and Ca^{2+} influx through P/Q-type channels of postsynaptic Purkinje neurons has also been reported (Fisyunov et al. 2006). In contrast, in the present study long-term maternal exposure to the cannabinoid receptor agonist WIN induced a significant enhancement of burst firing and Ca^{2+} channel current.

There are several possible explanations for this discrepancy. Firstly, prenatal WIN treatment may activate a neuronal target on the PC soma membrane with signaling distinct from CB1 receptors and thereby cause enhancement of the Ca^{2+} channel current. Although to our knowledge there is no comprehensive study focusing on the intrinsic electrophysiological consequences of long-term maternal exposure to cannabinoid receptor agonists, in HEK293 cells activation of GPR55, which is a type 3 endocannabinoid receptor, has been reported to increase Ca^{2+} channel currents (Lauckner et al. 2008).

Secondly, the increased calcium current in PCs obtained from maternally WIN-treated offspring could be due to the activation of transient receptor (TRP) channels, which play an important role in burst firing activity (Lee et al. 2013) and in the regulation of the intracellular calcium level in excitable cells (Gees et al. 2010). TRP channels are mostly permeable to both monovalent and divalent cations (Nilius et al. 2007) and have been reported to be activated by metabotropic glutamate receptor 1 (mGluR1) in cerebellar

Purkinje neurons (Chang et al. 2012). Although, Chang and colleagues indicated that the TRP current does not play an important role in endocannabinoid signaling in PCs, we believe that activation of TRP channels mediated by activation of mGluR1 may contribute to the enhancement of Ca^{2+} current in prenatally WIN-treated offspring since in the present study only ionotropic glutamate receptors were blocked by kynurenic acid. The impact of these channels on the pathogenesis of several diseases, including cerebellar ataxia, has been shown (Nilius et al. 2007; Becker et al. 2009; Bollimuntha et al. 2011).

Thirdly, another possible explanation for the enhanced Ca^{2+} current in WIN-treated rats could be inhibition of Na^{+} currents, including resurgent current (Nicholson et al. 2003; Theile and Cummins 2011), thereby unmasking Ca^{2+} channel currents. The residual inward current in both the control and WIN-treated groups, which was insensitive to Ca^{2+} channel blockers, may correspond to the R-type Ca^{2+} current. In the cerebellar cortex, prominent immunohistochemical staining for R-type voltage-gated Ca^{2+} was found in the soma of Purkinje neurons (Yokoyama et al. 1995). To date, there is only one observation of cannabinoid acute modulation of R-type Ca^{2+} channels, and in neurons of the nucleus tractus solitarius application of WIN did not significantly change the R-type current (Endoh 2006). However, further studies need to be conducted in order to investigate all these assumptions.

Together, these findings strongly suggest that prenatal exposure to cannabinoids may profoundly result in induction of long-term changes in burst firing pattern, which in turn may lead to massive alterations in neuronal output. In addition, these data suggest that exposure of animals to cannabinoids during pregnancy affects the calcium channel currents of offspring PCs and may thereby cause changes in neuronal burst firing activity, which plays an important role in the physiological function of neuron of PCs.

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Conflict of interest The authors declare no conflict of interest and that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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