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Modulacja farmakologiczna aktywności epileptycznych, badania na modelach zwierzęcych.

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Wykaz stosowanych skrótów

4-AP – 4-amniopirydyna

ADHD – *attention deficit hyperactivity disorder* (zespół niedoboru uwagi z nadpobudliwością)

DRE – *drug-resistant epilepsy* (padaczka lekooporna)

EPSP – *excitatory post-synaptic potential* (pobudzające potencjały post-synaptyczne)

GABA - *gamma-aminobutyric acid (kwas gamma-aminomasłowy)*

IED – *interictal epileptiform discharges* (wyładowania międzynapadowe)

IPSP - *inhibitory postsynaptic potentials* (hamujące potencjały post-synaptyczne)

NMDA - *N-methyl-d-aspartic acid (kwas n-metylo d aspartanowy)*

OUN – ośrodkowy układ nerwowy

TRPV – *transient receptor potential cation channel subfamily V (*receptor waniloidowy przejściowego potencjału)

VGSC - *voltage-gated sodium channels* (kanały sodowe bramkowane napięciem)

mPFC – *medial prefrontal cortex* (przyśrodkowa kora przedczołowa)

Streszczenie w języku polskim

Modulacja farmakologiczna aktywności epileptycznych, badania na modelach zwierzęcych

Celem niniejszej rozprawy doktorskiej było zaadaptowanie modelu zwierzęcego przy użyciu techniki patch-clamp do badania aktywności epileptycznej w korze przedczołowej, oraz jego zastosowanie do badania mechanizmów działania istniejących leków oraz oceny potencjału terapeutycznego nowych substancji. Szczególną uwagę poświęcono krótkotrwałym epizodom epileptycznym, nazywanym wyładowaniami międzynapadowymi (*Interictal Epileptiform Discharges,* IEDs), które obserwowano nie tylko w przypadku padaczki, ale także w innych zaburzeniach, takich jak zespół nadpobudliwości psychoruchowej z deficytem uwagi (*Attention Deficit Hyperactivity Disorder,* ADHD), choroba afektywna dwubiegunowa, czy zaburzenia ze spektrum autyzmu. Początkowo skupiono się na znanym leku przeciwpadaczkowym, kwasie walproinowym, a następnie na kapsaicynie, która może stanowić potencjalnie nową opcję terapeutyczną. Na koniec, wykorzystując model IEDs, zademonstrowano przeciwpadaczkowe właściwości guanfacyny, leku powszechnie stosowanego w leczeniu ADHD.

Kora przedczołowa to region mózgu zaangażowany w kilka funkcji poznawczych, koordynujący myśli i działania, aby były zgodne z wewnętrznymi celami. Wyładowania międzynapadowe w tym regionie opisano w kilku zaburzeniach psychiatrycznych, w tym w chorobie afektywnej dwubiegunowej. Pierwszy artykuł włączony do przedstawionej rozprawy doktorskiej to artykuł oryginalny pt. „Valproic acid potently inhibits interictal-like epileptiform activity in prefrontal cortex pyramidal neurons", opublikowany w *Neuroscience Letters*. Kwas walproinowy to dobrze znany lek przeciwpadaczkowy, którego mechanizm działania obejmuje nasilenie transmisji GABA-ergicznej oraz blokowanie kanałów sodowych zależnych od napięcia. Oprócz padaczki, kwas walproinowy jest powszechnie stosowany w chorobie afektywnej dwubiegunowej, chociaż jego mechanizm działania w tym kontekście nie jest całkowicie jasny. W tym badaniu użyliśmy metody indukowania IEDs w neuronach kory przedczołowej szczurów. Roztwór, w którym zanurzono skrawki mózgu szczura, nie zawierał jonów magnezu i miał podwyższone stężenie jonów potasu. Te warunki pozwoliły nam indukować IEDs czyli depolaryzacje krótsze niż 2 sekundy z wyzwalaniem potencjałów czynnościowych, stabilne przez kilkadziesiąt minut. Pokazaliśmy, że obserwowane depolaryzacje były zależne od receptora N-metylo-D-asparaginowego (NMDA), ponieważ znany inhibitor NMDA, AP-5, eliminował tę aktywność. Ponadto zademonstrowaliśmy, że terapeutyczne stężenia kwasu walproinowego eliminowały

zarówno krótkotrwałą aktywność epileptyczną w sposób zależny od stężenia leku, jak i spontaniczne pobudzające potencjały postsynaptyczne (*Excitatory Post-Synaptic Potentials,* EPSPs). Dodatkowo badaliśmy pobudliwość neuronów i stwierdziliśmy, że kwas walproinowy zmniejsza pobudliwość neuronów kory przedczołowej.

Drugie badanie wchodzące w skład przedstawionej rozprawy doktorskiej to artykuł oryginalny pt. „Capsaicin inhibits sodium currents and epileptiform activity in prefrontal cortex pyramidal neurons", opublikowany w *Neurochemistry International*. W tym przypadku zbadaliśmy potencjał terapeutyczny kapsaicyny – związku z rodziny waniloidów, najbardziej znanego jako aktywny składnik papryczek chili, odpowiedzialny za odczucie ostrego smaku. Związek ten od kilku lat jest stosowany w medycynie ze względu na swoje właściwości przeciwbólowe. W tym kontekście, obwodowego układu nerwowego, uważa się, że działa poprzez kanały kationowe TRPV1 (*transient receptor potential vanilloid*, TRPV1). Rozpoczęliśmy nasze badania od sprawdzenia wpływu kapsaicyny na pobudliwość neuronalną. Pokazaliśmy, że kapsaicyna znacząco hamowała częstotliwość potencjałów czynnościowych, a co więcej, amplitudy ostatnich potencjałów czynnościowych w serii były znacznie zmniejszone w obecności kapsaicyny. Sugerowało to modulację o typie zależnego od użycia blokowania kanałów sodowych. Aby dalej zbadać tą interakcję, przeprowadziliśmy badania prądów sodowych w konfiguracji *voltage-clamp* i pokazaliśmy, że kapsaicyna silnie hamowała prądy sodowe poprzez przesunięcie krzywej inaktywacji. Ponadto obserwowaliśmy, że związek ten silnie wzmacniał zależne od użycia blokowanie kanałów sodowych, potwierdzając nasze wcześniejsze obserwacje. Następnie przetestowaliśmy działanie kapsaicyny w trzech różnych modelach aktywności padaczkowej: IEDs, długich epizodach epileptycznych wywołanych za pomocą płynu bez magnezu i 4-aminopirydyny (4-AP), inhibitora kanałów potasowych, oraz epizodach epileptycznych o pośrednim czasie trwania wywołanych pikrotoksyną, antagonistą receptora GABA oraz płynem zewnątrzkomórkowym bez magnezu. Kapsaicyna całkowicie blokowała aktywność epileptyczną w pierwszych dwóch modelach, a w trzecim skracała czas trwania epizodów. Co ciekawe, wyładowania wywołane 4-AP były oporne na działanie kwasu walproinowego. Wszystkie powyższe odkrycia stanowią podstawę do rozważenia kapsaicyny jako strukturalnej ramy do opracowania nowych leków przeciwpadaczkowych.

Trzecia publikacja, wchodząca w skład przedstawionej rozprawy doktorskiej zatytułowana „Guanfacine inhibits interictal epileptiform events and sodium currents in prefrontal cortex

pyramidal neurons", została opublikowana w *Pharmacological Reports*. Krótkotrwałe epizody epileptyczne występują u osób chorujących na ADHD i mogą przyczyniać się do objawów choroby. Jednocześnie kora przedczołowa jest centrum badań nad patofizjologią ADHD. W prezentowanym artykule postawiliśmy hipotezę, że znany lek na ADHD – guanfacyna – może działać przez inhibicję IEDs w korze przedczołowej. Korzystając z modelu przyjętego w poprzednich badaniach, pokazaliśmy, że guanfacyna skutecznie redukowała IEDs czyli krótkotrwałą aktywność epileptyczną. Ponadto pokazaliśmy, że efekt był niezależny od receptora alfa-2, nominalnego miejsca działania guanfacyny, ponieważ w serii eksperymentów z idazoksanem – antagonistą receptora alfa-2-adrenergicznego efekt pozostał niezmieniony. Wreszcie pokazaliśmy, że guanfacyna silnie hamowała prądy sodowe. Co ciekawe struktura chemiczna guanfacyny przypomina tę inhibitorów kanałów sodowych stosowanych w znieczuleniach miejscowych. Podsumowując, sugerujemy, że modulacja aktywności kanałów sodowych może być dodatkowym mechanizmem działania guanfacyny w ADHD.

Ostatnia publikacja włączona do przedstawionej rozprawy doktorskiej to artykuł przeglądowy pt. „Beneficial Effects of Capsaicin in Disorders of the Central Nervous System", opublikowany w *Molecules*. Artykuł ten podsumował dostępne dowody naukowe na temat korzystnych efektów kapsaicyny na szeroki zakres zaburzeń neurologicznych, w tym chorobę Parkinsona i Alzheimera, udar i migrenę. Największy nacisk położono na dane opisujące rolę kapsaicyny w badaniach nad padaczką. Co ciekawe, opisano zarówno działania proepileptyczne, jak i przeciwpadaczkowe kapsaicyny. Większość istniejących badań koncentruje się na formacji hipokampa i działaniach poprzez receptory TRPV1. W przedstawionym artykule proponujemy nowe ramy do zrozumienia tych pozornie sprzecznych wyników. Sugerujemy, że kapsaicyna wywiera swoje działanie proepileptyczne poprzez oddziaływanie na receptory TRPV1 w formacji hipokampa, natomiast jej działanie przeciwpadaczkowe wynika z hamowania kanałów sodowych w neuronach korowych. W nielicznych badaniach, w których podawano kapsaicynę w sposób ogólnoustrojowy, obserwowano dominację mechanizmu przeciwpadaczkowego.

Podsumowując, badania włączone do rozprawy doktorskiej zastosowały kilka modeli aktywności epileptycznej *in vitro*, aby, przy użyciu techniki patch-clamp, pogłębić nasze zrozumienie mechanizmów działania znanych leków (kwasu walproinowego, guanfacyny) i dostarczyć dowodów *in vitro* na potencjał terapeutyczny nowego środka – kapsaicyny. Ponadto, przestudiowano efekty kapsaicyny w szerszym kontekście zaburzeń neurologicznych, ze

szczególnym uwzględnieniem jej miejsca w terapii przeciwpadaczkowej. Uzyskane wyniki mogą wzbogacić nasze zrozumienie patofizjologii wielu zaburzeń, jak również dostarczyć nowych opcji terapeutycznych.

Streszczenie w języku angielskim

Pharmacological modulation of epileptic activity, experiments on animals.

The aim of the presented dissertation was to develop a patch clamp animal model of epileptiform activity in the prefrontal cortex and use it to study the mechanisms of action of known medications and the therapeutic potential of novel agents. Special attention was given to short epileptiform activity, known as interictal epileptiform discharges (IEDs), which, apart from epilepsy, have been described in several other conditions including attention deficit hyperactivity disorder (ADHD), bipolar disorder, or autism spectrum disorder. Initially, the known antiepileptic medication, valproic acid, was studied, followed by the exploration of the potentially new therapeutic option, capsaicin. Lastly, using the model of IEDs, the antiepileptic properties of a known ADHD medication, guanfacine, were demonstrated.

The prefrontal cortex is a region of the brain involved in several cognitive functions, coordinating thoughts and actions to align with internal objectives. Interictal epileptiform discharges in this region have been described in several psychiatric disorders including bipolar disorder. The first article included in this thesis is an original article titled "Valproic acid potently inhibits interictal-like epileptiform activity in prefrontal cortex pyramidal neurons," published in *Neuroscience Letters*. Valproic acid is a well-known antiepileptic medication which mechanism of action involves GABA-transmission enhancement and voltage-gated sodium channels inhibition. Apart from epilepsy, valproate is commonly used in bipolar disorder, although its mechanism of action in this context is not entirely clear. In this study, we adopted a method of inducing IEDs in rat prefrontal cortex neurons. The solution in which brain slices were bathed contained no magnesium ions and an elevated concentration of potassium ions. This setup allowed us to induce depolarizations shorter than 2 seconds with firing of action potentials, stable for a period of up to an hour. We showed that the depolarization bursts were N-methyl-D-aspartic acid (NMDA) receptor-dependent, as a known NMDA blocker, AP-5, abolished this activity. Furthermore, we demonstrated that therapeutic concentrations of valproic acid abolished both the short epileptiform activity and the spontaneous excitatory postsynaptic potentials (EPSPs) in a concentration-dependent manner. Additionally, we studied the excitability of

prefrontal neurons and found that valproic acid reduced the excitability of prefrontal neurons in a dose-dependent manner.

The second study was an original article entitled “Capsaicin inhibits sodium currents and epileptiform activity in prefrontal cortex pyramidal neurons,” published in *Neurochemistry International*. Here, we investigated the therapeutic potential of capsaicin – a compound of the vanilloid family most famous for being an active ingredient of chili peppers, responsible for the hot sensation. The compound has been used in medicine for several years due to its analgesic properties. In this peripheral setting, it is believed to act via transient receptor potential vanilloid (TRPV) cation channels. We initiated our investigation by testing capsaicin's influence on neuronal excitability. We found that capsaicin significantly inhibited neuronal firing, and moreover, the amplitude of the last action potentials were markedly reduced in the presence of capsaicin. This suggested modulation of use-dependent blockade of sodium channels. To further investigate, we performed single-channel recordings in a voltage-clamp setting, and showed that capsaicin strongly inhibited sodium channels by shifting the inactivation curve of sodium channels towards hyperpolarization. Additionally, we observed that the compound strongly enhanced use-dependent blockade of sodium channels, confirming our previous observations. Furthermore, we tested the compound in three different models of epileptic activity: IEDs, long ictal events evoked with 4-aminopyridine (4-AP), a potassium channel inhibitor, and a solution without magnesium ions and intermediate-long events evoked with picrotoxin, a GABA receptor antagonist and a solution without magnesium ions. Capsaicin inhibited epileptiform activity in the first two models and shorten the duration of epileptic episodes in the third. Interestingly, the discharges evoked with 4-AP were resistant to valproate. All the findings above provide a basis for considering capsaicin as a structural framework for developing new antiepileptic medications.

The third publication, titled “Guanfacine inhibits interictal epileptiform events and sodium currents in prefrontal cortex pyramidal neurons,” was published in *Pharmacological Reports*. Several studies have described interictal discharges as prevalent in subjects suffering from ADHD and, possibly, contributing to the symptoms of the disease. At the same time, the prefrontal cortex is a center of studies investigating the pathology of ADHD.

In the presented article, we hypothesized that the known ADHD medication – guanfacine – may act by inhibiting the IEDs in the prefrontal cortex. Using the model adopted in previous studies, we showed that guanfacine potently reduced this short epileptiform activity. Furthermore, we showed that the effects were independent of the alpha-2 receptor, a nominal place of action of guanfacine, as in a series of experiments with idazoxan – an alpha-two antagonist – the effect remained unchanged. Finally, we showed that guanfacine potently inhibited sodium channels. Altogether, we point to the fact that modulating sodium channel activity may be an additional mode of action of guanfacine in ADHD.

The last publication included in the thesis is a review article entitled "Beneficial Effects of Capsaicin in Disorders of the Central Nervous System," published in *Molecules*. This article systematically summarized the available evidence on the beneficial effects of capsaicin on a wide range of neurological disorders including Parkinson's and Alzheimer's disease, stroke, and migraine. The strongest emphasis was placed on the data describing capsaicin's role in epilepsy research. Most existing research concentrates on TRPV1 actions and the hippocampal formation. Interestingly, both pro-epileptic and antiepileptic actions of capsaicin are described. In the presented article, we propose a new framework for understanding these seemingly contradictory results. In brief, we postulate that the pro-epileptic actions of capsaicin are mediated by its action on TRPV1 receptors in the hippocampal formation, whereas antiepileptic actions work via sodium channel inhibition in cortical neurons. In the few studies that used systemic application of capsaicin, the antiepileptic mechanism seemed to prevail.

In summary, the studies included in this dissertation applied several patch-clamp models of epileptiform activity to deepen our understanding of the mechanisms of action of known medications (valproate, guanfacine) and provide in vitro evidence for the therapeutic potential of a novel agent – capsaicin. Furthermore, the effects of capsaicin in the broader context of neurological disorders have been studied, with special attention given to its place in antiepileptic therapy. The obtained results may enhance our understanding of the pathologies of several disorders, as well as provide novel therapeutic options.

1. Wstęp

Padaczka jest najczęstszą przewlekłą chorobą neurologiczną, charakteryzującą się przede wszystkim napadami uogólnionymi lub ogniskowymi. W metaanalizie obejmującej 124 miliony osób oszacowano częstość występowania padaczki na 6,38 na 1000 osób, podczas gdy częstość występowania padaczki w ciągu całego życia wynosiła 7,60 na 1000 osób. W tym samym badaniu roczną zapadalność na padaczkę oszacowano na 67,77 na 100 000 osób [1]. Na przestrzeni ostatnich dziesięcioleci obserwuje się stopniowy wzrost częstości występowania padaczki, związany ze starzeniem się społeczeństwa, a także poprawą przeżywalności osób z chorobami współistniejącymi predysponującymi do padaczki, takimi jak pacjenci po udarze mózgu czy z demencją [2].

Patofizjologiczne mechanizmy leżące u podstaw padaczki i napadów są zróżnicowane, co przyczynia się do szerokiej gamy obserwowanych zaburzeń. Niemniej jednak wspólną cechą wszystkich jej fenotypów jest brak równowagi w aktywności synaptycznej. Zakłócona jest sygnalizacja pobudzająca, w której pośredniczy glutaminian, i sygnalizacja hamująca, w której pośredniczy kwas gamma-aminomasłowy (GABA), co prowadzi do wystąpienia napadów padaczkowych. Leczenie padaczki opiera się na lekach przeciwpadaczkowych, które działają głównie poprzez wpływ na kanały sodowe zależne od napięcia lub szlaki kwasu gamma-aminomasłowego. Niestety, blisko 15% przypadków nie reaguje na istniejące leki. Co ważne, wśród dzieci odsetek ten jest znacznie wyższy, a częstość występowania padaczki lekoopornej (*drug-resistant epilepsy,* DRE) wzrasta do jednego na cztery przypadki [3]. U pacjentów ze zdiagnozowaną padaczką lekooporną towarzyszące upośledzenie funkcji poznawczych występuje częściej i w większym nasilaniu [3]. Trwają wysiłki mające na celu opracowanie nowych leków, a 30 potencjalnych kandydatów przechodzi obecnie ocenę przedkliniczną lub kliniczną, chociaż ich bezpieczeństwo i skuteczność kliniczna nie zostały w pełni zbadane [4].

Ponadto, napady padaczkowe czy to uogólnione, czy ogniskowe, często towarzyszą innym chorobom neurologicznym i/lub psychiatrycznym. Wśród nich można wymienić depresję, chorobę afektywną dwubiegunową, zespół nadpobudliwości psychoruchowej z deficytem uwagi (ADHD) oraz zaburzenia ze spektrum autyzmu [5-7]. Tradycyjnie postrzegane jako powikłania padaczki, ostatnie badania wykazały dwukierunkową zależność między tymi schorzeniami a padaczką [8]. Oznacza to, że u osób z padaczką istnieje większe prawdopodobieństwo

wystąpienia tych zaburzeń psychicznych, podczas gdy u pacjentów z chorobą afektywną dwubiegunową, ADHD lub autyzmem również wzrasta ryzyko rozwoju padaczki. Szczególnie ADHD zwiększa ryzyko padaczki według kilku badań, niemal czterokrotnie [8]. Z drugiej strony ADHD jest częste wśród pacjentów z padaczką, dotykając ponad 25% z nich [9]. Te wzajemne powiązania między tymi patologiami sugerują istnienie wspólnych mechanizmów leżących u podstaw padaczki oraz zaburzeń psychicznych. Poznanie tych wspólnych mechanizmów patogennych może przyczynić się do lepszego zrozumienia neurobiologicznych podstaw tych zaburzeń.

W ostatnich latach większą uwagę zwraca się na międzynapadowe wyładowania padaczkowe (*interictal epileptifrom discharges*, IEDs), które są nieprawidłowymi wyładowaniami elektrycznymi rejestrowanymi w korze mózgowej i występującymi pomiędzy napadami padaczkowymi. Uważa się, że stanowią one istotny czynnik prowadzący do zaburzeń funkcji poznawczych w padaczce [10]. Wczesne badania wykazały, że około połowa osób chorych na padaczkę doświadczała przejściowych zaburzeń poznawczych, które korelowały z obecnością IEDs [11-13]. Zaobserwowano również efekt lateralizacji, gdzie wyładowania po lewej stronie mózgu wpływały na zadania werbalne (w związku z obecnością ośrodka Wernickiego i Brocki u większości osób po stronie lewej), a IEDs po prawej stronie wpływały na zadania wzrokowe (z uwagi na dominującą rolę prawej półkuli w procesowaniu zadań wzrokowych) [11-13]. Co więcej IEDs nie są charakterystyczne wyłącznie dla padaczki; stwierdzono je również w innych schorzeniach neuropsychiatrycznych, takich jak ADHD, zaburzenia ze spektrum autyzmu, choroba Alzheimera i choroba afektywna dwubiegunowa [6,13-15]. Mechanizm powstawania zaburzeń funkcji poznawczych towarzyszących aktywności typu IEDs jest wyjaśniany w różny sposób. Głównie podkreśla się znaczenie raptownego wzrostu stężenia jonów Ca^{2+} w nadaktywnych neuronach na skutek aktywacji potencjało-zależnych kanałów jonowych Ca^{2+}. Prowadzi to do destrukcji tych komórek na skutek cytotoksycznego działania jonów Ca^{2+} [16]. Aktywność IED może prowadzić do zaburzeń poznawczych również poprzez restrukturyzację obwodów neuronalnych lub zaburzenie procesów konsolidacji pamięci związanej ze snem [16]. Sugeruje się, że skuteczne redukowanie aktywności typu IEDs może łagodzić zaburzenia poznawcze [17,18].

Modele zwierzęce *in vivo* znacząco przyczyniły się do zrozumienia padaczki. Wykorzystują one podanie związków wywołujących drgawki ogólnoustrojowo lub bezpośrednio do struktur mózgu.

W ostatnich latach wprowadzono metodę *in vitro* do badania mechanizmów powstawania padaczki. W metodzie tej wykorzystuje się skrawki mózgu uzyskane od zwierząt doświadczalnych lub okazjonalnie skrawki pobrane od pacjentów poddawanych zabiegom neurochirurgicznym. Technika ta zapewnia kontrolowane środowisko do badania aktywności epileptycznej, przy jednoczesnym zachowaniu kluczowych obwodów neuronalnych [19]. W badanych skrawkach mózgu można wywoływać aktywności epileptyczne zbliżone lub identyczne do tych które występują u badanych zwierząt doświadczalnych in-vivo. Podobnie jak w badaniach *in vivo*, w izolowanych skrawkach z ośrodkowego układu nerwowego, można wywoływać aktywności typu epileptycznego różnymi metodami. Jedną z nich jest upośledzenie hamowania synaptycznego mediowanego przez receptory GABA, które można osiągnąć poprzez zastosowanie substancji takich jak penicylina, bikukulina lub pikrotoksyna [GABA inhibitory]. Alternatywnie, osłabienie potencjałów post-synaptycznych hamujących (*inhibitory postsynaptic potentials,* IPSP), gdzie pośredniczy receptor GABA, może wynikać z nagłego wycofania terapii aktywujących lub wzmacniających GABA. Podwyższoną pobudliwość można wywołać poprzez podniesienie poziomu potasu w płynie pozakomórkowym, co prowadzi do aktywności epileptycznej, poprzez depolaryzację błon neuronalnych. Ponadto, wzmocnione pobudzenie synaptyczne można osiągnąć przez zmniejszenie pozakomórkowego stężenia magnezu, co wzmacnia przewodnictwo glutaminergiczne mediowane przez NMDA ponieważ magnez jest wypłukiwany z receptora NMDA. Innym sposobem jest zablokowanie kanałów potasowych, co można osiągnąć za pomocą substancji takich jak 4-aminopirydyna (4-AP) lub dendrotoksyna, co zwiększa pobudliwość neuronów. Wreszcie, zmniejszenie stężenia jonów Ca^{2+} w płynie zewnątrzkomórkowym może prowadzić do zwiększenia pobudliwości neuronów. Uważa się, że zmniejszenie stężenia jonów Ca^{2+} w płynie zewnątrzkomórkowym prowadzi do zmniejszonej aktywacji Ca^{2+} zależnych kanałów jonowych K+ i tym samym zwiększenia pobudliwości neuronów [20]. Te różnorodne metody stanowią doskonałe pole do farmakologicznej modulacji aktywności padaczkowej, umożliwiając ocenę zarówno skuteczności leku, jak i zrozumienie mechanizmu jego działania.

Potencjałozależne kanały sodowe (*voltage-gated sodium channels*, VGSC) mają fundamentalne znaczenie dla wytwarzania skoordynowanej transmisji potencjałów czynnościowych w układzie nerwowym. Nie jest więc zaskoczeniem, że wykazano, że odgrywają one kluczową rolę w powstawaniu padaczki. Wiele powszechnie stosowanych leków przeciwpadaczkowych działa poprzez blokowanie VGSC. Szczególnie istotne klinicznie są tzw. blokery VGSC zależne od

użycia (*use-dependent blockers*). Takie związki hamują aktywność głównie tych neuronów, w których aktualnie dochodzi do powstawania potencjałów czynnościowych z dużą częstotliwością (tak jak to ma miejsce w napadzie padaczkowym) i mają nieznaczny wpływ na neurony cechujące się niską aktywnością. Na poziomie komórkowym mechanizm ten opisuje hipoteza modulowanego receptora (*modulated receptor hypothesis*), która głosi, że lek wiąże się z kanałami z różnym powinowactwem w zależności od stanu kanału [21]. Badania wykazały, że leki przeciwpadaczkowe, takie jak fenytoina preferencyjnie wiążą się z kanałem sodowym w stanie częściowo zinaktywowanym, który występuje, gdy błona jest zdepolaryzowana tak jak ma to miejsce w czasie napadu padaczkowego [22].

Przyśrodkowa kora przedczołowa (*medial prefrontal cortex*, mPFC) jest kluczowym ośrodkiem integracyjnym, zbierającym informacje z różnych obszarów mózgu i przekazującym je dalej do obszarów odpowiedzialnych za reakcje na bodźce. Badania dotyczące tego obszaru mózgu pokazały jego aktywny udział w regulacji emocji, podejmowaniu decyzji oraz analizie sytuacji społecznych [23,24]. Uszkodzenie mPFC, powodujące upośledzenie tych funkcji, powiązano z kilkoma zaburzeniami neurologicznymi i psychiatrycznymi, w tym depresją, zaburzeniami lękowymi, schizofrenią, zaburzeniami ze spektrum autyzmu, chorobą Alzheimera, chorobą Parkinsona, uzależnieniami i ADHD [23]. Jeżeli w czasie napadu padaczkowego dochodzi do zwiększonej aktywności neuronów zlokalizowanych w korze przedczołowej to kora ta nie może realizować swoich fizjologicznych funkcji. Ponadto powtarzajace się aktywnosci typu padaczkowego w jednym miejscu ośrodkowego układu nerwowego mogą prowadzic do trwałego uszkodzenia zaangażowanych neuronów lub ich destrukcji, w mechniźmie np. ekscytotoksyczności jonów wapnia. W związku z tym lokalizacja ogniska padaczkowego w obszarze kory przedczołowej może prowadzić wtórnie do zaburzenia funkcji tego obszaru kory z takimi objawami, które towarzyszą wyżej wymienionym chorobom neuropsychiatrycznym.

Celem przedstawionego cyklu prac było zbadanie wpływu i mechanizmu działania kwasu walproinowego, kapsaicyny i guanfacyny na aktywności typu padaczkowego wywołane w neuronach piramidowych kory przedczołowej. Badania przeprowadzono na neuronach korowych zlokalizowanych w skrawkach lub neuronach rozproszonych wyizolowanych z kory przedczołowej szczurów. Przedstawiona rozprawa doktorska stanowi monotematyczny cykl 4 publikacji. Doktorant jest pierwszym, wiodącym autorem w 3 z 4 tych prac. Łączna wartość współczynnika oddziaływania (impact factor) czasopism, w których artykuły zostały

opublikowane, wynosi 15.195, a sumaryczna liczba punktów MNiSW wynosi 450.

W przedstawionej rozprawie doktorskiej zastosowano modele aktywności epileptycznej *in vitro* w celu oceny skuteczności potencjalnego nowego kandydata na lek - kapsaicyny, a także rzucenie nowego światła na mechanizm działania dwóch znanych leków – kwasu walproinowego i guanfacyny. Ponadto przedstawiono artykuł przeglądowy opisujący działania kapsaicyny w zaburzeniach neurologicznych, ze szczególnym uwzględnieniem literatury dotyczącej wpływu kapsaicyny na aktywność epileptyczną. Zaprezentowane prace oryginalne stosowały zbliżoną metodologię. We wszystkich trzech pracach badano efekty testowanej substancji na wyładowania międzynapadowe (IEDs) indukowane przy użyciu roztworu bez jonów magnezu z podwyższonym stężeniem jonów potasu w konfiguracji *current-clamp*. Podobnie, we wszystkich trzech pracach oceniono wpływ badanych związków na pobudliwość (zdefiniowaną jako liczba potencjałów czynnościowych dla zadanego impulsu depolaryzującego) neuronów kory przedczołowej. W pracy dotyczącej kapsaicyny wykorzystano dodatkowe modele aktywności epileptycznej *in vitro*, aby dokładniej ocenić jej działanie przeciwpadaczkowe. Ponadto, w badaniach związanych z kapsaicyną i guanfacyną przeprowadzono rejestracje prądów sodowych w konfiguracji *voltage-clamp*, aby lepiej zrozumieć mechanizmy, przez które te związki wywierają swoje działanie.

W pierwszej z prac wchodzących w skład przedstawionej rozprawy doktorskiej pt. „Valproic acid potently inhibits interictal-like epileptiform activity in prefrontal cortex pyramidal neurons", opublikowanej w *Neuroscience Letters*, zbadaliśmy wpływ kwasu walproinowego na IEDs w piramidowych neuronach kory przedczołowej. Kwas walproinowy, powszechnie stosowany lek przeciwpadaczkowy, działa poprzez hamowanie pobudliwości neuronalnej. Osiąga to poprzez różne mechanizmy, w tym poprzez hamowanie napięciowo-zależnych kanałów sodowych i wapniowych oraz wzmacnianie neurotransmisji (GABA). Podczas gdy napady padaczkowe są charakterystycznym objawem padaczki, coraz częściej uznaje się, że związane z nimi zaburzenia poznawcze mogą istotnie wpływać na jakość życia pacjentów. Dysfunkcja poznawcza w padaczce często manifestuje się jako obniżone zdolności intelektualne i trudności w nauce, co stanowi przeszkodę w wykonywaniu codziennych czynności. Problem ten jest szczególnie powszechny wśród dzieci z padaczką, przy czym badania sugerują, że nawet do 80% z nich cierpi na współistniejące zaburzenia behawioralne lub deficyty poznawcze [25]. Liczne badania łączą IEDs z zaburzeniami poznawczymi u pacjentów z padaczką [10-12]. Pomimo skutecznego

hamowania napadów, wpływ kwasu walproinowego na aktywność IEDs, zwłaszcza w obszarach mózgu związanych z funkcjami poznawczymi, takimi jak kora przedczołowa, nie był badany.

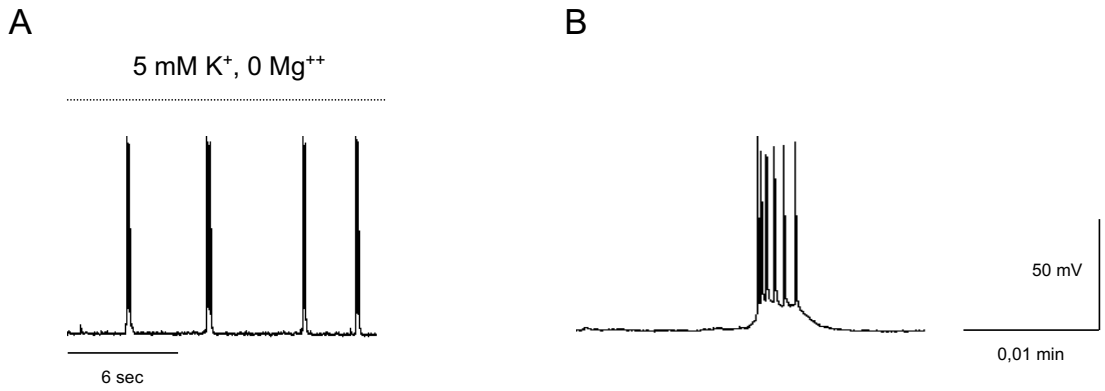
W doświadczeniach przedstawionych w tej publikacji aktywność typu IEDs wywoływano przez omywanie skrawków sztucznym płynem zewnątrzkomórkowym pozbawionym jonów Mg^{2+} i zawierającym podwyższone stężenie jonów K^{+}. Termin krótkotrwałe aktywności epileptyczne (IEDs) najczęściej kojarzony jest z rejestracjami in vivo. Zarówno w tej pracy, jak i w pozostałych pracach oryginalnych wchodzących w skład przedstawionej rozprawy, przyjęliśmy założenie, że krótkie depolaryzacje, którym towarzyszy seria potencjałów czynnościowych są odpowiednikiem *in vitro* IEDs rejestrowanych *in vivo* przy pomocy zbiorczych rejestracji zewnątrzkomórkowych typu elektrokortykogramu lub elektroencefalogramu. Takie założenie pojawia się w licznych wcześniejszych pracach [26-29]. Rejestracja aktywności epileptycznej wywołanej brakiem jonów magnezu jest możliwa wyłącznie in vitro (niemożliwe jest usunięcie jonów z organizmu). Natomiast, w jednej z prac badacze rejestrowali zewnątrzkomórkowe pole elektryczne na skrawkach zamoczonych w komorze bez jonów magnezu. Zrejestrowane krótkie aktywności epileptyczne, zostały zakwalifikowane jako IEDs [30].

Reprezentacyjny zapis IEDs przedstawiono na Rycinie 1. Prawdopodobny mechanizm odpowiedzialny za indukcję aktywności epileptycznej obejmuje aktywację receptorów NMDA, z uwagi na brak inhibicji przez jony magnezu oraz zwiększoną pobudliwość neuronów z powodu depolaryzacji związanej z wysokim stężeniem jonów potasu. W takich warunkach zwiększone spontaniczne uwalnianie glutaminianu otwiera odblokowane postsynaptyczne receptory/kanały NMDA, co prowadzi do aktywności epileptycznej. Jednak roztwór o niskiej zawartości magnezu może nie tylko nasilać aktywność receptorów NMDA, ale także obniżać próg aktywacji kanałów sodowych lub zmniejszać hamowanie wywołane przez GABA [31]. Roztwór o niskiej zawartości magnezu może także zwiększać presynaptyczne uwalnianie glutaminianu, co w konsekwencji może prowadzić do stymulacji receptorów innych niż NMDA i potencjalnie przyczyniać się do aktywności epileptycznej. Aby dokładniej zbadać mechanizmy występowania IEDs, wykonaliśmy serię eksperymentów z AP-5, inhibitorem receptora NMDA. Eksperymenty pokazały, że dodanie AP-5 do roztworu zewnątrzkomórkowego całkowicie zniosło aktywność epileptyczną, co wskazuje na zaangażowanie prądów receptora NMDA w powstawanie IEDs w stosowanym przez nas modelu.

Zasadnicza część pracy, obejmowała eksperymenty z kwasem walproinowym. Wykazaliśmy, że

kwas walproinowy istotnie zmniejsza częstość IEDs. Efekt ten był mocniej zaznaczany przy wyższych stężeniach leku, co sugeruje odpowiedź zależną od dawki. Na podstawie równania Nernsta można wywnioskować, że wyższe stężenia jonów potasu w przestrzeni zewnątrzkomórkowej prowadzą do większej depolaryzacji błony komórkowej. Postanowiliśmy sprawdzić wpływ kwasu walproinowego przy różnych stężeniach jonów potasu. Zaobserwowaliśmy wzmocniony efekt kwasu walproinowego na aktywność epileptyczną, gdy potencjał błony był bardziej zdepolaryzowany. Ponadto odnotowaliśmy, że kwas walproinowy hamuje spontaniczne potencjały postsynaptyczne pobudzające (*spontaneous excitatory post-synaptic potentials*, sEPSP). Te sEPSP reprezentują przejściowe depolaryzacje błony postsynaptycznej, wywołane napływem dodatnich jonów poprzez receptory glutaminergiczne. Występują one pomiędzy IEDs, co wskazuje na szerszy modulacyjny wpływ kwasu walproinowego na transmisję synaptyczną. Wreszcie, hamujący wpływ kwasu walproinowego na aktywność epileptyczną został dodatkowo potwierdzony przez jego zdolność do zmniejszenia pobudliwości neuronalnej w piramidowych neuronach kory przedczołowej.

Wyniki tego badania sugerują, że kwas walproinowy może być korzystny dla pacjentów z zaburzeniami poznawczymi związanymi z IEDs, nawet w przypadku braku napadów padaczkowych. Istnieją doniesienia sugerujące, że kwas walproinowy może poprawiać deficyty poznawcze związane z IEDs [32]. Warto zauważyć, że występowanie IEDs jest częste u osób z autyzmem, a badania sugerują, że kwas walproinowy może zmniejszyć drażliwość u dzieci i młodzieży z zaburzeniami ze spektrum autyzmu [33]. Hipotetyzuje się, że redukcja IEDs może przyczynić się do tego efektu. W tej pracy po raz pierwszy wykazano, że IEDs zależne od prądów NMDA mogą być indukowane w piramidowych neuronach kory przedczołowej. Postulujemy, że rejestracja takiej aktywności w korze przedczołowej stanowi obiecujący model do badania mechanizmów i farmakologicznej modyfikacji IEDs. Biorąc pod uwagę częste występowanie zaburzeń poznawczych u pacjentów z padaczką, zrozumienie efektów leków przeciwpadaczkowych, takich jak kwas walproinowy, na IEDs ma istotne znaczenie kliniczne. W dalszych badaniach konieczne jest wyjaśnienie długoterminowych efektów kwasu walproinowego na funkcję poznawczą oraz dokładniejsze zbadanie czy eliminacja IEDs za pomocą różnych środków farmakologicznych prowadzi do poprawy funkcji poznawczych.



Ryc 1. Przykład IED indukowanego płynem bezmagnezowym z podwyższonym stężeniem potasu. Panel A przedstawia kilka typowych wyładowań, panel B jedną depolaryzację z potencjałami czynnościowymi na rozszerzonej podstawie czasu. Na podstawie Szulczyk i wsp. [34]

W kolejnej pracy oryginalnej, będącej częścią niniejszej rozprawy doktorskiej i zatytułowanej „Capsaicin inhibits sodium currents and epileptiform activity in prefrontal cortex pyramidal neurons", opublikowanej w *Neurochemistry International*, badaliśmy wpływ kapsaicyny - substancji z rodziny waniloidów i aktywnego składnika papryki chili - na pobudliwość neuronalną oraz jej potencjał jako leku przeciwpadaczkowego. Kapsaicyna jest znana z wywoływania uczucia pieczenia, charakterystycznego dla ostrych potraw, poprzez aktywację kanałów TRPV1 (transient receptor potential vanilloid, TRPV1) w obwodowych neuronach czuciowych. Jest również stosowana jako lek przeciwbólowy. Jej działanie przeciwbólowe prawdopodobnie jest mediowane poprzez zależne od aktywacji receptora TRPV1 zużycie neuropeptydów uczestniczących w transmisji czuciowej, (aktywacja receptora TRPV1 blokuje również ich regenerację) oraz tzw. „defunkcjonalizację" zakończeń nerwowych [35,36]. Defunkcjonalizacja, związana z przeciążeniem jonami wapnia, które napływają przez kanały TRPV1, objawia się utratą funkcji mitochondrialnej, zahamowaniem metabolizmu oraz zakłóceniem integralności błony plazmatycznej, powodując upośledzenie funkcji zakończenia nerwowego bólowego [35,36].

Systemowe podawanie kapsaicyny może również oddziaływać na neurony ośrodkowego układu nerwowego (OUN). W naszym badaniu posłużyliśmy się techniką current-clamp w celu zbadania potencjału kapsaicyny jako leku przeciwpadaczkowego w neuronach piramidowych kory przedczołowej szczurów. Dodatkowo przeprowadziliśmy serię eksperymentów w konfiguracji voltage-clamp, aby zbadać wpływ kapsaicyny na prądy sodowe.

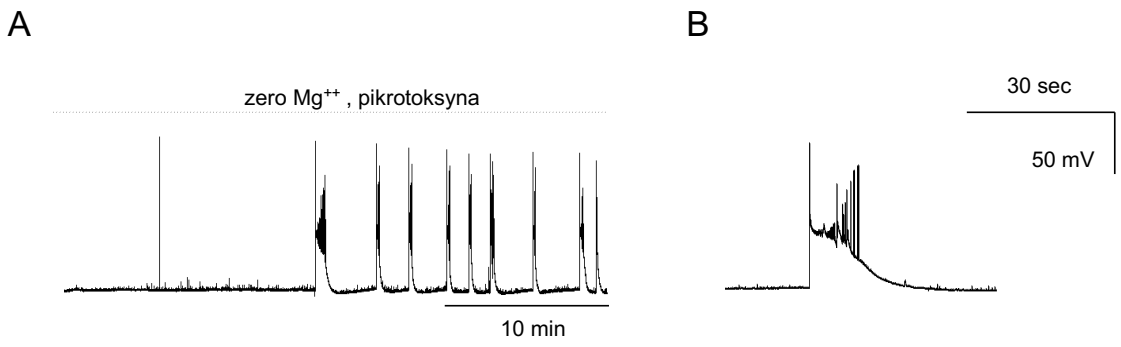
W pierwszej serii eksperymentów badaliśmy wpływ kapsaicyny na potencjał błonowy w konfiguracji *current-clamp*. Kapsaicyna nie wywołała żadnych zmian w potencjale błonowym. Następnie zbadaliśmy jej wpływ na pobudliwość neuronalną. Pokazaliśmy, że kapsaicyna znacząco hamowała częstotliwość potencjałów czynnościowych, a co więcej, amplitudy ostatnich potencjałów czynnościowych w serii były znacznie zmniejszone w obecności kapsaicyny. Sugerowało to możliwość modulacji o typie zależnego od użycia blokowania kanałów sodowych.

Następnie oceniliśmy potencjalne działanie przeciwpadaczkowe kapsaicyny w trzech różnych modelach aktywności epileptycznej. Na początku użyliśmy modelu indukowania IEDs, opartego na płynie zewnątrzkomórkowym bez jonów magnezu z podwyższonym stężeniem jonów potasu, wykorzystanego w poprzedniej pracy. Zaobserwowaliśmy silne hamowanie tego rodzaju aktywności przez kapsaicynę. Następnie użyliśmy dwóch modeli z dłuższymi aktywnościami epileptycznymi. W pierwszym, aktywność epileptyczna została wywołana pikrotoksyną - inhibitorem GABA oraz płynem bez jonów magnezu, co skutkowało epizodami trwającymi około 15 sekund (Rycina 2). W drugim jako prokonwulsant została zastosowana 4-aminopirydyna (4-AP), inhibitor kanałów potasowych oraz płyn bez jonów magnezu, wywołując dłuższe, trwające około 90 sekund epizody epileptyczne (Rycina 3). Kapsaicyna istotnie zmniejszyła czas trwania zdarzeń wywołanych pikrotoksyną, a ich częstość była mniejsza, choć nie było to istotne statystycznie. Ponadto, kapsaicyna całkowicie zniosła długą aktywność epileptyczną wywołaną przez 4-AP. W ramach dodatkowej serii eksperymentów postanowiliśmy ocenić wpływ kwasu walproinowego na długą aktywność epileptyczną indukowaną 4-AP. Dodanie kwasu walproinowego do płynu zewnątrzkomórkowego nie wykazało żadnego widocznego efektu na aktywność epileptyczną.

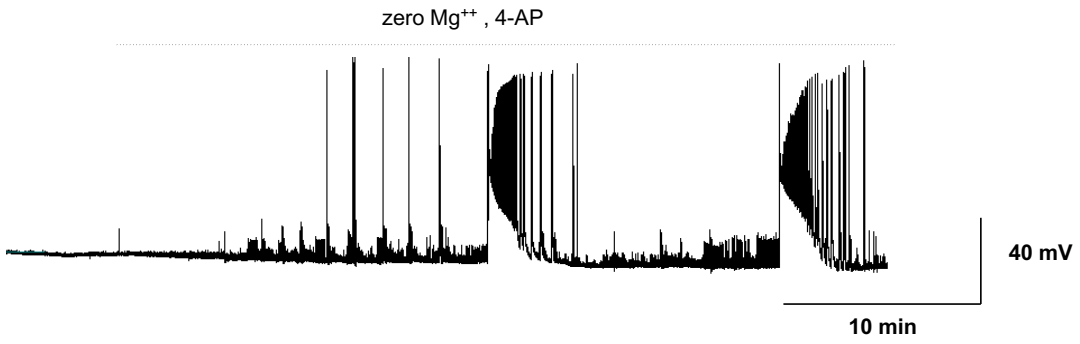
Na podstawie obserwacji sugerujących możliwość nasilenia zależnego od użycia blokowania kanałów sodowych przez kapsaicynę, w serii eksperymentów oceniających pobudliwość neuronalną przeprowadzonych w konfiguracji *current-clamp*, postanowiliśmy zbadać wpływ kapsaicyny na prądy sodowe, zakładając, że może ona hamować te prądy. Kapsaicyna nie miała wpływu na maksymalną amplitudę prądów sodowych. Co ciekawe, kapsaicyna istotnie zwiększyła blokadę zależną od użycia kanałów sodowych, co jest działaniem charakterystycznym dla wielu leków przeciwpadaczkowych. Co więcej, kapsaicyna

spowodowała zauważalne przesunięcie krzywej inaktywacji kanałów sodowych w kierunku ujemnych wartości napięcia, wskazując na zwiększoną inaktywację tych kanałów w spoczynku przy danym potencjale błonowym. Jest to zjawisko obserwowane także w przypadku innych leków przeciwpadaczkowych [37]. Co zaskakujące, zauważyliśmy, że kapsaicyna przesunęła również krzywą aktywacji prądów sodowych w kierunku hiperpolaryzacji. Oznacza to obniżenie progu potencjału błonowego dla otwarcia kanałów sodowych. Ten efekt potencjalnie może sprzyjać aktywności padaczkowej. Jednakże, biorąc pod uwagę wyniki naszych eksperymentów, w których kapsaicyna hamowała aktywności epileptyczne, postulujemy, że działanie przeciwpadaczkowe kapsaicyny przeważa nad jej jakimkolwiek proepileptycznymi efektami.

Podsumowując, niniejsza praca dostarcza dowodów na potencjał znacznego hamowania różnych form aktywności epileptycznej przez kapsaicynę, związku tradycyjnie niekojarzonego z zaburzeniami neurologicznymi, w neuronach piramidowych kory przedczołowej *in vitro*. Dodatkowo, pokazano, że kapsaicyna wzmacnia blokadę zależną od użycia kanałów sodowych. Sugerujemy, że struktura kapsaicyny może być przydatna do opracowania nowych leków przeciwpadaczkowych. Ponadto, długotrwała obecność kapsaicyny w ludzkiej diecie sugeruje korzystny profil bezpieczeństwa, co może ułatwić jej zastosowanie kliniczne. Przyszłe badania powinny się koncentrować na szczegółowych mechanizmach działania kapsaicyny, jej skuteczności w organizmach żywych oraz potencjalnych synergicznych efektach z istniejącymi lekami przeciwpadaczkowymi.



Ryc 2. Wyładowania epileptyczne uzyskane poprzez dodatnie pikrotoksyny do płynu zewnątrzkomórkowego bez jonów magnezu. Panel A przedstawia kilka typowych wyładowań, panel B jedną depolaryzację na rozszerzonej podstawie czasu. Na podstawie Pasierski i Szulczyk [38]



Ryc 3. Wyładowania epileptyczne uzyskane poprzez dodatnie 4-AP do płynu zewnątrzkomórkowego bez jonów magnezu. Na podstawie Pasierski i Szulczyk [38].

Trzecie z przedstawionych badań zatytułowane „Guanfacine inhibits interictal epileptiform events and sodium currents in prefrontal cortex pyramidal neurons" opublikowane w *Pharmacological Reports* miało na celu zbadanie wpływu guanfacyny, znanego leku na ADHD, na IEDs w mPFC u szczurów. Zespół nadpobudliwości psychoruchowej z deficytem uwagi to zaburzenie neurologiczne charakteryzujące się objawami takimi jak nieuwaga, nadpobudliwość i impulsywność. Często jest związane z nieprawidłowościami w mPFC. Osoby z ADHD mają czterokrotnie większe ryzyko padaczki w porównaniu z ogólną populacją, a IEDs są często obserwowane u pacjentów z ADHD [8,9]. Część prac sugeruje istnienie związku przyczynowo skutkowego pomiędzy IEDs a objawami choroby [18].

Leczenie ADHD u pacjentów z padaczką stanowi wyzwanie ze względu na potencjalne pogorszenie napadów padaczkowych przez leki na ADHD. Tradycyjne terapie ADHD często obejmują stymulanty, takie jak metylfenidat, ale obawy dotyczące działań niepożądanych, w tym potencjalnego obniżenia progu drgawkowego i zwiększenia częstotliwości napadów, ograniczają ich stosowanie w tej populacji. Guanfacyna, agonista receptora alfa-2A-adrenergicznego, jest często stosowana w leczeniu ADHD, chociaż dokładny mechanizm jej działania w mózgu nie jest w pełni zrozumiany. Zbadanie, w jaki sposób guanfacyna wpływa na IEDs, mogłoby dostarczyć wglądu w jej mechanizmy terapeutyczne w ADHD.

W omawianej pracy ponownie indukowaliśmy IEDs w skrawkach mózgu szczura, używając

proepileptycznego roztworu pozbawionego jonów magnezu z podwyższonym stężeniem jonów potasu, a następnie zbadaliśmy, jak guanfacyna wpływa na częstotliwość tych zdarzeń. Pokazaliśmy, że guanfacyna zmniejszała częstotliwość IEDs w neuronach piramidowych mPFC w sposób zależny od dawki. Dodatkowe eksperymenty z wykorzystaniem idazoksanu, blokera receptora alfa-2-adrenergicznego, wykazały, że efekt hamujący guanfacyny na IEDs nie był zależny od receptorów adrenergicznych alfa-2A ponieważ utrzymywał się po zablokowaniu tych receptorów. Sugeruje to, że działanie guanfacyny na IEDs wiąże się z mechanizmami innymi niż znane efekty na te receptory.

Aby zbadać mechanizm, w jakim guanfacyna wywiera swoje działanie, przeprowadziliśmy serię eksperymentów w konfiguracji *voltage-clamp*. Najpierw seria eksperymentów z wykorzystaniem NMDA wykazała, że guanfacyna nie wpływa na prądy NMDA. Następnie zbadaliśmy wpływ guanfacyny na prądy sodowe. W przedstawionej pracy oceniliśmy wpływ leku zarówno na wolno jak i szybko inaktywujące się prądy sodowe. Zaobserwowaliśmy, że guanfacyna silnie hamowała obydwa typy prądów sodowych. Na koniec zbadaliśmy wpływ leku na pobudliwość neuronalną, pokazując, że jest ona istotnie hamowana przez gunafacynę. Postawiliśmy hipotezę, że guanfacyna hamuje IEDs poprzez blokowanie prądów sodowych. Co ciekawe struktura chemiczna guanfacyny przypomina strukturę blokerów kanałów sodowych stosowanych w znieczuleniach miejscowych.

Przedstawione wyniki rzucą nowe światło na potencjalne mechanizmy leżące u podstaw terapeutycznych efektów guanfacyny w ADHD. Poprzez zmniejszanie pobudliwości neuronalnej i hamowanie aktywności epileptycznej w mPFC, guanfacyna może pomóc poprawić uwagę, kontrolę impulsów oraz funkcje poznawcze u osób dotkniętych przez ADHD.

Ostatnia publikacja zawarta w przedstawionej rozprawie doktorskiej to artykuł przeglądowy zatytułowany „Beneficial Effects of Capsaicin in Disorders of the Central Nervous System", opublikowany w *Molecules*. Opisuje ona dostępne dowody naukowe na potencjalne zastosowania terapeutyczne kapsaicyny w zaburzeniach OUN. Bazując na obiecujących wynikach z poprzednich badań, naszym celem było zebranie aktualnych danych dotyczących kapsaicyny, ze szczególnym uwzględnieniem badań nad padaczką, ale także jej potencjału terapeutycznego w innych zaburzeniach OUN.

W kontekście padaczki istniejące badania prezentują złożoną sytuację, w której przypisywane są zarówno mechanizmy proepileptyczne, jak i przeciwpadaczkowe kapsaicynie. Część badań wykazała, że kapsaicyna wpływa na kanały TRPV1 w OUN, znane z istotnej przepuszczalności dla jonów wapnia, które są uważane za istotne w patogenezie padaczki. Aktywacja kanałów TRPV1 prowadzi do depolaryzacji błony komórkowej i zwiększonej aktywności glutaminergicznej, jednocześnie zmniejszając uwalnianie GABA, co może prowadzić do efektów proepileptycznych [39]. Faktycznie, badania przeprowadzone na neuronach hipokampa wykazały, że aktywacja TRPV1 zaostrza aktywność padaczkową. Eksperymenty z użyciem pentylenotetrazolu, związku zwiększającego pobudliwość neuronalną poprzez hamowanie receptorów/kanałów GABA, wykazały, że iniekcje kapsaicyny do obszaru hipokampa skutkowały wyższym odsetkiem napadów u zwierząt [40]. Efekt został odwrócony przez wcześniejsze podanie antagonisty TRPV1, co wskazuje na bezpośrednie zaangażowanie receptorów TRPV1.

Niemniej jednak istnieją również doniesienia o TRPV1-niezależnych efektach przeciwpadaczkowych kapsaicyny, szczególnie w neuronach kory mózgowej. W naszej wcześniej przedstawionej pracy, pokazaliśmy, że kapsaicyna silnie blokuje różne typy aktywności padaczkowej w neuronach kory przedczołowej. Pokazaliśmy również, że blokuje ona prądy sodowe w tych neuronach. Niezależne od naszej pracy, badanie patch-clamp na neuronach korowych wykazało, że kapsaicyna hamowała aktywność padaczkową wywołaną stosowaniem antagonisty receptorów GABA, gabazyny. Ponadto, badacze zaobserwowali zmniejszenie zarówno częstotliwości, jak i maksymalnej amplitudy potencjałów czynnościowych, co sugeruje hamowanie prądów sodowych zależnych od napięcia przez kapsaicynę [41].

W przedstawionym artykule prezentujemy tezę, którą popieramy dowodami dostępnymi w literaturze, że, chociaż aktywacja TRPV1 w neuronach hipokampa wydaje się przyczyniać do efektów proepileptycznych kapsaicyny, jej działanie w neuronach kory mózgowej może obejmować bardziej złożone mechanizmy, potencjalnie obejmujące hamowanie kanałów sodowych i w efekcie działanie przeciwpadaczkowe. Nieliczne, dostępne badania, podczas których kapsaicyna była podawana ogólnoustrojowo, sugerują, że jej działanie przeciwpadaczkowe może przeważać. W jednym z nich kapsaicyna podawana podskórnie zmniejszyła częstość napadów drgawkowych wywołanych przez domięśniowe zastrzyki kwasu kainowego u myszy [42]. Te złożoności podkreślają konieczność dalszych badań w celu pełnego

wyjaśnienia mechanizmów działania kapsaicyny w padaczce.

Oprócz rozważań na temat wpływu kapsaicyny na leczenie padaczki, przedstawiliśmy również szereg badań dotyczących zastosowania kapsaicyny w innych schorzeniach układu nerwowego. W badaniach na myszach wykazano, że kapsaicyna stymuluje autofagię i zwiększa zdolność fagocytozy komórek mikrogleju, które odpowiadają za usuwanie blaszek beta-amyloidowych kluczowych w patogenezie choroby Alzheimera [43]. W konsekwencji, u myszy transgenicznych z chorobą Alzheimera obserwowano zmniejszenie osadzania się beta-amyloidu i poprawę funkcji poznawczych po podaniu kapsaicyny [43]. Ponadto, badania populacyjne sugerują, że spożywanie diety bogatej w kapsaicynę może korelować z niższym poziomem beta-amyloidu we krwi i lepszymi wynikami w testach kognitywnych [44]. W kontekście choroby Parkinsona również zaobserwowano potencjał terapeutyczny kapsaicyny. W modelach mysich, podanie kapsaicyny dootrzewnowo zwiększało liczbę neuronów dopaminergicznych w substancji czarnej, oraz powodowało redukcję reaktywnych form tlenu i cytokin prozapalnych uwalnianych przez aktywowany mikroglej jednocześnie poprawiając funkcję motoryczną [45]. Inne badania na modelach szczurzych wykazały, że kapsaicyna aktywowała receptory TRPV1 na astrocytach, co prowadziło do zwiększenia produkcji czynników neurotroficznych, zwiększając tym samym poziomy dopaminy i redukując stres oksydacyjny, co chroniło przed neurodegeneracją i upośledzeniem ruchowym [46]. W przedstawionej pracy opisaliśmy także potencjalne zastosowanie kapsaicyny w leczeniu migreny, powikłań udaru mózgu oraz depresji [47-49]. Podsumowując, kapsaicyna jest obiecującym kandydatem w leczeniu chorób układu nerwowego, jednak konieczne są dalsze badania przedkliniczne, aby w pełni zrozumieć jej mechanizmy działania oraz potencjalne zastosowania terapeutyczne.

Podsumowując, prace wchodzące w skład przedłożonej rozprawy doktorskiej wykorzystywały technikę patch-clamp do modelowania aktywności epileptycznej *in vitro*. Wszystkie oryginalne prace używały modelu opartego na zanurzeniu skrawków kory przedczołowej szczurów w płynie bez magnezu z podwyższonym stężeniem potasu, co pozwalało na ocenę wpływu badanych substancji na krótkotrwałe aktywności epileptyczne (IEDs). U ludzi IEDs występują u pacjentów chorych na padaczkę, gdzie korelują z upośledzeniem intelektualnym, jak również w innych zaburzeniach psychiatrycznych, w tym ADHD. Eksperymenty wykazały, że ta aktywność, zależna od receptorów NMDA, jest hamowana przez kwas walproinowy. Następnie wykazano, że kapsaicyna, substancja znajdująca się w papryczkach chili, silnie blokuje tę aktywność oraz

długie epizody epileptyczne wywołane obecnością 4-AP, które są oporne na działanie kwasu walproinowego. Dodatkowo, kapsaicyna zmniejszała pobudliwość neuronów kory przedczołowej. W eksperymentach voltage-clamp wykazano, że kapsaicyna silnie blokuje kanały sodowe, co może stanowić mechanizm odpowiedzialny za jej działanie przeciwpadaczkowe. Trzecia praca oryginalna pokazała, że guanfacyna, lek stosowany w leczeniu ADHD, silnie blokuje IEDs. Efekt ten był niezależny od receptorów alfa-2-adrenergicznych, które są uważane za typowy punkt uchwytu guanfacyny, ani od modulacji prądów NMDA. Wykazano, że guanfacyna silnie blokuje kanały sodowe, co może przyczyniać się do jej efektu przeciwpadaczkowego w badanym modelu. Badania wchodzące w skład przedstawionej rozprawy doktorskiej podkreśliły znaczenie zrozumienia działania leków na poziomie komórkowym, oferując obiecujące ścieżki interwencji terapeutycznej w stanach charakteryzujących się nieprawidłową aktywnością neuronalną.

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3. Założenia i cel pracy

U pacjentów z padaczką występują zaburzenia poznawcze. Takie zaburzenia są najprawdopodobniej spowodowane nieprawidłową funkcją neuronów kory przedczołowej. Jednocześnie, krótka aktywność epileptyczna wydaje się odgrywać istotną rolę w patogenezie nie tylko padaczki, ale również innych zaburzeń psychiatrycznych. Dlatego głównym celem niniejszej pracy doktorskiej było zbadanie wpływu leków już stosowanych w leczeniu padaczki i ADHD, jak i nowych substancji, na różne modele padaczki. Badania przeprowadzono *in vitro* na neuronach piramidowych wyizolowanych z kory przedczołowej zlokalizowanych w skrawkach oraz na neuronach rozproszonych. Poznanie mechanizmów działania leków przeciwpadaczkowych na neurony kory przedczołowej może przyczynić się do ograniczenia zaburzeń poznawczych, które stanowią znaczące powikłanie napadów padaczkowych.

Szczegółowe cele pracy obejmowały:

- Zbadanie roli receptorów NMDA w indukowaniu krótkiej aktywności epileptycznej w korze przedczołowej.
- Zbadanie wpływu kwasu walproinowego na krótkotrwałe aktywności epileptyczne w korze przedczołowej.
- Zbadanie wpływu kwasu walproinowego na pobudliwość (zdefiniowaną jako liczba potencjałów czynnościowych dla zadanego impulsu depolaryzującego) neuronów kory przedczołowej.
- Zbadanie wpływu kapsaicyny na potencjał błonowy i pobudliwość neuronów kory przedczołowej.
- Zbadanie wpływu kapsaicyny na aktywność epileptyczną w trzech różnych modelach aktywności epileptycznej.
- Zbadanie wpływu kapsaicyny na prądy sodowe w neuronach kory przedczołowej.
- Zbadanie wpływu guanfacyny na pobudliwość neuronów kory przedczołowej.
- Zbadanie wpływu guanfacyny na krótką aktywność epileptyczną w korze przedczołowej i ocena czy wpływ ten jest pośredniczony przez receptory alfa-2-adrenergiczne.
- Zbadanie wpływu guanfacyny na prądy sodowe w korze przedczołowej

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Research article

Valproic acid potently inhibits interictal-like epileptiform activity in prefrontal cortex pyramidal neurons

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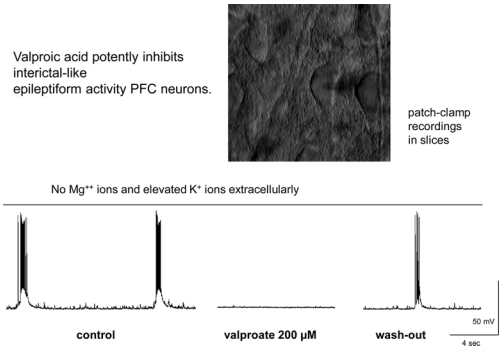
GRAPHICAL ABSTRACT



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ABSTRACT

Valproic acid has a long-standing reputation of effectively treating the symptoms of not only epilepsy but also psychiatric conditions. In the latter, the exact mechanism by which valproate exerts its effect remains unclear. In this study, epileptiform bursts were recorded from pyramidal neurons in the prefrontal cortex (the brain region thought to be involved in psychiatric disorders) using the patch-clamp technique. An extracellular solution with no magnesium ions and elevated potassium levels that is known to induce epileptiform activity in vitro was used. Because of their short durations, the epileptiform bursts were regarded as interictal-like epileptiform activity, which is believed to be involved in cognitive impairment. Interictal discharges occur in many neuropsychiatric disorders as well as in healthy population.

Epileptic activity in prefrontal cortex pyramidal neurons was potently inhibited by two therapeutic concentrations of valproic acid (20 µM and 200 µM). Moreover, valproate suppressed spontaneous excitatory postsynaptic potentials. Epileptiform bursts were fully inhibited by NMDA receptor antagonist, which suggests that epileptiform activity is driven by NMDA receptors. The inhibition of excitability in prefrontal cortex pyramidal neurons by valproate was also shown.

This study shows that it is possible to evoke NMDA-dependent epileptiform activity in prefrontal cortex pyramidal neurons in vitro.

We suggest that the prefrontal cortex is a good region for studying the influence of drugs on interictal epileptiform activity.

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1. Introduction

Epilepsy is a major neuropsychiatric disorder affecting approximately 70 million people worldwide [1]. Many different strategies aimed at suppressing seizures, including both pharmacological and non-pharmacological methods, have been developed. However, these strategies should focus not just on limiting seizure activity but also on managing coexisting psychiatric conditions. One of these psychiatric conditions is cognitive impairment [2,3]. It is characterized by decreased intellectual capabilities and learning difficulties [3]. It is highly prevalent, especially in the paediatric population; a population-based study of children with diagnosed epilepsy showed that 80% had either a behavioural disorder and/or cognitive impairment [4].

One of the reasons for cognitive dysfunction in epilepsy are interictal epileptiform discharges that occur between seizures in different brain regions, including the prefrontal cortex [2,3]. Some patients have no clinical seizures but present with cognitive disturbances associated with interictal epileptiform activity [5,6]. The prevalence of interictal discharges in the healthy population may be as high as 5% [7]. Interictal epileptiform activity has also been reported to occur in children with attention deficit hyperactivity disorder and may contribute to the symptoms of this disease [8]. Importantly, interictal epileptic discharges have been described in patients with autism spectrum disorder. The prevalence of such EEG abnormalities in patients with autism may reach 30% [9]. Moreover, interictal-like discharges have been recorded in an in vitro model of bipolar disorder [10].

Valproic acid is often used for the symptomatic treatment of epilepsy and other diseases due to its ability to reduce excessive neuronal firing. Different mechanisms of action, including the inhibition of voltage-gated sodium and calcium channels [11,12] as well as the stimulation GABA pathways [13] have been proposed for valproate. In vitro interictal epileptiform activity has been recorded mainly in hippocampal formation thus far. It has been shown that this type of epileptiform activity is not influenced by valproic acid in hippocampal [14] and entorhinal cortex neurons [15]. Since the prefrontal cortex is the brain region that is involved in cognitive functions such as reasoning, understanding and planning, it can be argued that cognitive impairment may be caused by interictal epileptic discharges in this area [2]. The aim of this study was to induce epileptiform activity in prefrontal cortex pyramidal neurons and assess the influence of valproate on this activity.

2. Materials and methods

The experimental procedures used in this study adhered to the institutional and international guidelines on the ethical use of animals.

2.1. Slices preparation and preincubation

Three-week-old rats were anaesthetized using ethyl chloride and decapitated. Slices (300 μM) were cut from the medial prefrontal cortex in an ice-cold extracellular solution of the following composition (in mM): NaCl (130), KCl (2.5), glucose (10), $NaHCO_3$ (25), NaH_2PO_4 (1.25), $MgCl_2$ (7), and $CaCl_2$ (0.5) (pH = 7.4, bubbled with carbogen). After being prepared, the slices were incubated (at room temperature) in ACSF of the following composition (in mM): NaCl (130), KCl (2.5), glucose (10), $NaHCO_3$ (25), NaH_2PO_4 (1.25), $MgCl_2$ (1), and $CaCl_2$ (2) (pH = 7.4, bubbled with carbogen).

2.2. Induction of epileptiform activity and composition of recording solutions

Spontaneous epileptiform discharges were recorded in the same solution as the one used to incubate the slices but without $MgCl_2$ and with a KCl concentration raised to 5 mM or 10 mM (see results). Spontaneous excitatory postsynaptic potentials (sEPSPs) were recorded in the same solution as the one used to incubate the slices but without magnesium ions and with a potassium ion concentration increased to 10 mM. Action potentials (shown in Fig. 5) were recorded in exactly the same solution as the one used to incubate the slices.

The intracellular solution in the patch pipette was composed of the following (in mM): potassium-gluconate (105), KCl (20), HEPES-Na + (10), EGTA (0.1), (pH = 7.4).

2.3. Recording techniques

Recordings were made from layer V pyramidal neurons in slices of the medial prefrontal cortex using the current-clamp technique, as described in our previous publication [16]. Neurons were visualized using DIC optics. Patch pipettes were fabricated from thick-walled borosilicate glass capillaries using a micropipette puller (P- 97, Sutter Instruments). The patch pipettes (resistances between 4 and 5 MΩ) were moved towards a selected pyramidal neuron using a micromanipulator (MPC 200, Sutter Instruments). Positive pressure was applied to the pipette to remove the extracellular matrix in the slice. After the pipette was pressed against the neuronal plasma membrane, slight negative pressure was applied to the pipette to form a gigaseal. After gigaseal formation, the patch membrane was ruptured by suction and/or by electrical stimulus.

Spontaneous epileptiform activity and sEPSPs were recorded using the membrane potential recording mode. Action potentials were evoked using rectangular 200 pA current steps lasting 2 s. Recordings were made using a Multiclamp 700A amplifier and Digidata 1440A (Molecular Devices). The recordings were analysed with pClamp software.

The recordings were obtained at 35 °C.

2.4. Analysis of sEPSPs and statistics

All of the results presented are shown as the mean ± S.E.M. Differences between three groups were evaluated using repeated measurements one-way ANOVA followed by Tukey's post hoc test (GraphPad InStat software v3.06) if the data passed the normality test. In experiments with AP-5 (Fig. 4), we used the Friedman test followed by Dunn's post hoc test because the distributions of the tested frequencies did not conform to normal distributions. Student's *t*-test was used to evaluate differences between two groups.

sEPSPs were detected by a threshold-crossing algorithm with the threshold set at 0.8 mV. One-way ANOVA and Tukey's test were used to compare the mean frequencies of the sEPSPs. Amplitude histograms were normalised and compared with the χ^2 test (control vs valproic acid; control vs washout; washout vs valproic acid). To account for multiple comparisons, the *P* values were adjusted using the Hochberg-Benjamini false discovery rate at q = 0.01.

In all figures, one recording was performed from one slice. Consequently, n is the number of slices that we used in each series of experiments. Slices for each series of experiments were obtained from 2 or 3 animals.

2.5. Chemical compounds

Valproic acid or AP-5 was applied to the entire bath for five minutes, and their effects were evaluated after two minutes of application. All chemical compounds, including valproic acid, were purchased from Sigma Aldrich.

3. Results

3.1. The influence of valproic acid on epileptiform activity evoked in extracellular solution with 5 mM potassium and 0 mM magnesium

First, membrane potential recordings were conducted in

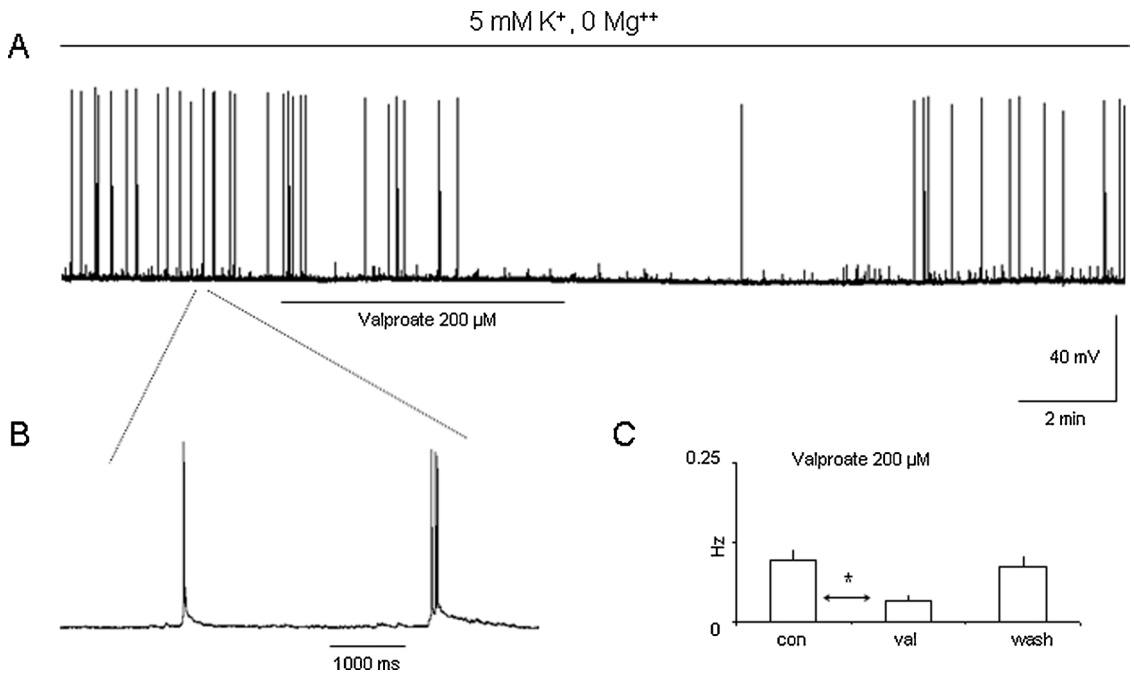

Fig. 1. Valproic acid (200 µM) inhibits epileptiform activity (recordings were made in an extracellular solution with zero magnesium and 5 mM potassium). **A** - Example recording showing the influence of valproate on epileptiform activity. **B** - Epileptiform discharges shown on an expanded time scale. **C** – Average frequency of burst events in control solution, in the presence of valproate and after washout.

extracellular solution with no magnesium ions and a potassium ion concentration of 5 mM. After 15–40 minutes of preincubation, spontaneous depolarizations (brief bursts) were recorded in this solution with one, two or three superimposed action potentials (2.2 ± 0.4 on average, the amplitude of the first action potential was 88.5 ± 2.7 mV). The amplitude of the depolarization that triggered action potentials was 13.3 ± 0.7 mV, and its duration was 458.5 ± 46.7 ms (Fig. 1A and B, n = 40). Moreover, the membrane potential was more depolarized (to -60.4 ± 1.6 mV) than that observed in the presence of the control extracellular solution (-68.2 ± 1.7 mV, Students' *t*-test, $p < 0.05$, n = 5), which contained 2.5 mM potassium ions and 1 mM magnesium ions.

The frequency of the spontaneous depolarizations was markedly reduced by bath application of 200 µM valproic acid (0.1 ± 0.02 Hz in control, 0.03 ± 0.01 Hz in the presence of 200 µM valproate and 0.09 ± 0.02 Hz after washout [Fig. 1A and C, control vs valprote, Tukey's test, p = 0.0157, n = 5]).

3.2. The influence of valproate on epileptiform activity evoked in extracellular solution with 10 mM potassium and 0 mM magnesium

It is easily concluded from the Nernst equation that the greater the extracellular concentration of potassium ions, the larger the membrane depolarization. This fact has been shown experimentally numerous times [17]. In the next series of experiments, we increased the potassium ion concentration from 5 mM to 10 mM to assess the influence of valproate on epileptiform activity at a more depolarized membrane potential. The extracellular solution did not contain magnesium ions.

After 10–30 minutes of preincubation, the membrane potential in this solution was depolarized to −55.9 ± 0.9 mV, whereas the membrane potential in control physiological solution was −68.2 ± 1.7 mV (see above, $p < 0.01$, n = 5), and spontaneously occurring bursts were recorded. The amplitude of the burst depolarization that triggered action potentials was 12.5 ± 0.6 mV. The events lasted 728.0 ± 53.9 ms. The number of action potentials in the burst was 14.4 ± 1.0, whereas the amplitude of the first action potential in the burst was 75.2 ± 0.9 mV (n = 104, Fig. 2A and B).

Spontaneous bursts were completely suppressed in the presence of a therapeutic concentration of valproic acid (200 µM). Their frequencies were 0.13 ± 0.004 Hz in control, 0.003 ± 0.002 Hz during valproate application and 0.1 ± 0.01 Hz after washout (Fig. 2A and D, n = 4, control vs valproate, Tukey's test p = 0.0001).

The influence of a lower therapeutic concentration of valproate (20 µM) was also tested in an extracellular solution with zero magnesium and 10 mM potassium. In this case, the frequency of the bursts was also markedly inhibited by the drug (0.03 ± 0.01 Hz) compared to that observed in control (0.18 ± 0.05 Hz, $p < 0.05$). As in previous experiments, it was possible to obtain washout (0.14 ± 0.04 Hz, Fig. 2C, n = 4, control vs valproate, Tukey's test, p = 0.0104).

When an extracellular solution with 0 mM magnesium and 10 mM potassium was used, the membrane potential was more depolarized (−55.9 ± 0.9 mV) than that observed in the presence of the 5 mM potassium solution (−60.4 ± 1.6 mV, n = 6, $p < 0.05$). The inhibitory effect of 200 µM valproate on the frequency of epileptiform events in a solution with 10 mM potassium and 0 magnesium was more pronounced (3 ± 2% of control) than the effect of valproate in an extracellular solution with 5 mM potassium and zero magnesium (32 ± 8% of control, n = 4, Students' *t*-test, $p < 0,05$, see Figs. 1 and 2). Epileptiform events had markedly different properties depending on the composition of the extracellular solution. In a solution with zero magnesium and 5 mM potassium, the epileptic bursts had a smaller number of action potentials (2.2 ± 0.4) than those in a solution with zero magnesium and 10 mM potassium (number of action potentials: 14.4 ± 1.0, Student's *t*-test, $p < 0.001$; compare Figs. 1B and 2 B). The amplitude of the first action potential in the burst was larger in the solution with 5 mM potassium (88.5 ± 2.7 mV) than in the extracellular solution with 10 mM potassium (75.2 ± 0.9 mV, Student's *t*-test, $p < 0.01$).

3.3. The influence of valproic acid on sEPSPs evoked in an extracellular solution with 10 mM potassium and 0 mM magnesium

Valproic acid also inhibited the frequency and amplitude of spontaneous excitatory postsynaptic potentials (sEPSPs) that occurred

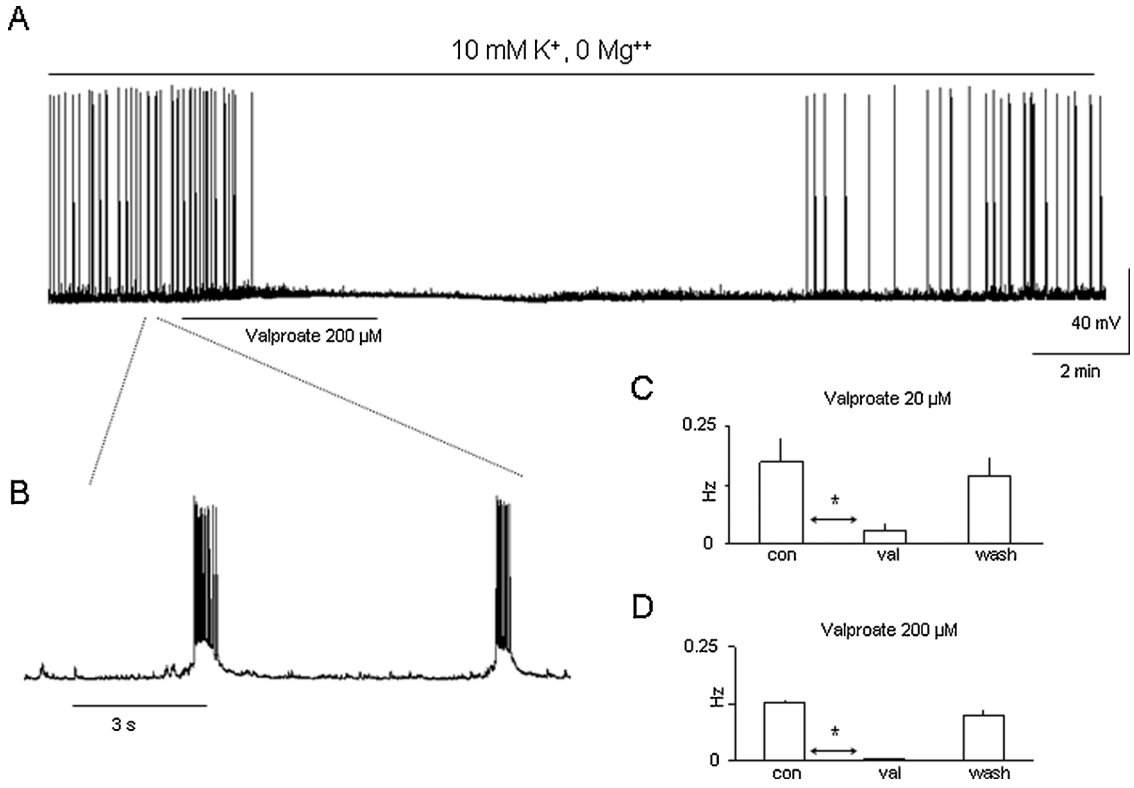

Fig. 2. Valproic acid inhibits epileptic bursts (in an extracellular solution with zero magnesium and 10 mM potassium). **A** - Example recording showing the influence of valproate on epileptiform activity. **B -** Burst events shown on an expanded time scale. **C** and **D** – Average frequency of epileptic bursts in control solution, in the presence of valproate (**C** - 20 µM and **D** - 200 µM) and after washout.

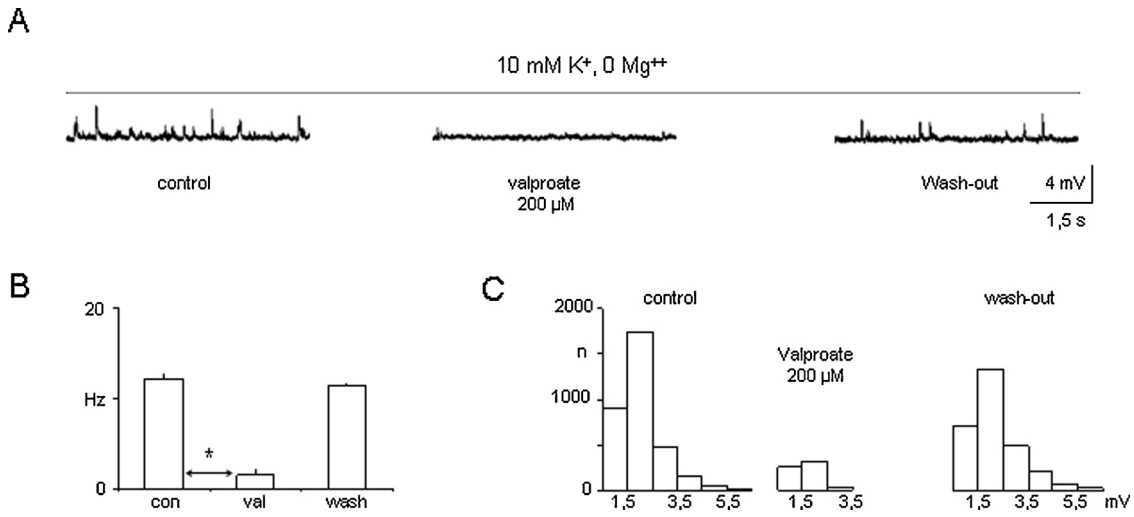

Fig. 3. Valproate suppresses the frequency and amplitude of sEPSPs that occur between epileptiform bursts. **A -** Example recordings of spontaneous excitatory postsynaptic potentials (sEPSPs) in control solution, after the application of 200 µM valproate and after washout (in an extracellular solution with zero magnesium and 10 mM potassium). **B** - Average frequency of sEPSPs in control solution, after the application of the tested compound and after washout. **C** - Histograms of sEPSP amplitudes; bin = 1 mV, events larger than 0.8 mV were analysed.

between the bursts (10 mM K^+ extracellular solution containing no magnesium ions was used), (Fig. 3). The frequency of the sEPSPs was 12.2 ± 0.6 Hz in control, 1.6 ± 0.7 Hz in the presence of valproic acid (200 µM) and 11.4 ± 0.2 Hz after recovery (Fig. 3A and B, control vs valproic acid, Tukey's test, p = 0,0001, n = 5). The average amplitude of the sEPSPs was 1.29 ± 0.06 mV in control, 1.06 ± 0.04 mV after the application of valproic acid (200 µM) and 1.45 ± 0.11 mV after washout (Fig. 3A and C, n = 5). To assess statistical significance, we compared the normalised distributions of the amplitudes of events that exceeded the threshold of 0.8 mV. The distribution obtained in the presence of valproic acid (200 µM) was different from the distribution obtained under control conditions and after washout (χ^2 test, $p < 0.01$). The amplitude distributions obtained under control conditions and during washout were similar (χ^2 test, $p > 0.05$).

3.4. The influence of NMDA inhibitor on epileptiform activity

Next, the influence of the NMDA receptor inhibitor AP-5 (100 µM) on burst events was assessed. Experiments with AP-5 were conducted in extracellular medium with an elevated (to 10 mM) potassium ion

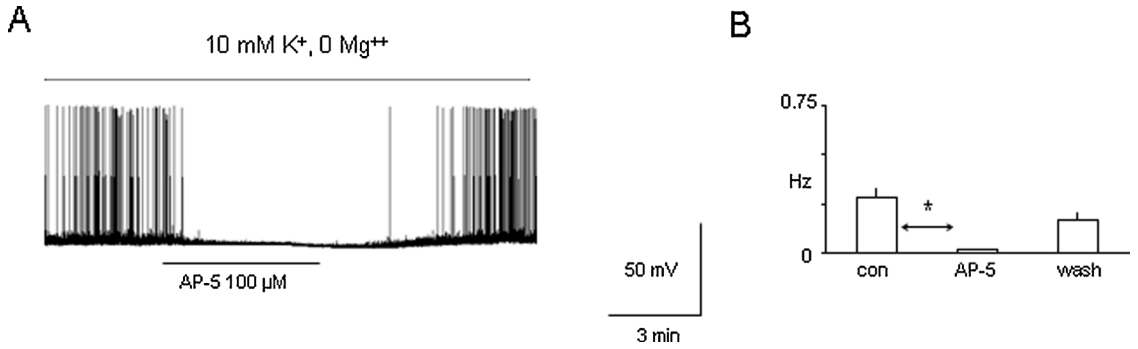

Fig. 4. AP-5 inhibits epileptiform activity. **A** - Example recording showing the influence of the NMDA receptor antagonist AP-5 (100 µM) on epileptic bursts (in an extracellular solution with zero magnesium and 10 mM potassium). **B** - Average frequency of epileptiform activity in control solution, in the presence of AP-5 and after washout.

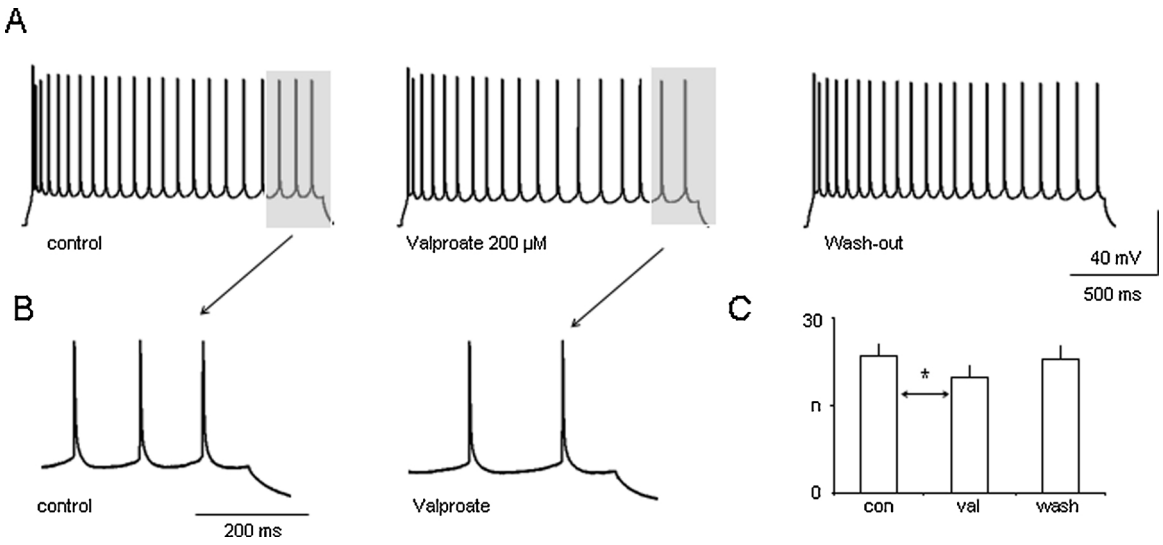

Fig. 5. Valproic acid inhibits excitability in prefrontal cortex pyramidal neurons. **A** – Example recordings of action potentials evoked by 200 pA current steps in control solution, after the application of 200 µM valproate and after recovery (in physiological extracellular solution). **B** - Action potentials marked in grey in Fig. A are shown on an expanded time scale to better visualize the difference in excitability. **C** – Average excitability (the number of action potentials) in control solution, in the presence of valproate and after washout.
In all figures, statistical significance is indicated by an asterisk (*).

concentration and in the absence of magnesium ions. The frequency of burst events was markedly decreased by AP-5 (0.28 ± 0.05 Hz in control, 0.01 ± 0.01 Hz in the presence of AP-5 and 0.16 ± 0.05 Hz after washout, n = 4, Dunn's test, p = 0,014, Fig. 4A and B). This result indicates that burst events are driven by NMDA receptor currents.

3.5. The influence of valproate on neuronal excitability in standard extracellular solution (1 mM magnesium and 2.5 mM potassium)

Furthermore, action potentials were evoked in standard extracellular solution containing 1 mM magnesium and 2.5 mM potassium ions (see methods). Excitability was defined as the number of action potentials per depolarization step (200 pA steps lasting 2000 ms were applied, Fig. 5). A therapeutic concentration of valproic acid (200 µM) inhibited the excitability of prefrontal cortex pyramidal neurons (Fig. 5). The average results are shown in Fig. 5C (22.7 ± 2.0 in control solution, 18.0 ± 1.87 in the presence of the drug and 22.9 ± 2.5 after washout, n = 15, Tukey's test, p = 0.018). As the effect was small, the action potentials are shown on an expanded time scale (Fig. 5B) to better show the inhibition of excitability exerted by valproic acid.

4. Discussion

As the epileptiform burst events recorded in this study were of short duration (less than 2–3 s), they may be regarded as interictal-like epileptiform discharges [18]. Interictal-like epileptiform discharges are not a model of seizures and may occur between seizures or even without any seizures [3]. It has been suggested that the consequence of this type of epileptic activity is cognitive impairment, especially in children [2,3]. It has been shown by other authors that valproic acid does not influence interictal-like epileptiform activity in vitro [14,15]. The therapeutic concentration of free valproate in the plasma is 35–200 µM and less than 100 µM in the extracellular fluid [19]. It has been found in a zero-magnesium model that 3 mM valproate (a concentration that greatly exceeds the therapeutic level) reduces the frequency of interictal epileptic events in entorhinal cortex neurons by approximately one half [20]. In a different study, the frequency of interictal discharges induced in hippocampal neurons in a low-calcium, elevated potassium model was reduced by valproic acid concentration as high as 5 mM [21]. The fact that authors applied such a high concentration of the drug suggests that lower (therapeutic) levels are not effective. Considering our results, it can be reasoned that valproic acid exerts different effects in the hippocampus and entorhinal cortex than those elicited in the prefrontal cortex.

The following mechanism may be proposed for the induction of epileptiform activity in a solution with high potassium and zero magnesium. A magnesium-free extracellular solution facilitates NMDA receptors, and a high-potassium solution depolarizes the neuronal membrane [17]. In such conditions, the increased spontaneous release of glutamate may potently open postsynaptic NMDA receptors/channels, which generate bursts [22]. However, a low-magnesium solution may not only facilitate NMDA receptors but also lower the threshold for sodium channel activation [17] and decrease GABA-induced inhibition [23]. This may lead to increased neuronal excitability and the generation of epileptiform discharges [17,23]. A low-magnesium solution may also increase presynaptic glutamate release and consequently non-NMDA receptor stimulation, which may contribute to epileptiform activity [24]. Our finding that an NMDA receptor inhibitor fully abolishes burst activity proves that NMDA receptors are effectively unblocked and that NMDA facilitation is the main mechanism by which epileptiform activity was produced in our study.

Valproate may inhibit epileptiform activity by different simultaneous mechanisms. It may inhibit presynaptic voltage-gated sodium and/or calcium channels, which are responsible for the exocytosis of glutamate [11,12]. In this case, postsynaptic NMDA receptors may be too weakly stimulated by glutamate to form epileptic discharges. It is also possible that valproate blocks postsynaptic glutaminergic receptors, which can contribute to epileptiform activity inhibition [25]. We also showed that valproic acid potently suppresses small amplitude spontaneous excitatory postsynaptic potentials (sEPSPs) that occur between epileptic bursts, which is an antiepileptic effect. These events may be mediated by AMPA and/or NMDA receptors. As in the case of interictal epileptiform activity suppression, valproate may reduce glutamate release by inhibiting presynaptic sodium or calcium channels or directly block postsynaptic glutamate receptors [11,12,25].

In our experiments, we showed that the frequency of epileptiform events was more strongly inhibited by valproate when the membrane potential was more depolarized (in 10 mM potassium solution compared to a 5 mM potassium solution, see results). This suggests that the effect of valproate is more pronounced at a more depolarized membrane potential, which is a feature of many antiepileptic drugs [26].

We found that valproic acid inhibits excitability (the number of action potentials) in prefrontal cortex pyramidal neurons, which is another mechanism of the antiepileptic action of this drug. This effect was weak compared with the strong inhibition of spontaneous epileptiform activity. The reason for this phenomenon may be that excitability was assessed using artificial rectangular current steps that do not occur under epileptic conditions. One can conclude that assessing the influence of valproate on excitability, as opposed to assessing the influence of this drug on spontaneous epileptiform activity, does not demonstrate its strong antiepileptic effects.

This study showed that therapeutic concentrations of valproic acid potently inhibit interictal-like activity in the prefrontal cortex in rats. This suggests that valproate may be beneficial for patients who have no seizures but present with interictal discharges associated with cognitive impairment. Further behavioural experiments should be conducted to prove this hypothesis. Studies have shown that valproate improves cognitive deficits associated with interictal epileptic discharges [6,27]. Importantly, interictal discharges frequently occur in patients with autism [9]. It has been found that valproic acid reduces irritability in children and adolescents with autism spectrum disorders [28]. The authors hypothesized that the reduction in epileptiform abnormalities contributes to this effect [28].

We suggest that prefrontal cortex pyramidal neurons are capable of generating NMDA-driven interictal-like epileptiform activity in vitro. Recording such activity in the prefrontal cortex seems to be a good method for studying the mechanisms and pharmacological modulation of interictal epileptiform activity.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Capsaicin inhibits sodium currents and epileptiform activity in prefrontal cortex pyramidal neurons

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ABSTRACT

Capsaicin, a compound found in chili peppers, causes burning sensations by acting on the peripheral sensory system. However, it has also been reported to exert substantial effects on central neurons. The aim of this patch-clamp study was to test the antiepileptic potential of capsaicin in prefrontal cortical pyramidal neurons.

Capsaicin at a concentration of 60 μM inhibited neuronal excitability. Moreover, later spikes in response to 50-s-long current steps were much smaller in amplitude in the presence of 60 μM capsaicin than in control solution. The tested compound did not influence the membrane potential. Voltage-clamp recordings showed that capsaicin markedly enhanced the use-dependent block of sodium channels (sodium currents were evoked at frequencies of 0,5 Hz and 10 Hz). The presence of the compound shifted the steady-state inactivation curve of sodium channels towards hyperpolarization, which suggests greater inactivation of sodium channels at rest in the presence of capsaicin.

Moreover, capsaicin inhibited epileptiform events evoked in three different proepileptic solutions. Capsaicin abolished interictal-like events lasting less than 1 s recorded in zero magnesium solution with an increased potassium ion concentration. The drug also abolished long ictal events evoked in zero magnesium solution containing 4-AP. Moreover, ictal events recorded in zero magnesium solution containing picrotoxin were substantially shortened in the presence of capsaicin.

We suggest that capsaicin exerts an antiepileptic effect. The important mechanism behind this phenomenon seems to be the inhibition of sodium channels, which is an effect of many antiepileptic drugs.

1. Introduction

Capsaicin is a compound found in chili peppers. The mechanism of its action on neural cells has not been fully elucidated. The effects of capsaicin on sensory receptors have been repeatedly described. It has been found that capsaicin stimulates peripheral sensory receptors via TRPV1 channel activation. This effect is responsible for the painful and burning sensations caused by capsaicin (Onizuka et al., 2011). Surprisingly, capsaicin also has pain-relieving effects. Consequently, this compound has been used as an analgesic in dermal patches (Anand and Bley, 2011). The mechanism of capsaicin-induced analgesia may be, among others, TRPV1 channel desensitization (Anand and Bley, 2011).

Surprisingly, the systemic administration of capsaicin affects central neurons, as capsaicin crosses the blood-brain barrier (Pezzoli et al., 2014). The antiepileptic actions of capsaicin have been tested with conflicting results (Lee et al., 2011; Pegorini et al., 2005). Both proepileptic and antiepileptic effects of capsaicin have been described. It has been shown that capsaicin prevents kainic acid-induced epileptogenesis (Lee et al., 2011). On the other hand, it has been demonstrated that capsaicin increases the severity of seizures in pentylenetetrazol-induced epilepsy (Carletti et al., 2017). The different results concerning the antiepileptic effects of capsaicin may be due to the different doses used and the different methods of application (Huang et al., 2017).

Other authors assessed the effects of capsaicin in the context of prefrontal cortex involvement in pain perception (Fierro et al., 2010; Lorenz et al., 2003). For example, it has been shown that transcranial magnetic stimulation of the prefrontal cortex in humans relieves capsaicin-induced pain (Fierro et al., 2010). In this study we assessed whether capsaicin influences sodium currents and action potentials in cortical neurons in vitro. Additionally, the effect of capsaicin on epileptiform activity was studied using three different proepileptic solutions. Interictal epileptiform activities (lasting less than 1 s) were evoked in extracellular solution with no magnesium ions and an increased potassium ion concentration (Szulczyk et al., 2019). Short ictal events were recorded in zero magnesium solution containing picrotoxin, whereas long ictal events were evoked in zero magnesium

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solution containing 4-AP (Brückner and Heinemann, 2000; D'Antuono et al., 2010; Sah et al., 2011). The aim of this study was to assess whether capsaicin influences sodium currents, action potentials and epileptiform activity in cortical neurons.

2. Material and methods

The experimental procedures used in this study adhered to the institutional and international guidelines on the ethical use of animals. All chemical compounds were purchased from Sigma Aldrich.

2.1. Slice preparation and preincubation

Three-week-old rats were anaesthetized using ethyl chloride and decapitated. Slices (300 μM) were cut from the medial prefrontal cortex in an ice-cold extracellular solution of the following composition (in mM): NaCl (130), KCl (2,5), glucose (10), $NaHCO_3$ (25), NaH_2PO_4 (1,25), $MgCl_2$ (7), and $CaCl_2$ (0,5) (pH = 7,4, bubbled with carbogen). After being prepared, the slices were incubated in standard ACSF of the following composition (in mM): NaCl (130), KCl (2,5), glucose (10), $NaHCO_3$ (25), NaH_2PO_4 (1,25), $MgCl_2$ (1), and $CaCl_2$ (2) (pH = 7,4, bubbled with carbogen).

2.2. Current-clamp recordings and induction of epileptiform activity

Recordings were made from layer V pyramidal neurons in slices of the medial prefrontal cortex.

The intracellular solution in the patch pipette was composed of (in mM): potassium-gluconate (105), KCl (20), HEPES-Na+ (10), EGTA (0,1), and MgATP (4), (pH = 7,4). After gigaseal formation, the patch membrane was ruptured by suction. The patch pipettes had resistances between 4 and 5 MΩ. The membrane potential and action potentials were recorded in an extracellular solution of the following composition (in mM): NaCl (130), KCl (2,5), glucose (10), $NaHCO_3$ (25), NaH_2PO_4 (1,25), $MgCl_2$ (1), and $CaCl_2$ (2) (pH = 7,4, bubbled with carbogen).

To determine the influence of capsaicin on the membrane potential and action potentials, control recordings were first made, and capsaicin was applied and washed out during the same recording. The influence of capsaicin (2 μM and 60 μM) on the membrane potential was assessed between the 2nd and 10th minute of capsaicin application, after which capsaicin was washed out. Action potentials were evoked using rectangular current steps of 300 pA lasting 2 or 50 s. Excitability was defined as the number of action potentials during 2 s when 2-s steps were applied and in the last 4 s when 50-s steps were applied. The influence of capsaicin (2 μM and 60 μM) was assessed after 2 min and after 10 min of the application of the tested compound.

Epileptic discharges were evoked in three different ways.

1 Interictal epileptiform discharges (IEDs) were evoked after 20–30 min of application of a proepileptic zero magnesium solution containing 5 mM potassium (Szulczyk et al., 2019). After recording control, capsaicin was applied for 10 min and washed out during the same recording (the proepileptic solution was applied throughout the whole recording).
2 Short ictal epileptiform discharges were evoked in zero magnesium solution containing 50 μM picrotoxin. The control neurons and capsaicin-treated neurons were different neurons. For capsaicin-treated neurons, capsaicin was preapplied in physiological solution for 10 min before proepileptic solution containing capsaicin was applied for 30 min.
3 Long ictal epileptiform discharges were evoked in zero magnesium solution containing 100 μM 4-AP. The control neurons and capsaicin-treated neurons were different neurons. For capsaicin-treated neurons, capsaicin was preapplied in physiological solution for 10 min before proepileptic solution containing capsaicin was applied for 40 min. Similarly, the control neurons and valproate-treated neurons were different neurons. For valproate-treated neurons, valproate was preapplied and was present throughout the recording in the same manner as capsaicin.

Capsaicin was dissolved in DMSO. The maximal concentration of DMSO was 0.07%. Lower DMSO concentrations were difficult to achieve due to the solubility threshold of capsaicin. Importantly, we added DMSO to all control experiments to ensure that the observed effects were not due to the potential actions of DMSO. Valproate is water soluble. For this reason, we did not add DMSO to the experiments with valproate.

Recordings were obtained at 35 °C. Recordings were made using a Multiclamp 700A amplifier and analysed with pClamp software. The flow rate was periodically checked and was 2 ml/min.

2.3. Voltage-clamp recordings

Sections of slices containing the medial prefrontal cortex were incubated with protease type XIV (1 mg/mL) for 20 min in a solution of the following composition (in mM): NaCl (135), HEPES-Cl (10), KCl (5), $MgSO_4$ (1), $CaCl_2$ (0,1), and glucose (10). After that they were mechanically dispersed with Pasteur pipettes, transferred to the recording chamber and placed on a stage of inverted Nikon microscope. The pipette solution contained the following components (in mM): CsF (110), NaCl (7), EGTA (11), HEPES-Cl (10), $MgCl_2$ (4), Na_2ATP (4) at pH 7,4 and osmolarity 290 mOsm.

The cells were continuously perfused with an external solution that was delivered to the whole bath at a rate of 2 ml/min. This solution contained the following components (in mM): NaCl (30), choline chloride (90), TEA-Cl (30), $CaCl_2$ (2), $MgCl_2$ (2), glucose (15), HEPES (10), and $CdCl_2$ (0,4) at pH 7,4. TEA-Cl and cadmium ions were added to the extracellular solution to block potassium and calcium currents, respectively.

The patch pipettes resistance was 4–5 MΩ. After a gigaseal was obtained, the membrane was ruptured either by suction or by an electrical stimulus. Pipette capacitance and membrane capacitance were compensated by the circuits of the Axopatch 1D amplifier (Axon Instruments, Foster City, CA, USA).

The recordings were performed at room temperature. Holding potential was −65 mV in all voltage-clamp recordings. Activation, inactivation and reactivation curves were analysed and fitted as shown in our previous study (Król et al., 2016). Capsaicin was applied to the whole-bath. Control neurons and capsaicin treated neurons were separate neurons. Recordings were made shortly after seal formation. Capsaicin was preapplied 8–12 min before seal formation. Control recordings were made in the extracellular recording solution without capsaicin.

2.4. Statistical analysis

All of the results presented are shown as the mean ± S.E.M. Differences between more than two groups were evaluated using ANOVA (Analysis of Variance), followed by Tukey's or Dunn's post hoc test (GraphPad InStat software v3.06). Paired or unpaired students t-test was used to evaluate differences between two groups.

3. Results

3.1. Effects of capsaicin on neural excitability and membrane potential

First, the influence of capsaicin on membrane potential of prefrontal cortical pyramidal neurons was tested. Capsaicin (2 μM and 60 μM) did not influence the membrane potential throughout the 10-min period of application. After 2 min of 2 μM capsaicin application, the membrane potential was −67,9 ± 1,7 mV whereas that of the control neurons was −68,1 ± 1,7 mV (n = 5, p > 0,05).

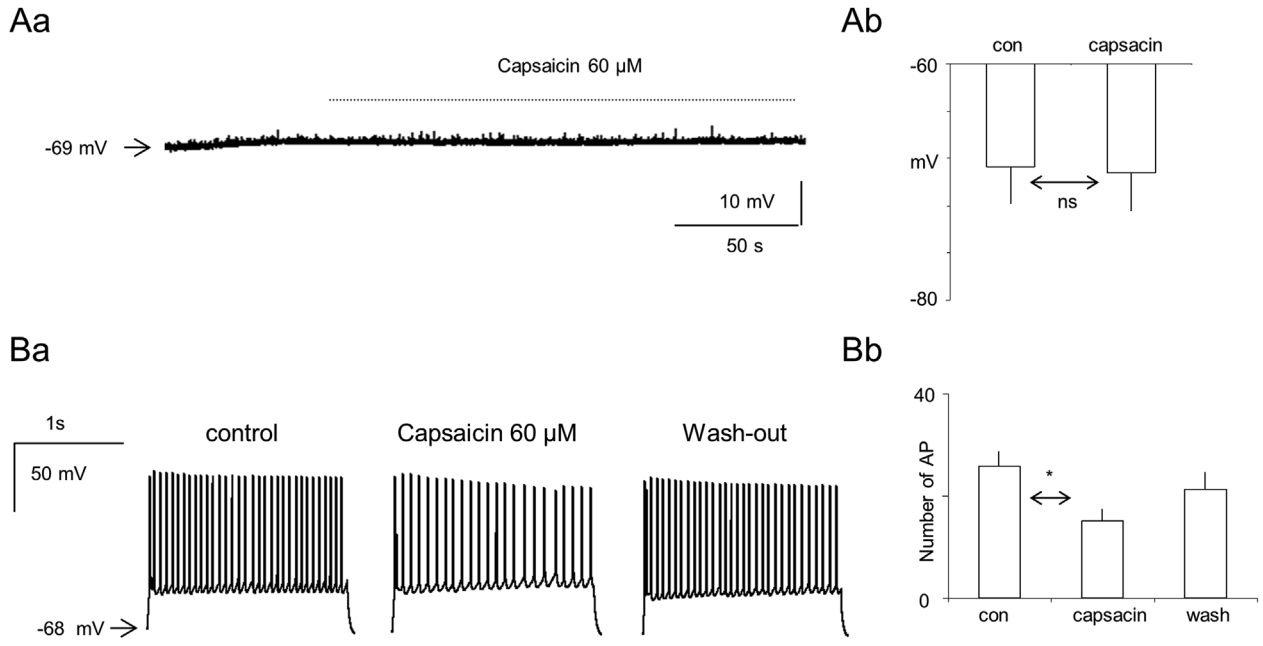

Fig. 1. A - Capsaicin 60 μM does not influence the membrane potential in PFC pyramidal neurons. **Aa** - example recordings of the membrane potential. **Ab** – averaged results in control and in the presence of capsaicin (2 min after capsaicin application).
B - Capsaicin 60 μM inhibits excitability in PFC pyramidal neurons. 2 s current steps were applied. **Ba** - example recordings of action potentials **Bb** – averaged results (number of action potentials per 2 s) in control, in the presence of capsaicin (10 min after application of the compound) and after wash-out.

Similarly, after 2 min of 60 μM capsaicin application, the membrane potential was the same as that of the control neurons (−69,2 ± 3,3 mV and −68,7 ± 3,0 mV, respectively; n = 5, p > 0,05, Fig. 1A).

Next, we assessed the influence of capsaicin on action potentials evoked by rectangular current steps of 300 pA lasting 2 s. Capsaicin at a concentration of 2 μM did not influence the excitability (number of action potentials per 2 s) of prefrontal cortical pyramidal neurons measured 2 min after capsaicin application (25,8 ± 4.0 in control neurons and 28,3 ± 4,4 in the presence of capsaicin; n = 5, p > 0.05) and did not have any influence after 10 min (data not shown). In contrast, 60 μM capsaicin significantly inhibited neuronal excitability after 10 min of application (25,8 ± 3,0 in control neurons, 15,2 ± 2,3 in the presence of the drug and 21,4 ± 3,3 after wash-out; n = 5, p < 0,05; Fig. 1B). However, after 2 min of 60 μM capsaicin application, the effect was not yet apparent (19,2 ± 4,2 in control neurons, 20,8 ± 3,3 in the presence of the drug; n = 5, p > 0,05).

Next, 50-s current steps were applied. The effect of capsaicin was assessed after 10 min of application. Neuronal excitability (number of action potentials during the last 4 s of a 50-s depolarization step) was strongly decreased in the presence of capsaicin (3,4 ± 2,5) compared to that in control neurons (38,4 ± 6,7). Wash-out was performed (15,6 ± 8,3; p < 0,05; n = 5, Fig 2A and C). The amplitudes of consecutive action potentials gradually decreased. The amplitude of the first action potential in response to a 50-s step was not influenced by capsaicin (81,6 ± 5,2 mV in control neurons, 82,5 ± 3,9 mV after capsaicin application and 81,9 ± 4,5 mV after wash-out; n = 5, p > 0,05, Fig. 2A). On the other hand, the amplitude of the last action potential was strongly inhibited by capsaicin (70,6 ± 3,9 mV in control neurons, 34,7 ± 7,1 mV in the presence of capsaicin and 52,3,0 ± 11,1 mV after wash-out; n = 5, p < 0,01, Fig. 2A and B). This suggests that capsaicin potently enhances the use-dependent block of fast voltage-gated sodium currents.

3.2. Effects of capsaicin on sodium currents

Next, we recorded sodium channels in the voltage-clamp configuration. The control neurons and capsaicin-treated neurons were different neurons. Capsaicin was preapplied 8–12 min before recordings. Sodium currents were evoked by several rectangular voltage steps from −60 mV to +10 mV in 10-mV increments. Capsaicin (60 μM) did not influence the maximal amplitude of sodium currents evoked by a −10 mV depolarization step (0,58 ± 0,08 nA in control neurons and 0,67 ± 0,15 nA in the presence of capsaicin; n = 5, p > 0,05, Fig. 3A). The normalized sodium channel activation curve was shifted towards hyperpolarization in the presence of capsaicin (the $V_{0,5}$ of activation were −17,8 ± 1,7 mV and −26,1 ± 2,9 mV mV in control neurons and in the presence of capsaicin, respectively; n = 5, p < 0,05, Fig. 3B). The K constants of activation were the same in control neurons (5,52 ± 0,58) and in the presence of the tested compound (5,89 ± 0,3; n = 5, p > 0,05, Fig. 3B). Steady-state inactivation was tested by a voltage step evoking maximal current, which was preceded by prepulses ranging from −110 mV to −10 mV and lasting 1000 ms. The $V_{0,5}$ of inactivation was shifted towards hyperpolarization in the presence of capsaicin (−65,1 ± 3,2 mV) compared to control neurons (−55,3 ± 2,7 mV; n = 5, p < 0,05, Fig. 3C). The K constants were not influenced by capsaicin (5,6 ± 0,25 in control neurons and 6,6 ± 0,42 in the presence of the tested compound; n = 5, p > 0,05, Fig. 3C). Recovery from inactivation was tested using two test pulses that evoke the maximal current with increased interpulse interval (from 0,5 ms–1024 ms). The tau constant of recovery from inactivation tended to be slower after the addition of capsaicin to the bath (31,6 ± 3,6 ms) compared to that of control neurons (26,0 ± 2,8 ms). The effect was not statistically significant (p > 0,05, n = 5, Fig. 3D).

The use-dependent block of sodium channels was markedly enhanced by capsaicin. Sodium currents were evoked at frequencies of 0.5 Hz and 10 Hz. In both cases, thirty current steps were used, and the currents were normalized to the current evoked by the first pulse. When use-dependent block was assessed at a frequency of 0,5 Hz, the relative current amplitude evoked by the last step was larger in control neurons (0,91 ± 0,05) than in the presence of capsaicin (0,67 ± 0,04; n = 6, p < 0,005, Fig. 4A). Similarly, when use-dependent block was assessed at a frequency of 10 Hz, the relative current amplitude evoked by the

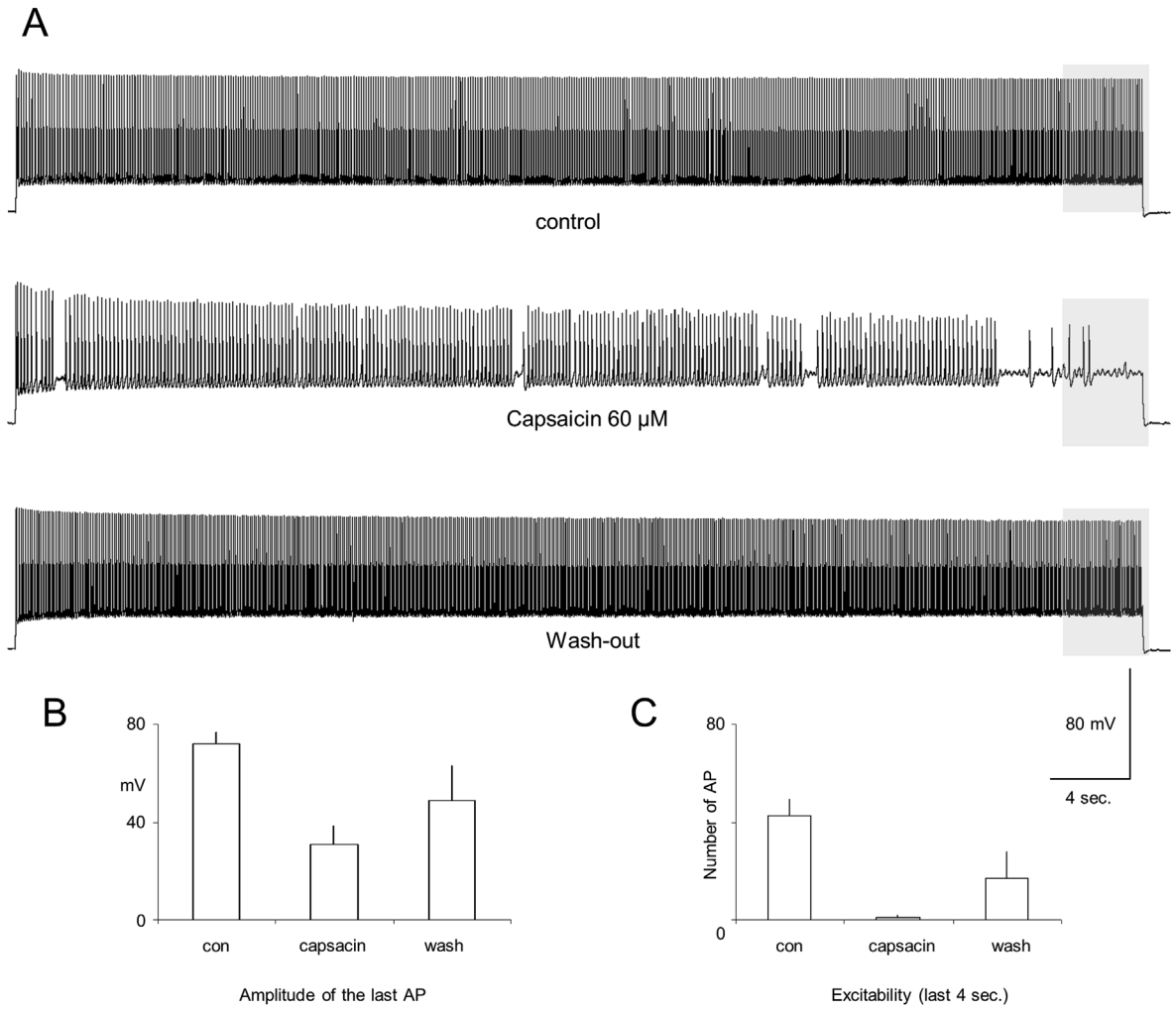

Fig. 2. A - Capsaicin 60 μM inhibits excitability and action potentials amplitude in PFC pyramidal neurons. 50 s current steps were applied. Example recordings of action potentials in control, in the presence of capsaicin (10 min after capsaicin application) and after wash-out, last 4 s of action potentials recordings have been marked in grey colour.
B - averaged amplitude of the last action potential in control, in the presence of capsaicin and after wash-out is shown. **C -** averaged excitability (number of action potentials during last 4 s of the 50- second depolarization step) is shown in control, after addition of tested compound and after wash-out.

last step was larger in control neurons (0,58 ± 0,03) than in the presence of capsaicin (0,24 ± 0,03; n = 6, p < 0,001, Fig. 4B). As 60 μM capsaicin influenced the use-dependent block and steady-state inactivation of sodium currents, the influence of a lower dose of capsaicin (2 μM) on these two parameters was tested. The use-dependent block assessed at 0,5 Hz was the same in the presence of 2 μM capsaicin as in control (the relative currents evoked by the last step were 0,91 ± 0,05 and 0,98 ± 0,02, respectively). Similarly, the use-dependent block of sodium channels assessed at 10 Hz was not changed by 2 μM capsaicin (0,58 ± 0,03 in control neurons and 0,65 ± 0,03 after the addition of the tested compound; n = 4, p > 0,05). Moreover, the properties of steady-state inactivation were not influenced by 2 μM capsaicin (data not shown).

3.3. Effects of capsaicin on epileptiform activity

Next, we showed that capsaicin inhibited three different types of epileptiform activity. First, we recorded the membrane potential in proepileptic extracellular solution containing no magnesium and elevated potassium ions, as described in our previous study (Szulczyk et al., 2019). In this solution, the membrane potential was depolarized (−59,4 ± 1,2 mV) compared to the membrane potential in the control physiological extracellular solution (−68,7 ± 3,0 mV; n = 5, p < 0,05).

Importantly, after the proepileptic solution was applied for 26,6 ± 5,3 min (n = 5), brief epileptiform events with action potentials were spontaneously evoked (Fig. 5A). These epileptiform events were interictal-like because they lasted less than 1 s (Fisher et al., 2014; Szulczyk et al., 2019). The properties of interictal-like discharges, such as the amplitude and duration, were characterized in our previous report (Szulczyk et al., 2019). This study showed that capsaicin (60 μM, applied for 10 min) almost fully inhibited interictal events (0,18 ± 0,06 Hz in control neurons, 0,01 ± 0,01 Hz after capsaicin application and 0,08 ± 0,03 Hz after wash-out; n = 5, p < 0,01, Fig. 5). We also found, in the same proepileptic solution, that 60 μM capsaicin depolarized the membrane potential 2 min after its application (−57,1 ± 1,2 mV) compared to control without capsaicin (−59,2 ± 1,1 mV). This depolarization was transient because after 5 min of capsaicin application (with capsaicin still in the bath) the membrane potential returned to control level (−59,2 ± 1,4; n = 6, p < 0,05).

Next, the membrane potential was recorded in zero magnesium extracellular solution containing 50 μM picrotoxin. In this solution, the membrane potential was the same as that in the control extracellular solution (data not shown). In the control, after the proepileptic solution was applied for 10,1 ± 0,5 (n = 5) minutes, short ictal events, which

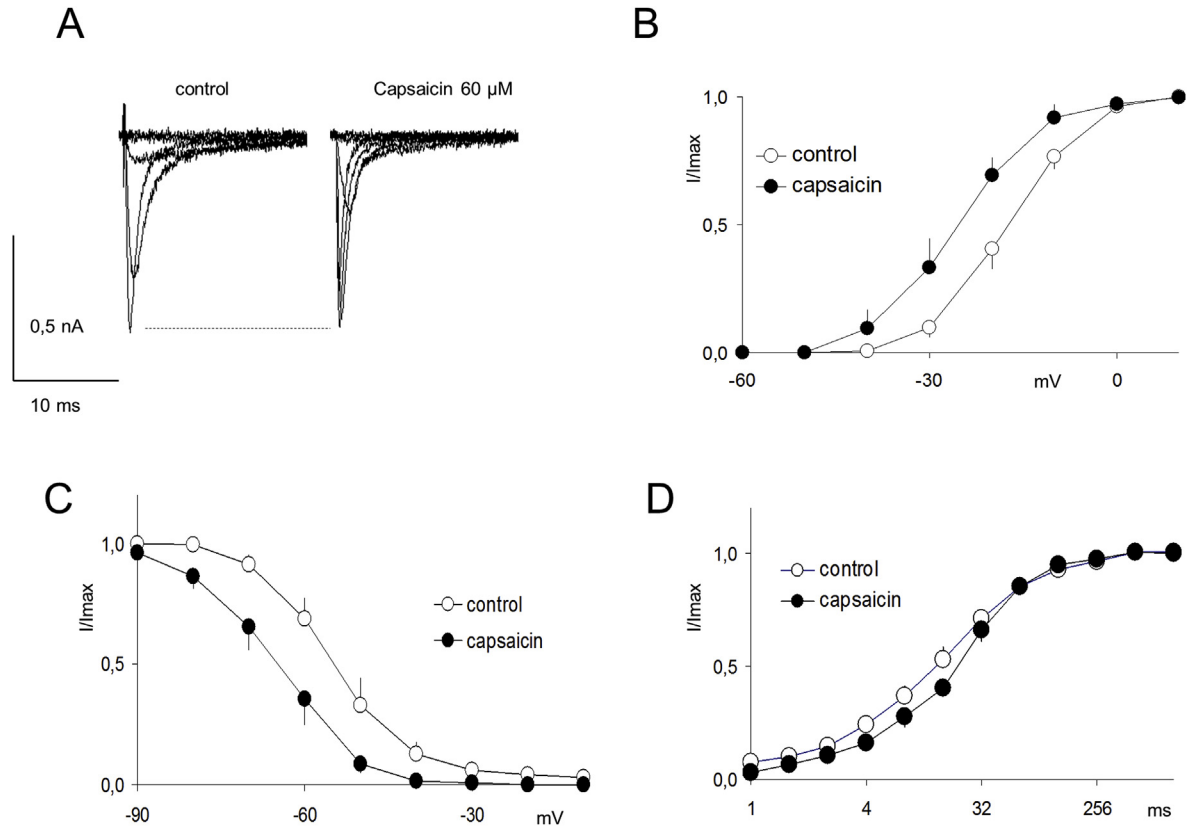

Fig. 3. The influence of capsaicin 60 μM on maximal amplitude and voltage-dependence of sodium currents.
A – example recordings of sodium currents activation show that tested compound did not influence maximal amplitude of sodium currents. Horizontal dotted line indicates maximal sodium currents evoked by −10 mV depolarization step. **B** – Normalized sodium currents activation curves are shifted towards hyperpolarization in the presence of capsaicin. Horizontal line shows depolarization steps in mV. **C** - tested compound shifts steady-state sodium channels inactivation curve towards hyperpolarization (Vertical axis shows normalized current amplitude and horizontal axis shows prepulse amplitude in mV). **D** – capsaicin exerts insignificant effect on sodium currents recover from inactivation (Vertical axis shows normalized current amplitude and horizontal axis shows interpulse interval in ms).

lasted 16,9 ± 0,24 s were observed. On top of these events, low amplitude action potentials were elicited. The average frequency of these control ictal events was 0,58 ± 0,08 per min (n = 5, Fig. 6A). The control and capsaicin-treated neurons were different neurons. Capsaicin was preapplied 10 min before proepileptic solution containing capsaicin was applied. In the constant presence of 60 μM capsaicin, epileptic events were observed after zero magnesium extracellular solution containing 50 μM picrotoxin was applied for 14,7 ± 2,0 min (n = 5), which was later than in the control. These values, however, were not significantly different (p > 0,05). Capsaicin markedly shortened the duration of the short ictal events (16,9 ± 0,24 s (n = 50) in control neurons compared to 7,5 ± 0,8 s (n = 36) in the presence of capsaicin; p < 0,001, Fig. 6). Capsaicin did not significantly influence the frequency of epileptic events (0,58 ± 0,1 per minute in control neurons

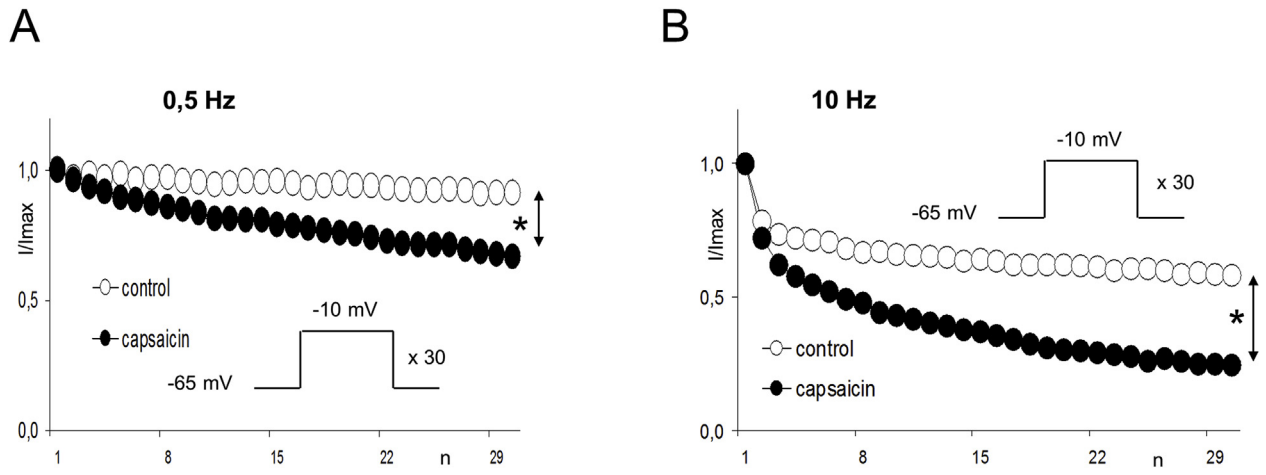

Fig. 4. The influence of capsaicin on use dependent block of sodium currents.
Use-dependent blockade was assessed with 30 pulses evoking a maximal current at a frequency of 0,5 Hz (**A**) and 10 Hz (**B**), holding potential was −65 mV. Vertical axis shows normalized current amplitude (**A** and **B**) and horizontal axis shows pulse number (**A** and **B**). Capsaicin 60 μM strongly enhanced use dependent block when sodium currents were evoked at a frequency of 0,5 Hz (**A**) and 10 Hz (**B**) as shown by an asterisk.

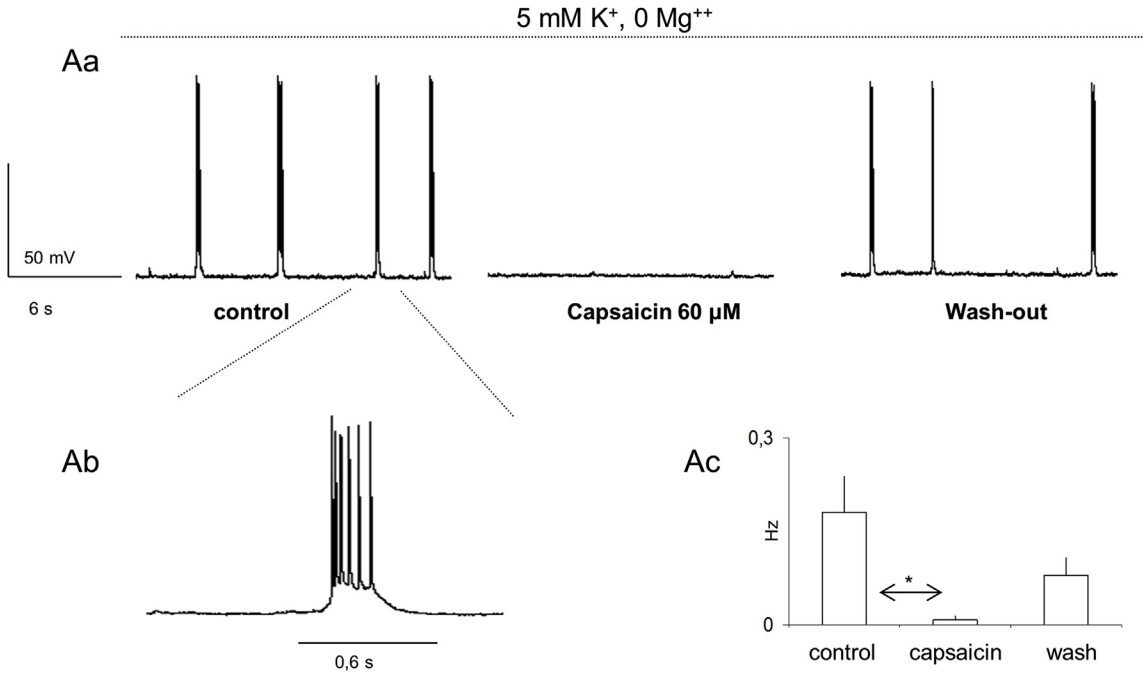

Fig. 5. Capsaicin inhibits interictal-like epileptiform activity in prefrontal cortex pyramidal neurons.
Aa - example recordings of epileptiform events in proepileptic solution containing no magnesium ions and increased potassium ions concentration. **Ab** – an epileptiform event is shown on an expanded time scale. **Ac** – averaged frequency of epileptiform events in control, in the presence of capsaicin (60 μM) and after washout.

and 0,5 ± 0,07 in the presence of capsaicin; n = 5, p > 0,05, Fig. 6B, C and D). The frequency, however, tended to be lower in capsaicin-treated neurons.

We also recorded long-lasting ictal events in proepileptic solution containing no magnesium ions and 100 μM 4-AP. In the control solution, without capsaicin, long ictal events were observed 25,8 ± 4,9 min after the onset of proepileptic solution application. The frequency of long ictal events was 0,05 ± 0,01 per minute (n = 6), and their duration was 140,3 ± 7,2 s (n = 12, Fig. 7A). The control and capsaicin-treated neurons were different neurons. Capsaicin was preapplied 10 min before proepileptic solution containing capsaicin was applied. In the solution containing 60 μM capsaicin, ictal events were not evoked. Instead, short-lasting epileptiform events were occasionally observed (Fig. 7B, n = 6). The presence of capsaicin in the proepileptic solution did not change the membrane potential (data not shown).

Finally, we assessed the influence of valproic acid (a reference antiepileptic drug) on long ictal events evoked in zero magnesium solution containing 4-AP. In our preparations, valproic acid (400 μM) did not influence the frequency of long ictal events (0,053 ± 0,01 per minute in control neurons and 0,051 ± 0,02 in the presence of valproate; n = 6, p > 0,05, Fig. 7C). Moreover, valproate did not change the duration of long ictal events (154,3 ± 18,5 s in control neurons and 139,2 ± 14,6 s in the presence of valproate; n = 12, p > 0,05, Fig. 7C). In the control group, long ictal events appeared 23,2 ± 4,1 min after the onset of proepileptic solution application, which was similar to the recordings in the presence of valproate (19,6 ± 1,9 min, n = 6, p > 0,05).

All recordings in Fig. 7C were performed without DMSO because valproate is water soluble, whereas all recordings in Fig. 7A and B (including control) were performed in the presence of DMSO because capsaicin is soluble in DMSO and not in water. The frequency and amplitude of control ictal events were not influenced by DMSO (control recordings in both series of experiments were almost identical - see paragraphs above).

4. Discussion

Our experiments showed that capsaicin inhibits neuronal excitability. Moreover, we showed that the amplitudes of the last action potentials evoked by long depolarization steps were strongly inhibited by capsaicin, whereas the amplitudes of the first action potentials were not. This suggests the use-dependent block of sodium channels. This was confirmed by voltage-clamp recordings of sodium currents, which showed that use-dependent block was potently enhanced by capsaicin. Additionally, we found that capsaicin shifted the steady-state inactivation curve of sodium channels towards hyperpolarization. Consequently, sodium channels were less available for activation at a given membrane potential. All results mentioned above indicate that the drug may directly inhibit voltage-gated sodium currents in a use-dependent manner. It has been repeatedly demonstrated that antiepileptic drugs enhance the use-dependent block of sodium currents (Jones et al., 2009; Król et al., 2016). Therefore, it may be suspected that the aforementioned effects of capsaicin contribute to its antiepileptic activity.

Surprisingly, we found that the sodium current activation curve was shifted towards hyperpolarization in the presence of capsaicin, which lowered the threshold for sodium channel opening. This effect is potentially proepileptic. The findings that capsaicin strongly inhibits epileptiform events (see below) strongly suggest, however, that the antiepileptic effects of capsaicin on sodium currents are more pronounced.

To prove that capsaicin inhibits epileptic events, we recorded epileptiform activity using three different models. First, interictal discharges were recorded in zero magnesium solution with an elevated potassium concentration; capsaicin was shown to have an inhibitory effect, strongly reducing the frequency of interictal discharges. Second, a solution without magnesium ions containing picrotoxin (a GABA receptor blocker) was used. The recorded events were considered ictal since they lasted more than 15 s in control solution (Fisher et al., 2014). Ictal events correlate with epileptic seizures (Fisher et al., 2014). Our experiments showed that capsaicin significantly reduced the duration of

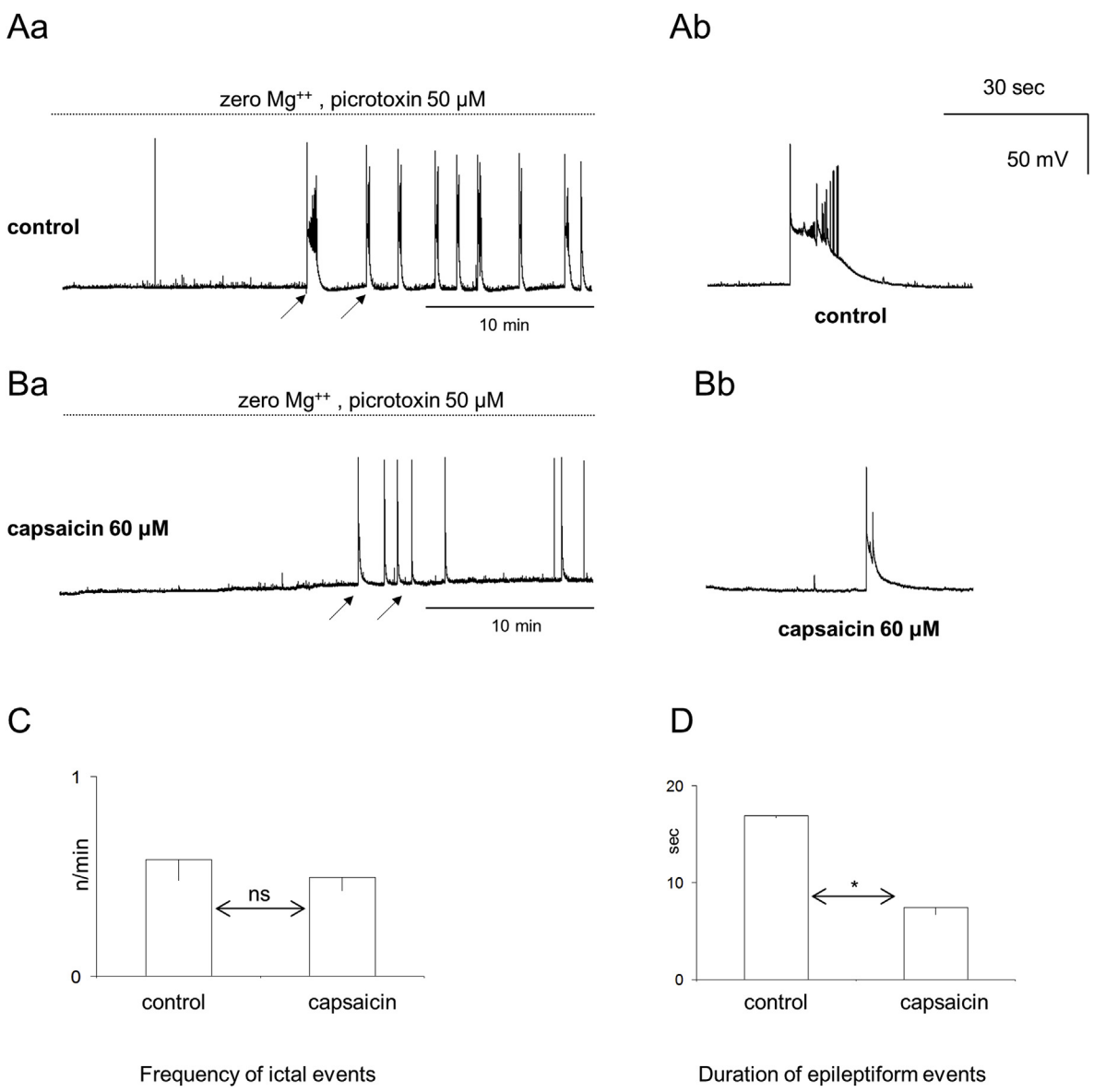

Fig. 6. Capsaicin 60 µM inhibits the duration of ictal epileptiform activity evoked in proepileptic solution containing no magnesium ions and picrotoxin 50 µM. **Aa** – example recording of epileptiform events in control proepileptic solution. **Ab** - single control epileptiform event is shown. **Ba** - example recording of epileptiform events in the same proepileptic solution with capsaicin 60 µM, it can be seen that that the duration of epileptiform events is reduced in the presence of capsaicin as shown by asterisks. Capsaicin was preapplied ten mintues before proepileptic solution containing capsaicin was applied. **Bb** – single epileptiform event is shown in the presence of capsaicin. **C** – averaged frequency of epileptiform events is not influenced by capsaicin. **D** – averaged duration of epileptiform events is markedly reduced by capsaicin.

these discharges. Third, we recorded long ictal events (duration longer than 150 s) in a solution without magnesium ions and containing 100 µM 4-AP (a potassium channel blocker). The application of capsaicin almost completely abolished this activity. These findings strengthen our conclusion that capsaicin exerts antiepileptic effects in PFC pyramidal neurons.

We also assessed the influence of valproate (a reference antiepileptic drug) on long ictal epileptiform events evoked in zero magnesium solution containing 4-AP. The therapeutic concentration of valproate is 35–200 µM (Vreugdenhil and Wadman, 1999). We found that valproate (400 µM, above the therapeutic range) did not influence the frequency or duration of long ictal events. Consequently, in our preparations, these events were resistant to valproate. In vitro models of epileptic events resistant to valproate have already been described (Călin et al., 2018). On the other hand, capsaicin fully inhibited long ictal events evoked in zero magnesium solution containing 4-AP (see above).

Our finding that capsaicin fully inhibits valproate-resistant ictal epileptic events strengthens our conclusion that the structure of capsaicin could be helpful in discovering new antiepileptic drugs.

Our study showed that capsaicin blocked interictal epileptiform discharges, which are caused by enhanced glutamate release (Szulczyk et al., 2019; Isaev et al., 2102; see above). Interictal activity is the name given to short discharges that last 2–3 s (Fisher et al., 2014). They do not cause seizures; however, several studies have linked interictal activity in humans with various psychiatric disorders and decreased cognitive abilities (Hernan et al., 2014; Leeman-Markowski and Schachter, 2016). Interictal epileptiform activity has been reported in patients with attention deficit hyperactivity disorder (Socanski et al., 2015). Moreover, interictal epileptic discharges have been recorded in patients with autism spectrum disorder. Such EEG abnormalities may be prevalent in up to 30% of these patients (Spence and Schneider,

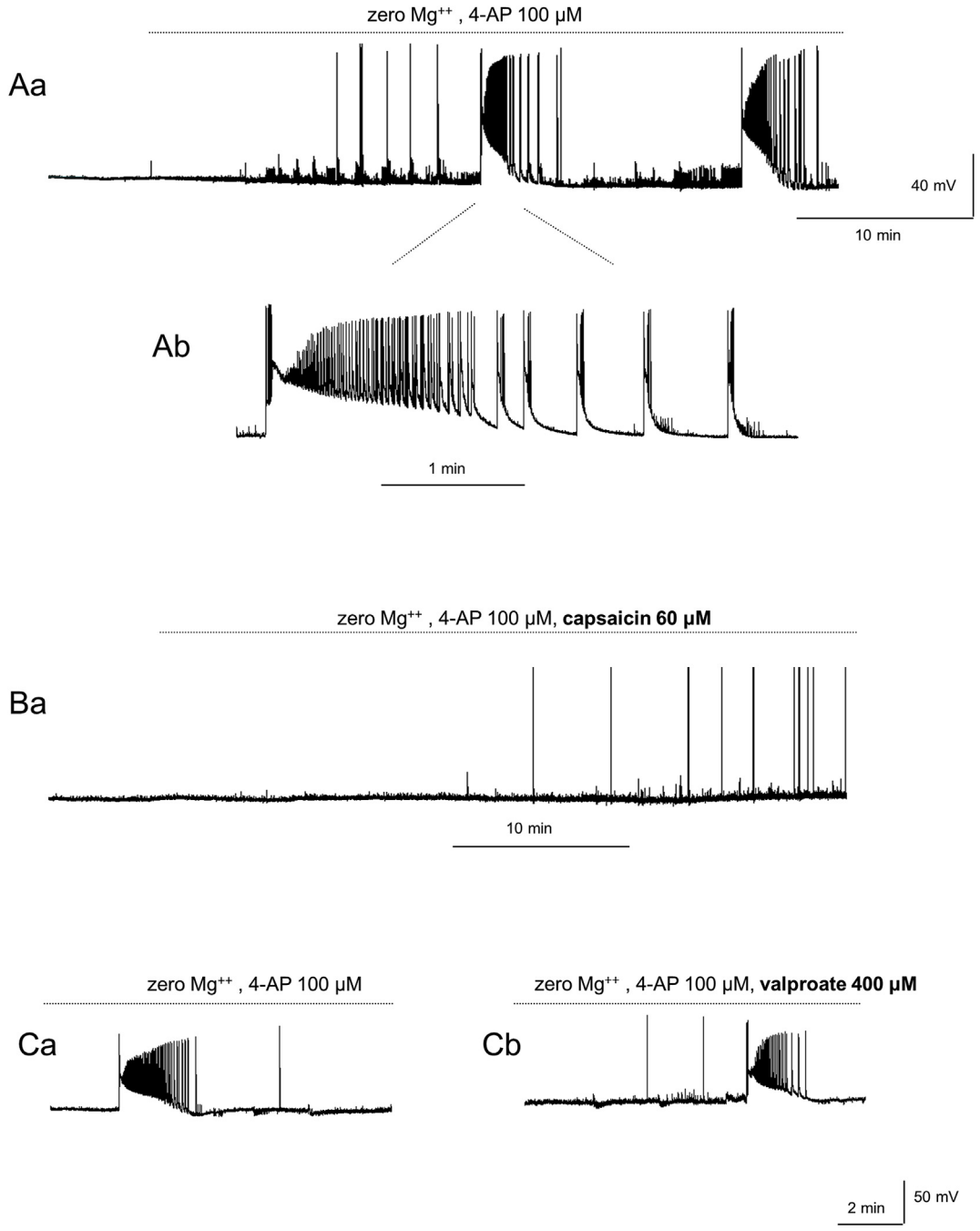

Fig. 7. Capsaicin 60 μM abolishes long lasting ictal epileptiform events evoked in proepileptic solution containing no magnesium ions and 4-AP 100 μM (A and B). Valproate 400 μM does not influence long ictal evoked in the same proepileptic solution (C).
Aa – example recording of epileptiform events in control proepileptic solution without capsaicin. **Ab** – control ictal event shown on an expanded time scale. **Ba** – example recording of the membrane potential in the same proepileptic solution containing capsaicin. Ictal events are abolished. Instead short-lasting interictal events are evoked. Capsaicin was preapplied ten mintues before proepileptic solution containing capsaicin was applied. Ca - example control recording of long ictal event evoked in zero magnesium solution. Cb – example recording of long ictal event in the same solution containing valproate 400 μM. Valproate was preapplied ten mintues before proepileptic solution containing valproate was applied. Vertical scale shown in Aa applies to A and B. In all figures statistical significance is indicated by an asterisk (*).

2009). In our recent publication (Szulczyk et al., 2019), we described the influence of valproate on interictal epileptiform discharges (IEDs) evoked in zero magnesium solution with high potassium and found that this drug almost completely inhibited IEDs in PFC pyramidal neurons obtained from young rats. Here, we showed using the same preparation that capsaicin almost completely inhibited IEDs. This suggests that capsaicin and valproate inhibit IEDs with similar potency.

Capsaicin is known to activate TRPV1 channels, which are sodium and calcium ion-permeable channels (Cao et al., 2007; Milesi et al., 2001; Onizuka et al., 2011). It cannot be excluded that the effects of capsaicin on action potentials and sodium channels are partially caused by TRPV1 channel activation. This notion, however, seems unlikely. It has been described that capsaicin in nanomolar and low micromolar concentrations depolarizes the membrane potential via TRPV1 channels shortly after its application (Cao et al., 2007; Milesi et al., 2001 Kitamura et al., 2018). In our study, neither a low concentration of

capsaicin (2 μM) not a high concentration of capsaicin (60 μM) depolarized the membrane potential shortly after capsaicin application in physiological extracellular solution, which suggests that under physiological conditions, TRPV1 channels were not functional in our preparations. Furthermore, in our study, low and high concentrations of capsaicin (2 μM and 60 μM) did not influence action potentials shortly after application (see results). This further suggests that the tested compound did not influence TRPV1 channels in physiological solution in our slices, as it has been described that capsaicin increases neuronal excitability in the initial period of its application (Zakharov et al., 2015).

It has been demonstrated that a low concentration of capsaicin (1 μM) inhibits voltage-gated sodium channels via TRP channels in sensory neurons (Cao et al., 2007). In our study, a low concentration of capsaicin (2 μM) did not influence the use-dependent block or steady-state inactivation of sodium channels, which suggests that TRP channels do not play a significant part in the effect of capsaicin on sodium currents.

The question arises whether TRPV1 channels contribute to the inhibition of ictal and interictal activities reported here. Recordings of such events required presynaptic glutamate release since they were evoked in zero magnesium solution, which enhances glutaminergic transmission (Isaev et al., 2012; Szulczyk et al., 2019). It has been described that capsaicin modulates synaptic transmission by influencing TRPV1 channels in presynaptic axons (Xu and Smith, 2015), which could potentially contribute to the inhibition of ictal and interictal epileptiform activities. The studies mentioned above, however, indicate that TRPV1 channels in presynaptic axons enhance and do not inhibit synaptic activity/neurotransmitter release, which is inconsistent with what seemed to be the case in our study (Bhaskaran and Smith, 2010; Jia et al., 2015). This enhancement of synaptic activity is caused by capsaicin-induced TRPV1 channel opening and the subsequent depolarization of the presynaptic membrane. In the studies cited above capsaicin was applied for a short period. In this study, we used a long application of capsaicin, which causes TRPV1 channel desensitization (Iannotti et al., 2014). We cannot exclude that TRPV1 channels located in presynaptic axons were desensitized by the drug, which may have reduced glutamate release and contributed to the ictal and interictal activity reduction seen in our experiments (Iannotti et al., 2014). It has been shown using multielectrode array recordings that capsaicin inhibits epileptiform activity evoked in zero magnesium solution in hippocampal neurons in vitro (Iannotti et al., 2014). The cited authors hypothesized that in zero magnesium solution, TRPV1 channels may become sensitized through increased phosphorylation and endogenously activated, which may contribute to epileptiform activity formation. The authors further suggested that capsaicin may desensitize TRPV1 channels through dephosphorylation, which may decrease epileptiform activity (Iannotti et al., 2014). We hypothesize that capsaicin may dephosphorylate and desensitize TRPV1 channels, which may be sensitized and endogenously activated in zero magnesium solution (Iannotti et al., 2014). Such a mechanism may contribute to epileptiform activity inhibition by capsaicin. Interestingly, we observed small, transient depolarization of the membrane potential shortly after capsaicin application in zero magnesium high potassium solution which may suggest the presence of functional TRPV1 channels in our preparation under epileptic conditions.

Several other important mechanisms could contribute to the inhibition of epileptiform discharges by capsaicin, with more than one likely being in play. As stated above, epileptiform discharges require glutamate release, which is regulated by voltage-gated sodium and calcium channels in presynaptic terminals (Rogawski et al., 2016). Since the effect of capsaicin on sodium channels was established in this study, it can be speculated that the compound inhibits presynaptic sodium currents, therefore inhibiting glutamate release. The inhibition of calcium currents in a similar manner is also possible (Hagenacker et al., 2011). The profound effect of capsaicin on epileptic discharges induced by 4-AP (a potassium channel inhibitor) suggests that the drug could also interact with potassium channels. There have been studies showing the influence of capsaicin on potassium currents (Yang et al., 2014). It can be speculated that this drug binds the potassium channels in a different place than 4-AP, making them unavailable for 4-AP. Additionally, capsaicin may enhance presynaptic potassium channels, therefore inhibiting neurotransmitter release (Rogawski et al., 2016). Capsaicin may also block postsynaptic NMDA and/or AMPA receptors, which contribute to epileptiform activity formation (D'Antuono et al., 2010; Isaev et al., 2012; Szulczyk et al., 2019). Finally, the action of capsaicin on the GABA pathway should be considered, however, taking into account the relatively small effect on epileptic activity induced by picrotoxin application, this hypothesis seems unlikely.

Epileptiform events are capped by trains of action potentials (see results; D'Antuono et al., 2010; Pezzoli et al., 2014). As we have shown, capsaicin enhances the use-dependent block of sodium currents. Therefore, it can be speculated that the drug enhances the use-dependent block of action potentials on top of epileptiform events, which could be another mechanism by which capsaicin exerts its antiepileptic effects.

It has been found that capsaicin exerts antiepileptic effects in vivo (Lee et al., 2011) and in vitro (Iannotti et al., 2014; Pezzoli et al., 2014). In the second study (Pezzoli et al., 2014), the effect was TRP-independent, while in the first study (Iannotti et al., 2014), capsaicin inhibited epileptic bursts in the hippocampus through TRPV1 desensitization. The results of a third study were inconsistent (Jia et al., 2015). The authors found that capsaicin injection into the hippocampus produced an antiepileptic effect but that the systemic administration of capsaicin increased the severity of epileptic seizures. Desensitization of TRPV1 channels has been proposed to be involved in antiepileptic effects (Jia et al., 2015). We suggest that capsaicin may reduce seizures not only through TRPV1 desensitization but also through sodium channel inhibition. It has been repeatedly described that antiepileptic drugs and experimental compounds that exert antiepileptic effects inhibit voltage-gated sodium channels (Jones et al., 2009; Król et al., 2016).

This study shows that capsaicin inhibits sodium channels, action potentials and epileptic events in cortical neurons.

Author statement

I declare that there are no conflicts of interest.

Declaration of competing interest

None.

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ARTICLE

Guanfacine inhibits interictal epileptiform events and sodium currents in prefrontal cortex pyramidal neurons

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Abstract

Background Guanfacine (an alpha-2A receptor agonist) is a commonly used drug with recognized efficacy in the treatment of attention deficit hyperactivity disorder (ADHD). This study aimed to assess the effects of guanfacine on short-lasting (interictal) epileptiform discharges in cortical neurons. Moreover, we assessed the effects of guanfacine on voltage-gated sodium currents.

Methods We conducted patch-clamp recordings in prefrontal cortex pyramidal neurons obtained from young rats. Interictal epileptiform events were evoked in cortical slices in a zero magnesium proepileptic extracellular solution with an elevated concentration of potassium ions.

Results Interictal epileptiform discharges were spontaneous depolarisations, which triggered action potentials. Guanfacine (10 and 100 μM) inhibited the frequency of epileptiform discharges. The effect of guanfacine on interictal events persisted in the presence of alpha-2 adrenergic receptor antagonist idazoxan. The tested drug inhibited neuronal excitability. Tonic NMDA currents were not influenced by guanfacine. Recordings from dispersed neurons showed that the tested drug (10 and 100 μM) inhibited persistent and fast inactivating voltage-gated sodium currents.

Conclusions This study shows that guanfacine inhibits interictal discharges in cortical neurons independently of alpha-2A adrenergic receptors. This effect may be mediated by voltage-gated sodium currents. Inhibition of interictal activity by guanfacine may be of clinical importance because interictal events often occur in patients with ADHD and may contribute to symptoms of this disease.

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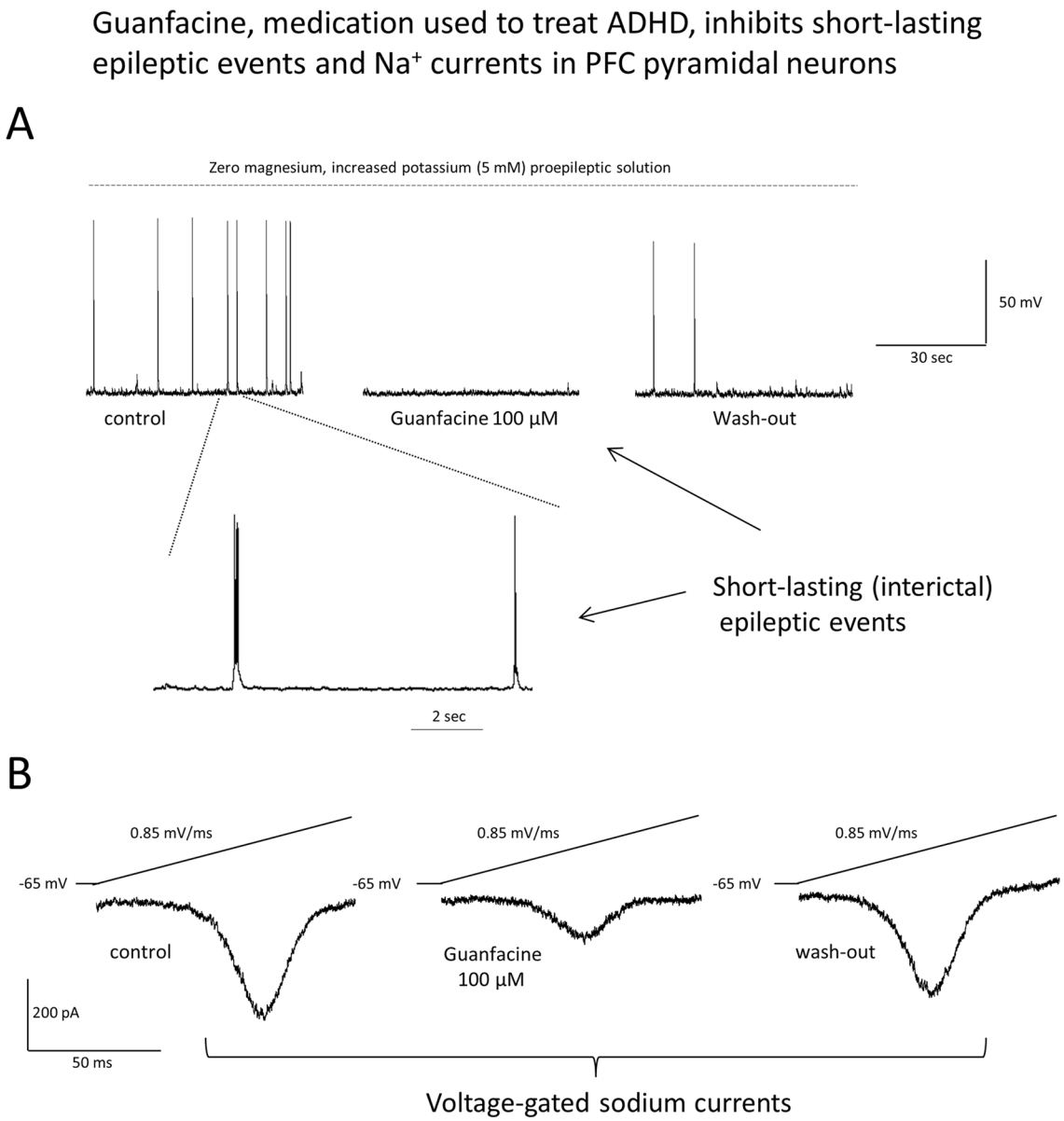
Graphical abstract



Keywords Attention deficit hyperactivity disorder · Interictal discharges · Guanfacine · Sodium channels · Patch-clamp

Introduction

Attention deficit hyperactivity disorder (ADHD) is described as a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development [1]. Emerging evidence points to the involvement of the prefrontal cortex (PFC) in the pathogenesis of ADHD [2]. One study showed that children with ADHD present with a slowed or impaired development of the right lateral PFC [3]. Other authors have described a smaller volume of grey matter in the PFC of boys with ADHD as compared to age-matched controls [4]. Consequently, the PFC is a target for ADHD medications [5]

Guanfacine, an alpha-2A-adrenergic receptor agonist, is an approved medication for ADHD, both in adults and children [6]. It has been shown that guanfacine and other ADHD medications such as methylphenidate improve cognitive functions which are impaired in ADHD [5, 6]. In the PFC, guanfacine enhances cognition by stimulating post-synaptic alpha-2A-adrenergic receptors located in pyramidal neurons.

This leads to the inhibition of nearby cAMP-dependent K^+ channels, which strengthens network connectivity and working memory [6]. Besides ADHD, guanfacine has been used "off-label" in several other conditions associated with prefrontal cortex pathology, such as autism spectrum disorders (ASD), schizophrenia, substance abuse, and post-traumatic stress disorder [6].

Several reports have linked epileptic seizures with a decline in cognitive performance, which is also a feature of attention and social disorders [7, 8]. Less attention has been focused on interictal epileptiform discharges (IEDs) or subclinical epileptiform activity which occurs in epileptic patients between seizures [7, 9]. These types of epileptiform events may also be present in patients without seizures [10]. Interictal epileptiform discharges are a relatively common finding in patients diagnosed with ADHD and are presumed to have a causal relationship with the symptoms of the disease [10–15]. Cognitive impairment is a common symptom of ADHD. From a pathophysiological standpoint, increased excitability associated with IEDs may hinder cognition through calcium excitotoxicity, remodeling of neuronal circuitry, or disruption of sleep-related memory consolidation processes [7] There have been reports suggesting that targeting epileptiform activity may alleviate cognitive problems [7, 11, 12].

Our previous publication showed that a common antiepileptic drug—valproate, a sodium channel blocker and a GABA receptor agonist, inhibits interictal activity in the prefrontal cortex [16]. In the current report, we aimed to assess the effect of guanfacine on interictal activity in the rat's prefrontal cortex in vitro. Furthermore, we sought to investigate the mechanism of this effect.

Materials and methods

The experimental procedures used in this study adhered to the Polish and international guidelines on the ethical use of animals (Directive 2010/63/EU, Polish Legislation for the protection of animals used for scientific or educational purposes 2015). Male Wistar Rats (3 week-old) were purchased from the Medical University of Warsaw animal house. The total number of animals used in this study was 20. Rats were bred at room temperature (3 rats per cage, 12 h/12 h light/dark cycle) and fed with a standard laboratory chow. After decapitation, the brain was gently removed. Decapitation was the only procedure performed on animals in this study. Slices (300 µM) of the prefrontal cortex were prepared exactly the same way as shown in our previous publication [16]. After cutting, slices were incubated in a physiological artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl (130), KCl (2.5), glucose (10), $NaHCO_3$ (25), NaH_2PO_4 (1.25), $MgCl_2$ (1), and $CaCl_2$ (2), pH = 7.4, bubbled with carbogen. For the experiments in slices this solution was heated to 32 °C for the first 20 min of incubation and after that was maintained at room temperature. For the recordings from dispersed neurons, the incubating solution was maintained at room temperature for the duration of the experiment (6–8 h).

Recordings in slices

Recordings were made from layer V pyramidal neurons in slices of the medial prefrontal cortex.

Action potentials and IEDs were recorded in the current-clamp configuration. Action potentials were evoked once every 60 s by 250 pA rectangular current steps lasting 3 s in physiological ACSF (see above). Interictal epileptiform discharges (IEDs) were recorded in zero magnesium/5 mM potassium proepileptic extracellular solution which contained (in mM): NaCl (130), KCl (5), glucose (10), $NaHCO_3$ (25), NaH_2PO_4 (1.25), and $CaCl_2$ (2), pH = 7.4, bubbled with carbogen. IEDs were spontaneous discharges and were recorded in membrane potential recording mode.

Tonic NMDA currents were recorded in the voltage-clamp configuration in zero magnesium extracellular solution which contained (in mM): NaCl (130), KCl (2.5), glucose (10), $NaHCO_3$ (25), NaH_2PO_4 (1.25), and $CaCl_2$ (2), glycine (0.05), pH = 7.4, bubbled with carbogen. Magnesium ions were omitted and glycine was added to facilitate NMDA receptors. Moreover, this solution contained, tetrodotoxin (TTX) 0.25 µM, DNQX (6,7-Dinitroquinoxaline-2,3-dione) 10 µM and picrotoxin 50 µM to block synaptic transmission. NMDA 2 µM was applied to the bath. After stable NMDA current was evoked, NMDA 2 µM and guanfacine 100 µM were coapplied (see results).

For all slice recordings, the intracellular solution in the patch pipette was composed of (in mM): potassium-gluconate (105), KCl (20), HEPES-Na + (10), EGTA (0, 1), MgATP (4), GTP (0.5), pH = 7.4. Neurons were visualized in DIC optics. Slice recording techniques were the same as in our previous study [16]. Positive pressure was applied to the pipette tip to blow away extracellular debris. After gigaseal formation, the patch membrane was ruptured. Recordings were made using a Multiclamp 700A amplifier and analyzed with pClamp software (Axon Instruments, USA). Patch-pipettes had resistances between 4 and 5 MΩ. Recordings were obtained at 35 °C. Guanfacine was applied to the bath.

Recordings in dispersed neurons

Sections of slices containing the prefrontal cortex were enzymatically dispersed using protease type XIV (0.5 mg/ml) and mechanically dispersed using Pasteur pipettes exactly the same way as in our previous publication [17]. Dispersed neurons were transferred to a recording chamber.

Recordings were made from pyramidal neurons which were visualized under an inverted microscope.

Persistent voltage-gated sodium currents were recorded in an external solution that contained the following (in mM): NaCl (120), $CaCl_2$ (2), $MgCl_2$ (2), TEA-Cl (30), 4-AP (3), HEPES (10), glucose (15), $CdCl_2$ (0.4), $LaCl_3$ (0.005), pH 7.4. Fast activating and fast inactivating voltage-gated sodium currents were recorded in an external solution of the following composition (in mM): NaCl (30), choline chloride (90), TEA-Cl (30), $CaCl_2$ (2), $MgCl_2$ (2), glucose (15), HEPES (10), $CdCl_2$ (0.4) and $LaCl_3$ (0.005), at pH 7.4. Voltage-gated calcium currents were blocked by cadmium and lanthanum ions in the extracellular solution. Voltage-gated potassium currents were blocked by TEA-CL in the extracellular solution. Moreover, potassium ions were absent in the intracellular solution. The pipette (intracellular) solution was the same for fast and persistent sodium currents and contained the following (in mM): CsF (110), NaCl (7), EGTA (3), HEPES-Cl (10), $MgCl_2$ (2), Na_2ATP (4), pH was 7.4.

Recording techniques were exactly the same as in our previous study [17]. After gigaseal formation, the membrane was ruptured. The access resistance ranged from 5 to 7 MΩ. A series resistance compensation of 80% was applied. The currents were leak subtracted. All recordings were performed at room temperature (21–22 °C). Currents were recorded using an Axopatch 1D amplifier and analyzed with pClamp software (Axon Instruments, USA). Guanfacine was applied to the bath.

Guanfacine was purchased from Sigma-Aldrich (product number G1043). DNQX, picrotoxin NMDA and idazoxan were also purchased from Sigma-Aldrich (product numbers D0540, P1675, M3262 and I6138, respectively). Tetrodotoxin (TTX) was purchased from Abcam (product number ab120055). Other chemical compounds were purchased from Polskie Odczynniki Chemiczne Avantor or from Sigma-Aldrich.

Statistical analysis

Normally distributed values are presented as means ± SEM, whereas non-normally distributed values are as medians [IQR]. Differences between more than two groups were evaluated using one-way ANOVA for repeated measures followed by the Tukey post hoc test if the data passed the normality test. If the data did not pass the normality test nonparametric equivalent of one-way ANOVA for repeated measures (Friedman's test) followed by Dunn's post hoc test was used. Depending on the results of the normality test, the Students *t* test or Wilcoxon matched-pairs test were used to evaluate differences between the two groups.

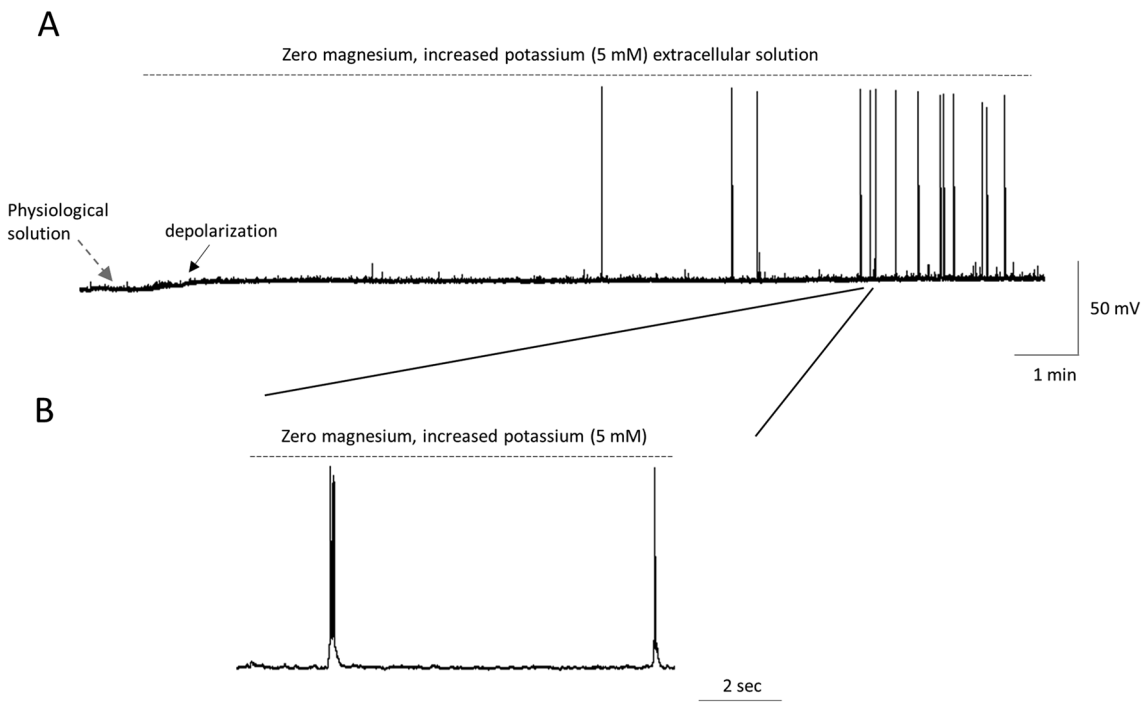


Fig. 1 Induction of interictal epileptiform events (IEDs) in prefrontal cortex pyramidal neurons. **A** IEDs were induced in a zero magnesium and high potassium proepileptic extracellular solution as shown by a dashed line. The membrane potential was initially recorded in physiological artificial cerebrospinal fluid, as shown by the grey dashed arrow. Application of the proepileptic extracellular solution depolarized the membrane potential because of an increased potassium ions concentration, as shown by the black solid arrow. **B** Two IEDs are shown on an expanded time scale. The same vertical scale is used for (**A**) and (**B**)

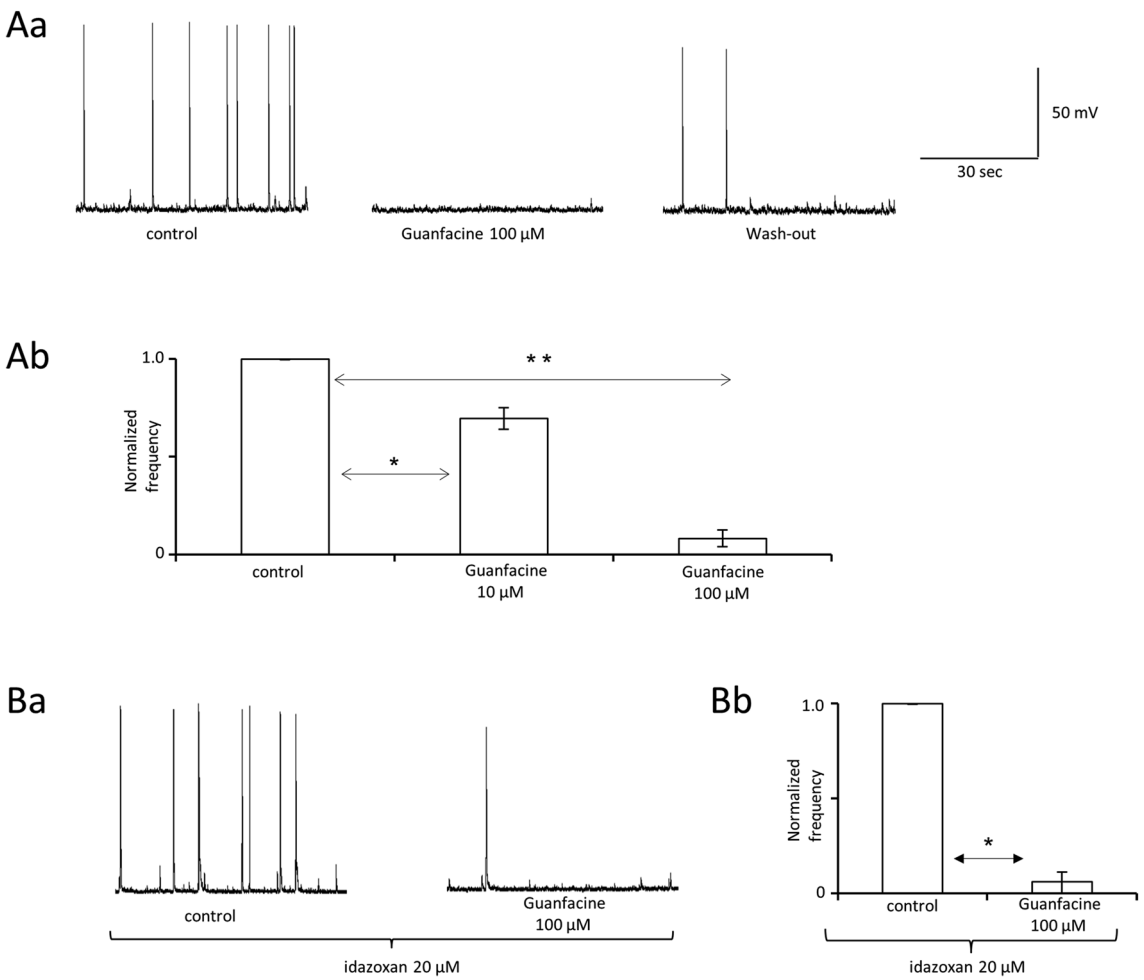


Fig. 2 Guanfacine inhibits interictal epileptiform events (IEDs) in prefrontal cortex pyramidal neurons. **Aa** Original recordings of IEDs in control, in the presence of guanfacine 100 µM and after wash-out. **Ab** Normalized frequency of IEDs in the control and in the presence of guanfacine: 10 µM (paired t test [control vs drug application], $p < 0.05$) and 100 µM (one-way ANOVA for repeated measures [control, drug application, wash-out], $p < 0.0001$, followed by Tukey's post hoc test [control vs drug application], $p < 0.001$). Bars represent means and whiskers represent SEM. $*p < 0.05$ and $**p < 0.001$. **Ba** Original recordings of IEDs in the control and after application of guanfacine 100 µM (alpha-2 adrenergic receptor antagonist idazoxan 20 µM was present in all extracellular solutions). **Bb** Normalized frequency of IEDs in the control and in the presence of guanfacine. Idazoxan was present in all extracellular solutions (Friedman's test [control, drug application, wash-out], $p = 0.0046$, followed by Dunn's post hoc test [control vs drug application], $p < 0.05$). Inset in (**Aa**) applies to (**Ba**). Bars represent medians and whiskers represent IQR. $*p < 0.05$

Kolmogorov–Smirnov test was used to assess normality (GraphPad InStat software v3.06).

Results

Induction of interictal epileptiform events in PFC pyramidal neurons

Interictal epileptiform discharges (IEDs) were evoked in a zero magnesium, high potassium proepileptic extracellular solution similar to our previous study [16]. Firstly, the membrane potential was stabilized in a physiological ACSF for a few minutes. After switching to the proepileptic solution, the membrane potential depolarised because of increased potassium ions concentration (-66.6 ± 2.3 mV and -59.8 ± 3.2 mV in a physiological and proepileptic solution, respectively, $n = 6$ recordings, the paired Students t test, $p = 0.0362$, $t_5 = 2.8$, as shown by the black solid arrow in Fig. 1A). After 10–30 min of applying the zero magnesium, high potassium proepileptic extracellular solution, IEDs were evoked which were brief depolarisations capped by action potentials (Fig. 1A and B). The events lasted less than 2 s [9, 16]. Two single epileptiform events are shown on an expanded time scale in Fig. 1B.

Guanfacine inhibits interictal epileptiform events in PFC pyramidal neurons

After a steady control frequency of the IEDs was recorded, the influence of guanfacine on the membrane potential and the frequency of interictal events were assessed. Guanfacine was applied for 7–10 min. The tested compound (100 µM) did not change the membrane potential (-58.8 ± 2.3 mV in the control and -59.7 ± 2.7 mV after the application of the drug, $n = 5$ recordings, the paired Students t test, $p > 0.05$, $t_4 = 1.04$).

The control frequency of the IEDs was 0.12 ± 0.02 Hz ($n = 15$). Guanfacine inhibited the frequency of the IEDs. Example recordings are shown in Fig. 2Aa. The normalized frequency of the IEDs was 1.0 ± 0.0 in the control, 0.08 ± 0.04 in the presence of guanfacine (100 µM), and 0.33 ± 0.04 after wash-out (3 animals, $n = 6$ recordings, one-way ANOVA for repeated measures, $F_{3,17} = 324.72$ ($p < 0.0001$) followed by Tukey's post hoc test ($p < 0.001$ control vs guanfacine), Fig. 2Ab). As expected, a lower concentration of guanfacine (10 µM) inhibited the normalized frequency of the IEDs to a smaller extent (1.0 ± 0.0 in the control compared to 0.69 ± 0.05 after the application of the drug, $n = 5$ recordings, 2 animals, the paired Students t test, $p = 0.0042$, $t_4 = 5.9$, Fig. 2Ab).

The most commonly described mechanism of action of guanfacine is the stimulation of alpha-2A adrenergic receptors [18]. For this reason, we assessed the influence of guanfacine 100 µM on the frequency of IEDs in the constant presence of the alpha-2 adrenergic receptor antagonist idazoxan in all extracellular solutions. With idazoxan (20 µM) in the bath, guanfacine 100 µM inhibited the frequency of the IEDs to the same extent as without idazoxan in the bath (see above). Example recordings are shown in Fig. 2Ba and normalized results are shown as medians in Fig. 2Bb 1.0 [1.0–1.0] in the control, 0.06 [0.00–0.11] after the application of guanfacine 100 µM and 0.36 [0.26–0.52]) after wash-out, 2 animals, $n = 4$ recordings, nonparametric repeated measures ANOVA (Friedman's test), Friedman's statistic = 8, $p = 0.0046$ followed by Dunn's post hoc test ($p < 0.05$, control vs guanfacine)). Thus, the effect of guanfacine on IEDs in PFC pyramidal neurons is independent of alpha-2A adrenergic receptors.

Guanfacine inhibits neuronal excitability and voltage-gated sodium currents in PFC pyramidal neurons

In the next series of experiments, we recorded action potentials in PFC pyramidal neurons in physiological ACSF. Excitability was defined as the number of action potentials per depolarisation step lasting 3 s. We found that guanfacine 100 µM inhibited neuronal excitability (29.0 [26.5–32.3] in the control, 14.0 [8.0–19.0] in the presence of the tested drug and 27.0 [24.5–30.5] after wash-out, 2 animals, $n = 4$ recordings, nonparametric repeated measures ANOVA (Friedman's

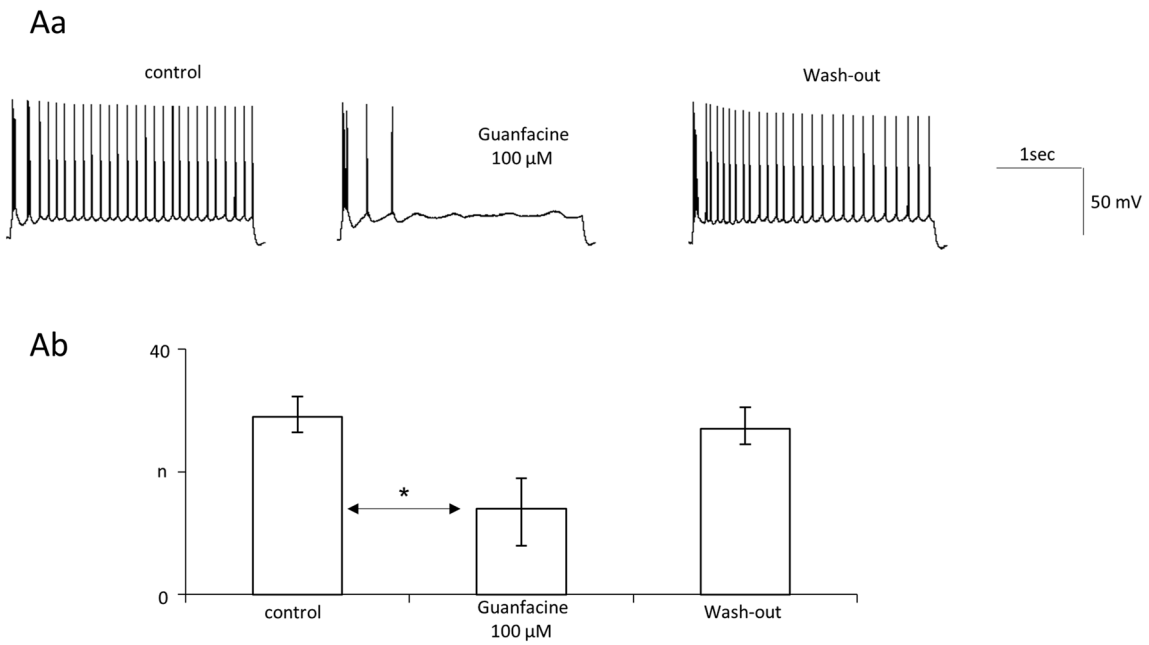


Fig. 3 Guanfacine inhibits neuronal excitability in prefrontal cortex pyramidal neurons. **Aa** Example recordings of action potentials in the control, in the presence of guanfacine 100 µM and after wash-out. **Ab** Excitability (number of action potentials per current step) in the control, after application of the tested drug and after wash-out (Friedman's test [control, drug application, wash-out], $p = 0.0046$, followed by Dunn's post hoc test [control vs drug application], $p < 0.05$). Bars represent medians and whiskers represent IQR. *$p < 0.05$

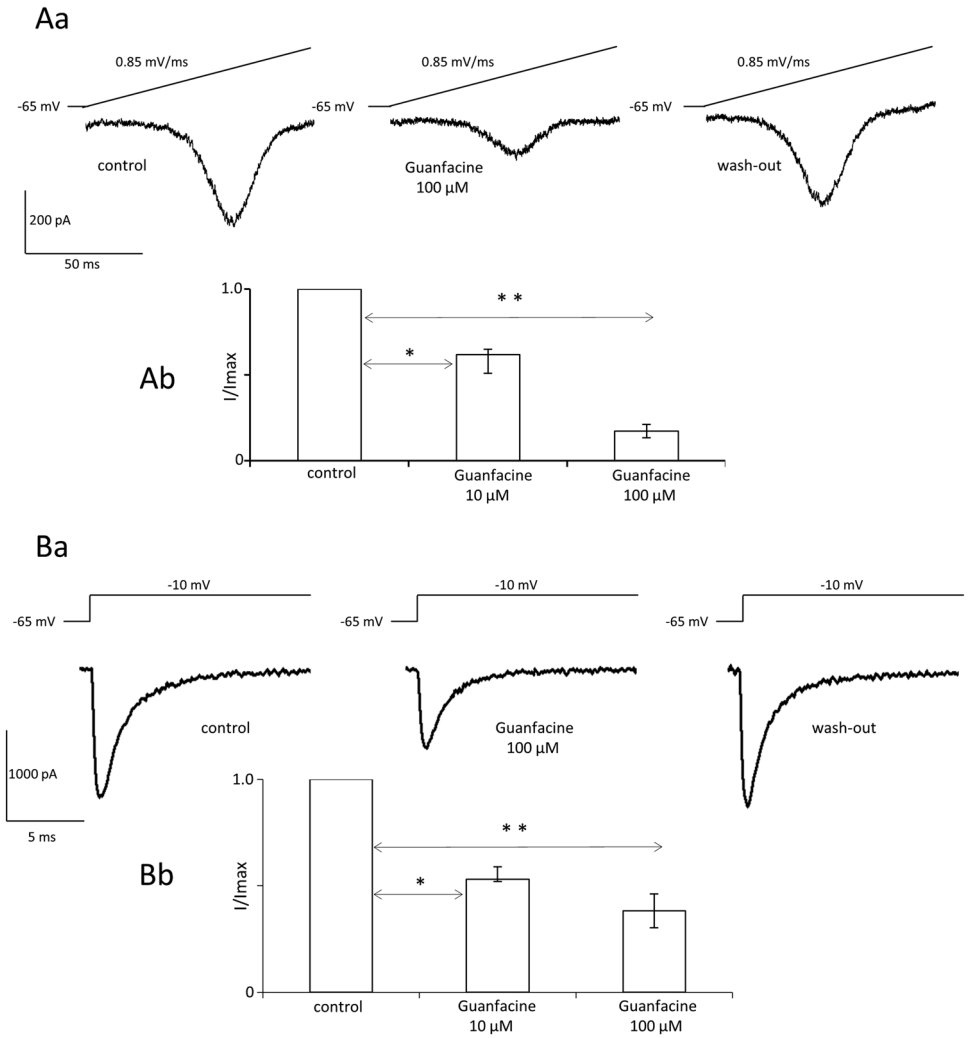


Fig. 4 Guanfacine blocks persistent and fast inactivating sodium currents in prefrontal cortex pyramidal neurons. **Aa** Example recordings of slowly inactivating (persistent) sodium currents in the control, in the presence of guanfacine 100 µM and after wash-out. Persistent sodium currents were evoked by ramp depolarizations shown above current traces. **Ab** Normalized maximal persistent sodium current amplitudes in the control and after application of two concentrations of guanfacine: 10 µM (Friedman's test [control, drug application, wash-out], $p = 0.0046$, followed by Dunn's post hoc test [control vs drug application], $p < 0.05$, bars represent medians and whiskers represent IQR) and 100 µM (One-way ANOVA for repeated measures [control, drug application, wash-out], $p < 0.0001$, followed by Tukey's post hoc test [control vs drug application], $p < 0.001$, bars represent means and whiskers represent SEM). *$p < 0.05$ and **$p < 0.001$. **Ba** Example recordings of fast inactivating (transient) sodium currents in the control, after application of guanfacine 100 µM and after wash-out. Sodium currents were evoked by rectangular voltage steps shown above current traces. **Bb** Normalized maximal transient sodium current amplitudes in the control and after application of two concentrations of guanfacine: 10 µM (Friedman's test [control, drug application, wash-out], $p = 0.0046$, followed by Dunn's post hoc test [control vs drug application], $p < 0.05$, bars represent medians and whiskers represent IQR) and 100 µM (One-way ANOVA for repeated measures, [control, drug application, wash-out] $p < 0.0001$, followed by Tukey's post hoc test [control vs drug application], $p < 0.001$, bars represent means and whiskers represent SEM). *$p < 0.05$ and **$p < 0.001$

test), Friedman's statistic = 8, $p = 0.0046$ followed by Dunn's post hoc test ($p < 0.05$, control vs guanfacine)). Example recordings and averaged results are shown in Fig. 3Aa and b, respectively.

We hypothesized that guanfacine may inhibit persistent sodium currents because it has been found that the inhibition of these currents substantially contributes to decreasing excitability in central neurons [19]. Persistent voltage-gated sodium currents were evoked once every 20 s by short ramp depolarisations from − 65 mV to 10 mV lasting 100 ms. Control recordings were conducted for 2 min, guanfacine was applied for 3 min and after that wash-out was recorded. A dose-dependent effect was observed. A higher concentration of the tested drug (100 µM) inhibited persistent sodium currents (example recordings are shown in Fig. 4Aa).

Normalized, maximal current amplitudes were 1.0 in the control, 0.17 ± 0.04 in the presence of guanfacine 100 µM and 0.66 ± 0.08 after wash-out (3 animals, $n = 7$ recordings, one-way ANOVA for repeated measures, $F_{3,20} = 119.44$ ($p < 0.0001$) followed by Tukey's post hoc test ($p < 0.001$ control vs guanfacine)). Furthermore, normalized maximal current amplitudes were 1.0 [1.0–1.0], 0.62 [0.51–65] and 0.78 [0.55–0.82] in the control, after the application of guanfacine 10 µM and after wash-out, respectively (2 animals, $n = 4$ recordings, nonparametric repeated measures ANOVA (Friedman's test), Friedman's statistic = 8, $p = 0.0046$ followed by Dunn's post hoc test ($p < 0.05$, control vs guanfacine)). Normalized results are depicted as medians in Fig. 4Ab.

In the next series of experiments, the influence of guanfacine on fast-activating and fast-inactivating voltage-gated sodium channels was tested. The currents were evoked once every 10 s by rectangular voltage steps to − 10 mV. Control recordings were conducted for 2 min, the tested drug was applied for 2 min and after that wash-out was recorded. Example recordings of fast inactivating sodium currents are shown in Fig. 4Ba. After the application of guanfacine 100 µM, the maximal, normalized sodium current amplitude was 0.38 ± 0.08 as compared to control 1.0 (Fig. 4Bb). It was possible to obtain wash-out (0.73 ± 0.08, 2 animals, $n = 5$ recordings, one-way ANOVA for repeated measures, $F_{3,14} = 40.95$ ($p < 0.0001$) followed by Tukey's post hoc test ($p < 0.001$ control vs guanfacine)). A lower concentration of guanfacine (10 µM) was also tested and the maximal, normalized sodium current amplitudes were 1.0 [1.0–1.0] in the control, 0.53 [0.52–0.59] after application of the tested drug and 0.66 [0.64–0.71] after wash-out (2 animals, $n = 4$ recordings, nonparametric repeated measures ANOVA (Friedman's test), Friedman's statistic = 8, $p = 0.0046$ followed by Dunn's post hoc test ($p < 0.05$, control vs guanfacine), Fig. 4Bb). We also assessed the time-dependent inactivation of fast voltage-gated sodium currents. Tau constants of time-dependent inactivation were not significantly different in control and in the presence of guanfacine 100 µM (1.6 ± 0.12 ms and 1.74 ± 0.22 ms, respectively, $n = 4$ recordings, paired t test $p > 0.05$, $t_3 = 0.6$).

Guanfacine does not influence tonic NMDA currents in PFC pyramidal neurons

Recordings were conducted in an extracellular solution that contained no magnesium ions, glycine 50 µM, TTX 0.25 µM, DNQX 10 µM and picrotoxin 50 µM (see Methods). NMDA 2 µM without guanfacine was applied for 8–10 min. After evoking stable NMDA currents, NMDA 2 µM and guanfacine 100 µM were coapplied for 7 min. The amplitude of the control NMDA currents was 127.0 [99.3–146.3] pA, as shown by the left grey arrow in Fig. 5Aa. The amplitude of the NMDA currents after the application of guanfacine 100 µM was 139.5 [117.0 -162.0] pA as shown by the right

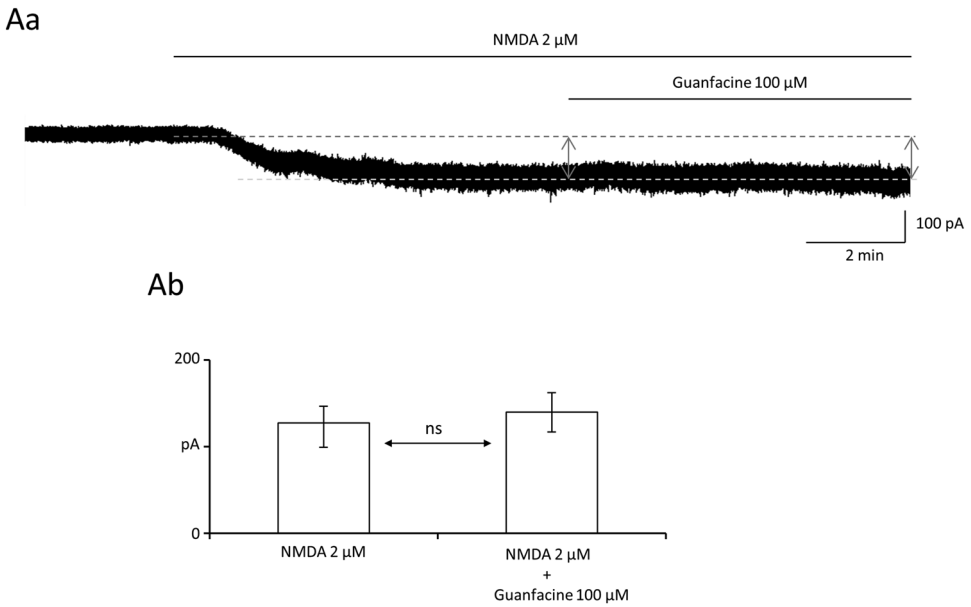


Fig. 5 Guanfacine does not influence tonic NMDA currents. **Aa** Example recording of NMDA current evoked by application of NMDA 2 µM to the whole bath. Dashed lines indicate control current and current after application of NMDA 2 µM. Left vertical arrow indicates the NMDA current before the application of guanfacine and the right vertical arrow indicates the NMDA current after the application of guanfacine 100 µM. **Ab** NMDA current without guanfacine and NMDA current after application of guanfacine 100 µM (Wilcoxon's matched-pairs test [control vs drug application], $p > 0.05$). Bars represent medians and whiskers represent IQR. *ns* nonsignificant

grey arrow in Fig. 5Aa, which was not significantly different from the control NMDA currents (Fig. 5Ab $n=4$ recordings, 2 animals, Wilcoxon's matched-pairs test, $p>0.05$). It was shown in our previous study [20] that tonic NMDA currents were fully inhibited by a selective NMDA inhibitor, AP-5.

Discussion

The epileptiform discharges recorded in this study may be regarded as interictal events because of their short duration (less than 2 s) [9]. They do not cause seizures but are often recorded in EEG between seizures in epilepsy patients [9]. They can also be present in non-epileptic patients with different neuropsychiatric disorders [7, 10, 13–15].

In this study, IEDs were recorded with the use of the patch-clamp technique in PFC pyramidal neurons in a zero magnesium, elevated potassium pro-epileptic solution. Such composition of the extracellular solution enhances the effects of glutamate on NMDA receptors/channels because magnesium ions are removed from the NMDA channels pore. Furthermore, more glutamate is released from presynaptic terminals in the presence of increased potassium concentration because presynaptic axons are depolarized. Thus, the zero magnesium, elevated potassium extracellular solution increases the glutaminergic transmission that generates IEDs [16, 21].

We found that guanfacine blocks IEDs. The tested drug, however, did not exert this effect via the inhibition of glutaminergic NMDA receptors/channels, because we showed that they were not influenced by guanfacine. We recorded both synaptic and extra-synaptic NMDA currents simultaneously since the recordings were made from the whole plasma membrane, and NMDA was applied to the whole bath [22].

Glutamate release is caused by the opening of presynaptic voltage-gated sodium and calcium channels [23, 24]. Consequently, guanfacine may inhibit IEDs by targeting presynaptic voltage-gated sodium and calcium channels, thus lowering increased glutamate release, which generates IEDs. Additionally, guanfacine may block IEDs by suppressing postsynaptic sodium and calcium channels, as they were also reported to be involved in the generation of IEDs [25, 26]. There are two types of voltage-gated sodium currents: fast inactivating (transient) and slowly inactivating (persistent) sodium currents [19]. In this study, we recorded both fast and persistent voltage-gated sodium channels from dispersed PFC pyramidal neurons and found that guanfacine inhibits these channels, which may substantially contribute to the blocking of IEDs by the tested drug.

Few reports assess guanfacine's influence on the electrophysiological properties of neurons. It was found that guanfacine suppressed excitatory postsynaptic currents in PFC pyramidal neurons [18, 27]. Similarly, in vivo experiments showed that the application of guanfacine reduced field excitatory post-synaptic potentials in PFC neurons [18]. The reports cited above suggest that guanfacine inhibits glutaminergic transmission in PFC neurons via the α2A adrenergic receptors [18, 27]. The authors hypothesized that this mechanism may improve PFC functioning (working memory) during excessive stress. Different authors performed in-vivo experiments and found that guanfacine improved working memory by enhancing neuronal activity in the PFC during the delay period of a working memory task [28]. This effect was also abolished by the alpha-2 adrenergic receptor antagonist. The authors suggested that this mechanism may explain guanfacine's beneficial effects in treating ADHD [28].

As stated above guanfacine enhances neuronal activity in the PFC during the delay period of a working memory task via alpha-2 adrenergic receptors [28]. It may be argued that mentioned result contradicts our study that shows that guanfacine inhibits neuronal excitability and IEDs via direct inhibition of sodium channels. It may, however, be hypothesized that a lower concentration of guanfacine may enhance neuronal activity via alpha-2 adrenergic receptors as shown previously [28] and a higher concentration of the tested drug may inhibit sodium channels and consequently block IEDs and neuronal excitability, as shown in the present study. The concentrations of guanfacine that we used were 10 µM and 100 µM and were higher than the therapeutic plasma concentration of guanfacine [29]. They were, however, similar to previous patch-clamp studies [18, 30, 31].

The most commonly described mechanism of action of guanfacine is stimulating G-protein-coupled alpha-2A adrenergic receptors [18, 27, 28]. Guanfacine, however, may also have other, alpha-2 adrenergic receptor-independent mechanisms of action. In other words, guanfacine may influence ionic channels either directly or via G-protein-coupled alpha-2 adrenergic receptors. We hypothesize that in our experiments, the effects of guanfacine were mediated via direct action on ionic channels due to the following reasons. Firstly, our experiments in slices showed that the tested drug inhibited IEDs in the presence of the selective alpha-2 adrenergic receptor antagonist. Secondly, in our experiments in dispersed neurons, guanfacine most likely directly inhibited sodium channels since fluoride ions in the patch pipette disrupted G-protein-mediated signalling [32]. The important finding of this study is that guanfacine may act not only by stimulating alpha-2A adrenergic receptors but also by an additional mechanism, which is the direct inhibition of sodium channels. Interestingly, the chemical structure of guanfacine, with an aromatic ring linked to an amine group by an amide bond, resembles local anaesthetics (sodium channel inhibitors). This strengthens our hypothesis that guanfacine directly

influences sodium channels. There are reports showing that other alpha-2 adrenergic receptor agonists such as clonidine and dexmedetomidine block sodium channels in peripheral neurons and in cell lines in an adrenergic receptor-independent fashion [33–35].

IEDs occur more often in patients with ADHD and may contribute to symptoms of this disease [10–15]. There are clinical studies suggesting that antiepileptic drugs (sodium and calcium channel inhibitors) reduce ADHD symptoms. For example, it was found that the calcium channel inhibitor levetiracetam inhibits IEDs and reduces symptoms of ADHD in children suffering from this disease [11, 12]. Another study showed that sodium channel inhibitor lamotrigine decreases ADHD symptoms in epileptic patients with ADHD. This effect correlated with EEG normalization and a reduction of epilepsy symptoms [36]. It was also found that sodium channel inhibitor carbamazepine inhibits IEDs in children with ADHD. This effect correlated with clinical improvement [37]. It could be speculated that in some patients guanfacine may reduce ADHD symptoms by inhibiting interictal epileptic events. Thus, guanfacine may exert beneficial effects in ADHD not only by stimulating alpha-2 adrenergic receptors as shown previously [6] but also in an additional mechanism which is the inhibition of sodium channels and consequently inhibition of IEDs.

This study shows that guanfacine inhibits IEDs in prefrontal cortex pyramidal neurons independently of alpha-2A adrenergic receptors. Sodium channel blockade by guanfacine is likely involved in this effect. This novel mechanism may be important clinically as inhibition of IEDs by guanfacine may reduce symptoms of ADHD.

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Authors' contributions MP: performing experiments, data analysis, figure preparation and manuscript writing. WK: performing experiments. BS: devising the concept of the study, manuscript writing.

Data availability Raw data are provided in a supplementary file.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

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Review

Beneficial Effects of Capsaicin in Disorders of the Central Nervous System

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Abstract: Capsaicin is a natural compound found in chili peppers and is used in the diet of many countries. The important mechanism of action of capsaicin is its influence on TRPV1 channels in nociceptive sensory neurons. Furthermore, the beneficial effects of capsaicin in cardiovascular and oncological disorders have been described. Many recent publications show the positive effects of capsaicin in animal models of brain disorders. In Alzheimer's disease, capsaicin reduces neurodegeneration and memory impairment. The beneficial effects of capsaicin in Parkinson's disease and depression have also been described. It has been found that capsaicin reduces the area of infarction and improves neurological outcomes in animal models of stroke. However, both proepileptic and antiepileptic effects of capsaicin in animal models of epilepsy have been proposed. These contradictory results may be caused by the fact that capsaicin influences not only TRPV1 channels but also different molecular targets such as voltage-gated sodium channels. Human studies show that capsaicin may be helpful in treating stroke complications such as dysphagia. Additionally, this compound exerts pain-relieving effects in migraine and cluster headaches. The purpose of this review is to discuss the mechanisms of the beneficial effects of capsaicin in disorders of the central nervous system.

Keywords: capsaicin; neurodegenerative diseases; stroke; epilepsy; depression

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1. Introduction

Capsaicin is a natural compound, often used in many countries during food preparation, responsible for the spiciness of chili peppers. Capsaicin belongs to a group of compounds called vanilloids [1,2]. The widely-described mechanism of action of capsaicin is the activation of calcium-permeable TRPV1 channels. These receptors are also activated by other stimuli, for example, noxious temperature, acidic pH, and endogenous chemical activators such as endocannabinoids [1]. Capsaicin exerts its effects on different organs because TRPV1 channels are expressed in different cell types, for example, neuronal, muscular, immune, and epithelial cells as well as adipocytes [1]. Moreover, other molecular targets for capsaicin such as voltage-gated sodium and calcium channels have been described [3,4].

Capsaicin exerts beneficial effects in many disorders [2]. It was reported, for example, that capsaicin exerts anti-obesity effects by changing gut-microbiota compositions [5]. Moreover, capsaicin induces apoptosis in different types of cancer cell lines, as shown by in vitro experiments. In vivo studies have shown that capsaicin reduces the growth of many tumors in animal models of oncological disorders [6]. In the vascular system, capsaicin stimulates TRPV1 receptors expressed on endothelial cells and increases the production of vasodilating factors [7]. This process reduces ischemic injury which contributes to the pathogenesis of cardiovascular and neurological disorders [7,8].

In the peripheral nervous system, capsaicin acts on pain receptors (nociceptors) in the skin and mucosa and evokes burning sensations by opening TRPV1 channels. Surprisingly, capsaicin also exerts analgesic effects and, consequently, this compound is used in treating

neuropathic pain. It has been suggested that capsaicin relieves pain by causing desensitization of TRPV1 channels expressed in peripheral pain receptors [9]. Moreover, capsaicin inhibits voltage-gated sodium currents which reduces the excessive activity of sensory neurons and contributes to the pain-relieving effects of this compound [10]. Capsaicin also influences brain neurons and glial cells via TRPV1-dependent and independent mechanisms. Many different positive effects of capsaicin in neurodegenerative diseases, epilepsy, stroke, and depression have been described in animal and human studies [3,11–14]. The aim of this review is to describe the beneficial effects of capsaicin in disorders of the central nervous system.

2. Physico-Chemical and Pharmacokinetic Properties of Capsaicin

Capsaicin is an odorless, colorless, crystalline compound (its chemical formula is represented in Figure 1). It has a melting point of about 63 Degrees Celsius and its molar mass is 305.4 g/mol. It is not soluble in water; however, it is well soluble in organic solvents such as ethanol or DMSO [15].

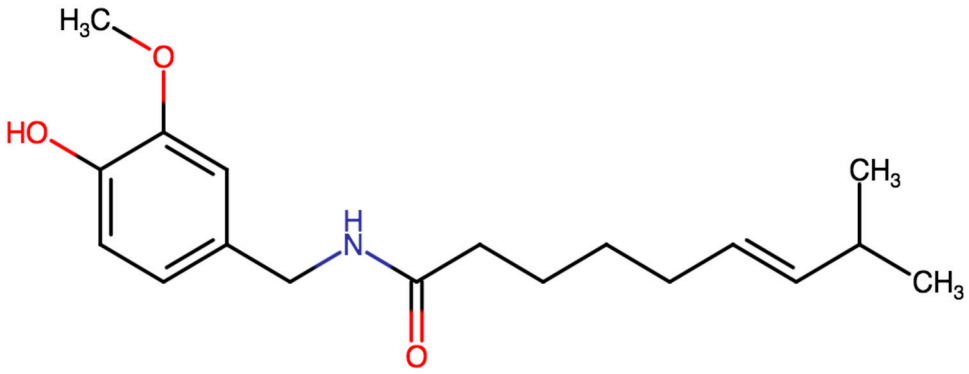

Figure 1. Chemical structure of capsaicin.

Animal studies show that the absorption of capsaicin after oral intake varies from 50 to 90% [15]. In one study conducted on humans, after oral administration of 5 g of chili peppers (which amounted to 26.6 mg of pure capsaicin), the peak plasma concentration was 2.47 ± 0.13 ng/mL, time to peak concentration was 47.1 ± 2.0 min and $t_{1/2}$ was 24.9 ± 5.0 min [16].

Capsaicin crosses the blood–brain barrier efficiently, as shown by animal studies. After intravenous administration, 5-fold higher concentrations of the compound in the brain and spinal cord were reported compared with serum [17]. Additionally, it was shown in animal studies that capsaicin could be detected in the plasma, brain, and spinal cord after subcutaneous administration [15].

3. Alzheimer's Disease

Alzheimer's disease is a common neurodegenerative disease. Its main histopathological features are beta-amyloid accumulation, tau hyperphosphorylation, and the death of brain cells. The main symptoms of this disease are memory loss and psychiatric disturbances [18]. Certain drugs such as cholinesterase inhibitors are administered to slow the progress of this disorder [19]. Moreover, a proper diet may be beneficial in Alzheimer's disease because healthy food contributes to cognitive health during aging [20]. Transgenic mice expressing a mutated human amyloid precursor protein are often used as a model of Alzheimer's disease [18].

3.1. Beneficial Effects of Capsaicin in Animal Models of Alzheimer's Disease

Microglia are immune cells of the central nervous system. An important function of these cells is phagocytosis of the beta-amyloid plaques in the early phases of Alzheimer's disease. This function is then impaired in more advanced diseases [21–23]. Autophagy is a process of intracellular degradation of organelles and toxic molecules which is involved

in the aging process [23]. It was reported that treatment of microglial cultures with capsaicin (10 μM) induced autophagy and increased the phagocytic capacity of microglial cells. Consequently, beta-amyloid was more efficiently removed from the culture medium. TRPV1 receptors expressed in microglia were involved in this effect. It was shown in the same study that intraperitoneal administration of capsaicin (1 mg/kg daily for one month) reduced beta-amyloid deposition and improved learning and memory in transgenic mice with Alzheimer's disease. The authors concluded that capsaicin ameliorated symptoms of Alzheimer's disease by inducing autophagy in microglial cells and by increasing their phagocytic capacity [23]. In a different study, it was found that the metabolic and phagocytic function of microglia was impaired by beta-amyloid. The authors demonstrated that this impairment was reduced after treatment of microglia cultures with capsaicin (10 μM). Thus, the phagocytosis of beta-amyloid by microglial cells was more efficient after treatment with capsaicin. This beneficial effect was dependent on TRPV1 channels. The same authors, in behavioral experiments, found that dietary capsaicin (0.01% in a chow) improved memory in a mouse model of Alzheimer's disease [21].

It was found in mice with Alzheimer's disease, that dietary capsaicin (0.01% in a chow) reduced beta-amyloid plaque formation and tau phosphorylation in different brain areas, and attenuated neurodegeneration and cognitive impairment [23]. Beta-amyloid is a protein produced by enzymatic cleavage of the amyloid precursor protein. Cited authors provided evidence that in capsaicin-fed mice with Alzheimer's disease, an enzyme alpha-secretase was up-regulated which cleaved amyloid precursor protein within the beta-amyloid domain. This reduced beta-amyloid production [23]. The beneficial effects of capsaicin in Alzheimer's disease are shown in Figure 2 in simplified form.

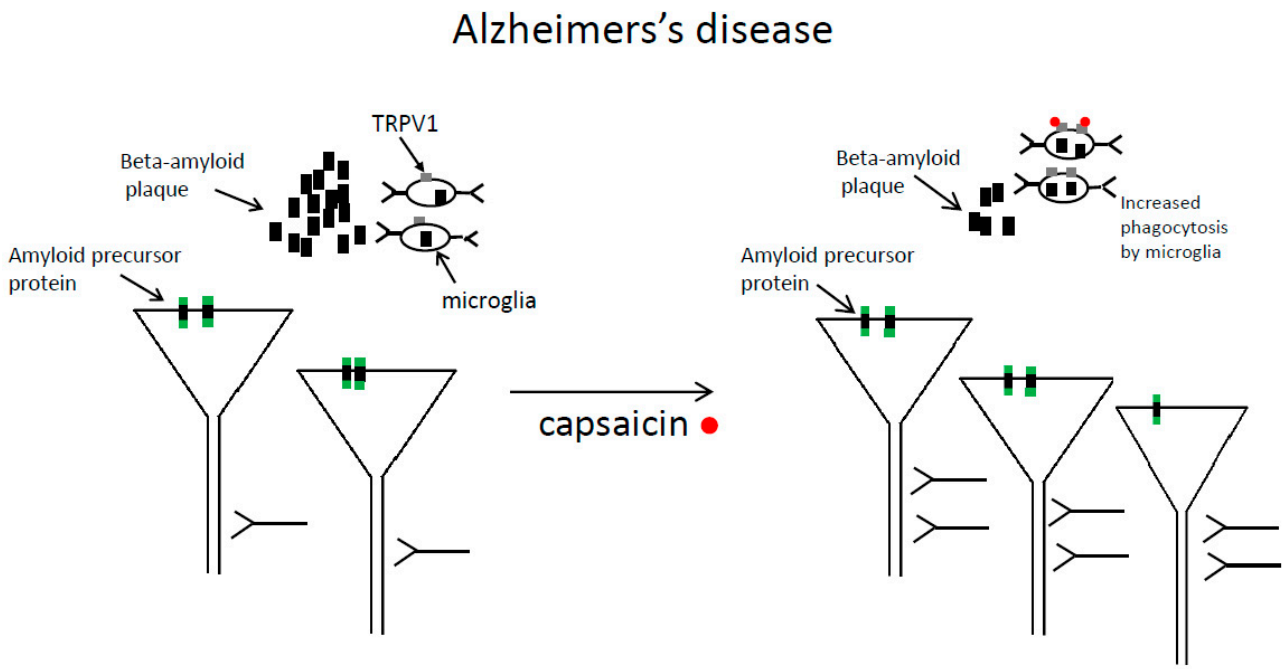

Figure 2. The beneficial effects of capsaicin in Alzheimer's disease are shown in simplified form. Capsaicin treatment increases the number of cortical pyramidal neurons and reduces synapse loss. Additionally, capsaicin decreases beta-amyloid deposition in the extracellular space. One of the mechanisms is that capsaicin treatment enhances phagocytosis of beta-amyloid plaques by microglial cells (capsaicin binds TRPV1 receptors expressed on microglial cells). Left panel—before capsaicin treatment, right panel—after capsaicin treatment.

Long-term potentiation (LTP) is the persistent increase in synaptic strength evoked by high-frequency synaptic stimulation. In other words, the rising slope and/or maximal amplitude of excitatory postsynaptic potential is increased after high-frequency synaptic stimulation. This phenomenon is often recorded in hippocampal and cortical slices obtained

from animals. Long-term potentiation (LTP) is the electrophysiological correlate of certain forms of memory. Consequently, LTP is impaired in Alzheimer's disease [24,25].

In one study, pathological changes resembling Alzheimer's disease were induced by intracerebral microinjection of beta-amyloid in mice. Electrophysiological experiments showed impaired hippocampal long-term potentiation (LTP) whereas behavioral experiments revealed impaired spatial memory and learning. These alterations were accompanied by a decreased number of synapses in beta-amyloid-treated mice as assessed by electron microscopy techniques. The authors reported that intraperitoneal capsaicin (1 mg/kg) improved spatial memory, enhanced LTP, and reduced synapse loss in tested animals [24].

In a different publication, it was found that intraperitoneal administration of TRPV1 agonist capsaicin (1 mg/kg) reduced spatial learning and memory impairments in a mouse model of Alzheimer's disease [25]. The authors also reported that genetic upregulation of TRPV1 receptors reduced beta-amyloid deposition in the same model of Alzheimer's disease. Similarly, to the study by Chen and colleagues [24], histological and behavioral experiments were confirmed by electrophysiological studies. The authors found that genetic upregulation of TRPV1 receptors ameliorated hippocampal long-term potentiation (LTP) impairment in mice with Alzheimer's disease. The authors also presented evidence that TRPV1 upregulation reduced LTP impairment by inhibiting AMPA receptor endocytosis [25].

Neuronal network gamma oscillations are correlated with cognitive processes. These EEG patterns decrease in Alzheimer's disease patients [26]. An interesting electrophysiological study showed protective effects of capsaicin (10 μM) against neuronal gamma oscillations impairment induced by beta-amyloid. Gamma oscillations were recorded using extracellular recordings in hippocampal slices. The authors found that the application of beta-amyloid strongly reduced the amplitude of gamma oscillations and that this reduction was almost abolished in the presence of capsaicin. This positive effect of capsaicin was absent in TRPV1 knock-out mice. Additionally, the co-application of TPV1 antagonist with capsaicin did not protect gamma oscillations from beta-amyloid-induced impairment. Thus, the effect of capsaicin depended on TRPV1 channels [27].

One publication has suggested that capsaicin may deteriorate symptoms of Alzheimer's disease. In this study, the level of beta-amyloid was assessed in cell lines expressing amyloid precursor protein. It was found that the level of beta-amyloid increased after preincubation with capsaicin (10 μM). Moreover, capsaicin impaired the degradation of beta-amyloid [28]. The authors suggested the limitation of their study was that tested cells did not express TRPV1 channels and were incubated with a high concentration of capsaicin for quite a short period of time (24 h). On the other hand, beneficial dietary capsaicin intake may last many years and provides exposure to low concentrations of capsaicin. This may be an explanation for why the authors [28] presented different conclusions than many other studies, which suggested beneficial effects of capsaicin in Alzheimer's disease (see above).

3.2. Capsaicin Reduces the Risk of Alzheimer's Disease

It was also reported in both human and animal subjects that capsaicin reduces the risk of Alzheimer's disease. In western regions of China, chili peppers are more often consumed and there is a smaller number of people with dementia than in other regions where dietary capsaicin intake is lower [29]. It was found in healthy humans that a capsaicin-rich diet correlates with reduced levels of serum beta-amyloid which indicates a lower risk of Alzheimer's disease. In the same subjects, it was also found that a capsaicin-rich diet improved cognition [29]. In a different study, it was shown that dietary capsaicin (0.01% in a chow) reduced the risk of Alzheimer's disease in rats with type 2 diabetes, which is a risk factor for Alzheimer's disease. One of the mechanisms of this effect was that the capsaicin-rich diet reduced tau protein phosphorylation in the hippocampus of diabetic rats. Additionally, the authors found that dietary capsaicin decreased blood glucose concentration in diabetic rats which suggests that this compound may be beneficial in treating diabetes [30].

The publications presented above combine to suggest that capsaicin exerts beneficial effects in animal models of Alzheimer's disease. Moreover, capsaicin intake may reduce the risk of Alzheimer's disease. Some of the mechanisms are presented in Figure 2 in simplified form.

4. Beneficial Effects of Capsaicin in Parkinson's Disease

The main symptoms of Parkinson's disease are tremor, rigidity, and bradykinesia, all of which are caused by the degeneration of tyrosine hydroxylase positive dopaminergic neurons in the substantia nigra. Consequently, there is less dopamine in the striatum because substantia nigra neurons project to the striatum. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and its active metabolite MPP+ (1-methyl-4-phenylpyridinium), are toxic chemical compounds that cause neurodegeneration of substantia nigra neurons. Therefore, MPTP and MPP+ treated animals are used as models of Parkinson's disease [31,32].

Different interconnected mechanisms cause substantia nigra neurodegeneration in Parkinson's disease, for example, alpha-synuclein aggregation, mitochondrial dysfunction, and microglial activation [31,32]. It was found that activated microglia contribute to the neurodegeneration process in the substantia nigra by producing oxidants and proinflammatory cytokines [31].

It was repeatedly found that capsaicin reduced neurodegeneration and motor impairment in animal models of Parkinson's disease [12,31,33–36]. This compound exerted beneficial effects on Parkinson's disease by decreasing microglial activation and reducing neuroinflammation [12,31,35,37]. These findings are summarized in Figure 3.

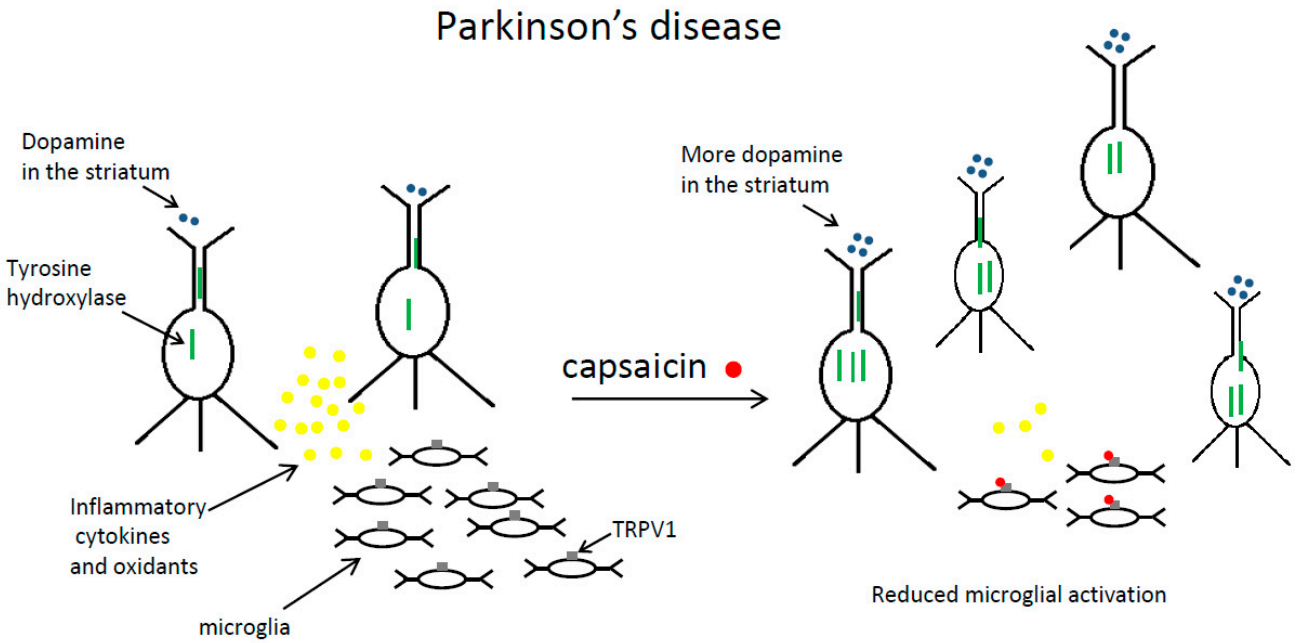

Figure 3. The beneficial effects of capsaicin in Parkinson's disease are shown in simplified form. Capsaicin administration increases the number of dopaminergic neurons in the substantia nigra and the expression of tyrosine hydroxylase in these neurons. Consequently, there is more dopamine in the striatum. Moreover, after capsaicin treatment, microglial cells in the substantia nigra produce fewer oxidants and inflammatory cytokines. Left panel—before capsaicin treatment, right panel—after capsaicin treatment.

In an MPTP mouse model of Parkinson's disease, it was found that intraperitoneal application of capsaicin (0.5 mg/kg) increased the number of dopaminergic (tyrosine hydroxylase positive) neurons in the substantia nigra. This effect was dependent on TRPV1 channels. The authors also found that capsaicin improved motor impairment and suggested that one of the mechanisms of these beneficial effects was that this compound decreased

the production of reactive oxygen species and proinflammatory cytokines (TNF-α and IL-β) by activated microglia [31]. Similar results were obtained by a different laboratory in a lipopolysaccharide rat model of Parkinson's disease. The authors found that intraperitoneal capsaicin (1 mg/kg) reduced neurodegeneration in the substantia nigra. In the presence of the tested compound, microglial cells shifted from a proinflammatory to an anti-inflammatory state and produced fewer oxidants and proinflammatory cytokines (IL-1β and IL-6). These neuroprotective effects were dependent on TRPV1 channels because they were reduced after administration of a selective TRPV1 inhibitor, capsazepine [35].

Not only microglial cells but also astrocytes (a different type of glial cell) are influenced by capsaicin in Parkinson's disease [36]. It was found in an MPP+ animal model that capsaicin applied intraperitoneally (1 mg/kg; a single injection/day for 7 days) activated TRPV1 receptors on astrocytes in the substantia nigra. TRPV1 receptors activation enhanced the production of a ciliary neurotrophic factor by astrocytes which increased tyrosine hydroxylase activity in the substantia nigra and dopamine levels in the striatum [36]. Capsaicin also caused behavioral recovery in MPP+ animals. In a different study from the same laboratory, it was found in the same model of Parkinson's disease that activated microglia produced fewer reactive oxygen species in the substantia nigra after intraperitoneal administration of capsaicin (1 mg/kg). Microglia-derived oxidative stress was reduced by a ciliary neurotrophic factor produced by capsaicin-stimulated astrocytes in the substantia nigra [12]. This effect of capsaicin reduced neurodegeneration and decreased motor impairment in MPP+ rats.

Alpha-synuclein deposition is an important feature of Parkinson's disease [32]. A protective role of dietary capsaicin (20, 40, 80 and 100 μM for 24 days) against Parkinson's disease was reported in flies expressing human alpha-synuclein. It was found that capsaicin increased dopamine content, reduced oxidative stress markers, and enhanced free radical scavenging potential in brains obtained from flies with Parkinson's disease [38].

To summarize, capsaicin treatment reduces neurodegeneration and improves behavioral outcomes in different animal models of Parkinson's disease. The important mechanism of this effect is that this compound reduces oxidants and proinflammatory cytokines production by activated microglia. This process is shown schematically in Figure 3.

5. Effects of Capsaicin in Animal Models of Epilepsy

Epilepsy is the most prevalent chronic neurological disease. The main symptoms are generalized or focal seizures. The backbone of therapy is antiseizure medications which help control symptoms, acting mainly through influencing voltage-gated sodium channels or gamma-aminobutyric acid pathways [39–41]. One in four epilepsy cases is resistant to currently available pharmacologic therapy and sadly, drug-resistant epilepsy is more prevalent among children [42]. Numerous efforts are made to design new pharmaceuticals, with 30 drug candidates currently at the preclinical or clinical stage [43], however, their safety profile is yet to be established.

The literature on capsaicin effects in epilepsy is sparse and, at least at first glance, appears conflicting because both pro- and antiepileptic mechanisms of action are described [44–46]. Most of the studies focused on the effects of capsaicin on the TRPV1 channels. Calcium ions are widely believed to play a major role in epilepsy pathogenesis [47,48] and, therefore, TRPV1 channels, with their high permeability to calcium ions, are an attractive target for drug action. Activation of the TRPV1 channel also leads to membrane depolarization and to an increase in glutaminergic activity [49,50]. Moreover, some studies showed that the activation of TRPV1 channels decreases GABA release [51]. All these mechanisms suggest that TRPV1 activation could have proepileptic effects, which has been shown in several studies [45,52].

Pentylenetetrazol (PTZ) potently increases neuronal excitability by inhibiting GABA receptors/channels. It was found that injections of this compound evoked seizures in higher percentages of animals after intra-cerebrovascular application of capsaicin (1 or 10 μg/mouse). This effect was abolished by pretreatment with a synthetic TRPV1 antago-

nist, capsazepine. Moreover, intra-cerebrovascular application of TRPV1 antagonist alone protected against PTZ-induced seizures [52]. Different authors studied the effects of capsaicin in vivo in acute rat models of temporal lobe epilepsy. Epileptic activity was evoked by a stimulating electrode in the hippocampal perforant path. The recording electrode measuring the extracellular potential was placed in the hippocampal formation. The authors reported that intraperitoneal application of capsaicin (10 mg/kg) increased epileptic activity. Capsazepine, applied without capsaicin, showed antiepileptic effects which indicated that TRPV1 receptors were involved [45]. This finding also suggested that in hippocampal neurons, TRPV1 receptors/channels were tonically active under epileptic conditions.

In a different study, in vitro patch-clamp recordings of excitatory postsynaptic currents (EPSCs) were conducted in hippocampal slices in a proepileptic extracellular solution containing no magnesium and a GABA antagonist—bicuculline. The experiments showed that capsaicin (1–10 μM) enhanced the frequency of EPSCs in slices obtained from mice with pilocarpine-induced epilepsy compared to control animals. The proepileptic action of capsaicin was mediated via increased glutamate release. In the same study, using the Western blotting technique, it was found that the expression of TRPV1 protein was increased in hippocampal neurons obtained from epileptic mice. The authors concluded that capsaicin acted on newly expressed TRPV1 channels in presynaptic axons which enhanced glutamate release in epileptic mice [53]. Similarly, different authors reported an increased expression of TRPV1 receptors in hippocampal pyramidal neurons in rats with pilocarpine-induced epilepsy [54].

In another study, capsaicin (10 μM and 100 μM) enhanced epileptiform activity in hippocampal slices in vitro, with epileptiform events evoked in the presence of a potassium channel inhibitor 4-aminopyridine (4-AP). In the same in vitro preparation, TRPV1 antagonist capsazepine applied without capsaicin suppressed epileptic activity. This result suggested a tonic activation of TRPV1 channels in hippocampal neurons under epileptic conditions. The authors also conducted in vivo recordings and obtained similar conclusions. Capsazepine, a TRPV1 antagonist, when applied subcutaneously, inhibited epileptic activity evoked by intrahippocampal administration of 4-AP. The recording electrode was placed in the hippocampus [55].

The above-mentioned studies suggested that TRPV1 channels activation in hippocampal neurons is involved in the proepileptic action of capsaicin. However, TRPV1-independent antiepileptic effects of capsaicin were reported in two in vitro studies in cortical neurons. In our previous publication, we recorded ictal epileptic events lasting more than 100 s using the patch-clamp technique in zero magnesium solution containing 4-AP in prefrontal cortex pyramidal neurons (Figure 4). Additionally, we recorded interictal epileptiform events lasting less than 3 s. Capsaicin (60 μM) potently inhibited both types of epileptic events [3]. We hypothesized that sodium channels, and not TRPV1 channels, were involved in the antiepileptic action of capsaicin, as this compound inhibited voltage-gated sodium channels and action potentials in cortical neurons in our study [3]. Such effects are exerted by many antiepileptic drugs and drug candidates [56–58]. Different authors also studied the effects of capsaicin in neocortical pyramidal neurons in vitro. Patch-clamp recordings showed that capsaicin inhibited epileptiform events evoked by applying a proepileptic solution containing GABA receptor antagonist gabazine. The authors also reported that capsaicin (25 μM) reduced the number of action potentials and decreased the maximal amplitude of single-action potential which indicated that the inhibition of voltage-gated sodium currents may have been involved in the antiepileptic action of capsaicin [44].

Thus, in the in vitro studies postulating antiepileptic effects of capsaicin, including our study, epileptiform events were induced in cortical neurons and a TRPV1-independent mechanism of action was most likely. On the other hand, the influence of capsaicin on TRPV1 channels expressed in hippocampal neurons consistently showed the proepileptic effects of this compound (Figure 4). Antiepileptic effects of TRPV1 antagonist capsazepine were also described in hippocampal neurons [45,55]. It can be hypothesized that in certain brain areas such as the hippocampus, proepileptic actions of capsaicin prevail

whereas, in different brain areas such as the cerebral cortex, antiepileptic actions of capsaicin predominate.

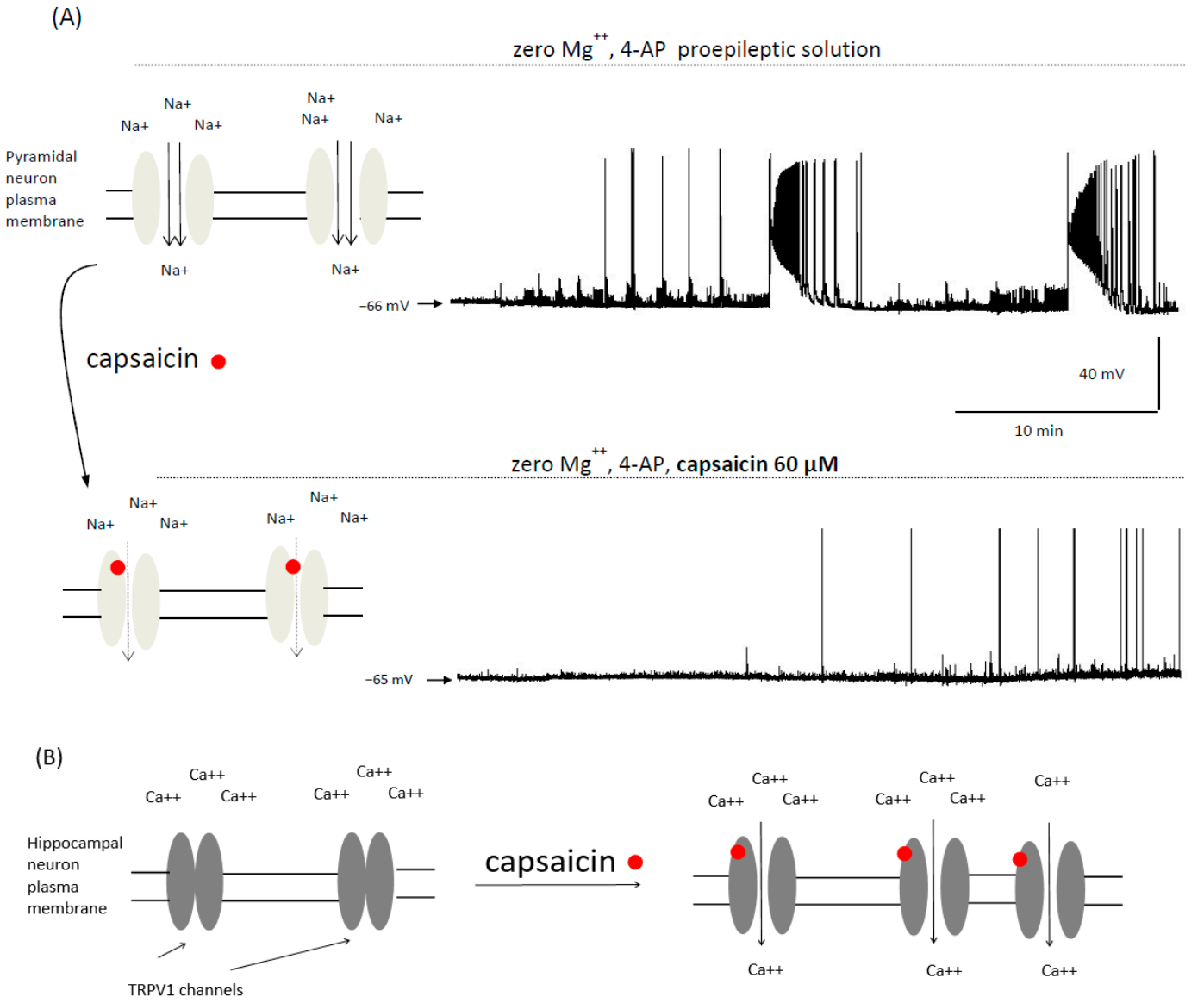

Figure 4. Animal studies show that capsaicin exerts both anti- and proepileptic effects. (**A**)—the top panel shows a schematic drawing of voltage-gated sodium channels in the plasma membrane of a brain cortical neuron. Example epileptic events recorded from a cortical neuron in vitro are also shown. A patch-clamp recording was made in proepileptic extracellular solution containing zero magnesium and potassium channels inhibitor 4-AP. The bottom panel shows that capsaicin potently inhibits epileptic activity [3]. The blockading of sodium channels by capsaicin removes depolarization and contributes to the antiepileptic effect of capsaicin. Please note that a number of other channels contribute to the generation of epileptic seizures such as calcium channels which may also be blocked by capsaicin. The recordings shown in Figure 4A were presented in our previous publication [3]. (**B**)—schematic drawing showing that capsaicin exerts proepileptic effects by the opening of calcium-permeable TRPV1 receptor/channels in hippocampal neurons [53,55]. Some of these channels are newly expressed under epileptic conditions. Calcium inflow causes depolarization of the membrane potential and exerts a proepileptic effect. If this process occurs in presynaptic axons, glutamate release is enhanced which may evoke seizures.

Indeed, it was reported that capsaicin applied subcutaneously (1 mg/kg) decreased behavioral seizures induced by intraperitoneal kainic acid injections in mice [59]. Capsaicin

acted on different brain areas because systemic application of kainic acid induces epileptic activity in different brain regions [60]. It may be hypothesized that capsaicin suppressed seizures because the antiepileptic actions of capsaicin were more pronounced than the proepileptic effects [59]. The authors did not conduct experiments aiming to indicate a specific molecular target responsible for the antiepileptic effects of capsaicin.

To summarize, both proepileptic and antiepileptic effects of capsaicin were described in animal models. This is shown in Figure 4 in simplified form. Studies assessing the effects of dietary capsaicin in human epileptic subjects are, therefore, needed.

6. Stroke

Stroke is a common, life-threatening neurological condition. There are two types of stroke: ischemic and hemorrhagic. In the first type, a blood clot forms in an atherosclerotic cerebral artery causing inadequate blood flow and ischemia in the brain area supplied by the blocked artery. Consequently, thrombolytic agents are often used to treat acute ischemic stroke. In the second type of stroke, a certain brain area is damaged because a rupture of a cerebral artery occurs which causes bleeding into the brain. Ischemic stroke is more frequent than hemorrhagic stroke [61]. A low cholesterol diet is essential in preventing ischemic stroke because atherosclerotic plaques that narrow cerebral arteries are mainly composed of cholesterol. There is a variety of symptoms in acute stroke and many serious long-term complications such as motor impairment, speech disorders, and dysphagia. The common animal model of ischemic stroke is cerebral artery occlusion followed by reperfusion [61].

6.1. Beneficial Effects of Capsaicin in Animal Models of Stroke

There are a number of publications showing the neuroprotective effect of capsaicin in experimental models of stroke [62,63]. It was found in a middle cerebral artery occlusion/reperfusion model in rats, that capsaicin (1 nmol or 3 nmol) injected into the peri-infarct area decreased infarct volume and improved neurological deficits. The authors conducted cell culture experiments and proposed that capsaicin (3 μM and 10 μM) caused TRPV1 dependent downregulation of NMDA receptors in cortical neurons, which reduced calcium inflow through NMDA receptors and consequently decreased excitotoxicity which contributes to cell death during stroke [62]. It was also found in Mongolian gerbils that capsaicin decreased neuronal cell death caused by brain hypoxia which was induced by internal carotid occlusion for 10 min. Capsaicin (0.01, 0.025, 0.05, 0.2 and 0.6 mg/kg) was injected subcutaneously 5 min after recirculation. The authors hypothesized that capsaicin provided neuroprotection by desensitizing neuronal TRPV1 channels which reduced TRPV1-mediated calcium inflow and decreased excitotoxicity [64].

Capsaicin exerts beneficial effects in stroke models not only by enhancing neuroprotection but also by influencing cerebral vasculature. In one study, the effects of the intraperitoneal application of capsaicin (0.2 mg/kg or 2.0 mg/kg) were assessed in young rats in carotid artery occlusion followed by a global hypoxia stroke model. It was found that capsaicin pretreatment reduced infarct size. The authors suggested that capsaicin treatment caused better oxygenation of brain tissue because myogenic autoregulation of cerebral blood flow was improved after the application of the tested compound [63]. Moreover, it was reported that dietary capsaicin (0.02% in a chow) delays the onset of stroke in stroke-prone rats with hypertension. The authors presented evidence that capsaicin activated TRPV1 receptors/channels expressed in the endothelium of cerebral arteries. Activation of TRPV1 channels increased the activity of endothelial nitric oxide synthase which enhanced the production of relaxing factor nitric oxide. Increased synthesis of nitric oxide enhanced relaxation of the cerebral arteries and prevented stroke [8].

6.2. Capsaicin Is Helpful in Reducing Stroke Complications in Humans

Dysphagia is a common complication after stroke [65]. It was shown that capsaicin improves the swallowing function in stroke patients presenting clinical signs of dysphagia.

Capsaicin (150 μM) was applied to drinking water. Additionally, oropharyngeal mucosa was touched with a swab soaked with capsaicin. The authors speculated that capsaicin activated TRPV1 channels in sensory receptors in the oropharyngeal mucosa which improved sensory input to the swallowing center [65]. In a different study, the swallowing function was compared in two groups of patients with dysphagia after stroke. In the first group, the palate and tongue were stimulated twice a day for several weeks with ice made of normal saline. In the second group of patients, the same stimulation was performed with the exception that the ice was made of saline with capsaicin (150 μM). Swallowing function improved in both groups, but the effect was significantly stronger in the capsaicin-treated patients [66].

In a randomized trial, the effects of oral capsaicin (10 μM) dissolved in tomato juice were compared with transcutaneous sensory electrical stimulation in patients suffering from dysphagia. Both therapies improved swallowing, however, the response rate was higher in patients treated with oral capsaicin. The dysphagia was caused by stroke, advanced age, or other diseases [67]. In a different randomized trial, the same authors assessed the effects of oral capsaicin and other neurorehabilitation strategies on swallow response and motor cortex excitability in patients with post-stroke dysphagia. Although capsaicin (10 μM dissolved in water) had no effect on the biomechanics of swallowing (neither did other interventions), it significantly enhanced pharyngeal and primary motor cortex excitability. Capsaicin had the strongest effects out of all investigated strategies. These results suggest that capsaicin could rapidly induce functional changes in the pharyngeal motor cortex responsible for swallowing. No adverse events of capsaicin were observed [68]. There is currently a phase II clinical CADYS study underway (Capsaicin for Post-stroke Dysphagia) which randomizes stroke patients with dysphagia to receive either a 1% oral solution of capsaicin or a placebo. The assessment of the swallowing function will be performed with standardized tests and the results should be available in 2023 (number of clinical trial NCT04470752).

The clearance of airway secretions is often impaired after stroke. It was found that nebulized capsaicin (0.49 μM and doubling in dose up to a maximum of 1000 μM) enhanced coughing in a group of non-tracheotomized stroke patients [69]. Moreover, it was reported that capsaicin nebulization (62.5 μM) combined with routine care enhanced sputum excretion in patients tracheotomized after a hemorrhagic stroke. Capsaicin treatment, however, did not significantly improve cough function [70].

Different authors have studied the potential of capsaicin to increase cerebral blood flow, a feature that could be helpful in the setting of ischemic stroke. Capsaicin was tested on healthy volunteers with doses ranging from 33 to 165 μM. During and after the study, there were no side effects. All the tested doses showed the same pattern, which consisted of an increase in the middle cerebral artery mean velocity and a reduction in the middle cerebral artery pulsatility index. This result suggests that capsaicin enhanced cerebral blood flow, which warrants further studies in stroke patients [71].

In summary, it was found in animal stroke models that capsaicin reduced the infarct area and improved neurological deficits. There are few clinical studies that have suggested positive effects of capsaicin in the treatment of stroke complications such as dysphagia.

7. Antidepressant Effects of Capsaicin

The main symptoms of depression are depressed mood, slow thinking, and suicidal tendencies. Several drugs are administered to alleviate the symptoms of this disease such as serotonin reuptake inhibitors. It has also been shown that dietary interventions are helpful in reducing depressive symptoms [14,72]. Immobilization stress or lipopolysaccharide injections are often used as models of depression in animal studies [14,73]. The severity of depressive symptoms and the effectiveness of antidepressive drugs are evaluated in the forced swimming test and tail suspension test [73–75]. These tests are used in animal models of depression and in healthy animals because healthy subjects present depressive symptoms under stressful situations such as forced swimming [74].

Several reports have shown the antidepressant effects of capsaicin in animal models. In one interesting study, mice were fed a chow containing capsaicin (0.005% for four months) and, after that, depressive symptoms were evoked by lipopolysaccharide injections. Behavioral tests such as a forced swimming test and tail suspension test showed that depressive symptoms were less pronounced in animals on a capsaicin-rich diet as compared to control. The authors also found that lipopolysaccharide injections changed the composition of gut microbiota and decreased serum serotonin levels. Importantly, these changes were partially reversed by dietary capsaicin [14].

It was reported that intraperitoneal administration of capsaicin produced an antidepressant effect in rats and mice. The authors assessed the antidepressant effects of capsaicin in forced swimming tests [73–75]. Interestingly, capsaicin not only exerted antidepressive effects but also enhanced the effectiveness of commonly used antidepressants. It was found that low, subthreshold doses of capsaicin (1 pg/kg, 1 ng/kg, and 0.001 mg/kg, intraperitoneally) combined with a subthreshold dose of amitriptyline induced an antidepressant effect [74]. It was also found that coadministration of capsaicin (0.002 mg/kg, intraperitoneally) and selective serotonin reuptake inhibitor citalopram exerted synergistic antidepressive effects in rats. Moreover, combined administration of the two compounds reduced side-effects of citalopram such as impairment of spatial memory and learning [75]. Different authors found that not only capsaicin (0.1, 1 and 2.5 mg/kg, intraperitoneally) but also a different TRPV1 agonist, olvanil, reduced depressive behavior caused by nicotine treatment or immobilization stress. The authors suggested that TRPV1 channels are involved in the antidepressant effect of capsaicin and olvanil [73]. It was also reported that intracerebroventricular administration of capsaicin (10 µg/mouse) reduced depressive behavior caused by amphetamine withdrawal. The effect was shown to be dependent on TRPV1 channels [76].

Stress may exacerbate symptoms of depression and other psychiatric disorders [77]. Long-term potentiation (LTP) is activity-dependent synaptic plasticity which is an electrophysiological correlate of certain forms of memory such as spatial memory [24,25,77]. It was found that acute stress evoked by placing animals on an elevated platform suppressed LTP and that this effect was prevented by capsaicin. LTP was recorded in vitro in hippocampal slices obtained from young, stressed rats. Capsaicin (1 mM) was applied in vitro before LTP induction. In the same report, it was found that acute stress impaired spatial memory retrieval. This negative effect was prevented by intrahippocampal infusion of capsaicin (1 mM). Thus, in vitro electrophysiological experiments correlated with behavioral experiments. The beneficial effects of capsaicin were mediated by TRPV1 channels [77]. The authors suggested that activating TRPV1 channels may reverse stress-evoked spatial memory impairment.

Other reports, however, have suggested that inhibition of TRPV1 channels reduced the effects of stress which is in contrast to the study presented above. For example, it was found that injections of the TRPV1 antagonist capsazepine to midbrain periaqueductal grey matter reduced behavioral effects of stress in rats exposed to a predator [78].

In essence, capsaicin reduces depressive behaviors in animal models as shown by behavioral tests. Moreover, studies show that capsaicin may enhance the anti-depressive effects of commonly used antidepressants.

8. Beneficial Effects of Capsaicin in the Treatment of Headaches

Migraine is a common disorder that is characterized by disabling headaches and many other symptoms such as increased sensitivity to light and psychiatric disturbances. A migraine is either episodic or chronic [79]. A cluster headache is a different type of headache that is shorter in duration than a migraine attack [79–81]. It was found that calcitonin gene-related peptide (CGRP), a potent vasodilator, contributes to the pathogenesis of migraine and cluster headaches. This peptide is released from trigeminal fibers innervating cerebral vasculature and causes the dilation of cerebral blood vessels which evokes pain. Consequently, CGRP and CGRP receptors are new therapeutic targets for drugs used in

the treatment of migraine and cluster headaches [79]. The beneficial effects of capsaicin in these two types of headaches were previously described [82–85].

People suffering from migraines may experience pain at pressure points on the scalp arteries between attacks [85]. In a small clinical study, it was found that capsaicin jelly (0.1%) applied to painful arteries reduced their tenderness in the interictal period in the majority of patients. Moreover, the same method of capsaicin application reduced the severity of migraine attacks [85]. It was also shown that intranasal application of capsaicin (10 mM) reduced pain intensity in chronic migraine patients compared to placebo treatment. Burning sensations evoked by capsaicin were well-tolerated. Moreover, they decreased after each consecutive capsaicin application because the effect was desensitized. In placebo-treated patients, burning sensations of decreasing intensity were evoked by repeated intranasal application of citric acid solution (its pH was increased after each application) [82].

It was also shown that intranasal capsaicin (10 mM) application exerted pain-relieving effects in cluster headache patients. The authors reported that intranasal application of capsaicin on the painful side of the head (ipsilateral side) gave better results than capsaicin application on the contralateral side [80].

In a different study, it was found that intranasal application of capsaicin (10 mM) in cluster headache patients reduced the frequency of pain attacks. Cerebral circulation was likely involved in this effect because middle cerebral arteries were narrowed in a group of healthy humans after a single application of capsaicin, measured with a Doppler device. In the same report, it was shown in animal studies that intranasal capsaicin reduced the immunoreactivity to substance P and CGRP in sensory fibers innervating nasal mucosa. The authors suggested that capsaicin may exert the same effect in cluster headache patients, which could contribute to the pain-relieving effects of the tested compound [81].

To summarize, few clinical studies show pain-relieving effects of topical capsaicin in migraine and cluster headaches. One of the limitations of these studies, however, was the small number of patients involved.

9. Concluding Remarks

Several recent studies performed on animals assessed the effects of capsaicin in brain disorders using histological, behavioral, genetic, and electrophysiological techniques. Most publications described the effects of capsaicin in neurodegenerative diseases, stroke, and epilepsy. It was reported that capsaicin decreased neurodegeneration in Alzheimer's and Parkinson's diseases. Moreover, it was found that capsaicin pretreatment reduced the area of infarction in experimental models of stroke. In the case of epilepsy, both beneficial and adverse effects were described. The tested compound exerted proepileptic effects by opening TRPV1 channels, and antiepileptic effects by inhibiting voltage-gated sodium channels. Capsaicin reduced symptoms of depression in animal models. There are also human studies suggesting the analgesic effects of topical capsaicin in different kinds of headaches. Capsaicin was helpful in treating post-stroke dysphagia. Moreover, nebulized capsaicin enhanced airway clearance in stroke patients.

Capsaicin is a safe compound because it is often consumed in different countries. Spices containing capsaicin are relatively cheap. We suggest that chili peppers should be added to meals regularly. Considering that, besides capsaicin, they contain precursors of vitamin A, vitamin C, and other antioxidants [86]. Chili peppers may be helpful not only in neurological but also in cardiovascular and oncological diseases [6,7,12,13]. Chili peppers may also help obese people to lose weight [5,86]. People with epilepsy should eat them with caution, however, because some studies have shown that capsaicin may enhance epileptic symptoms.

Without a doubt, the health effects of capsaicin are mostly positive. Further preclinical research aiming to elucidate the effects of capsaicin in brain disorders is required. For example, modifying the chemical structure of capsaicin may improve the pharmacological properties of this compound. More studies involving larger groups of patients are needed to assess the full therapeutic potential of capsaicin in brain disorders.

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8. Podsumowanie i wnioski

W ramach niniejszej pracy doktorskiej przeprowadzono badania nad wpływem trzech substancji – walproinianu, kapsaicyny i guanfacyny – na aktywność epileptyczną w korze przedczołowej u szczurów. Badania te przyczyniły się do głębszego zrozumienia mechanizmów farmakologicznych wpływających na krótkotrwałą aktywność epileptyczną. Wykazano, że wszystkie trzy badane substancje hamują krótkotrwałe aktywności epileptyczne in vitro, a kapsaicyna hamuje również długotrwałe aktywności epileptyczne.

Szczegółowe wnioski wynikające z pracy doktorskiej to:

- Krótka aktywność epileptyczna wywołana płynem bez magnezu z podwyższonym stężeniem potasu w korze przedczołowej jest zależna od receptorów NMDA.
- Walproinian, kapsaicyna i guanfacyna redukują pobudliwość neuronalną w neuronach kory przedczołowej.
- Walproinian redukuje krótkotrwałą aktywność epileptyczną w neuronach kory przedczołowej co sugeruje możliwość poprawy deficytów poznawczych.
- Kapsaicyna znacząco redukuje zarówno krótkie jak i długotrwałe aktywności epileptyczne w neuronach kory przedczołowej. W związku z tym jej struktura może być pomocna w konstrukcji nowych leków przeciwpadaczkowych. Hamowanie kanałów sodowych może być mechanizmem odpowiedzialnym za działanie przeciwpadaczkowe kapsaicyny.
- Guanfacyna redukuje krótkotrwałą aktywność epileptyczną w neuronach kory przedczołowej prawdopodobnie poprzez blokowanie potencjałozależnych kanałów sodowych. Efekt ten może przyczyniać się do korzystnego działania guanfacyny w ADHD.

Oświadczenia współautorów publikacji

Warszawa, 11.01.2024
(miejscowość, data)

Bartłomiej Szulczyk
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. **"Valproic acid potently inhibits interictal-like epileptiform activity in prefrontal cortex pyramidal neurons"** oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

zaplanowanie eksperymentów, przeprowadzenie eksperymentów, opracowanie statystyczne danych eksperymentalnych, przygotowanie manuskryptu

Mój udział procentowy w przygotowaniu publikacji określam jako 50 %.

Wkład Michała Pasierskiego w powstawanie publikacji określam jako 45 %,

obejmował on: przeprowadzenie eksperymentów, przygotowanie manuskryptu

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Pasierskiego

(imię i nazwisko kandydata do stopnia)

B. Szulczyk

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Michał Pasierski Warszawa 11.01.2024

OŚWIADCZENIE

Jako współautor pracy pt. “Valproic acid potently inhibits interictal-like epileptiform activity in prefrontal cortex pyramidal neurons” oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

przeprowadzenie eksperymentów, przygotowanie manuskryptu

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Mój udział procentowy w przygotowaniu publikacji określam jako 45%.

Michał Pasierski

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 11.01.2024
(miejscowość, data)

Ewa Nurowska
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. **"Valproic acid potently inhibits interictal-like epileptiform activity in prefrontal cortex pyramidal neurons"** oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

opracowanie statystyczne danych eksperymentalnych

Mój udział procentowy w przygotowaniu publikacji określam jako 5 %.

Wkład Michała Pasierskiego w powstawanie publikacji określam jako 45 %,

obejmował on: przeprowadzenie eksperymentów, przygotowanie manuskryptu

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Pasierskiego

(imię i nazwisko kandydata do stopnia)

ADIUNKT
Katedra i Zakład Farmakoterapii i Opieki Farmaceutycznej
dr hab. Ewa Nurowska

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Michał Pasierski Warszawa 11.01.2024

OŚWIADCZENIE

Jako współautor pracy pt. “Capsaicin inhibits sodium currents and epileptiform activity in prefrontal cortex pyramidal neurons” oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

zaplanowanie eksperymentów, przeprowadzenie eksperymentów, opracowanie statystyczne danych eksperymentalnych, przygotowanie manuskryptu

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Mój udział procentowy w przygotowaniu publikacji określam jako 85%.

Michał Pasierski

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 11.01.2024
(miejscowość, data)

Bartłomiej Szulczyk
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. "**Capsaicin inhibits sodium currents and epileptiform activity in prefrontal cortex pyramidal neurons** " oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
zaplanowanie eksperymetów, przeprowadzenie eksperymentów, przygotowanie manuskryptu
Mój udział procentowy w przygotowaniu publikacji określam jako 15 %.
Wkład Michała Pasierskiego w powstawanie publikacji określam jako 85 %,
obejmował on: zaplanowanie eksperymentów, przeprowadzenie eksperymentów, opracowanie statystyczne danych eksperymentalnych, przygotowanie manuskryptu
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Pasierskiego
(imię i nazwisko kandydata do stopnia)

B. Szulczyk
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Michał Pasierski Warszawa 11.01.2024

OŚWIADCZENIE

Jako współautor pracy pt. Guanfacine inhibits interictal epileptiform events and sodium currents in prefrontal cortex pyramidal neurons oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

zaplanowanie eksperymentów, przeprowadzenie eksperymentów, opracowanie statystyczne danych eksperymentalnych, przygotowanie manuskryptu.

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Mój udział procentowy w przygotowaniu publikacji określam jako 80%.

Michał Pasierski

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 11.01.2024
(miejscowość, data)

Bartłomiej Szulczyk
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. "**Guanfacine inhibits interictal epileptiform events and sodium currents in prefrontal cortex pyramidal neurons**" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
zaplanowanie eksperymetów, przeprowadzenie eksperymentów, przygotowanie manuskryptu
Mój udział procentowy w przygotowaniu publikacji określam jako 15 %.
Wkład Michała Pasierskiego w powstawanie publikacji określam jako 80 %,
obejmował on: zaplanowanie eksperymentów, przeprowadzenie eksperymentów, opracowanie statystyczne danych eksperymentalnych, przygotowanie manuskryptu
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Pasierskiego
(imię i nazwisko kandydata do stopnia)

B. Szulczyk
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 11.01.2024
(miejscowość, data)

Weronika Kołba
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. "**Guanfacine inhibits interictal epileptiform events and sodium currents in prefrontal cortex pyramidal neurons**" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

przeprowadzenie eksperymentów

Mój udział procentowy w przygotowaniu publikacji określam jako 5 %.

Wkład Michała Pasierskiego w powstawanie publikacji określam jako 80 %,

obejmował on: zaplanowanie eksperymentów, przeprowadzenie eksperymentów, opracowanie statystyczne danych eksperymentalnych, przygotowanie manuskryptu

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Pasierskiego

(imię i nazwisko kandydata do stopnia)

Weronika Kołba

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Michał Pasierski Warszawa 11.01.2024

OŚWIADCZENIE

Jako współautor pracy pt. “Beneficial Effects of Capsaicin in Disorders of the Central Nervous System” oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

przygotowanie manuskryptu

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Mój udział procentowy w przygotowaniu publikacji określam jako 75%.

Michał Pasierski

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 11.01.2024
(miejscowość, data)

Bartłomiej Szulczyk
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. **"Beneficial Effects of Capsaicin in Disorders of the Central Nervous System"** oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

przygotowanie manuskryptu

Mój udział procentowy w przygotowaniu publikacji określam jako 25 %.

Wkład Michała Pasierskiego w powstawanie publikacji określam jako 75 %,

obejmował on: przygotowanie manuskryptu

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Pasierskiego

(imię i nazwisko kandydata do stopnia)

B. Szulczyk

.....

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Analiza bibliometryczna dorobku publikacyjnego

WARSZAWSKI
UNIWERSYTET
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BIBLIOTEKA UCZELNIANA

Nr referencyjny
BIBG/Punktacja/150/2024/DP

Warszawa, 03.04.2024

Sz. Pan
Michał Pasierski

ANALIZA BIBLIOMETRYCZNA PUBLIKACJI
PANA MICHAŁA PASIERSKIEGO,
WCHODZĄCYCH W SKŁAD CYKLU PUBLIKACJI STANOWIĄCYCH ROZPRAWĘ DOKTORSKĄ

Lp.	Opis bibliograficzny	Impact Factor	MNiSW
Artykuły			
1.	Szulczyk B, **Pasierski M**, Nurowska E. Valproic acid potently inhibits interictal-like epileptiform activity in prefrontal cortex pyramidal neurons. Neuroscience Letters. 2019;708:1-7 [rodzaj pracy: praca oryginalna]	2,274	70
2.	**Pasierski M**, Szulczyk B. Capsaicin inhibits sodium currents and epileptiform activity in prefrontal cortex pyramidal neurons. Neurochemistry International. 2020;135:1-10 [rodzaj pracy: praca oryginalna]	3,921	100
3.	**Pasierski M**, Szulczyk B. Beneficial Effects of Capsaicin in Disorders of the Central Nervous System. Molecules. 2022;27(8):1-16 [rodzaj pracy: praca poglądowa]	4,6	140
4.	**Pasierski M**, Kołba W, Szulczyk B. Guanfacine inhibits interictal epileptiform events and sodium currents in prefrontal cortex pyramidal neurons. Pharmacological Reports. 2023;75(2):331-341 [rodzaj pracy: praca oryginalna]	4,4	140
	Łącznie:	**15,195**	**450**
Książki			
1.	-		
Rozdziały w książkach			
1.	-		

p.o. DYREKTOR
Biblioteki Uczelnianej
Warszawskiego Uniwersytetu Medycznego
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BIBLIOTEKA UCZELNIANA

Nr referencyjny
BIBG/Punktacja/151/2024/DP

Warszawa, 03.04.2024

Sz. Pan
Michał Pasierski

ANALIZA BIBLIOMETRYCZNA CAŁOKSZTAŁTU DOROBKU PUBLIKACYJNEGO
PANA MICHAŁA PASIERSKIEGO
W POSTĘPOWANIU O NADANIE STOPNIA NAUKOWEGO DOKTORA

Lp.	Opis bibliograficzny	Impact Factor	MNiSW
I. Artykuły opublikowane w czasopismach naukowych lub w recenzowanych materiałach z konferencji międzynarodowych ujętych w aktualnym wykazie MNiSW[1]			
1.	Szulczyk B, **Pasierski M**, Nurowska E. Valproic acid potently inhibits interictal-like epileptiform activity in prefrontal cortex pyramidal neurons. Neuroscience Letters. 2019;708:1-7 [rodzaj pracy: praca oryginalna]	2,274	70
2.	**Pasierski M**, Szulczyk B. Capsaicin inhibits sodium currents and epileptiform activity in prefrontal cortex pyramidal neurons. Neurochemistry International. 2020;135:1-10 [rodzaj pracy: praca oryginalna]	3,921	100
3.	Kowalewski M, **Pasierski M**, Litwinowicz R, Zembala M, Piekuś-Słomka N, Tobota Z, Maruszewski B, Suwalski P, KROK Investigators. Multiple versus single arterial coronary arterial bypass grafting surgery for multivessel disease in atrial fibrillation. Seminars in Thoracic and Cardiovascular Surgery. 2021;33(4):974-983 [rodzaj pracy: praca oryginalna]	2,410	40
4.	Szulczyk B, **Pasierski M**, Gawlak M. Prefrontal cortex pyramidal neurons express functional Nav1.8 tetrodotoxin-resistant sodium currents. Clinical and Experimental Pharmacology and Physiology. 2022;49(3):350-359 [rodzaj pracy: praca oryginalna]	2,9	100

[1] Wykaz sporządzony zgodnie z przepisami wydanymi na podstawie art. 267 ust. 2 pkt 2 lit. b Ustawy z dnia 20 lipca 2018 r. - Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2022 r., poz. 574 z późn. zm.). Wykaz stanowi załącznik do komunikatu MNiSW z 5 stycznia 2024 r. w sprawie wykazu czasopism naukowych i recenzowanych materiałów z konferencji międzynarodowych.

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5.	Kowalewski M, **Pasierski M**, Finke J, Kołodziejczak M, Staromłyński J, Litwinowicz R, Filip G, Kowalówka A, Wańha W, Bławat P, Łoś A, Stefaniak S, Wojakowski W, Jemielity M, Rogowski J, Deja M, Jagielak D, Bartus K, Sierakowska K, Mariani S, Li T, Ravaux JM, Matteucci M, Ronco D, Jiritano F, Fina D, Martucci G, Meani P, Raffa GM, Malvindi PG, Lorusso R, Suwalski P, Thoracic Research Centre. Permanent pacemaker implantation after valve and arrhythmia surgery in patients with preoperative atrial fibrillation. Heart Rhythm. 2022;19(9):1442-1449 [rodzaj pracy: praca oryginalna]	5,5	140
6.	**Pasierski M**, Staromłyński J, Finke J, Litwinowicz R, Filip G, Kowalówka A, Wańha W, Kołodziejczak M, Piekuś-Słomka N, Łoś A, Stefaniak S, Wojakowski W, Jemielity M, Rogowski J, Deja M, Jagielak D, Bartus K, Mariani S, Li T, Matteucci M, Ronco D, Jiritano F, Fina D, Martucci G, Meani P, Raffa GM, Słomka A, Malvidni PG, Lorusso R, Zembala M, Suwalski P, Kowalewski M. Clinical Insights to Complete and Incomplete Surgical Revascularization in Atrial Fibrillation and Multivessel Coronary Disease. Frontiers in Cardiovascular Medicine. 2022;9:1-9 [rodzaj pracy: praca oryginalna]	3,6	40
7.	**Pasierski M**, Czarnecka K, Staromłyński J, Litwinowicz R, Filip G, Kowalówka A, Wańha W, Kołodziejczak M, Piekuś-Słomka N, Łoś A, Stefaniak S, Wojakowski W, Jemielity M, Rogowski J, Deja M, Jagielak D, Bartus K, Mariani S, Li T, Lorusso R, Suwalski P, Kowalewski M. Total arterial revascularization coronary artery bypass surgery in patients with atrial fibrillation. Polish Heart Journal / Kardiologia Polska. 2022;80(11):1119-1126 [rodzaj pracy: praca oryginalna]	3,3	100
8.	Kowalewski M, Wańha W, Litwinowicz R, Kołodziejczak M, **Pasierski M**, Januszek R, Kuźma Ł, Grygier M, Lesiak M, Kapłon-Cieślicka A, Reczuch K, Gil R, Pawłowski T, Bartuś K, Dobrzycki S, Lorusso R, Bartuś S, Deja MA, Smolka G, Wojakowski W, Suwalski P. Standalone left atrial appendage occlusion for thromboembolism prevention in nonvalvular atrial fibrillation Disease registry (SALAMANDER): protocol for a prospective observational nationwide study. BMJ Open. 2022;12(9):1-8 [rodzaj pracy: praca poglądowa]	2,9	100
9.	**Pasierski M**, Szulczyk B. Beneficial Effects of Capsaicin in Disorders of the Central Nervous System. Molecules. 2022;27(8):1-16 [rodzaj pracy: praca poglądowa]	4,6	140
10.	**Pasierski M**, Kołba W, Szulczyk B. Guanfacine inhibits interictal epileptiform events and sodium currents in prefrontal cortex pyramidal neurons. Pharmacological Reports. 2023;75(2):331-341 [rodzaj pracy: praca oryginalna]	4,4	140
11.	Szułdrzyński K, Kowalewski M, Jankowski M, Staromłyński J, Prokop J, **Pasierski M**, Chudziński K, Drobiński D, Martucci G, Lorusso R, Wierzba W, Zaczyński A, Król Z, Suwalski P. Effects of adding the second drainage cannula in severely hypoxemic patients supported with VV ECMO due to COVID-19-associated ARDS. Artificial Organs. 2023;47(10):1622-1631 [rodzaj pracy: praca oryginalna]	2,4	100

12.	Kowalewski M, Dąbrowski EJ, Kuźma Ł, Jasiński M, **Pasierski M**, Widenka K, Hirnle T, Deja M, Bartuś K, Lorusso R, Tobota Z, Maruszewski B, Suwalski P, KROK Investigators. Tricuspid intervention for less-than-severe regurgitation simultaneously with minimally invasive mitral valve surgery in patients with atrial fibrillation. Polish Heart Journal / Kardiologia Polska. 2023;81(10):990-997 [rodzaj pracy: praca oryginalna]	3,3	100
13.	Kowalewski M, **Pasierski M**, Makhoul M, Comanici M, Dąbrowski EJ, Matteucci M, Litwinowicz R, Kowalówka A, Wańha W, Jiritano F, Fina D, Martucci G, Raffa GM, Malvindi PG, Kuźma Ł, Suwalski P, Lorusso R, Meani P, Lazar H; Thoracic Research Centre. Topical vancomycin for sternal wound infection prophylaxis. A systematic review and updated meta-analysis of over 40,000 cardiac surgery patients. Surgery. 2023;174(5):1102-1112 [rodzaj pracy: praca oryginalna]	3,8	100
14.	Kowalewski M, Raffa GM, **Pasierski M**, Kołodziejczak M, Litwinowicz R, Wańha W, Wojakowski W, Rogowski J, Jasiński M, Widenka K, Hirnle T, Deja M, Bartus K, Lorusso R, Tobota Z, Maruszewski B, Suwalski P, KROK Investigators. Prognostic impact of preoperative atrial fibrillation in patients undergoing heart surgery in cardiogenic shock. Scientific Reports. 2023;13:1-11 [rodzaj pracy: praca oryginalna]	4,6	140
15.	Kowalewski M, **Pasierski M**, Kołodziejczak M, Litwinowicz R, Kowalówka A, Wańha W, Łoś A, Stefaniak S, Wojakowski W, Jemielity M, Rogowski J, Deja M, Bartuś K, Mariani S, Li T, Matteucci M, Ronco D, Massimi G, Jiritano F, Meani P, Raffa GM, Malvindi PG, Zembala M, Lorusso R, Cox JL, Suwalski P. Atrial fibrillation ablation improves late survival after concomitant cardiac surgery. The Journal of Thoracic and Cardiovascular Surgery. 2023;166(6):1656-1668.e8 [rodzaj pracy: praca oryginalna]	6,0	140
16.	**Pasierski M**, Batko J, Kuźma Ł, Wańha W, Jasiński M, Widenka K, Deja M, Bartuś K, Hirnle T, Wojakowski W, Lorusso R, Tobota Z, Maruszewski BJ, Suwalski P, Kowalewski M, KROK Investigators. Surgical ablation, left atrial appendage occlusion or both? Nationwide registry analysis of cardiac surgery patients with underlying atrial fibrillation. European Journal of Cardio-Thoracic Surgery. 2024;65(3):1-10 [rodzaj pracy: praca oryginalna]	3,4	100
17.	Suwalski P, Dąbrowski EJ, Batko J, **Pasierski M**, Litwinowicz R, Kowalówka A, Jasiński M, Rogowski J, Deja M, Bartus K, Li T, Matteucci M, Wańha W, Meani P, Ronco D, Raffa GM, Malvindi PG, Kuźma Ł, Lorusso R, Maesen B, La Meir M, Lazar H, McCarthy P, Cox JL, Rankin S, Kowalewski M. Additional bypass graft or concomitant surgical ablation? Insights from the HEIST registry. Surgery. 2024;175(4):974-983 [rodzaj pracy: praca oryginalna]	3,8	100
18.	Witkowski G, Szulczyk B, Nurowska E, Jurek M, **Pasierski M**, Lipiec A, Charzewska A, Dawidziuk M, Milewski M, Owsiak S, Rola R, Sienkiewicz Jarosz H, Hoffman-Zacharska D. Functional Characteristics of the Nav1.1 p.Arg1596Cys Mutation Associated with Varying Severity of Epilepsy Phenotypes. International Journal of Molecular Sciences. 2024;25(3):1-17 [rodzaj pracy: praca oryginalna]	5,6	140

Łącznie:	**68,705**	**1890**
II. Artykuły opublikowane przed 1.01.2019 r. w czasopismach ujętych w wykazie czasopism MNiSW z dnia 25.01.2017 r., o ile czasopismo uzyskało co najmniej 10 pkt.		
Łącznie:	-	-
III. Pozostałe artykuły		
Łącznie:	-	-
Łącznie (cz. I- III):	**68,705**	**1890**
IV. Monografie naukowe/rozdziały w monografiach wydane przez wydawnictwa ujęte w wykazie MEiN[2] lub jednostki organizacyjne podmiotów, których wydawnictwa są ujęte w tym wykazie		
Brak		
V. Pozostałe monografie lub rozdziały w monografiach		
Brak		
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Brak		

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[2] Wykaz sporządzony zgodnie z przepisami wydanymi na podstawie art. 267 ust. 2 pkt 2 lit. a Ustawy z dnia 20 lipca 2018 r. - Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2022 r., poz. 574 z późn. zm.). Wykaz ogłoszony komunikatem MEiN z dnia 22 lipca 2021 r. w sprawie wykazu wydawnictw publikujących recenzowane monografie naukowe.

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