UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

mgr Tomasz Bielawski

Chroniczny stres a funkcjonowanie poznawcze jednostki w zwierzęcym modelu traumy

Rozprawa na stopień doktora nauk medycznych

Promotor: prof. dr hab. n. med. Dorota Frydecka

Promotor pomocniczy: dr n. med. Bartłomiej Stańczykiewicz, prof. uczelni

Wrocław 2022

Składam serdeczne podziękowania prof. dr hab. Dorocie Frydeckiej, której wytrwałość w prowadzeniu badań naukowych jest dla mnie źródłem nieustającej inspiracji. Dziękuję dr. Bartłomiejowi Stańczykiewiczowi za pomoc i wsparcie w trakcie realizacji badań.

Spis treści

Streszczenie:

Doświadczenia traumatyczne są ważnym czynnikiem etiologicznym wielu zaburzeń psychicznych. Takie doświadczenia wpływają na objawy prezentowane przez pacjentów, między innymi pogarszając funkcje poznawcze osób, które ich zaznały. Doświadczenia traumatyczne, rozumiane jako ekspozycja na silny stres przekraczający zdolności radzenia sobie jednostki, mogą być spowodowane wieloma czynnikami, między innymi: zaniedbaniem opiekunów, znęcaniem i zastraszanie w miejscu pracy lub w rodzinie, ekspozycją na przemocą fizyczną, emocjonalną lub seksualną. Doświadczenia traumatyczne mogą być szczególnie szkodliwe, gdy przyjmują wymiar chroniczny, czyli długotrwały. Współczesna literatura poświęca coraz więcej uwagi zmianom neurobiologicznym zachodzącym pod wpływem traumy oraz stresu. W ostatnich latach pojawia się coraz więcej doniesień o epigenetycznych zmianach wywoływanych przez chroniczny stres i doświadczenia traumatyczne oraz o specyficznej, wyciszającej funkcji układu endokannabinoidowego, względem nadmiernego pobudzenia osi podwzgórze-przysadka-nadnercza (ang. hypothalamic-pituitary-adrenal axis, HPA). Literatura przedmiotu wykazuje negatywny wpływ doświadczeń traumatycznych na funkcje poznawcze zarówno w grupie pacjentów z zaburzeniami psychicznymi, jak i w grupie osób zdrowych. Testy oparte o uczenie się z nagród i kar (na przykład Probabilisitc Selection Task, PST), to proste narzędzia pozwalające badać funkcje poznawcze jednostki, szeroko wykorzystywane w badaniach ludzi oraz w modelach zwierzęcych. Neurobiologiczne podłoże uczenia się z nagród i kar jest dobrze opisane - dzięki temu wiemy, które części mózgu wykazują wzmożoną aktywnością podczas badania PST. Struktury te okazują się być istotnymi miejscami działania osi HPA, układu endokannabinoidowego oraz miejscami intensywnych zmian epigenetycznych inicjowanych przez stres. Istnieje potrzeba opracowania protokołu umożliwiającego badanie doświadczeń traumatycznych wywołanych przez ekspozycję na chroniczny stres, z wykorzystaniem modelu zwierzęcego, który daje znacznie więcej możliwości kliniczno-laboratoryjnych niż opisowy model ludzki. W literaturze przedmiotu większość badań przeprowadzana jest z wykorzystaniem jednorazowej ekspozycji na ostry stres (ang. acute stress), brakuje protokołów umożliwiających badanie uczenia się z nagród i kar podczas ekspozycji na stres. Opracowany w ramach pracy doktorskiej protokół różni się od innych, opisywanych w literaturze przedmiotu, wprowadzając chroniczną ekspozycję na zapach drapieżnika (12 dni) oraz codzienny pomiar uczenia się na podstawie nagród i kar za pomocą badania PST. W protokole wykorzystano także test behawioralny interakcji z nowym osobnikiem (ang. Social Interaction Test, SIT), badający wycofanie społeczne wywołane przez lęk.

Cele pracy:

Celem pracy było 1) opracowanie nowego protokołu badawczego na modelu zwierzęcym, pozwalającego badać konsekwencje traumy wywołanej przez chroniczną ekspozycją na stres,

2) zaprojektowanie i zbudowanie innowacyjnego urządzenia badającego uczenie się zwierząt na podstawie nagród i kar w sposób identyczny, jak badania PST u ludzi, z wykorzystaniem ekranu dotykowego wyświetlającego bodźce,

3) zbadanie wpływu traumy, wywołanej chronicznym stresem, na uczenie się na podstawie nagród i kar (PST)oraz zbadanie wpływu traumy na behawior związany z interakcjami społecznymi (SIT), w ramach eksperymentu z użyciem opracowanego protokołu badawczego,

4) opisanie neurobiologicznych zmian, zachodzących pod wpływem traumy, w kontekście rozwoju zaburzeń psychicznych, ze szczególnym uwzględnieniem struktur odpowiedzialnych za funkcje poznawcze.

Materiały i metody:

W badaniu wykorzystano n=20 osobników szczura wędrownego linii Wistar Rat (*Rattus Norvegicus*), grupa eksperymentalna (n=10) została poddana chronicznej ekspozycji na zapach drapieżnika. W trakcie okresu traumatyzacji przez ekspozycję (12 dni), zwierzęta były poddawane procedurze badawczej funkcji poznawczych przy użyciu testu uczenia się z nagród i kar (ang. Probabilistic Selection Task, PST). W trakcie wykonywania testu PST, na ekranie dotykowym wyświetlano symbole, których wybór, za pomocą dotknięcia ekranu, powodował otrzymanie nagrody (kropli słodkiego płynu) lub kary (braku nagrody). Zbudowane w ramach pracy doktorskiej urządzenie do badania funkcji poznawczych mierzyło postęp uczenia się w trakcie postępującego procesu traumatyzacji. By ocenić wpływ traumatyzacji na behawior, wykorzystano Test Społecznej Interakcji (ang. Social Interaction Test, SIT). W trakcie SIT dokonywano pomiaru czasu spędzanego przez każdego osobnika w tunelu, na eksploracji otwartej przestrzeni oraz w obszarze kontaktu z nowym, nieznanym osobnikiem. Procedura eksperymentalna całego badania uwzględniała: etap przyuczania zwierząt do swobodnego korzystania z urządzenia (w którym otrzymywały kary i nagrody w zamian za wybór symboli na ekranie dotykowym), etap badania zdolności uczenia się na podstawie wzmocnień bez ekspozycji na chroniczną traumę, etap ekspozycji na traumę, badanie po zakończonej ekspozycji oraz dwa badania behawioralne SIT. Grupa kontrolna (n=10) została poddana identycznej procedurze badawczej, z wyłączeniem ekspozycji na traumatyzujący bodziec.

Wyniki:

W grupie eksperymentalnej odnotowano istotne statystycznie pogorszenie zdolności uczenia się, na podstawie wzmocnień pozytywnych i negatywnych, mierzonych za pomocą PST w pierwszym dniu ekspozycji oraz dalsze pogorszenie po 11 dniach chronicznej ekspozycji, w porównaniu do wyników tej samej grupy przed traumatyzacją. Grupa kontrolna nie wykazała pogorszenia zdolności uczenia się na podstawie wzmocnień mierzonych za pomocą PSTw kolejnych dniach badania. W porównaniach międzygrupowych nie odnotowano statystycznie istotnej różnicy w funkcjach poznawczych na etapie przed-ekspozycyjnym. W tracie chronicznej ekspozycji grupa eksperymentalna osiągała znacznie gorsze wyniki w PST niż grupa kontrolna, która nie była eksponowana. Grupa eksperymentalna , mimo ekspozycji na traumę, nie wykazała pełnoobjawowej anhedonii ani katatonii (ang. freezing behawior) podczas PST.W badaniach behawioralnych z użyciem SIT, osobniki traumatyzowane wykazywały preferencję do izolacji i wycofania z interakcji społecznych, w porównaniu do zachowania przed-ekspozycyjnego. W pracy teoretycznej opisano procesy epigenetyczne, które w trakcie pojawienia się traumy psychicznej, przyczyniają się do powstawania licznych psychopatologii, między innymi zaburzają uczenie się z nagród oraz wywołują objawy wycofania społecznego. Przedstawiono także ochronną rolę układu endokannabinoidowego względem długotrwałej i szkodliwej ekspozycji na doświadczenia traumatyczne. Wyniki eksperymentu, wraz z wynikami prac teoretycznych, pozwalają

wnioskować o użyteczności opracowanego protokołu w badaniach zmian w funkcjach poznawczych oraz behawioru wywoływanego przez chroniczną ekspozycję na traumę.

Wnioski:

1) proces chronicznej traumatyzacji prowadzi do pogorszenia funkcji poznawczych w zakresie uczenia się na podstawie nagród i kar u szczurów oraz powoduje wycofanie z kontaktów społecznych straumatyzowanych zwierząt,

2) opracowany protokół, wykorzystujący ekran dotykowy, umożliwia badanie funkcji poznawczych podczas ekspozycji na traumę na tej samej grupie osobników, rejestrując zmiany w uczeniu się na podstawie nagród i kar u szczurów *in vivo*, podczas traumatyzacji,

3) opracowany protokół, mimo wykorzystania ekspozycji na silny i chroniczny stres, nie wywołał anhedonii ani katatonii (ang. freezing behawior),która uniemożliwiłaby badanie funkcji poznawczych i behawioru związanego z interakcjami społecznymi,

4)wykazano potencjał translacyjny z modelu ludzkiego na model zwierzęcy - szczury nauczyły się korzystania z ekranów dotykowych, które wyświetlają symbole, dokonują wyborów poprzez dotknięcie odpowiedniego pola, korzystają z procedury PST identycznej do tej, wykorzystywanej na modelu ludzkim.

5) ekspozycja na zdarzenia traumatyczne wywołuje zmiany na poziomie epigenetycznym, które obniżają funkcje poznawcze oraz powodują objawy wycofania społecznego (wniosek pracy teoretycznej).

6) układ endokannabinoidowy pełni funkcję ochronną względem nadmiernego pobudzenia osi HPA wywołanego traumą, a niektóre ligandy receptorów kannabinoidowych mogą pełnić funkcję biomarkerów przeżytych traum (wniosek pracy teoretycznej).

7) w przyszłości, opracowany i opisany w ramach pracy doktorskiej protokół, może być użyteczny w badaniu zmian epigenetycznych wywoływanych chroniczną traumą oraz w badaniu biologicznych systemów aktywnych chronicznym stresem, takich jak układ endokannabinoidowy czy oś HPA.

Abstract

Traumatic experiences are important etiological factors of many mental disorders. They disturb cognitive functions of those who have experienced them, and thus affect symptoms of numerous mental disorders. Traumatic experience, defined as an exposure to severe stress beyond one's ability to cope with, can be caused by many factors, including: caregiver neglect, bullying and victimization in the workplace or in the family, exposure to physical, emotional or sexual violence. Traumatic experiences can be particularly harmful when they are chronic. Growing body of research focuses on neurobiological changes evoked by traumatic experiences and chronic stress. In recent years, there have been an abundance of reports regarding epigenetic changes caused by chronic stress and traumatic experiences, and about specific, inhibitory functions of the endocannabinoid system against excessive hypothalamic-pituitary-adrenal (HPA) axis stimulation. There are many studies that report the negative impact of traumatic experiences on cognitive functions in patients with mental disorders, as well as in healthy individuals. Tests based on learning from rewards and punishments (for example, Probabilistic Selection Task, PST) are simple tools that enable assessment of one's cognitive functions. They are widely used in human research, as well as in animal models. Neurobiology of reward learning is well-described, thus we know which parts of the brain are activated during PST. Interestingly, these structures are heavily influenced by epigenetic changes during stress exposure, and are targeted by HPA axis and endocannabinoid system activity. There is a need for an experimental protocol that allows the study of traumatic experiences caused by chronic stress exposure with an animal model that offers more laboratory possibilities than (mostly) descriptive human models. In the literature, most of the research is carried out using one-time acute stress exposure, there are no protocols enabling measurement of reward learning during stress exposure. The protocol developed as part of the doctoral dissertation differs from the others described in the literature, due to usage of chronic exposure to predator odor (12 days), and daily measurement reinforcement learning via PST, as the trauma progresses. Moreover, in our experiment, we used the Social Interaction Test (SIT), as it examines anxiety-induced social withdrawal.

The aim of the study was 1) to develop a new experimental protocol that allows to study the consequences of trauma, caused by chronic exposure to stress, in an animal model,

2) to design and build an innovative device that measures reward learning via PST, using a touch screen that displays stimuli, in an approach identical with human PST testing,

3) to investigate the effects of chronic stress and trauma on reward learning (via PST) and investigate the effects of chronic stress on social interaction behaviour (via SIT) using novel protocol,

4) to describe neurobiological changes induced by trauma in brain structures responsible for cognitive functions, in the context of mental disorders development.

Materials and methods:

N=20 Wistar Rat (Rattus norvegicus) were used in the study. Experimental group (n=10) was subjected to chronic exposure to predator odor. During that period (12 days), animals were subjected to Probabilistic Selection Task (PST). During the PST, symbols were displayed on the touch screen, selection was done via touch or nose-poke, and resulted in a reward (a drop of sweet liquid) or a punishment (lack of reward). The device, built as part of the doctoral dissertation, measured the progress of reinforcement learning via PST, during the process of traumatization. To assess the impact of trauma on one's behaviour, the Social Interaction Test (SIT) was used. During SIT, time spent by each individual in every part of the cage was measured (tunnel, open space, area of an interaction with an unknown rat). The experimental procedure included: accommodation to the device that displays PST, PST testing without exposure to chronic trauma, PST testing with parallel chronic trauma exposition, post-exposure PST measurement, pre- and post- trauma SIT measurement. The control group (n=10) was subjected to the identical test procedure, except for exposure to a traumatizing stimulus.

Results:

In the experimental group, there was a statistically significant deterioration in PST during the first day of exposure. Further deterioration in PST was observed after 11 days of chronic exposure, in comparison to the pre- trauma performance. The control group did not present deterioration in reinforcement learning with PST throughout the experiment. In the intergroup comparisons, there was no statistically significant difference in PST performance at the pre-stress exposure stage. During chronic exposure, the experimental group performed significantly worse in PST in comparison with the control group. The experimental group, despite exposure to trauma, did not show full-blown anhedonia or catatonia during PST. In SIT, traumatized individuals revealed a preference for isolation and withdrawal from social interactions, in comparison with pre- stress exposure behavior. Review paper presents epigenetic processes which, during the onset of psychological trauma, contribute to the formation of numerous psychopathologies, disturb reinforcement learning and induce social withdrawal. The protective role of the endocannabinoid system against long-term and harmful exposure to traumatic experiences is presented in another review paper.

Conclusions:

1) chronic trauma deteriorates reinforcement learning in rats, and induces social withdrawal,

2) novel protocol enables to measure PST performance during exposure to chronic trauma on animal model,

3) novel protocol does not induce full-blown anhedonia nor freezing behaviour,

4) the translational scope of the protocol has been demonstrated - rats learn to use touch screen monitors that displayed stimuli, similarly to human PST-procedure,

5) exposure to traumatic experience evokes epigenetic changes that disrupts cognitive functions and causes symptoms of social withdrawal (theoretical work conclusion),

6) endocannabinoid system has a potent, protective function against trauma-induced excessive activation of the HPA axis, ligands of cannabinoid receptors may act as biomarkers of trauma (theoretical work conclusion),

7) protocol may be useful in the future research of epigenetic changes induced by chronic trauma, as well as in the future research of biological systems involved during chronic stress activation, such as the endocannabinoid system or the HPA axis.

Wstęp

1.Trauma

Medyczne rozumienie psychicznej traumy jest ściśle związane z rozpoznaniem zespołu stresu pourazowego (ang. post-traumatic stress disorder, PTSD)[1], podczas gdy w rozumieniu psychoanalitycznym, trauma jest bardzo silnym bodźcem, z którym psychika nie jest w stanie sobie poradzić,w obliczu którego pojawia się uczucie utraty (na przykład zdrowia, wolności, bliskiej osoby) oraz bezsilności wobec zaistniałej sytuacji[2]. W naukach eksperymentalnych przed-klinicznych, wykorzystujących modele zwierzęce, traumą określany jest każdy silny lub chroniczny stres, który wywołuje wyraźne i utrzymujące się zmiany behawioralne, podobne do objawów PTSD u ludzi (na przykład wycofanie społeczne, lękliwość, hiperaktywność)[3].Przytoczone definicje są zgodne w rozumieniu traumy jako formy ekstremalnego stresu, który wywołuje gwałtowne zmiany w psychice. W naszej pracy używamy sformułowania "doświadczenia traumatyczne", jako określenia na silny i chroniczny stres, który może potencjalnie wywołać traumę, zgodnie z omówionych wyżej definicjami. U ludzi traumę opisuje się najczęściej w odniesieniu do wydarzeń takich jak śmierć bliskiej osoby, utrata zdrowia, doświadczenie przemocy fizycznej, seksualnej, ekonomicznej, emocjonalnej lub jako efekt zaniedbania dzieci przez opiekunów. We współczesnej psychiatrii obserwujemy wzrost zainteresowania tematem traumy głównie ze względu na to, iż wydarzenia traumatyczne zdają się leżeć u podłoża bardzo wielu zaburzeń psychicznych i trudności psychologicznych[4,5], oraz wpływają na nasilenie objawów prezentowanych przez pacjentów[6,7]. Co więcej, doświadczenia traumatyczne pogarszają funkcje poznawcze[8], upośledzają działanie systemu immunologicznego organizmu[9], wywołują strukturalne i funkcjonalne zmiany w mózgu[10]. Chroniczna ekspozycja na traumę, szczególnie w wieku dziecięcym, jest wyjątkowo szkodliwa, przyczynia się do powstawania wyraźnych deficytów rozwojowych oraz zaburzeń psychicznych w późniejszym życiu[11]. Co ciekawe, ekspozycja na chroniczny stres (który nie zawsze spełnia kryterium traumy), przyczynia się do powstawania łagodnych objawów PTSD[12]. Badania epidemiologiczne wykazują, iż współczesny model życia, sprzyjający ekspozycji na chroniczny stres, w znaczącym stopniu przyczynia się do uzależnień od substancji, otyłości, cukrzycy, rozwoju zaburzeń psychicznych oraz psychologicznych deficytów, generując olbrzymie koszty społeczne[13–17].

2. Wpływ traumy na rozwój zaburzeń psychicznych

Przyjmuje się, że w trakcie życia większość ludzi doświadcza przynajmniej jednej potencjalnie traumatycznej sytuacji, mimo to, tylko niewielki procent populacji wykształca pełnoobjawowe PTSD[18,19]. Rozpowszechnienie tego zaburzenia psychicznego szacowane jest obecnie na 7% w skali populacji[20], jednak w grupach szczególnie narażonych na silny stres ta liczba wzrasta. W grupie amerykańskich weteranów to około 10% [21], a najnowsze dane z Niemiec, wykazały, że aż 60% nieletnich uchodźców leczonych ambulatoryjnie, z powodu różnych somatycznych dolegliwości, cierpi równolegle na PTSD[22]. Trauma jest wskazywana jako istotny czynnik predestynujący do rozwoju psychoz[5], co więcej, osoby, które doświadczyły wczesnodziecięcej traumy posiadają trzy razy większe ryzyko zachorowania na schizofrenię niż osoby bez takiego obciążenia[23]. Współczesne doniesienia wskazują także na związek między traumą

a zaburzeniami nastroju, szczególnie chorobą afektywną dwubiegunową[4], oraz ścisły związek między traumą a rozwojem niektórych zaburzeń osobowości, w szczególności zaburzenia osobowości typu borderline[24].

3.Neurobiologia traumy

Doświadczenia traumatyczne mają istotny wpływ na funkcje poznawcze jednostki, takie jak pamięć robocza, uczenie się na podstawie kar i nagród, zdolność do utrzymania uwagi, funkcje wykonawcze, czy orientacja wizualno-przestrzenna[25]. Pacjenci z historią traum prezentują osłabienie funkcji poznawczych w porównaniu z pacjentami o identycznym rozpoznaniu, lecz bez doświadczeń traum[26,27]. Podobne zjawisko obserwowane jest w grupie osób zdrowych z doświadczeniami traum, w porównaniu z grupą kontrolną, która nie zgłaszała takich doświadczeń[25,28]. Chroniczna ekspozycja na doświadczenia traumatyczne osłabiają funkcje poznawcze poprzez wzmożoną aktywność osi stresu (przysadka-podwzgórze-nadnercza, HPA) oraz poprzez molekularne zmiany zachodzące na poziomie epigenetycznym.

3.1.Oś HPA

Oś HPA odpowiada za bezpośrednią reakcję na stres, wpływa na proces konsolidacji szlaków pamięciowych, od jej aktywności zależy proces odzyskiwania homeostazy po intensywnym pobudzeniu. Do aktywacji osi HPA potrzebna jest sekrecja kortykoliberyny przez neurony w podwzgórzu, ale także przez neurony w jądrze migdałowatym, w części grzbietowej hipokampa oraz w prążku końcowym. Sekrecja kortyzolu wywołana stresem pobudza receptory glukokortykoidowe oraz mineralokortykoidowe, a utrzymujący się stan chronicznego pobudzenia obniża odpowiedź immunologiczną organizmu, utrudnia proces formowania szlaków pamięciowych, wpływa na stany emocjonalne wywołując objawy depresyjne[29]. Badania na zwierzęcych modelach PTSD wykazały strukturalne zmiany ubytkowe w hipokampie osobników traumatyzowanych[30], oraz zmiany ubytkowe w korze przedniej u osobników eksponowanych na chroniczny stres[31,32]. Co ciekawe, badania neurobiologiczne ludzi z grupy ryzyka rozwoju zaburzeń psychotycznych wykazują związek między zmianami zubożeniowymi w hipokampie a wysokim stężeniem kortyzolu[33]. Badania kliniczne dostarczają coraz większej ilości danych na temat zmian w osi HPA u osób z doświadczeniem traumy i cierpiących jednocześnie na zaburzenia afektywne dwubiegunowe, schizofrenię[34] oraz PTSD[35].

3.2.Procesy epigenetyczne

Doświadczenia traumatyczne wywołują także zmiany na poziomie molekularnym w obrębie procesów epigenetycznych, regulujących dostępnością materiału genetycznego, gotowego do translacji i transkrypcji[36,37]. W kontekście psychiatrycznym badania epigenetyczne dotyczą najczęściej procesów metylacji DNA lub acetylacji histonów, na przykład w schizofrenii[38–40]. Badania zwierzęcego modelu chronicznej ekspozycji na traumę porażki społecznej (ang. chronic social defeat stress)wykazały zmiany aktywności w ATP-zależnych kompleksach remodulujących chromatynę w jądrze półleżącym[41,42]. Te białkowe kompleksy są znacznie rzadziej badane w kontekście rozwoju zaburzeń psychicznych, mimo że ich aktywność jest opisywana w neuronach hipokampa[43], kory przedczołowej[44], szlaku wydzielniczego dopaminy wiodącego z pola brzusznego nakrywki (ang. ventral tegmental area, VTA)[45], czy jądra migdałowatego[46]. Wykorzystanie zwierzęcych modeli pokazuje, że ekspozycja na psychiczną traumę może wywoływać zmiany epigenetyczne, zmieniają ekspresję niektórych genów, uniemożliwiając konstruowanie białek i enzymów potrzebnych do konstruowania szlaków

pamięciowych, ograniczając tworzenie nowych połączeń synaptycznych w wyżej wymienionych strukturach[43,47]. Współcześnie brakuje prac teoretycznych, porządkujących wiedzę z obszaru zmian epigenetycznych powodowanych doświadczeniami traumatycznymi, w kontekście zaburzeń funkcji poznawczych oraz rozwoju psychopatologii u ludzi.

3.4.Układ endokannabinoidowy

Badania wpływu doświadczeń traumatycznych na działanie osi HPA są potrzebne. Nie mniej, istnieje potrzeba badania systemów biologicznych, pełniących funkcję ochronną względem chronicznego pobudzenia osi HPA oraz przyczyniających się do odzyskiwania homeostazy po ekspozycji na stres. Układ endokannabinoidowy, opisany na modelu ludzkim po raz pierwszy w połowie lat 90, może okazać się takim właśnie systemem[48]. W ostatnich latach endokannabinoidy cieszą się coraz większym zainteresowaniem badaczy, głównie ze względu na działania neuro-homeostatyczne. Odnotowano wysokie stężenie receptorów endokannabinoidowych w obszarach mózgu szczególnie narażonych na szkodliwe działanie kortyzolu takich jak hipokamp, jądro migdałowate oraz przyśrodkowa część kory przedczołowej[49,50]. Ostatnie lata to także wzrost liczby doniesień o zmianach w stężeniu neuroprzekaźników związanych z układem endokannabinoidowym w kontekście zaburzeń psychicznych[51,52]. Z powodu rosnącej liczby publikacji w literaturze tematu pojawia się wyraźna potrzeba prac poglądowych, porządkujących wyniki badań nad układem endokannabinoidowym, w kontekście zaburzeń psychicznych oraz wpływu endokannabinoidów na funkcje poznawcze.

4. Pomiar funkcji poznawczych w paradygmacie uczenia się na podstawie kar i nagród

Testy oparte na uczeniu się na podstawie wzmocnień(ang. reinforcement learning) powstały, by badać funkcje poznawcze ludzi i zwierząt. W trakcie PST prezentuje się osobie badanej (“graczowi”) nieznane dotychczas symbole, które wygrywają z ustalonym wcześniej (nieznanym dla gracza) prawdopodobieństwem. Kolejne wybory skutkują nagrodami lub karami, na podstawie których gracz uczy się, które symbole preferować, a których unikać, konstruując własną strategię gry[53]. Konstrukcja testu pozwala na badanie behawioralnych manifestacji neuronalnej aktywności konkretnych struktur w mózgu. Gracz korzysta z pamięci krótkotrwałej (przyśrodkowa część kory przedniej), a na podstawie nagród i kar szacuje wartość wybieranych symboli (struktury podkorowe oparte na aktywności dopaminergicznej-prążkowie, pole brzuszne nakrywki). Badania z wykorzystaniem pozytonowej tomografii emisyjnej (PET) oraz funkcjonalnego rezonansu magnetycznego (fMRI) potwierdziły skuteczność PST w aktywacji wymienionych powyżej struktur mózgowych[54,55]. Badania z użyciem PST wykazały zmiany w uczeniu się z nagród i kar osób chorujących na PTSD[56,57], Parkinsona[53]oraz schizofrenię[58,59].

W czasie badań PST na ludziach symbole (bodźce), dające kary i nagrody, wyświetlane są na ekranie komputera, a wybór dokonywany jest poprzez wciśnięcie klawisza lub wybór na ekranie dotykowym[19,57]. Modele zwierzęce wykorzystują proste sygnały świetlne i dźwiękowe jako bodźce, a wybór dokonywany jest dzięki dźwigniom (nacisk ciała zwierzęcia) lub dzięki otworom (wybór przez dotknięcie nosem)[60,61]. Badania z użyciem PST u zwierząt wykorzystywane były, między innymi do opracowania protokołu symulującego schizofrenię (poprzez społeczną izolację), gdzie wykazano zmiany w hamowaniu przed-impulsowym (prepulse inhibition), podobne do tego obserwowanego w modelu ludzkim[61]. PST używany był

także w modelu zwierzęcym PTSD, gdzie wykazano pogorszenie uczenia się ze wzmocnień osobników traumatyzowanych pojedynczą ekspozycją na silny stresor [60].

5. Nowy protokół badawczy

W literaturze brakuje protokołów eksperymentalnych pozwalających na badanie PST w kontekście chronicznej ekspozycji na doświadczenia traumatyczne. Podczas gdy PST pozwala skutecznie badać aktywność kory przedniej oraz prążkowia w trakcie uczenia się na podstawie kar i nagród, włączenie do procedury ekspozycji na chroniczny stres pozwoliłoby na uwzględnienie nowego, neurobiologicznego kontekstu tego zjawiska. Taki protokół mógłby służyć badaniu aktywności struktur w mózgu omówionych w pracy (prążkowie, kora przedczołowa, jądro migdałowate, hipokamp, jądro półleżące). Co więcej, chroniczna (kilkudniowa) ekspozycja na doświadczenia traumatyczne mogłaby umożliwić badanie zmian epigenetycznych, ujawniających się w dłuższej perspektywie czasowej (doby) po ekspozycji na bodziec. Badania traumy na modelach ludzkich mają swoje ograniczenia, dotyczą bowiem pomiarów i opisów efektu ekspozycji na traumę, która wystąpiła w przeszłości. Modele zwierzęce dają możliwość badania zmian zachodzących w funkcjach poznawczych pod wpływem doświadczeń traumatycznych *in vivo*, jednak aby uzyskać wysoki potencjał translacyjny, należy: 1) zbliżyć procedurę badania PST u ludzi do procedury badania PST u zwierząt, 2) zastosować procedurę traumatyzacji nie powodującą całkowitej katatonii i/lub pełnoobjawowej anhedonii u zwierząt, która uniemożliwiłaby dobrowolne badanie funkcji poznawczych.

Cel i założenia pracy

Część pierwsza:

Bielawski T., Misiak B., Moustafa A., Frydecka D.: Epigenetic mechanisms, trauma, and psychopathology: targeting chromatin remodeling complexes. *Reviews in the Neurosciences* 2019; 30: 595–604

Głównym celem była analiza prac badawczych dotyczących zmian epigenetycznych w ramach ATP-zależnych kompleksów re-modulujących chromatynę po ekspozycji na traumę w kontekście rozwoju zaburzeń psychicznych. Zmiany epigenetyczne w obrębie metylacji DNA oraz acetylacji histonów są opisywane w literaturze tematu, jednak literatura dotycząca ATP-zależnych kompleksów w kontekście zaburzeń psychicznych jest niewielka. Celem pracy było przedstawienie wyników badań na modelach ludzkich i zwierzęcych, ze szczególnym uwzględnieniem zwierzęcych modeli chronicznej ekspozycji na traumę. Kolejnym celem pracy było przedstawienie hipotezy dotyczącej wpływu wywołanych przez traumę zmian na poziomie epigenetycznym, na funkcje poznawcze jednostki, oraz przedstawienie potencjalnych molekularnych mechanizmów tego zjawiska.

Część druga:

Bielawski T., Albrechet-Souza L., Frydecka D.: Endocannabinoid system in trauma and psychosis: distant guardian of mental stability.*Reviews in the Neurosciences* 2021.32(7)707-722

Celem pracy było kompleksowe przedstawienie układu endokannabinoidowego jako neuro-modulującego systemu odpowiedzi na silny stres (traumę), która jest uznanym czynnikiem zwiększającym ryzyko rozwoju zaburzeń psychicznych. W pracy wykorzystano badania na ludziach oraz na modelach zwierzęcych, by odpowiedzieć na pytanie, jak wyglądają czynnościowe (fizjologiczne) zmiany wywoływane przez traumę oraz jak wygląda odpowiedź organizmu (w tym układu endokannabinoidowego) na chroniczne pobudzenie osi stresu HPA. Głównym celem pracy było przedstawienie wyników w kontekście zmian neurobiologicznych, zachodzących pod wpływem traumatyzacji w obrębie struktur mózgowych, determinujących funkcje poznawcze takich jak hipokamp i kora przedczołowa. Celem dodatkowym było przedstawienie potencjalnych biologicznych markerów przeżytych traum.

Część trzecia:

Bielawski T., Drapała J., Krowicki P., Stańczykiewicz B., Frydecka D.: Trauma disrupts reinforcement learning in rats - a novel animal model of chronic stress exposure.*Frontiers In Behavioral Neuroscience* 2022; 185

Celem pracy było opracowanie oryginalnego protokołu badawczego wykorzystującego model zwierzęcy do badania zmian funkcji poznawczych, powstających podczas procesu traumatyzacji pod wpływem chronicznej ekspozycji na stres. Współczesna literatura zwraca uwagę na negatywny wpływ traumy na funkcje poznawcze w grupie osób zdrowych [25,28], jak i w grupie pacjentów leczonych psychiatrycznie[27]. Eksperymentalne badania na ludziach obarczone są oczywistymi ograniczeniami etycznymi, dlatego wykorzystano model zwierzęcy, który pozwala na pomiar funkcji poznawczych przed ekspozycją na stres oraz w trakcie zachodzącego procesu traumatyzacji. Do pomiaru uczenia się na podstawie kar i nagród wykorzystano zadanie PST, wyświetlane na ekranie dotykowym, szeroko stosowane w badaniach na modelu ludzkim. Otrzymany wynik PST może stanowić behawioralny wskaźnik aktywności neuronalnej w przyśrodkowej części kory przedczołowej, jądrze półleżącym, prążkowiu, polu brzusznym nakrywki - obszarach odpowiedzialnych za uczenie się na podstawie kar i nagród. By zwiększyć potencjał translacyjny badania, opracowano i zbudowano innowacyjną maszynę dostosowującą narzędzie PST do modelu zwierzęcego. Opracowany protokół wykorzystuje zapach drapieżnika, który w literaturze tematu jest dobrze zbadanym bodźcem traumatyzującym gryzonie. W badaniu wykorzystano także test zachowania SIT, by oszacować poziom lęku oraz zakres interakcji społecznych po ekspozycji na stres - oba parametry są behawioralnymi wskaźnikami aktywności neuronalnej w obrębie pola brzusznego nakrywki oraz jądra migdałowatego.

Materiały i metody badań

W niniejszym podrozdziale zaprezentowano zarys metodologii przeprowadzonych badań. Szczegółowy opis zawarty jest w załączonych publikacjach, na podstawie których opracowano pracę doktorską.

Materiał: w badaniu wykorzystano 20 samców szczura wędrownego linii Wistar (*Rattus norvegicus*), w wieku 39-42 dni w momencie rozpoczęcia eksperymentu. Badania otrzymały zgodę Lokalnej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach przy Instytucie Immunologii i Terapii Doświadczalnej im. Ludwika Hirszfelda PAN we Wrocławiu (zgoda numer 14/2019 udzielona w dniu 11.03.2019.)

Protokół: Każdy osobnik został poddany procedurze aklimatyzacji (7 dni) oraz handlingowi (7dni). Etap ''P0'' polegał na akomodacji do maszyny testującej oraz przyuczania procedury PST, w czasie której pojawiały się nagrody i kary (10 dni). Następny etap (''P1'') trwał 12 dni, w czasie których odbywały się codzienne badania PST w maszynie testującej. Ostatni etap (''P2'') wyglądał identycznie jak poprzedni dla grupy kontrolnej, z kolei grupa eksperymentalna była eksponowana na zapach drapieżnika (Lynxrufus; Maine Outdoor Solutions, Hermon, ME, USA) w trakcie PST. Badania PST odbywały się codziennie na przestrzeni wszystkich etapów, badanie SIT odbyło się jeden raz w trakcie każdego etapu.

Badanie PST polegało na wyświetleniu jednej pary symboli na ekranie dotykowym. Symbole wyświetlane były w losowych kombinacjach, po losowej stronie (lewa lub prawa strona ekranu). Każdy symbol wygrywał z ustalonym wcześniej prawdopodobieństwem (90%, 10%,50%, 50%). Wygrana skutkowała nagrodą w postaci kropli słodkiego płynu (wysokobiałkowy napój truskawkowy NUTRICA). Karą był brak nagrody. O wygranej decydował rachunek prawdopodobieństwa, to znaczy: symbol wygrywający z prawdopodobieństwem 90% raz na 10 wyborów dostarczał karę[53]. Badanie PST zostało przeprowadzone w zbudowanej w ramach grantu maszynie, opatrzonej w ekran dotykowy, podajnik ze słodkim napojem oraz mikrokomputer sterowany z poziomu aplikacji w środowisku Android. Działaniem maszyny sterowało oprogramowanie, które gromadziło dane o wyborach każdego osobnika w trakcie trwania PST. Maszyna do badania została opisana i zgłoszona do Urzędu Patentowego RP przez Centrum Innowacji i Transferu Technologii UMW (nr wniosku P.440017 z dnia 29.12.2021). Badanie behawioralne SIT zostało przeprowadzone w zbudowanej do tego celu komorze testowej, zgodnie z zaleceniami opisanymi w literaturze[62]. Analizy behawioru przeprowadzono z wykorzystaniem nagrań wideo zachowań prezentowanych przez każdego osobnika w sytuacji testowej.

Metody statystyczne: Wyniki badań, które wykorzystują PST, często analizowane są przy użyciu modelowania matematycznego, na przykład modeli Q-learning'u[63,64]. Takie metody analizy, oparte na zaawansowanych założeniach teoretycznych (modele obliczeniowe), niosą ryzyko wystąpienia rozbieżności między wynikami surowymi a wynikami otrzymanymi po analizie zgodnie z założeniami modelu - tak zwany *mathematical bias* może utrudnić weryfikację uzyskanych danych przez osoby recenzujące oraz utrudnić walidację eksperymentu. Z powodu, iż praca przedstawia nowatorską procedurę badawczą, zdecydowano przedstawić wyniki używając prostych, matematycznych wyliczeń, niezależnych od modeli obliczeniowych. Wyliczenia statystyczne opierały się na kilku prostych założeniach:

wynik każdego badania PST (*Test Score*) opisany był prostym stosunkiem liczby nagród do liczby wszystkich wyświetlonych par. Miara Test Score służyła następnie wyliczeniu indywidualnego progresu każdego osobnika podczas danej fazy (*WinRatio*) oraz do porównań międzygrupowych i wewnątrzgrupowych.

Do porównań wyników PST tego samego osobnika podczas P1 i P2 wykorzystano test dwustronny Wilcoxona. W porównaniach międzygrupowych PST wykorzystano test U-Manna-Whitney'a. Wyniki behawioralne SIT zostały przeanalizowane używając testu Wilcoxona przy porównaniu wyników sprzed i po traumatyzacji, test U-Manna-Whitney'a został wykorzystany do porównań międzygrupowych. Za granicę istotności statystycznej przyjęto $p<0,05$. Statystyczne analizy zostały przeprowadzone z użyciem biblioteki scipy.stats należącej do ekosystemu języka programowania Python[1].

[1] 1https://docs.scipy.org/doc/scipy/reference/stats.html

Cykl publikacji

Wyniki pracy badawczej prowadzonej przez doktoranta zostały ujęte w cyklu trzech publikacji, stanowiących podstawę niniejszej rozprawy doktorskiej:

- Bielawski T., Misiak B., Moustafa A., Frydecka D.: Epigenetic mechanisms, trauma, and psychopathology: targeting chromatin remodeling complexes. *Reviews in the Neurosciences* 2019; 30: 595–604 (IF 4.3, 70 punktów MNiSW)

- Bielawski T., Albrechet-Souza L., Frydecka D.: Endocannabinoid system in trauma and psychosis: distant guardian of mental stability. *Reviews in the Neurosciences* 2021. 32(7) 707-722 (IF 4.3, 70 punktówMNiSW)

- Bielawski T., Drapała J., Krowicki P., Stańczykiewicz B., Frydecka D.: Trauma disrupts reinforcement learning in rats - a novel animal model of chronic stress exposure. *Frontiers In Behavioral Neuroscience* 2022; 185(IF 3.34, 100punktówMNiSW)

Sumaryczny Impact Factor Cyklu: 11.94

Sumaryczna liczba pkt. MNiSW/KBN cyklu: 240

Tomasz Bielawski*, Blazej Misiak, Ahmed Moustafa and Dorota Frydecka

Epigenetic mechanisms, trauma, and psychopathology: targeting chromatin remodeling complexes

https://doi.org/10.1515/revneuro-2018-0055
Received June 4, 2018; accepted October 19, 2018

Abstract: Environmental pressure affects the genotype throughout different epigenetic processes. There is currently ample evidence on the role of epigenetics in developing various mental disorders. A burden of environmental pressure, such as psychological trauma, and its influence on genotype can lead to a variety of psychopathologies. Thus, this study focuses on the epigenetic activity of the complex protein machinery operating on chromatin – the ATP-dependent chromatin remodeling complexes. Although there are several recent studies on the molecular structure, functions, and taxonomy of ATP-dependent chromatin remodeling complexes, the focus of this paper is to highlight the importance of those 'protein machines' in developing psychiatric disorders. Data were obtained from human preclinical and clinical studies. The results of this review indicate an importance of ATP-dependent chromatin remodeling complexes in the interaction between environmental factors, including traumatic events, and genetic vulnerability to stress. Several studies indicate that ATP-dependent chromatin remodeling complexes play a crucial role in the development and consolidation of memory, in neurodevelopmental processes, and in etiology depressive-like behavior. Thus, the activity of those 'protein machines' emerges as a key factor in the pathophysiology of various psychiatric diseases. It can also be concluded that the limitations of clinical studies may be explained by inappropriate laboratory methods and research paradigms due to the delayed timeframe of biochemical responses to environmental stimuli. Future research in this field may enable a better understanding of the pathophysiology of psychiatric diseases and contribute to the development of novel molecular treatment targets.

Keywords: ATP-dependent chromatin remodeling complex; epigenetics; psychiatry; psychopathology; trauma.

***Corresponding author: Tomasz Bielawski,** Department of Psychiatry, Wroclaw Medical University, 10 Pasteur Street, 50-367 Wroclaw, Poland, e-mail: tomaszbielawski90@gmail.com
Blazej Misiak: Department of Genetics, Wroclaw Medical University, 1 Marcinkowski Street, 50-368 Wroclaw, Poland
Ahmed Moustafa: School of Social Sciences and Psychology, Western Sydney University, Sydney, New South Wales 2000, Australia; and Department of Social Sciences, Qatar University Ringgold Standard Institution, Ad Dawhah, Doha, Qatar
Dorota Frydecka: Department of Psychiatry, Wroclaw Medical University, 10 Pasteur Street, 50-367 Wroclaw, Poland

Introduction

There is a growing body of evidence that epigenetic processes play an important role in the development of psychopathologies. Historically, epigenetics was defined as an interaction between different molecular pathways, which determines the phenotype under the influence of the environment (Waddington, 1968). However, Jullien and Berger (2009) defined epigenetics as follows: 'An epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence'. The following processes have been recognized as epigenetic mechanisms: DNA methylation, modification of histones (e.g. methylation, acetylation, and phosphorylation), regulation of DNA expression by microRNA species, and modification via ATP-dependent protein complexes. The effects of these mechanisms as well as the regulatory enzymes involved in biochemical pathways are key epigenetic processes (Ptashne, 2007; Tsankova et al., 2007). Over the past few years, the role played by epigenetic processes in the development of psychiatric disorders became widely accepted. Throughout the human life, the environment puts pressure on individual genotype, initiating various epigenetic mechanisms. One such clinically important environmental pressure is psychological trauma. Recent reports lend support to the considerable impact of traumatic events on epigenetic processes, which contribute to the development of psychopathologies (Sun et al., 2015; Misiak et al., 2017). By targeting epigenetics in psychiatry research, several studies have focused on DNA methylation and histone acetylation (Table 1), yet nucleosome remodeling via ATP-dependent complexes in developing psychopathologies remains an unexplored area. These protein complexes control the histone arrangement

Table 1: Selected papers targeting epigenetic processes other than ATP-dependent chromatin remodeling complexes.

Epigenetic process	Title	Authors
Methylation	Balancing histone methylation activities in psychiatric disorders	Peter and Akbarian, 2011
	DNA methylation and antipsychotic treatment mechanisms in schizophrenia: Progress and future directions	Ovenden et al., 2018
	DNA methylation in schizophrenia	Pries et al., 2017
	Increased serotonin transporter gene (SERT) DNA methylation is associated with bullying victimization and blunted cortisol response to stress in childhood: a longitudinal study of discordant monozygotic twins	Ouellet-Morin et al., 2013
	The role of DNA methylation in the central nervous system and neuropsychiatric disorders	Feng and Fan, 2009
	Recently evolved human-specific methylated regions are enriched in schizophrenia signals	Banerjee et al., 2018
Acetylation	Exercise-induced modulation of histone H4 acetylation status and cytokines levels in patients with schizophrenia	Lavratti et al., 2017
	Chemogenomic analysis reveals key role for lysine acetylation in regulating Arc stability	Lalonde et al., 2017

and thereby alter the chromatin structure (Vignali et al., 2000). A tightly packed heterochromatin is unavailable to translation, contrary to a loose form of euchromatin, which enables the initiation of translation because of easy access to genes located within. ATP-dependent complexes use ATP hydrolysis to steer a dynamic process of DNA accessibility. Thus, they are pivotal for adequate temporal and spatial gene expression (Zaghlool et al., 2016). ATP-dependent complexes can be grouped according to a unique 'taxonomy' that refers to the specificity of distinct ATPase subunits (Vignali et al., 2000; Becker and Hörz, 2002). Indeed, four groups of ATP-dependent complexes, including switch/sucrose non-fermentable (SWI/SNF), imitation switch (ISWI), chromodomain helicase DNA-binding (CHD), and inositol (INO80), play a role in genomic homeostasis during the lifespan (Zaghlool et al., 2016). Every complex is a 'protein machinery' built around ATPase, with many multifunctional protein subunits. The INO80 group is important for DNA repair, checkpoint regulation, DNA replication, telomere maintenance, and chromosome segregation (Morrison and Shen, 2009). Although INO80 is crucial for many processes involved in developmental processes and homeostasis, its impact on human neurobiology is either minor or not yet known. For this reason, this paper focuses on two protein families – the SWI/SNF and ISWI complexes. The protein family CHD is included in this review only in reference to the increased number of reports, suggesting the involvement of CHD proteins in the etiology of autism spectrum disorder (ASD). The aim of this review paper is to both (a) provide basic and initial information about selected epigenetic 'complex protein machines' operating on chromatin – ATP-dependent chromatin remodeling complexes – and (b) highlight the relationship between traumatic stress and those proteins. A detailed description of the molecular and physicochemical properties of ATP-dependent chromatin remodeling complexes is beyond the scope of this article.

Materials and methods

To conduct our search, we have used the following databases: PubMed, Scopus, Medline, Google Scholar, and Web of Science, using the following keywords: 'epigenetic', 'trauma', 'chromatin remodeling complexes', 'psychopathology', 'SWI', 'SNF', 'ISWI', 'ATP dependent chromatin remodeling complexes', 'CSDS', 'chronic social defeat stress', 'psychopathology', 'autism', 'schizophrenia', 'bipolar disorder', 'depression', and 'BAF'. Keywords were used in various combinations to find articles regarding the role of ATP-dependent chromatin remodeling complexes in the development of various psychopathologies. Furthermore, the references section of the publications we found based on the criteria described above was manually reviewed for relevant articles. The following publication records were included: (1) publications in English, (2) publications from peer-reviewed journals or books, and (3) review articles or original studies of human subjects or animal models, presenting data of clinical/psychiatric importance.

Results

After conducting the preliminary search with defined keywords as mentioned above, we obtained 65 articles on

the given topic. We excluded six of these articles based on the following exclusion criteria: (1) articles concerning strictly molecular structure and/or physicochemical properties of ATP-dependent chromatin remodeling complexes, (2) articles concerning the function of ATP-dependent chromatin remodeling complexes outside the nervous system, and (3) articles concerning the role of epigenetic mechanisms other than ATP-dependent chromatin remodeling complexes in the development of mental disorders. A total number of 59 articles were included in this review; 31 of them were experimental studies.

SWI/SNF family

A complex taxonomy of SWI/SNF chromatin remodeling complexes is based on the conserved ATPase subunit that represents the SWI2/SNF2 protein family. In humans, the most common ATPase subunits are hBRM and BRG1 (Vignali et al., 2000; Becker and Hörz, 2002). A complicated machinery of different protein subunits is formed around the ATPase subunit (Table 2). One of those proteins are the BRG1 or HBRM-associated factors (BAF) complexes. The mutations of the polymorphic BAF complexes are often associated with altered cognitive functions. Notably, BAF complexes are expressed by postmitotic neurons [known as neuronal BRG1 or HBRM-associated factors (nBAF)]. Mutations in the neuron-specific subunit known as BAF53b (actin-related protein) result in long-term memory deficits and altered hippocampal synaptic plasticity (Vogel-Ciernia et al., 2013). Transgenic mice with a deletion of the hydrophobic domain of BAF53b perform significantly worse than healthy control mice on the Object Location Memory Test (OLM) and Object Recognition Memory Test (ORM) as well as in a long-term memory version of both tests. In the short-term memory versions of OLM and ORM, mutants did not differ significantly from wild-type (control) individuals. Similar results were obtained in fear conditioning tasks in the lateral amygdala neurons (Yoo et al., 2017). Genetic manipulation in the BAF53b subunit results in an altered gene expression and leads to an impairment of hippocampal synaptic plasticity (Vogel-Ciernia et al., 2013) and a blunted dendritic outgrowth in the hippocampus (Wu and Liu, 2007). The overexpression of BAF53b does not enhance the already existing memory but plays a crucial role in consolidating and forming a memory trail. BAF53b operates in a late phase of memory creation (after up to 24 h after training) and plays an essential role in creating long-term memory as found in the paradigm of fear conditioning (Yoo et al., 2017). Important recent reports by Marom et al. (2017) confirm the key role of the BAF complex in developing intact cognitive functioning. A pathogenic variant of the *actin-like 6A* (*ACTL6A*) gene (which encodes another protein component of the BAF complex) was described in three subjects with developmental disabilities, affecting mainly language and memory (Marom et al., 2017). Although the size of a group does not allow us to draw unequivocal conclusions, this report is part of a series of studies confirming the relevance of ATP-dependent complexes and their genetic impact on cognitive functions. Altered hippocampal cytoarchitecture, especially among dendrites, is also observed during the manipulation in other components of BAF, especially the *SWI/SNF-related, matrix-associated, actin-dependent regulator chromatin A2* (*SMARCA2*) gene. Some polymorphisms of the *SMARCA2* gene are linked to the animal model of schizophrenia (Loe-Mie et al., 2010) and human schizophrenia (Walsh et al., 2008), whereas mutations in *SMARCA2* are linked to the Coffin-Siris syndrome (Santen et al., 2012; Tsurusaki et al., 2012), Nicolaides-Baraitser syndrome (Van Houdt et al., 2012), and general intellectual disability (Hoyer et al., 2012). In addition, *SMARCA2* (and its protein product) seems to be one of the 'network centers' among the SWI/SNF (BAF) complexes. The *SMARCA2* gene is modified by the down-regulation of REST/RFSF, which results in alterations of other interactors and consequences in the development of abnormal dendritic spines. Mouse models revealed impaired social interaction and prepulse inhibition in *Smarca2* knockout mice. Importantly, deficits in both domains – social interaction and prepulse inhibition – are observed in patients with schizophrenia. Moreover, it is possible to manipulate the expression of the *SMARCA2* gene by the application of psychoactive drugs that down-regulate this complex and antipsychotic drugs that in turn up-regulate it. Therefore, *SMARCA2* is considered to be involved in the pathophysiology of schizophrenia (Koga et al., 2009; Loe-Mie et al., 2010). Several mutations in the *SMARCA2* gene seem to be related to the Coffin-Siris syndrome, which is characterized by intellectual disability, growth deficiency, microcephaly, and

Table 2: Few of many genes and protein units/subunits of the SWI/SNF group ATP-dependent chromatin remodeling complexes.

Examples of genes involved in coding units and subunits	Protein units and subunits	SWI/SNF ATPase unit
ACTL6A	BAF	BRG1
ACTL6A	BAF53a	BRG1
SMARCA2	nBAF	hBRM
SMARCA2	BAF53b	hBRM

a number of dysmorphic features (Tsurusaki et al., 2012). Loe-Mie et al. (2010) revealed how these schizophrenia-related genes, and their protein products, are evolutionary novel. Risk alleles in the *SMARCA2* gene are derived from a point mutation in the conservative region. This mutation leads to altered protein trafficking in the cytoplasm-nucleus pathway. This mechanism is hypothesized to influence both robust primate evolution and schizophrenia. Finally, there is a positive selection among primates and humans toward the *SMARCA2* risk alleles, which reinforce the appearance of this evolutionally novel molecular phenomena (Loe-Mie et al., 2010).

Epigenetic mechanisms regulated by ATP-dependent chromatin remodeling structures within the SWI/SNF family are being affected by other ongoing epigenetic processes or even occur in coordination with other chromatin remodeling complexes (Zaghlool et al., 2016). Gozes (2016) suggested that there is a possible interaction between activity-dependent neuroprotective protein (ADNP) and the SWI/SNF complexes. An increased activity of ADNP (at the mRNA and protein level) has been reported in peripheral blood lymphocytes of patients with schizophrenia as well as in individuals with mild cognitive impairment (Gozes, 2016). The ADNP level has been associated with cognitive functions in patients with Alzheimer's disease (Magen and Gozes, 2014) and correlates with the level of τ protein in an animal model of Alzheimer's disease (Schirer et al., 2014). The altered interaction between ADNP and SWI/SNF is proposed as one of the potential indicators of ASD (Vandeweyer et al., 2014). Helsmoortel et al. (2014) showed that mutations in the *ADNP* gene occur in at least 0.17% of patients with ASD. This estimation may seem irrelevant, but in fact it is among the most prevalent ASD genes, taking into account the fact that no single gene is found to be mutated in more than 1% of patients with autism (Vandeweyer et al., 2014). These findings provide evidence of complex protein-protein and multigene interactions that could be a consequence of epigenetic mechanisms. A biochemical analysis performed by Mandel et al. (2008) revealed that proteins form the SWI/SNF chromatin remodeling complex family (BRG1, BAF250a, and BAF170) immunoprecipitated with ADNP. Furthermore, ADNP binds to SMARCA2, SMARCA4, and SMARCC2 by its C-terminal end and possibly positions the whole protein complex in the appropriate DNA location by its zinc finger and homeobox domain. This crucial relationship explains the impact of epigenetic processes, involving nBAF complexes and concomitant enzymes on altered cognitive abilities, intellectual disabilities, and ASD. A specific protein architecture among nBAF complexes regulates the functional characteristics and tissue specificity during neuronal development. Location shift of 3 of 15 subunits of BAF components initiate dendritic outgrowth and axonal development. Thus, mutations of the nBAF protein components (SAMRCB1, SMARCA4, SMARCA2, SMARCE1, ARID1A, and ARID1B) are reported in syndromic intellectual disability disorders (Santen et al., 2012; Vandeweyer et al., 2014). To date, the reports of exome sequencing analyses of patients with ASD reveal even more important mutations in genes encoding the nBAF proteins, including BAF155, BAF170, BAF180, BAF250b, and BAF100a (O'Roak et al., 2012; Basak et al., 2015).

ISWI family

A complex taxonomy of the ISWI chromatin remodeling complexes is based on the conserved ATPase subunit that belongs to the ISWI protein family. In humans, the most common ATPase subunit is SMARCA5 (hSNF2h; Vignali et al., 2000; Becker and Hörz, 2002). A complex machinery of protein is formed around the ATPase subunit. The most widely studied protein complex is ATP-utilizing chromatin assembly and remodeling factors (ACF) and its subunit bromodomain adjacent to zinc finger domain 1A (BAZ1A; Table 3). Animal models imply an overexpression of the *ACF* (and *BAZ1A*) gene in the nucleus accumbens (NAc), which results in greater reductions of social interaction and lower sucrose preference. As much as 65% of experimental mice with up-regulated *ACF* exhibit depression-related behavioral abnormalities, including social avoidance. The up-regulation of *BAZ1A* correlates with a greater susceptibility to social defeat stress in animal models (Sun et al., 2015). This leads to a disruption in nucleosome location and gene silencing (Sananbenesi and Fischer, 2009). The chronic social defeat stress (CSDS) experiment demonstrates the importance and impact of epigenetic processes on the development of pathological behavior (Golden et al., 2011; Sun et al., 2015). It is undeniable that some individuals are characterized by genetic vulnerability to develop depression-like behavior. However, this genetic background is not sufficient; thus,

Table 3: Gene and subunit of the ISWI group AIP-dependent chromatin remodeling complexes.

Example of gene involved in coding units and subunits	Protein subunit	ISWI ATPase unit
BAZ1A	ACF	SMARCA5

an interaction with disadvantageous environment is obviously required for the development of depression (e.g. mice placed in a cage with dominant and an aggressive individuals with high position in the hierarchy). In this experimental paradigm, epigenetic changes are observed, which includes upstream regulation of the ISWI family subunit (BAZ1A), likely due to altered ventral tegmental area neuronal projections to NAc as well as the activation of the BDNF protein (Sun et al., 2015). In the NAc, BAZ1A alters the activity of chromatin remodeling complexes. ATP-dependent 'protein machinery' is more likely to position nucleosomes within the transcription start sites (TSS regions), blocking translation and silencing genes (Sananbenesi and Fischer, 2009). Therefore, depressive-like behavior can be observed not due to the activation of some defective or mutated genes but due to epigenetic alterations of ATP-dependent chromatin remodeling complexes (Figure 1).

The BAZ1B protein, with a similar structure to BAZ1A, works contradictory to BAZ1A and heightens responses to rewarding stimuli as well as promotes adaptive responses to aversive stimuli. BAZ1B appears to be closely related to chronic salient stimuli: cocaine administration and CSDS. Importantly, behavioral effects caused by cocaine and CSDS last longer than remodeled chromatin complex.

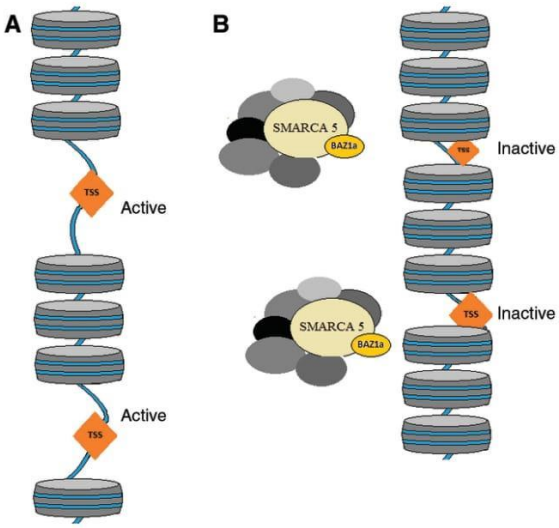


Figure 1: Euchromatin (A) changes to heterochromatin (B) in NAc. ATP-dependent chromatin remodeling complexes position nucleosomes close to the TSS region (B) and therefore unable transcription. In an animal model of CSDS, Sun et al. (2015) showed that such a phenomenon occurs more often among individuals susceptible to CSDS, and it leads to development depression-like phenotype.

BAZ1B levels return to control values within hours after cocaine administration or stress exposure, whereas behavioral repercussions of these treatments can be still observed for a long time afterward. Thus, it is hypothesized that epigenetic changes in chromatin induce further alterations in various genes, which exert longer behavioral effects (Sun et al., 2015). BAZ1A contribute to stress susceptibility maintenance of depressive-like phenotypes, whereas BAZ1B promotes adaptive responses to aversive stimuli, being hypothesized to serve as a stress resilience indicator. Other studies have revealed an interaction between the ACF (ISWI family) and BAF (SWI/SFN) components, particularly between *BAZ1B* and *SMACRA* genes, which can have clinical implications for future studies (Zaghlool et al., 2016). BAZ1B plays a pivotal role in neuron differentiation from pluripotent stem cells. Moreover, the haploinsufficiency of the *BAZ1B* gene plays a crucial role in the development of Williams syndrome (Lalli et al., 2016).

CHD family

In the CHD family, SNF2-like ATPase domain is located in the center of a protein network and the tandem chromodomains are located in the N-terminal region (Marfella and Imbalzano, 2007). There are several human diseases associated with the CHD protein family (e.g. dermatomyositis, Hodgkin's lymphoma, neuroblastoma, and the CHARGE syndrome) and some reports suggest that it has an anticancer properties (Rother and van Attikum, 2017), but due to the scope of this article we focus on the *CHD8* gene, which is strongly associated with ASD. This gene encodes a protein with the ATP-dependent chromatin remodeling activity. CHD8 binds to posttranslationally trimethylated histone H3 at active promoters and modulates the interaction between DNA and histone (Thompson et al., 2008; Cotney et al., 2015). Krumm et al. (2014) revealed that *CHD8* represents genes with *de novo* loss-of-function mutations in large-scale resequencing studies of ASD. Combined with reports of the CHD8 role in the neurodevelopment of midfetal cortex, these findings suggest an important role of the CHD protein family in the development of cognitive impairments. Other research has confirmed that CHD8 activity targets risk genes for ASD. Indeed, a loss of the *CHD8* gene significantly deregulates the function of ASD-related genes (Cotney et al., 2015). An important role of CHD8 in genomic homeostasis during neurodevelopment, along with reports on BAF protein and the ADNP enzymatic role (discussed in the 'SWI/SNF family' section), implies that understanding and

explaining the pathophysiology of ASD might be achieved using epigenetic approaches.

Memory of psychological trauma and ATP-dependent chromatin remodeling complexes

The definition of epigenetic processes emphasizes its crucial role in the mediation between environmental pressure and genotype (Waddington, 1968; Ptashne, 2007). Stressful and traumatic events, as part of environmental burden, have large impact on the development of the phenotype, somatic and mental health state, psychosocial functioning, and general environmental fitness (Weiner, 1992). Clinical studies reveal a number of posttranslational alterations in protein architecture that result in signaling cascade forming short-term memory. Then, epigenetic processes contribute to the conversion of unstable short-term memory to robust long-term memory trail. Thus, new gene expression is hypothesized to be crucial in creating long-lasting memory trail of a stressful event (Barrett and Wood, 2008; Kwapis and Wood, 2014). Animal studies of memory consolidation often use trauma paradigm or fear conditioning due to the necessity of strong stimulus appliance. There are several lines of evidence revealing how various traumatic events affect epigenetic processes, mainly due to histone acetylation, histone phosphorylation, and methylation, but there are also reports about DNA methylation and nucleosome remodeling complexes (Vogel-Ciernia et al., 2013; Kwapis and Wood, 2014; Sun et al., 2015). Epigenetic mechanisms of ATP-dependent chromatin remodeling complexes take part in memory consolidation through the nBAF complex and its BAF53b subunit. Although BAF53b seems to be pivotal for the consolidation of, particularly, visually contextual fear memory, it does not participate in the consolidation of auditory fear (Kwapis and Wood, 2014). The nBAF complexes play a crucial role in the consolidation of the hippocampus-related memory trails, whereas auditory amygdala-dependent trails are autonomous from the BAF53b complex. In animal studies, the CSDS paradigm is used to develop depressive-like features, manifesting in anxiety, anhedonia, and social avoidance in rodents (Golden et al., 2011). Considering the CSDS protocol, results obtained with this paradigm can easily be categorized as consequences of an exposure to trauma. In this protocol, a smaller mouse is placed in a cage with a bigger and more aggressive male. Interindividual interference that lasts for 5–10 min is pivotal for the protocol because, after the defeat of the smaller mouse, both rodents are separated by Plexiglass, allowing visual and olfactory, but not physical, contact for the following 24 h. The procedure is repeated for 10 consecutive days, each day with a different aggressor (Golden et al., 2011; Sun et al., 2015). The CSDS procedure attunes with modern trauma definitions that imply the occurrence of various types of traumatic events (Sideli et al., 2012; Misiak et al., 2017). Another important factor is interindividual interference (presenting in abuse, neglect, wars, human-made disaster, technological disasters, or assaults), considering there is a lower prevalence of posttraumatic stress disorder as an aftermath of natural disasters (Galea et al., 2005; Neria et al., 2008). Thus, taking into account all the variables discussed above, we here provide a crucial information about molecular alterations emerging after trauma. In animal models, applying the CSDS paradigm results in alterations among all four families of the ATP-dependent protein complexes (SWI/SNF, ISWI, CHD, and INO80) in the NAc. Particularly robust up-regulation of the BAZ1A protein was observed among stress-susceptible individuals, and the interaction between *BAZ1A* and *SMARCA5* genes in executing transcriptional regulations was noted. Interestingly, the up-regulation of BAZ1A mRNA is observed in the NAc in postmortem brain examinations in individuals with depression (Sun et al., 2015). This allows us to hypothesize similar epigenetic mechanisms among CSDS-susceptible people and animal (rodent) models. Moreover, a significant up-regulation in the prefrontal cortex of *BAZ1A* genes was observed in genome-wide expression profiling of schizophrenia patients performed by Mistry et al. (2013). This is in agreement with several studies showing an increased risk of psychosis in individuals with a history of childhood trauma (Varese et al., 2012). In addition, novel theoretical models consider childhood trauma (and concomitant epigenetic alterations) to be essential components of the etiology of psychosis (Misiak et al., 2017). Early-life adversities cause significant alterations in NAc activity (Tidey and Miczek, 1996; Gambarana et al., 2001; Salamone and Correa, 2012), which is a structure of great dependency from the ATP-dependent chromatin remodeling complexes (Sun et al., 2015).

Discussion

Epigenetic processes may not be a primary cause of psychiatric diseases, but their alterations can have an important downstream effect on mental health. Recent reports suggest a presence of an interrelation between chromatin remodeling complexes and even alcohol

dependence. Animal studies of the nematode *Caenorhabditis elegans* reveal a relationship between the SWI/SNF chromatin remodeling complexes and acute behavioral response to ethanol (Mathies et al., 2015). The results are significant for human studies mainly due to a similar and conservative molecular mechanism in mammals and worms. This relationship can be observed in clinical research, where an association among ATP-dependent chromatin remodeling complexes, antisocial behavior, and alcohol dependence has been shown (Mathies et al., 2015). It is highly plausible that ATP-dependent chromatin remodeling complexes assist in many neural processes and possibly even play a crucial role in the development of pathological mechanisms, but they are difficult to examine due to their subtle and intermediary functions. A mediation on the molecular level between environmental stimuli and an adequate genetic response is difficult to be investigated mainly because our methods and research paradigms may not be appropriate. Yoo et al., 2017 showed that ATP complexes that are pivotal in consolidating memory trials can peak up to 24 h after stimulation. If other ATP-dependent chromatin remodeling complexes work in a similar manner, we have to change our testing paradigms and stop looking for direct and rapid stimulus-based effects. The complexity of multiprotein constructions, diversity in subunit functions, and family 'taxonomy', along with a complex cooperation between various ATP-dependent chromatin remodelers, dims overall the picture of what we know about those molecules. Some studies (Larrieu et al., 2017) provide important reports about the neurochemical modifications and metabolic alterations in distinct brain regions, such as the NAc, which is known for being widely regulated by ATP-dependent chromatin remodelers (Sun et al., 2015). Extending our knowledge about epigenetic processes may contribute to explain these discrepancies. Novel theoretical models on the development of psychosis among people with childhood trauma emphasize the importance of environmental factors in developing psychopathology (Misiak et al., 2017). It seems inevitable that epigenetic mechanisms, such as chromatin remodeling complexes, play a pivotal role in the interdependence between environmental factors (like trauma) and genetic vulnerability, further leading to biological alterations that result in pathological and psychological mechanisms and psychiatric diseases. ATP-dependent chromatin remodeling complexes may predetermine mental disorders and constitute triggering factors for psychiatric diseases. Furthermore, epigenetic alterations of genes involved in the regulation of ATP-dependent chromatin remodeling complexes and schizophrenia pathophysiology are observed in our ancestral, phylogenetic tree. This validates the relevance and utility of psychiatric animal model studies. It is not difficult to hypothesize about the potential clinical importance of more robust research in this area. Examples of translating epigenetic mechanisms into clinical trials can be found in some diseases associated with neurodegeneration, such as Huntington's disease. Although it is a hereditary and fatal disorder caused by dynamic mutations, epigenetic modifications are critical in the development of Huntington's disease. Promising drugs that are being investigated in preclinical studies inhibit neurodegeneration in Huntington's disease via modifications of chromatin (Kim and Kaang, 2017). The majority of mental disorders cannot be described as monogenic phenotypes and one disease approach. The 'multiprotein machinery' of ATP-dependent chromatin remodeling complexes, alongside different regulatory enzymes, is involved in complex protein-protein networks and multigene systems. Improving our knowledge in this field may be challenging, but the complexity of environmental factors and genetic vulnerability creates a vast range of methods for both the prevention and management of various psychopathologies. Recent reports of epigenetic mechanisms, other than ATP-dependent chromatin remodeling complexes (like histone or DNA methylation and histone modifications), imply that substantial molecular changes can be induced by various lifestyle factors. Changes in the physical activity of schizophrenia patients may induce hypoacetylation of histone 4 as well as down-regulation of inflammatory processes in the peripheral blood (Lavratti et al., 2017). Another important relationship was pointed out by Alam et al. (2017), who highlighted the relationship among diet, gut microbiota, and different epigenetic processes (like DNA methylation), with relevance to mental disorders (Malan-Muller et al., 2018). It can be hypothesized that similar, yet undiscovered, relationships between lifestyle-dependent environmental factors and significant molecular alterations may be induced by epigenetic mechanisms related to ATP-dependent chromatin remodeling complexes. It seems evident that we are facing an uncharted field, where the association between environmental stress and genetic adaptation can be observed at the molecular level *in vivo*. Some authors hypothesized epigenetics to carry the potential of transgenerational inheritance (Woodhouse, 2018; Yeshurun and and Hannan, 2018). It is possible that we are yet to discover a whole new 'layer' of mechanisms that not only simply control gene expression but also serve as potential treatment and diagnostic targets.

Main findings

Our review of the current literature provides a framework to better understand the role of epigenetic mechanisms of ATP-dependent chromatin remodeling complexes in developing psychopathologies. Environmental pressure, manifesting in various forms, via epigenetic processes, interacts with genetic vulnerability and plays an important role in the development of psychiatric disorders. 'The protein machinery' of ATP-dependent chromatin remodeling complexes, as well as different accompanying enzymes, steers a dynamic process of DNA accessibility, up-regulate and down-regulate gene expression, and affect neuronal development. Three 'families' of proteins – SWI/SNF, ISWI, and CHAD, representing ATP-dependent chromatin remodeling complexes – play a crucial role in the development of various psychopathologies that are often accompanied by cognitive impairment. In this article, we reviewed the role of ATP-dependent chromatin remodeling complexes in forming long-term memory trails and attuned these findings to modern models of psychological trauma. We reasoned that psychological phenomena, including helplessness, forced submission, and low in-hierarchy position, lead to epigenetic changes. We showed evidence of the crucial role of chromatin remodeling complexes in the development of depressive-like behavior in the social defeat paradigm. We also provided potential limitations of laboratory methods (examining the molecular background of memory trials) due to the delayed timeframe of biochemical responses to environmental stimuli. The role of ATP-dependent chromatin remodeling complexes in neurodevelopmental disorders, including schizophrenia, ASD, and various intellectual disabilities, was described.

Finally, we noted that the multidependency of environmental factors and genetic vulnerability creates a vast range of therapeutic methods for both the prevention and management of different psychopathologies. We hypothesized that research in this field can not only broaden our knowledge in the area of pathophysiology of mental diseases but may also contribute to the development of swift diagnostic procedures as well as novel treatment targets.

References

Alam, R., Abdolmaleky, H.M., and Zhou, J.R. (2017). Microbiome, inflammation, epigenetic alterations, and mental diseases. AJGM: Neuropsychiatr. Genet. *174*, 651–660.

Banerjee, N., Polushina, T., Bettella, F., Giddaluru, S., Steen, V.M., Andreassen, O.A., and Le Hellard, S. (2018). Recently evolved human-specific methylated regions are enriched in schizophrenia signals. BMC Evol. Biol. *18*, 63.

Barrett, R.M. and Wood, M.A. (2008). Beyond transcription factors: the role of chromatin modifying enzymes in regulating transcription required for memory. Learn. Mem. *15*, 460–467.

Basak, A., Hancarova, M., Ulirsch, J.C., Balci, T.B., Trkova, M., Pelisek, M., and Dyment, D.A. (2015). BCL11A deletions result in fetal hemoglobin persistence and neurodevelopmental alterations. JCI. *125*, 2363–2368.

Becker, P.B. and Hörz, W. (2002). ATP-dependent nucleosome remodeling. Annu. Rev. Biochem. *71*, 247–273.

Cotney, J., Muhle, R.A., Sanders, S.J., Liu, L., Willsey, A.J., Niu, W., and Reilly, S.K. (2015). The autism-associated chromatin modifier CHD8 regulates other autism risk genes during human neurodevelopment. Nat. Commun. *6*, 6404.

Feng, J. and Fan, G. (2009). The role of DNA methylation in the central nervous system and neuropsychiatric disorders. Int. Rev. Neurobiol. *89*, 67–84.

Galea, S., Nandi, A., and Vlahov, D. (2005). The epidemiology of post-traumatic stress disorder after disasters. Epidemiol. Rev. *27*, 78–91.

Gambarana, C., Tolu, P.L., Masi, F., Rinaldi, M., Giachetti, D., Morazzoni, P., and De Montis, M.G. (2001). A study of the antidepressant activity of *Hypericum perforatum* on animal models. Pharmacopsychiatry *34*, S42–S44.

Golden, S.A., Covington, H.E., Berton, O., and Russo, S.J. (2011). A standardized protocol for repeated social defeat stress in mice. Nat. Prot. *6*, 1183–1191.

Gozes, I. (2016). The cytoskeleton as a drug target for neuroprotection: the case of the autism-mutated ADNP. Biol. Chem. *397*, 177–184.

Helsmoortel, C., Vulto-van Silfhout, A.T., Coe, B.P., Vandeweyer, G., Rooms, L., Van Den Ende, J., and Yntema, H.G. (2014). A SWI/SNF-related autism syndrome caused by *de novo* mutations in ADNP. Nat. Genet. *46*, 380.

Hoyer, J., Ekici, A.B., Endele, S., Popp, B., Zweier, C., Wiesener, A., and Zweier, M. (2012). Haploinsufficiency of ARID1B, a member of the SWI/SNF-a chromatin-remodeling complex, is a frequent cause of intellectual disability. Am. J. Hum. Genet. *90*, 565–572.

Jullien, P.E. and Berger, F. (2009). Gamete-specific epigenetic mechanisms shape genomic imprinting. Curr. Opin. Plant Biol. *12*, 637–642.

Kim, S. and Kaang, B.K. (2017). Epigenetic regulation and chromatin remodeling in learning and memory. Exp. Mol. Med. *49*, e281.

Koga, M., Ishiguro, H., Yazaki, S., Horiuchi, Y., Arai, M., Niizato, K., and Ozaki, N. (2009). Involvement of SMARCA2/BRM in the SWI/SNF chromatin-remodeling complex in schizophrenia. Hum. Mol. Genet. *18*, 2483–2494.

Krumm, N., O'Roak, B.J., Shendure, J., and Eichler, E.E. (2014). A *de novo* convergence of autism genetics and molecular neuroscience. Trends Neurosci. *37*, 95–105.

Kwapis, J.L. and Wood, M.A. (2014). Epigenetic mechanisms in fear conditioning: implications for treating post-traumatic stress disorder. Trends Neurosci. *37*, 706–720.

Lalli, M.A., Jang, J., Park, J.H.C., Wang, Y., Guzman, E., Zhou, H., and Tutulan-Cunita, A.C. (2016). Haploinsufficiency of BAZ1B contributes to Williams syndrome through transcriptional dysregulation of neurodevelopmental pathways. Hum. Mol. Genet. *25*, 1294–1306.

Lalonde, J., Reis, S.A., Sivakumaran, S., Holland, C.S., Wesseling, H., Sauld, J.F., and Haggarty, S.J. (2017). Chemogenomic analysis reveals key role for lysine acetylation in regulating Arc stability. Nat. Commun. *8*, 1659.

Larrieu, T., Cherix, A., Duque, A., Rodrigues, J., Lei, H., Gruetter, R., and Sandi, C. (2017). Hierarchical status predicts behavioral vulnerability and nucleus accumbens metabolic profile following chronic social defeat stress. Curr. Biol. *27*, 2202–2210.e4.

Lavratti, C., Dorneles, G., Pochmann, D., Peres, A., Bard, A., de Lima Schipper, L., and Elsner, V.R. (2017). Exercise-induced modulation of histone H4 acetylation status and cytokines levels in patients with schizophrenia. Physiol. Behav. *168*, 84–90.

Loe-Mie, Y., Lepagnol-Bestel, A.M., Maussion, G., Doron-Faigenboim, A., Imbeaud, S., Delacroix, H., and Moalic, J.M. (2010). SMARCA2 and other genome-wide supported schizophrenia-associated genes: regulation by REST/NRSF, network organization and primate-specific evolution. Hum. Mol. Genet. *19*, 2841–2857.

Magen, I., and Gozes, I. (2014). Davunetide: Peptide therapeutic in neurological disorders. Curr. Med. Chem. *21*, 2591–2598.

Malan-Muller, S., Valles-Colomer, M., Raes, J., Lowry, C.A., Seedat, S., and Hemmings, S.M. (2018). The gut microbiome and mental health: implications for anxiety-and trauma-related disorders. OMICS *22*, 90–107.

Mandel, S., Spivak-Pohis, I., and Gozes, I. (2008). ADNP differential nucleus/cytoplasm localization in neurons suggests multiple roles in neuronal differentiation and maintenance. J. Mol. Neurosci. *35*, 127–141.

Marfella, C.G. and Imbalzano, A.N. (2007). The Chd family of chromatin remodelers. Mutat. Res. *618*, 30–40.

Marom, R., Jain, M., Burrage, L.C., Song, I.W., Graham, B.H., Brown, C.W., and Gavrilova, R.H. (2017). Heterozygous variants in *ACTL6A*, encoding a component of the BAF complex, are associated with intellectual disability. Hum. Mutat. *38*, 1365–1371.

Mathies, L.D., Blackwell, G.G., Austin, M.K., Edwards, A.C., Riley, B.P., Davies, A.G., and Bettinger, J.C. (2015). SWI/SNF chromatin remodeling regulates alcohol response behaviors in *Caenorhabditis elegans* and is associated with alcohol dependence in humans. Proc. Natl. Acad. Sci. *112*, 3032–3037.

Misiak, B., Krefft, M., Bielawski, T., Moustafa, A.A., Sąsiadek, M.M., and Frydecka, D. (2017). Toward a unified theory of childhood trauma and psychosis: a comprehensive review of epidemiological, clinical, neuropsychological and biological findings. Neurosci. Biobehav. Rev. *75*, 393–406.

Mistry, M., Gillis, J., and Pavlidis, P. (2013). Genome-wide expression profiling of schizophrenia using a large combined cohort. Mol. Psychiatry *18*, 215–225.

Morrison, A.J. and Shen, X. (2009). Chromatin remodeling beyond transcription: the INO80 and SWR1 complexes. Nat. Rev. Mol. Cell Biol. *10*, 373–384.

Neria, Y., Nandi, A., and Galea, S. (2008). Post-traumatic stress disorder following disasters: a systematic review. Psychol. Med. *38*, 467–480.

O'Roak, B.J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., Coe, B.P., and Turner, E.H. (2012). Sporadic autism exomes reveal a highly interconnected protein network of *de novo* mutations. Nature *485*, 246.

Ouellet-Morin, I., Wong, C.C.Y., Danese, A., Pariante, C.M., Papadopoulos, A.S., Mill, J., and Arseneault, L. (2013). Increased serotonin transporter gene (SERT) DNA methylation is associated with bullying victimization and blunted cortisol response to stress in childhood: a longitudinal study of discordant monozygotic twins. Psychol. Med. *43*, 1813–1823.

Ovenden, E.S., McGregor, N.W., Emsley, R.A., and Warnich, L. (2018). DNA methylation and antipsychotic treatment mechanisms in schizophrenia: progress and future directions. Prog. Neuro-Psychopharmacol. Biol. Psychiatry *81*, 38–49.

Peter, C.J. and Akbarian, S. (2011). Balancing histone methylation activities in psychiatric disorders. Trends Mol. Med. *17*, 372–379.

Pries, L.-K., Gülöksüz, S., and Kenis, G. (2017). DNA methylation in schizophrenia. Adv. Exp. Med. Biol. *978*, 211–236.

Ptashne, M. (2007). On the use of the word 'epigenetic'. Curr. Biol. *17*, R233–R236.

Rother, M.B. and van Attikum, H. (2017). DNA repair goes hip-hop: SMARCA and CHD chromatin remodellers join the break dance. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *372*, 1–13.

Salamone, J.D. and Correa, M. (2012). The mysterious motivational functions of mesolimbic dopamine. Neuron *76*, 470–485.

Sananbenesi, F. and Fischer, A. (2009). The epigenetic bottleneck of neurodegenerative and psychiatric diseases. Biol. Chem. *390*, 1145–1153.

Santen, G.W.E., Aten, E., Sun, Y., Almomani, R., Gilissen, C., Nielsen, M., and Wessels, M.W. (2012). Mutations in SWI/SNF chromatin remodeling complex gene ARID1B cause Coffin-Siris syndrome. Nat. Genet. *44*, 379–380.

Schirer, Y., Malishkevich, A., Ophir, Y., Lewis, J., Giladi, E., and Gozes, I. (2014). Novel marker for the onset of frontotemporal dementia: early increase in activity-dependent neuroprotective protein (ADNP) in the face of tau mutation. PLoS One *9*, e87383.

Sideli, L., Mule, A., La Barbera, D., and Murray, R.M. (2012). Do child abuse and maltreatment increase risk of schizophrenia? Psychiatry Investig. *9*, 87–99.

Sun, H., Damez-Werno, D.M., Scobie, K.N., Shao, N.Y., Dias, C., Rabkin, J., and Cahill, M.E. (2015). ACF chromatin-remodeling complex mediates stress-induced depressive-like behavior. Nat. Med. *21*, 1146–1153.

Thompson, B.A., Tremblay, V., Lin, G., and Bochar, D.A. (2008). CHD8 is an ATP-dependent chromatin remodeling factor that regulates β-catenin target genes. Mol. Cell. Biol. *28*, 3894–3904.

Tidey, J. and Miczek, K.A. (1996). Social defeat stress selectively alters mesocorticolimbic dopamine release: an *in vivo* microdialysis study. Brain Res. *721*, 140–149.

Tsankova, N., Renthal, W., Kumar, A., and Nestler, E.J. (2007). Epigenetic regulation in psychiatric disorders. Nat. Rev. Neurosci. *8*, 355.

Tsurusaki, Y., Okamoto, N., Ohashi, H., Kosho, T., Imai, Y., Hibi-Ko, Y., and Fukushima, Y. (2012). Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome. Nat. Genet. *44*, 376–378.

Van Houdt, J.K.J., Nowakowska, B.A., Sousa, S.B., van Schaik, B.D., Seuntjens, E., Avonce, N., and Castori, M. (2012). Heterozygous missense mutations in SMARCA2 cause Nicolaides-Baraitser syndrome. Nat. Genet. *44*, 445–449.

Vandeweyer, G., Helsmoortel, C., Van Dijck, A., Vulto-van Silfhout, A.T., Coe, B.P., Bernier, R., and Wilson, M. (2014). The transcriptional regulator *ADNP* links the BAF (SWI/SNF) complexes with autism. Am. J. Med. Genet. Pt. C Semin. Med. Genet. *166*, 315–326.

Varese, F., Smeets, F., Drukker, M., Lieverse, R., Lataster, T., Viechtbauer, W., and Bentall, R.P. (2012). Childhood adversities

increase the risk of psychosis: a meta-analysis of patient-control, prospective- and cross-sectional cohort studies. Schizophr. Bull. *38*, 661–671.

Vignali, M., Hassan, A.H., Neely, K.E., and Workman, J.L. (2000). ATP-dependent chromatin-remodeling complexes. Mol. Cell. Biol. *20*, 1899–1910.

Vogel-Ciernia, A., Matheos, D.P., Barrett, R.M., Kramár, E.A., Azzawi, S., Chen, Y., and Jia, Y. (2013). The neuron-specific chromatin regulatory subunit BAF53b is necessary for synaptic plasticity and memory. Nat. Neurosci. *16*, 552–561.

Waddington, C.H. (1968). Towards a theoretical biology. Nature *218*, 525.

Walsh, T., McClellan, J.M., McCarthy, S.E., Addington, A.M., Pierce, S.B., Cooper, G.M., and Stray, S.M. (2008). Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. Science *320*, 539–543.

Weiner, H. (1992). Perturbing the Organism: The Biology of Stressful Experience (University of Chicago Press).

Woodhouse, R.M., Buchmann, G., Hoe, M., Harney, D.J., Low, J.K., Larance, M., and Ashe, A. (2018). Chromatin modifiers SET-25 and SET-32 are required for establishment but not long-term maintenance of transgenerational epigenetic inheritance. Cell Rep. *25*, 2259–2272.

Wu, L. and Liu, Y.J. (2007). Development of dendritic-cell lineages. Immunity *26*, 741–750.

Yeshurun, S., and Hannan, A.J. (2018). Transgenerational epigenetic influences of paternal environmental exposures on brain function and predisposition to psychiatric disorders. Mol. Psychiatry, *1*. https://doi.org/10.1038/s41380-018-0039-z [Epub ahead of print].

Yoo, M., Choi, K.Y., Kim, J., Kim, M., Shim, J., Choi, J.H., and Han, J.H. (2017). BAF53b, a neuron-specific nucleosome remodeling factor, is induced after learning and facilitates long-term memory consolidation. J. Neurosci. *37*, 3686–3697.

Zaghlool, A., Halvardson, J., Zhao, J.J., Etemadikhah, M., Kalushkova, A., Konska, K., and Feuk, L. (2016). A role for the chromatin-remodeling factor *BAZ1A* in neurodevelopment. Hum. Mutat. *37*, 964–975.

Tomasz Bielawski*, Lucas Albrechet-Souza and Dorota Frydecka

Endocannabinoid system in trauma and psychosis: distant guardian of mental stability

https://doi.org/10.1515/revneuro-2020-0102
Received September 6, 2020; accepted January 8, 2021;
published online March 1, 2021

Abstract: Central endocannabinoid system (eCBS) is a neuromodulatory system that inhibits potentially harmful, excessive synaptic activation. Endocannabinoid receptors are abundant among brain structures pivotal in different mental disorders development (for example, hippocampus, amygdala, medial-prefrontal cortex, hypothalamus). Here, we review eCBS function in etiology of psychosis, emphasizing its role in dealing with environmental pressures such as traumatic life events. Moreover, we explore eCBS as a guard against hypothalamic-pituitary-adrenal axis over-activation, and discuss its possible role in etiology of different psychopathologies. Additionally, we review eCBS function in creating adaptive behavioral patterns, as we explore its involvement in the memory formation process, extinction learning and emotional response. We discuss eCBS in the context of possible biomarkers of trauma, and in preclinical psychiatric conditions, such as at-risk mental states and clinical high risk states for psychosis. Finally, we describe the role of eCBS in the cannabinoid self-medication-theory and extinction learning.

Keywords: biomarkers; endocannabinoid system; HPA; psychosis; trauma.

Introduction

Endocannabinoid (eCB) neurotransmitters, specific cannabinoid receptors and eCB degradation enzymes constitute eCB system (eCBS)—an axis steering and co-regulating subcortical emotional processes, memory formation, after-stress homeostasis restoration—all of which contribute to the general behavioral adaptiveness. Discovery of cannabinoid receptors located in rat brain tissue (Devane et al. 1988) led to identification of similar receptors in humans (Matsuda et al. 1990). First cannabinoid receptors agonists, synthesized by the human body, were discovered in the early 90s of the XX century (Devane et al. 1992; Sugiura et al. 1995). Endocannabinoid receptor type 1 (CB1R) is expressed in the central nervous system, while endocannabinoid receptor type 2 (CB2R) is abundant in peripheral tissue and neuroglia (Scheller and Kirchoff, 2016). The 2-arachidonoylglycerol (2-AG) and *N*-arachidonoyl-ethanolamine (AEA) are main eCB agonists of CB1R and CB2R. The 2-AG and AEA display higher agonist efficacy at CB1R than CB2R, while 2-AG is more potent than AEA. Exposure to environmental pressure (stress) reduces AEA level which boosts anxiety, but simultaneously increases 2-AG that results in adaptation and termination of stress. Both molecules respond differently, but are strongly involved in a psychological outcome: anxiety-like behavior development (Hill et al. 2013). Neurotransmitters differ in terms of biochemical properties; 2-AG and AEA have different affinities toward CB1R and CB2R. Moreover, both present the ability to influence synaptic plasticity. While 2-AG is highly involved in hippocampal synaptic plasticity regulation, AEA plays a lesser role in this process (Augustin and Lovinger 2018; Luchicchi and Pistis 2012). These findings imply that albeit similar in structure, eCB agonists are not interchangeable and present highly specialized, different roles. eCBS comprise eCB degrading-enzymes: fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), as well as enzymes that are responsible for eCB synthesis—diacylglycerol lipases (DAGL) and N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD).

Triggered by environmental stress, anterograde synaptic activity in neurons induces post-synaptic release of eCB. Unique, retrograde signaling of eCB occurs among neurons that express cannabinoid receptors on axon terminals. The eCBS is stress-related, “antioverload” neuronal system that displays on-demand activity, without explicit diurnal pattern. This mechanism inhibits neuronal

***Corresponding author: Tomasz Bielawski**, Department of Psychiatry, Wroclaw Medical University, 10 Pasteur Street, 50-367 Wroclaw, Poland; and Department of Physiology, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA, E-mail: tomaszbielawski90@gmail.com.
Lucas Albrechet-Souza, Department of Physiology, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA; and Alcohol & Drug Center of Excellence, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA
Dorota Frydecka, Department of Psychiatry, Wroclaw Medical University, 10 Pasteur Street, 50-367 Wroclaw, Poland

activation, which in excessive or prolonged form is potentially harmful. Inhibition occurs among cells that are functionally capable to present opposing roles, for example neurons with both GABAergic and glutamatergic receptors (Zanettini et al. 2011). In our article, we review the neuroprotective function of eCBS against stress, in the light of the to-date reports of psychiatric relevance, in both human and animal models. Modern literature presents eCBS as a "distant guard" against harmful hypothalamic-pituitary-adrenal (HPA) over-activation. We present eCBS as an important factor in psychosis development, and as a system opposing harmful environmental stress. We discuss exogenous CB1R agonist phytocannabinoid tetrahydrocannabinol (THC) role in the context of cannabis self-medication theory. Function of peripheral eCBS, i.e. endocannabinoids and cannabinoid receptors located external to the central nervous system, is beyond the scope of our study.

Clinical background

Psychotic experience is a sensation of disconnection from reality, while a psychosis is a clinical syndrome of mental disorder, composed of delusions, hallucinations and thought disorder (Gaebel and Zielasek 2015). Number of genetic and environmental factors (socioeconomic status, substance abuse, low income, childhood trauma, urban environment, immigration) may contribute to development of mental disorders (Carr et al. 2000; Misiak et al. 2017; Yung et al. 2007). Genetically vulnerable individuals exposed to numerous detrimental environmental factors are At-Risk-Mental-States (Yung et al. 2007). People who present early signs and symptoms of psychosis (but do not fulfill the criteria of the mental disorder) are within prodromal stage of psychosis (Ferrarelli and Mathalon 2020). Clinical High Risk-group (CHR) are individuals who are at high risk of conversion from Prodromal Phase to a full-blown schizophrenia (Addington et al. 2011). First episode psychosis (FEP) refers to those who present psychotic symptoms at a clinical setting for the first time, and thus receive initial medical treatment (Breitborde et al. 2009). Finally, schizophrenia is the most common medical diagnosis when a patient is psychotic, neurological illnesses and/or substance abuse are excluded; it has three categories of symptoms: positive, where psychosis constitutes the main feature, negative, and cognitive (Althwanay et al. 2020).Changes within endocannabinoid system can be found on each level of the transition process from being at At-Risk-Mental-States to full-blown schizophrenia (see Figure 1).

It is important to note, literature lacks coherent and clear distinction between ARMS, CHR, prodromal states, thus we propose a simplified model that helps only to visualize the process of transition throughout consecutive stages of mental disorder. Many authors debate if ARMS is synonymous with prodromal state (van Os and Guloksuz 2014), or CHR (Fusar-Poli 2017). On the other hand, it is estimated that less than 25% of people in ARMS will transition to psychosis, with one study showing 4% of ARMS

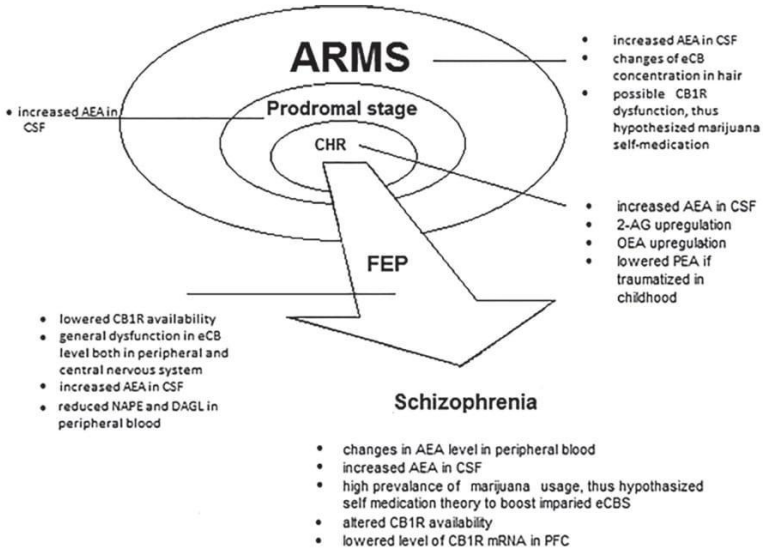


Figure 1: Selected changes in the eCBS throughout consecutive stages of schizophrenia development. ARMS, at-risk-mental-states; CHR, clinical high risk; cerebrospinal fluid (CSF); FEP, first episode psychosis; eCB, endocannabinoid; CB1R, endocannabinoid receptor type 1; AEA, anandamide; OEA, oleoylethanolamine; 2-AG, 2-arachidonoylglycerol; NAPE, N-acyl phosphatidylethanolamine phospholipase D; DAGL, diacylglycerol lipases; eCBS, endocannabinoid system; PFC, prefrontal cortex; PEA, N-palmitoylethanolamine.

turning into FEP (Allan et al. 2020) That is why, in our simplified model, we used ARMS as the widest category of which only a few will develop psychotic symptoms. Debate about clinical criteria distinguishing ARMS, CHR, prodromal stage is beyond the scope of our paper, as we use terminology originally applied in studies we review.

Traumatic experience (TE) captures a range of severe adverse experiences (traumas) such as physical, sexual or emotional abuse, neglect, parental death, bullying, while childhood traumas are harm, potential harm of threat of a harm resulting from commission or omission by child's caregivers (Misiak et al. 2017). TE contributes to development of numerous disorders such as anxiety disorders, schizophrenia, post-traumatic stress disorder (PTSD), depression. PTSD affects people who were exposed to potentially traumatic experience and re-experience it through intrusive memories, flashbacks, and nightmares, they suffer from active avoidance of external and internal reminders of the trauma and hyperarousal (Brewin et al. 2017). Exposure to trauma, especially during childhood, impacts immune system and might lead to pro-inflammatory state in the adulthood (Mehta et al. 2020; Misiak et al. 2017). Elevated level of tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) are related to physical and sexual abuse while parental absence influences C-reactive protein (CRP) level in blood (Baumaister et al. 2016). In adult PTSD patients, elevated levels of IL-6, IL-1β and interferon-γ (IFN-γ) are found (Passos et al. 2015). Interestingly, studies suggest CB2R stimulation reduces peripheral level of IFN-γ (Gibson et al. 2020) and IL-6 (Keen et al. 2014). Moreover, IL-6, IL-1β and TNF-α upregulations elevate mRNA expression of both CB1R and CB2R (Jean-Gilles et al. 2015). It can be hypothesized that eCBS actively mitigates ongoing inflammatory processes, thus prevalent cannabis abuse among people with PTSD and psychosis (Fowler et al. 1998; Rosen et al. 2006). History of trauma disrupts neuroanatomy of brain regions that develop during postnatal period—hippocampus, amygdala, prefrontal cortex, corpus callosum (Assogna et al. 2020; Teicher et al. 2003). Changes are more prominent when trauma occurs at earlier developmental stages, thus it has long-term developmental consequences that contribute to psychopathologies like psychosis (Assogna et al. 2020, Misiak et al. 2017). Brain structures sensitive to trauma are densely "coated" with eCBS that provides neuroprotection. While most people experience at least one TE during a lifetime, only a minority develops full-blown PTSD (Knipscheer et al. 2020). Thus we hypothesize that capacity and resistance of this "neuroprotective shield" significantly contributes to development of mental disorders such as PTSD and psychosis.

Cannabinoid receptor 1 in psychosis and trauma

In the brain, limbic structures are rich in CB1R, thus eCBS plays a profound role in emotional response and cognitive performance. Lasting impairment in CB1R results in depression-like symptoms (Parolaro et al. 2010) and vulnerability to chronic stress and anhedonia (Martin et al. 2002; Uriguen et al. 2004). In accordance with these findings, the CB1R antagonist (and antiobesity agent) rimonabant was withdrawn due to undesirable side effects of increased anxiety, depression and increased risk of suicide (Di Marzo and Després 2009). Similar results were observed in animal studies where rimonabant induced depression-like phenotypes (Beyer et al. 2010). CB1R is widely distributed among structures implicated in schizophrenia and PTSD development, such as prefrontal cortex (PFC), basal ganglia (BG), hippocampus, anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC) (Glass and Felder 1997). Difference in eCB binding to CB1R in ACC among patients with schizophrenia was first revealed by Zavitsanou et al. (2004). Lower level of CB1R mRNA was found in the PFC of schizophrenia patients (probably to increase GABA transmission when GABA synthesis is impaired) (Eggan et al. 2008, 2012), while CB1R density in PCC was increased among patients with schizophrenia, likely due to reduced amygdala connectivity and PCC cytoarchitecture (Newell et al. 2006). There is a growing evidence that among patients with schizophrenia, cannabidiol (CBD), a negative allosteric modulator of the CB1R (Laprairie et al. 2015), reduces psychotic symptoms both in a monotherapy (Leweke et al. 2012) and as an adjunctive treatment (McGuire et al. 2018). It has been suggested that antipsychotic treatment increases CB1R availability. Medicated patients with schizophrenia have been shown to have CB1R availability closer to that of healthy controls in comparison to unmedicated patients, suggesting that antipsychotic treatment may normalize CB1R availability in the whole brain (Ceccarini et al. 2013; Ranganathan et al. 2016). A recent review on positron emission tomography (PET) studies on eCB signaling shows global, aberrant CB1R binding in schizophrenia (Sloan et al. 2019). There are three human PET studies on CB1R binding in schizophrenia so far, two of them showing increase in CB1R availability in schizophrenia (Ceccarini et al. 2013; Wong et al. 2010), while the third one found reductions in global CB1R availability, in both medicated and unmedicated patients (Ranganathan et al. 2016). Contradictory results are possibly due to PET study limitations, since radiotracers used to probe the CB1R cannot discriminate whether

altered radiotracer binding is due to changes in receptor density, occupancy, or affinity. Moreover, tobacco consumption, prevalent among individuals with psychiatric disorders, lowers CB1R availability (Sloan et al. 2019). Additionally, receptor availability is often affected by individual genetic ancestry (Hirvonen et al. 2012). eCBs are involved in a number of neuronal developmental processes prenatally (Richardson et al. 2016). Interestingly, it seems there may be a "window of vulnerability" at the onset of puberty—a period of brain maturation when it is the most vulnerable toward the development of psychosis. Some hypothesize that cannabis use during this period may contribute to development of psychosis (Radhakrishnan et al. 2014), as THC consumption induces psychotic symptoms and affects dopamine regulation processes (discussed in "ARMS and self-medication theory" paragraph).

Endocannabinoids in psychosis and trauma

In recent years there has been growing interest in eCB changes in different mental disorders, mainly in the context of developing psychosis, depression and PTSD (Table). AEA concentration in blood and cerebrospinal fluid (CSF) is significantly higher among individuals with schizophrenia, in comparison to healthy controls. Elevated level of AEA in CSF was found in every stage of illness, from the first episode to chronic states, and was stable in time (independent of diurnal cycle) and independent of antipsychotic medication (Minichino et al. 2019). AEA serum level is increased in schizophrenia, while clinical remission is associated with AEA serum decrease (Koethe et al. 2019). Blood level of AEA is elevated among schizophrenia patients in comparison to healthy controls, while individuals in prodromal states of psychosis present higher CSF levels of AEA (Koethe et al. 2009). Interestingly, general increase of AEA was found to decline severity of positive and negative symptoms and has an impact on cognitive functioning (Minichino et al. 2019). It is hypothesized that an increase in AEA synthesis is a compensatory mechanism aimed to counter dopaminergic hyperactivity, observed during psychotic states. It may be hypothesized that AEA increase is an early reaction against psychosis development. Administration of antipsychotic drugs that antagonize dopamine receptors (such as D_2 antagonist—haloperidol and atypical $5HT_{2A}/D_2$—risperidone, clozapine) reduces psychotic symptoms and causes reduction of AEA blood levels (Giuffrida et al. 2004).

Vast interest in AEA function in context of psychiatric states is mainly due to AEA potency (Hillard 2000), but

Table 1: Summary of selected findings of eCB in human studies.

Source	eCB	Tissue	Illness stage	eCB in comparison to healthy control
Koethe et al. 2019 systematic review	AEA	CSF	Schizophrenia	Upregulation
	AEA	Serum	schizophrenia	Upregulation
	AEA	CSF	Schizophrenia prodromal states	Upregulation
Hill et al. 2009 systematic review	AEA	Serum	Major depression	Downregulation
	2-AG	Serum	Major depression	Downregulation
Appiah-Kusi et al. 2019	2-AG	Plasma	CHR	Upregulation
	AEA	Plasma	CHR	Upregulation
	OEA	Plasma	CHR	Upregulation
Hill et al. 2013	2-AG	Plasma	PTSD	Downregulation
Wilker et al. 2016	OEA PEA SEA	Hair	PTSD	Downregulation

AEA, anandaminde; 2-AG, 2-arachidonoylglycerol; OEA, N-oleoylethanolamine; PEA, N-palmitoylethanolamine; SEA, stearoylethanolamide; CHR, clinical high risk for psychosis; PTSD, post-traumatic stress disorder; CSF, cerebrospinal fluid.

AEA is a partial agonist of CB1R and a weak agonist of CB2R (Reggio and Traore 2000). 2-AG is much more abundant in the human brain, and thus is the most efficient agonist of both CB1R and CB2R (Reggio and Traore 2000; Stella 1997). Basal serum concentrations of both 2-AG and AEA are significantly lower in women with major depression, in comparison to healthy controls (Hill et al. 2009). In the hippocampus 2-AG is a neuromodulator of synaptic plasticity (Luchicchi and Pistis 2012), while its high level in peripheral blood reduces anxiety-like behavior and stress related behavior (Choukèr et al. 2010). Genetic disruption of 2-AG synthesis induces anxiety and depressive behavior (Hill et al. 2013; Shonesy et al. 2014), decrease in peripheral blood is found among PTSD and major depressive disorder (MDD) patients (Hill et al. 2013). 2-AG mediates long-term depression and heterosynaptic long-term potentiation among neurons in different regions of the hippocampus, therefore is pivotal in learning and memory formation. Moreover, preclinical findings suggest improved cognitive performance among individuals with higher levels of 2-AG (Pan et al. 2011), other authors imply its involvement in cognitive dysfunctions in animal model of schizophrenia (Vigano et al. 2009) and extinction learning (Marsicano et al. 2002). Moreover, 2-AG regulates excitatory afferents to dopamine neurons in the ventral tegmental area (VTA) (Melis et al. 2004), and mediates firing activity of nucleus

accumbens (NAc) neurons (Seif et al. 2011). Recent study by Appiah–Kusi was the first to find an increase in AEA synthesis in clinical high-risk (CHR) for psychosis group (Appiah-Kusi et al. 2019).

There are several structural analogues to 2-AG and AEA, such as N-palmitoylethanolamine (PEA), N-oleoylethanolamine (OEA), stearoylethanolamide (SEA). Altered levels of OEA and PEA are found in peripheral blood in animal studies of trauma (Holman et al. 2014). In human studies, OEA and PEA levels increase immediately after exposure to psychosocial stress (Dlugos et al. 2012) and decline in recovery phase (Hill et al. 2009). Moreover, hair concentration of PEA, OEA and SEA, may serve as a novel biomarker of distant traumas (Wilker et al. 2016). Single study done by Koethe et al. (2019) found elevated levels of PEA among twin-pairs discordant for schizophrenia. Research done by Appiah-Kusi et al. (2019) found an increase in OEA CHR individuals.

To summarize, altered levels of eCB are found in PTSD, different stages of schizophrenia and depression. While AEA levels in CSF seems to be stable markers of schizophrenia, independent of antipsychotic medication and stage of the illness, blood levels of eCB measured in peripheral blood vary in regard to stages of illness and applied pharmacotherapy. Moreover, eCB levels seem to influence symptomatology of mental disorders, probably via intensified inhibition of neuronal excitation of various structures in the brain (NAc, VTA).

Fatty acid amide hydrolase enzyme in psychosis

Fatty acid amide hydrolase (FAAH) is an integral membrane enzyme that hydrolyzes the AEA and related amidated signaling lipids. FAAH activation decreases eCB activity, facilitating HPA axis stimulation (Hill et al. 2009). Treatment with FAAH inhibitor increases concentration of AEA (without alternation of 2-AG) in the brain, thus contributes to non-depressive-like phenotype with low anxiety and low avoidance (Kathuria et al. 2003). Similar results were obtained during administration of pharmacological AEA reuptake inhibitor AM404. Administration of AM404 contributes to endogenous anandamide concentration (De Marchi et al. 2003; Naderi et al. 2008; Zanettini et al. 2011) and increases 2-AG signaling in the rat brain (Wiskerke et al. 2012). High level of FAAH inhibitor and AM404 administration increases levels of eCB signaling, decreases anxiety (Lisboa et al. 2008). Interestingly, this mechanism possibly contributes to exacerbation of psychotic symptoms in humans. Recent human PET studies confirm that hypothesis—lower FAAH (and thus higher AEA levels) predicted positive psychotic symptoms severity among schizophrenia patients (Watts et al. 2020). We hypothesize that high AEA obtained due to inhibition of FAAH (or due to AM404 administration in animals model) significantly contributes to low-anxiety, low-depression outcome but it also elevates positive symptoms in schizophrenia (such as delusions or hallucinations). In accordance, low AEA (via FAAH activity or AM404 withdrawal) contributes to high-anxiety and depression, elevates negative symptoms of schizophrenia (such as flattened affect or reduced speech) and reduces positive symptoms. Interestingly, there are studies that associates FAAH polymorphism with substance abuse (Bioque et al. 2019; Chiang et al. 2004; Hindocha et al. 2020), and a single study that associates polymorphisms of NAPE-PLD and FAAH genes with schizophrenia (Si et al. 2018). Future studies should investigate other eCB enzymes like MAGL or DALG in the context of psychosis development.

The HPA axis and eCBS

eCBS is strongly involved in regulation of the HPA axis that takes part in immediate response to stress, memory formation and afterward homeostasis resumption. HPA axis activation is mediated by corticotropin-releasing hormone (CRH) that expresses neurons in the paraventricular nucleus (PVN) of the hypothalamus. CRH is also expressed in nonhypothalamic brain regions, including the amygdala, bed nucleus of the stria terminalis (BNST) and dorsal hippocampus. PVN responds and consolidates information from amygdala, hippocampus, medial prefrontal cortex, brain stem (Hillard 2000). CRH upregulates adrenocorticotropic hormone (ACTH) which acts as agonist of ACTH receptors (MC-2 receptors) and induces glucocorticoid synthesis. Glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) are found in a great number in the prefrontal cortex, hypothalamus as well as limbic regions, including the hippocampus, amygdala and septum. Cortisol release targets GR and MR and is responsible for a vast number of after-stress alterations like suppression of immune system, changes in memory, mood alterations or increased food intake (Joëls et al. 2012).

Some structures, abundant in CB1R, serve as “distant gates” of HPA activation control—pituitary gland (Pagotto et al. 2001), adrenal glands (Ziegler et al. 2010) and PVN (Wamsteeker et al. 2010; Wittman et al. 2007). CB1R can both reduce and increase HPA activity, an effect depends

on the dose of CB1 agonist release, neuronal population affected by the eCB and tissue maturation (Steiner and Wotjak 2008). Individuals with lower AEA baseline level show greater rise in cortisol immediately after stress in comparison to those with higher AEA baseline level (Dlugos et al. 2012), animal studies reveal that complete loss of CB1R results in increased glucocorticoids release in response to acute stress (Cota 2007). These lines of evidence support a hypothesized inhibitory role of eCBS on the HPA axis (Hill et al. 2010). Inhibition can occur on many different levels of HPA-eCB co-regulation. CB1 receptors activation on glutaminergic outputs restricts PVN CRH neurons stimulation and reduces glucocorticoids release. Activation can derive in the hippocampus, where endocannabinoids find a robust number of CB1 receptors (Wise et al. 2009). Hippocampus is strongly involved in HPA activity (Hillard 2018), potent eCB signaling in this region serves as a protection buffer from prolonged cortisol activity that has known harmful effects on cognitive functioning (Hillard 2018; Pruessner et al. 2017). Thus, dense CB1R population serve as a counterbalance to the great number of corticosteroid receptors present in the hippocampus. Schizophrenia and bipolar disorder patients with positive history of childhood traumas have elevated hair cortisol level and present poor cognitive functioning, all of which can be associated with HPA axis alterations (Aas et al. 2019). Similar results were obtained in PTSD survivors of atrocious war in Uganda (Wilker et al. 2016). Prolonged PVN activation can be inhibited by medial prefrontal cortex, another structure rich in CB1R that serves as a distant gate of HPA activation (Hill et al. 2011). Moreover, high level of eCB in basolateral amygdala inhibits HPA axis activation and affects acute stress reaction in animal models (Ganon-Elazar and Akirav 2009) as well as memory formation in humans (Hill et al. 2009). AEA release from amygdala targets CB1R, eCBS inhibits glutamate release which suppress excitatory afferents to the amygdala, in a self-regulating mechanism (Hill et al. 2009). Moreover, pituitary gland CB1R activation lowers levels of ACTH and affects adrenal steroidogenesis (Ziegler et al. 2010) and thus, epinephrine release (Niederhoffer 2001). On the contrary, severe or prolonged stress increases CRH and *CRH*-mRNA levels in amygdala and in BNST, activating HPA. Thus, eCBS gates not only all components of the HPA axis, but also structures closely involved (and targeted) by its activity (hippocampus, amygdala, medial prefrontal cortex, hypothalamus).

Individuals with PTSD exhibit numerous changes in HPA axis functioning, with abnormally low levels of cortisol and chronic increase in CRH in comparison with controls (Sbarski and Akirav 2020). Moreover, there is a cortisol dysregulation that occurs within hours after trauma, among people who experienced traumatic events, in comparison with traumatized individuals without PTSD (McFarnalne et al. 2011). It is hypothesized that excessive CRH release results in abnormally low levels of cortisol, but also enhanced sensitivity and greater number of glucocorticoid receptors. This leads to greater cortisol binding and HPA axis overactivation (Szeszko et al. 2018), that can be downregulated by eCBS (Hill and Tasker 2012). PTSD patients present altered CRH and *CRH*-mRNA levels in amygdala and PVN. In blood, elevated levels of cortisol has been observed among psychotic patients, as well as increased levels of serum cortisol in first episode psychosis (FEP) patients (Pruessner et al. 2017). Study done by Walker et al. (2013) revealed that high baseline cortisol level predicts transition to psychosis among youths at-risk. Moreover, patients suffering from major depression with psychosis have significantly elevated evening cortisol level in comparison to individuals with nonpsychotic major depression and healthy control. In the high-cortisol level group, diminished cognitive performance was observed (Keller et al. 2017). Higher cortisol baseline level is elevated among healthy individuals with high risk for psychosis (Pruessner et al. 2017). Among children at risk of schizophrenia, altered cortisol level associates with disruption in cognitive abilities (Cullen 2014). This may partly derive from neuroanatomical changes common in psychosis, as well as prefirst episode changes, including reduced volume of matter in different brain regions, including hippocampus, pituitary glands, amygdala. Interestingly, all of these regions are crucial for HPA axis activity, but also rich in eCB system receptors and neurotransmitters. Great reduction of hippocampus volume is one of the most consistently reported structural alterations in psychosis (Adriano et al. 2012), moreover shrinkage seems to progress as the disease advances in time (Velakoulis et al. 1999). Noteworthy, the burden of chronic traumatic stress can induce brain structural abnormalities, especially in CA1 hippocampus region, that is reported in animal models (Schoenfeld et al. 2019). Interestingly, volume reduction of CA1 region is also considered to correlate with positive symptoms in patients with schizophrenia (Kühn et al. 2012). While some studies suggest volume shrinkage has a genetic background, Pruessner et al. (2015) revealed an association between cortisol level and shrinkage of the left hippocampus in FEP and CHR. These results corroborate a study done by Mondelli et al. (2010), that reports a relationship between smaller hippocampus volume and high cortisol diurnal level among patients with psychosis. Animal models reveal stress-induced chronic HPA axis activation can cause cell damage in hippocampus and medial PC (Cerqueria et al.

2008; Sapolsky et al. 2000), both structures with great density of endocannabinoid CB1 receptors (Hill et al. 2011).

In this paragraph, we presented eCBS neuroprotective function against HPA overstimulation. Detrimental cortisol augmentation affects numerous brain structures and impairs cognitive abilities (Walder et al. 2000), thus advances development of mental disorders. eCBS controls HPA axis on many levels and thus preserves numerous brain structures against prolonged, stress induced neuronal excitation. Animal studies present results complementary to human studies and suggest interdependence between eCB level and dopamine activity. Some authors propose HPA axis-targeted, pharmaceutical treatment strategy that blocks CRH neurons in hypothalamic PVN that prevents cortisol upregulation (Wolkowitz et al. 2009). Wolkowitz's theoretical model was focused on minimizing HPA activation on multiple levels, we believe it may be with benefit to approach this concept with consideration of eCBS properties discussed above.

Cognitive abilities

Short- and long-term memory

First attempts to estimate the influence of eCBS on cognitive abilities were based on the reports of great density of CB1R in hippocampus, hippocampal formation and basal ganglia—structures strongly involved in memory and learning (Herkenham et al. 1991; Zanettini et al. 2011). Animal models showed appliance of CB1R agonists (via intraperitoneal injection) effectively reduced spatial memory acquisition during aversive trials, negative effects were predominantly reversed after antagonist administration (Da Silva and Takahashi 2002). Interestingly, appliance of CB1R agonist in animals with well-established long-term memory trails did not reduce their performance (Mishima et al. 2001). Thus, it is hypothesized that increased activity that targets CB1R hampers an active process of spatial memory trail acquisition (and possibly consolidation), but not spatial memory retrieval. Intrahippocampal increase in CB1R signaling results in impaired object recognition (OR) and hippocampal CB1R knockout mice are characterized by enhanced OR (Maccarrone et al. 2002; Quinn et al. 2008). Animal models provide ample evidence for impaired working memory after administration of CB1R exogenous agonists—marijuana (Mishima et al. 2001; Varvel et al. 2001). This phytocannabinoid alters activity of hippocampus (Zanettini et al. 2011) and dopamine signaling in PC (de Melo Rodrigues et al. 2011). In FEP individuals, peripheral eCB synthesizing enzymes NAPE and DAGL are reduced in comparison with healthy controls. Moreover, in the subgroup of FEP cannabis users, additional increase of FAAH and MAGL (both eCB degrading enzymes) becomes relevant in relation to healthy controls (Bioque et al. 2013). Decrease in eCB synthesis and increase in peripheral eCB degradation seem to contribute cognitive decline among FEP individuals. Bioque et al. (2016) reveal correlation between lower scores in verbal and working memory with low levels of NAPE and DAGL, while taking into account phytocannabinoid usage. Moreover, increased FAAH level correlated with decline in verbal memory (Bioque et al. 2016). Obtained result is supported by animal studies, where it has been shown that FAAH inhibition improves hippocampal-dependent memory formation (Rivera et al. 2018).

Extinction learning in PTSD

The term "extinction" refers to the gradual decline of learned response, when the element that induced learning is gone. Thus, it can be termed "unlearning" or "learning new developmental patterns". Studies imply that CB1R knockout in animals causes impairment in extinction learning. Similar results were obtained after administration of CB1R antagonist (rimonabant) (Nissen et al. 2010). During the process of memory extinction, appearance of previously conditioned stimulus (auditory stimulus that once preceded foot-shock) effects with AEA and 2-AG release, which enhances extinction learning (Marsicano et al. 2002). Noteworthy, eCB levels increase during extinction learning occurs mainly in amygdala, while eCB levels in mPFC remain unaltered. Animals with functioning eCBS extinguished freezing behavior, while CB1R knockout presented prolonged freezing in exposure to previously conditioned stimulus (Marsicano et al. 2002). Moreover, high concentration of AEA (via increase of AEA intake inhibitor—FAAH) promotes extinction of both startle and freezing behavior during aversive tests (Zanettini et al. 2011). Interestingly, FAAH inhibitors seem to enhance both extinction learning and new memory acquisition. This is likely due to FAAH regulating not only AEA level in the brain, but also other eCB concentration—OEA and PEA (Murillo-Rodríguez et al. 2006; Varvel et al. 2006). It may be that eCBS plays a crucial role in overcoming stressful events and prevents antiadaptational, ruminations—symptoms common in PTSD and depression. PTSD disrupts numerous brain structures, neurotransmitters and hormones, most of which are affected by eCBS activity (Sbarski and Akirav 2020). Administration of synthetic CB1 agonist

(Nabilone) reduced PTSD symptoms severity in humans (Cameron et al. 2014), similar results were obtained with CBD administration in the subgroup of PTSD patients (Elms et al. 2019). Animal studies revealed advantageous effects of CB1R agonist administration during traumatic memories extinction (Lisboa et al. 2019). Treatment that stimulates CB1R potentiates extinction learning in the presence of stressful stimuli in animal models (Sbarski and Akirav 2020). Recent meta-analysis (Raymundi et al. 2020) reveals dronabinol administration (synthetic version of THC) potentates extinction learning in healthy individuals, while in the group of PTSD patients subsequent THC administration reduced anxiety and improved global reduction of PTSD symptoms severity (Roitman et al. 2014). Noteworthy, anxiolytic effect shifts toward an anxiogenic state with increased dose of THC. It can be prevented when CBD is administered concurrently in a dose similar or higher than THC (Raymundi et al. 2020). Intrusive and aversive memories constitute PTSD symptomatology, thus eCB may not only alleviate symptoms, but enable the process of extinction learning on maladaptive patterns, acquired as a result of exposure to traumatic experience. This is reinforced by studies that show antidepressants are efficient in treating symptoms of mood disorder and anxiety in PTSD, but fail to reverse fear memory dysfunction (Lin et al. 2016). On the other hand, animal models provide ample evidence of dopamine involvement in extinction learning in rodent PTSD models. DA is a crucial component of the fear circuit that regulates memory formation after traumatic stress. Key structures involved in this process; amygdala, hippocampus, mPFC, striatum are all highly dependent on dopamine system response (Lin et al. 2016), but also are key structures of eCBS. Moreover, dopamine receptor 1 (DR1) modulates acquisition of memory extinction and DA receptor 2 (DRD2) modulates consolidation of memory extinction (Madsen et al. 2017). Rich in DA, striatum consolidates cortical information promoting memory formation and reward learning, but DA activity is dependent on striatal synaptic plasticity regulated by eCBS. Concisely, DA system responds in accordance with wide range of signaling patterns received via rich in CB1R neuronal pathways. eCBS mediates long-term neuronal depression (eCB-LTD), that provides plasticity and prevents excessive neuronal activation in striatum (Xu et al. 2018). Abnormalities in DA system are exposed both in animal models (Enman et al. 2015; Lin et al. 2020), and human studies (Torrisi et al. 2019). It is our belief, that interaction between DA and eCBSin the context of extinction learning is crucial to understand PTSD pathophysiology and symptomatology.

Endocannabinoids as biomarkers of mental disorders

Some authors hypothesize both eCB concentration and eCB receptors density in different tissues may serve as possible biomarkers of psychiatric conditions (Bioque 2016). Recent studies present ample evidence to support this assumption. AEA peripheral blood serum level is associated with binocular depth inversion illusion (BDII) among patients with schizophrenia and in ARMS individuals (Reuter et al. 2017). BDII is a perception of a convex surface, even though observer receives concavity-consistent stereoscopic information. This cognitive impairment is frequently observed in schizophrenia and ARMS. Reuter and colleagues found association between AEA peripheral serum levels and binocular BDII score in schizophrenia patients in regard to healthy control. This implies peripheral AEA may serve as a biomarker of eCBS activity in the brain. On the other hand, AEA levels in peripheral blood differ from AEA level in CSF, in the subgroup of psychotic patients (Minichino et al. 2019). It is possibly due to rich in FAAH blood–brain barrier that differentiates AEA levels in central nervous system and peripheral blood (Minichino et al. 2019; Zhuang et al. 2013). It is hypothesized that despite blood-brain barrier, AEA may still be a valid biomarker of eCBS activity in the brain due to abundant and direct blood transfer via dural venous sinuses from frontal region of the brain to the bloodstream. In FEP, similar dysregulation in eCB levels circulating in peripheral and central nervous system occurs, noteworthy, it is not reported in healthy individuals (Dickens et al. 2020). Moreover, CB1R availability is lower among unmedicated FEP individuals, in relation to healthy individuals (Borgan et al. 2019).

AEA level in CSF in schizophrenia patients is significantly increased in comparison to healthy control (Giuffrida et al. 2004). In regard to trauma, there is an alteration in blood levels of AEA, PEA and OEA after stress exposure. Moreover, AEA concentration is negatively correlated with anxiety baseline among healthy individuals after acute trauma (Dlugos et al. 2012; Holman et al. 2014). Elevated level of anandamide in CSF among psychotic patients is independent from the current stage of disease, cannabis use and pharmacological treatment, thus it may serve as a biomarker (Minichino et al. 2019). Unexpected, novel biomarkers of psychiatric significance may be located in human hair (Aas et al. 2019; Wilker et al. 2016). Study done on PTSD survivors of Ugandan war reveals relationship between PTSD symptoms and eCB neurotransmitters concentration in human hair. A negative relationship between

PTSD severity and concentration of OEA, PEA and SEA was observed. Intrusion, avoidance and hyperarousal were negatively associated with eCB levels in hair. Moreover, a negative relationship was observed between the number of traumatic exposures and OEA, PEA and SEA hair concentration. In the Wilker et al. (2016) study, hair samples were no more than 3 cm in length, analysis showed no association between potentially traumatic experiences that occurred during 12 months preceding the study, and endocannabinoids concentrations. This makes the method potentially useful and relevant to study past traumas. Another research revealed correlation between traumatic events (childhood maltreatment) in patients with schizophrenia and bipolar disorder and elevated level of hair cortisol concentrations (HCC) (Aas et al. 2019). Patients with childhood maltreatments had higher HCC in comparison to patients without a history of abuse, and in comparison to healthy controls. Moreover, higher HCC reflected disrupted cognitive functioning in the domain of working memory. Perhaps the most prominent factor of hair analysis method is substantial resistance to diurnal-rhythm concentration of different toxicology-important substrates. Blood, saliva, urine are commonly used in modern toxicology due to high sensitivity to sudden fluctuation (variation) of analyzed agent concentration, but are not suitable for evaluation of chronic states (Stalder and Kirschbaum 2012). Hair analysis may serve as a better, noninvasive indicator of distant and prolonged trauma, enabling retrospective assessment. Incorporation of different substances (like cortisol or eCB neurotransmitters) into the hair proceeds via blood during formation of the hair shaft, but also during continuous, everyday routine of sweat and sebum absorption. HCC seems to be advantageous when it comes to prediction of future trauma development, as lower concentration of baseline HCC may be a risk marker of PTSD development (Steudte-Schmiedgen et al. 2015).

Both studies (Aas et al. 2019; Wilker et al. 2016) display a potentially novel method of upregulated HPA activity measurement via eCB and HCC in regard to delayed traumatic events. Thus, that approach may be a novel ARMS diagnostic opportunity. Higher blood levels of AEA, 2-AG and PEA are found among individuals with a history of childhood maltreatment (Appiah-Kusi et al. 2019). Interestingly, the same study implies the relationship between PEA level and childhood trauma in the context of CHR of schizophrenia. Individuals within CHR who never encountered trauma during childhood, had lower levels of PEA in comparison with healthy controls. In contrast, those with a positive history of childhood trauma and in the CHR group, had higher levels of PEA in comparison to both, healthy control and individuals with only one traumatic event (Appiah-Kusi 2019). Individuals with mental exhaustion caused by work-related stress (clinically burnout patients) are characterized by lower AEA concentration in hair (Gao et al. 2020).

ARMS and self-medication theory

Influence of endocannabinoids on anxiety seems to be ambiguous, due to the opposed effect of high and low doses of the CB1R agonists (Raymundi et al. 2020; Zanettini et al. 2011). Low to medium dose of THC enhances anxiolytic state, while high doses reinforce anxiogenic state. Animal studies present comparable results: while low doses of external eCB agonists often elicit lower levels of avoidance and anxiety, high doses result in increased avoidance and anxiety (Haller et al. 2007). Consumption of an exogenous CB1R agonist, phytocannabinoid THC can activate HPA and enhance anxiety (Steiner and Wotjak 2008), these results seem to confirm a common statement formulated by marijuana users, that the effect of THC depends on dose, environmental factors like already existing level of stress, mood and individual attitude toward experience. Most people self-administer marijuana to achieve a state of relaxation and joy (Moreira and Wotjak 2009) but THC can cause opposite effect, in the presence of stressful conditions, likely due to increased striatal DA uptake (Bloomfield et al. 2014; MacLean and Littleton 1977). Numerous studies report prevalence of marijuana usage among ARMS (Rosen et al. 2006) and people with schizophrenia (Fowler et al. 1998). Both stress (Norman and Malla 1993) and cannabis (Thornicroft 1990) contribute to acceleration in psychotic symptoms (Mathers and Ghodse 1992) and increased likelihood of psychosis development (Arseneault et al. 2004). Pre-existing genetic vulnerability overlaps with a number of environmental risk factors such as trauma, low socioeconomic status or substance abuse. This results in biological alternations and specific psychological mechanisms development that contributes to psychosis development (Misiak et al. 2017). Important question arise, if marijuana can be exploited as a self-medication among people exposed to severe stress, patients with PTSD and individuals with malfunctioning biological systems, that are unable to adequately react to detrimental HPA overstimulation. Childhood traumas, stressful environment and substance abuse may impair eCBS (Mizrahi 2016) that protects against HPA overstimulation. It is hypothesized that self-medication (exogenous cannabis introduction via marijuana consumption) upregulates defective eCBS, enabling adequate stress

response. Stimulation of eCB receptor via eCBagonist (AEA), or exogenous cannabinoid (THC) blunts HPA activation, therefore dampens DA response (Mizrahari et al. 2014; Pruessner et al. 2017). This mechanism is highly desirable among ARMS and those with schizophrenia, for whom even a short increase in dopamine release may lead to an episode of psychosis. Over-sensitization of DA receptor is a well-known trigger of psychosis (Seeman 2006). It is proposed that DA sensitization is a genetic pre-existing vulnerability that exacerbates one's reaction to environmental pressure, for example traumatic life stressors (Myin-Germeys et al. 2005). Studies done by Mizrahi et al. (2014) revealed those with schizophrenia and CHR show increased DA release in response to psychosocial stress. Noteworthy, clinical high risk for schizophrenia cannabis users showed reduced DA, stress-induced response in comparison to cannabis-abstinent-schizophrenia patients (Mizrahi et al. 2014). It is possible that marijuana consumption in the subgroup of ARMS may serve as a self-medication among individuals with malfunctioning eCBS, that is incapable to protect against cortisol increase and psychosis induced dopamine burst. An example may be FAAH polymorphism, associated with problematic drug use (Chiang et al. 2004). Other researchers reveal polymorphism in gene (*CNR1 rs1049353*) coding CB1R and polymorphism in enzyme FAAH (*rs32442*) may contribute to cannabis use disorder (Hindocha et al. 2020). Moreover, in his recent work, Bioque et al. (2019) reports tenfold higher probability of psychotic episode among relevant cannabis users with FAAH *rs2295633* gene polymorphism. Noteworthy, self-medication may be a "double edge sword"—robust eCB signaling decreases anxiety but may contribute to development of delusions and hallucination (Lisboa et al. 2008; Watts et al. 2020), as we discussed earlier in the text.

Conclusions

Relationship between trauma, HPA axis and susceptibility to stress is mediated by the endocannabinoids system that mitigates stress-related activation. A complex network of cannabinoid receptors, transmitters and enzymes enfolds important brain structures, "guard them" against prolonged and detrimental corticosteroids rise. We emphasized eCBS role in reestablishing homeostasis among structures (hippocampus, medial prefrontal cortex, amygdala) involved in different psychopathologies. eCBS plays an important role in behavior regulation, emotional response and memory formation—processes disrupted by profound or prolonged stress exposure. This contributes to development of trauma, PTSD, substance abuse—all of which are multilayer risk factors of psychosis (Misiak et al. 2017). The holistic approach may seem indistinct and complex, but it captures a wide range of eCBS activity (see Figure 2). Abundance of eCB receptors within brain structures implicated in schizophrenia, as well as robust alternations of eCB transmitters in schizophrenia patients and CHR, confirms eCBS role in the development of psychosis. Moreover, patients with schizophrenia present altered CB1R availability in comparison with healthy individuals. Antipsychotic administration affects CB1R function, while external CB1R cannabinoid agonists influence symptomatology in psychosis. It seems that eCBS is involved in symptomatology and pathophysiology of PTSD and depression, also by its involvement in dopamine regulation processes in striatum and mPFC.

eCBS influences human cognitive abilities and plays an important role in extinction learning—a process of

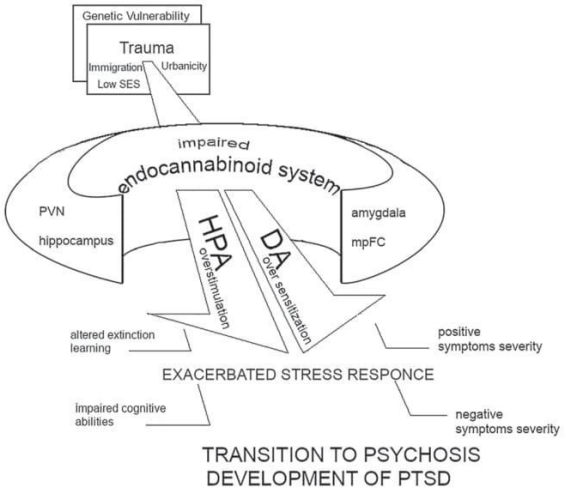


Figure 2: Impaired eCBS fails to protect vital structures against HPA hyperactivity that results in exacerbated stress and psychopathologies, all of which may lead to development of mental disorders like PTSD or psychosis. Genetic vulnerability (specific individual genotype) interacts with a number of high-environmental pressures: traumas, low SES (socioeconomic status), urbanicity, immigration (Misiak et al. 2017; Mizrahi, 2016). Prolonged environmental adversities impair endocannabinoid system f. It is unable to adequately guard pivotal brain structures (hypothalamic PVN, hippocampus, amygdala, mpFC) from stress-related, neuronal overload (Hill and Tasker, 2012). This results in exacerbated stress response and number of psychopathologies (psychotic symptoms development, cognitive impairments, altered extinction learning). Harmful HPA overstimulation mediate dopaminergic activity (Mizrahari 2016; Pruessner et al. 2017) crucial in psychosis development (Seeman et al. 2006), but also important in PTSD and substance dependence etiology (Gilpin et al. 2015; Lin et al. 2020).

"overwriting" maladaptive behavioral patterns. Further studies in this subject may be crucial for psychotherapy of PTSD, trauma, anxiety and depression. Further research in regard to eCBS shall expand our knowledge of altered behavior and among PTSD patients, especially in the context of substance abuse, a common comorbidity of trauma. Phytocannabinoid consumption, prevalent among ARMS and schizophrenia patients, influences dopamine level and affects symptomatology among clinical high risk for schizophrenia cannabis users. This confirms eCBS involvement in DA signaling and draws attention toward self-medication theory. It is hypothesized that numerous profound environmental challenges impair eCBS activity, that fails to protect against stress, thus need for an external "boost" of CB1R activity, in the form of THC consumption. This results with low-anxiety and low-depression outcome ("self-medication" against negative symptoms), but contributes to development of positive symptoms of schizophrenia.

It is beneficial to explore numerous factors involved in mental disorder development, but it is our belief that we need to research factors that contribute maintenance of mental health as well. Agents that reestablish homeostasis and prevent from developing mental disorders are important to study. Current literature implies that eCBS may be this anticipated, restorative agent. Future research should evaluate endocannabinoid system efficacy in the context of psychosis development and after-trauma restoration. Holistic approach improves our knowledge not only of eCBS role in numerous mental disorders, but may contribute to discovery of new trauma biomarkers and novel agents of pharmaceutical significance.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Research funding: None declared.
Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

Aas, M., Pizzagalli, D.A., Laskemoen, J.F., Reponen, E.J., Ueland, T., Melle, I., and Andreassen, O.A. (2019). Elevated hair cortisol is associated with childhood maltreatment and cognitive impairment in schizophrenia and in bipolar disorders. Schizophr. Res. 213: 65–71.

Addington, J., Cornblatt, B.A., Cadenhead, K.S., Cannon, T.D., McGlashan, T.H., Perkins, D.O., and Heinssen, R. (2011). At clinical high risk for psychosis: outcome for nonconverters. Am. J. Psychiatry 168: 800–805.

Adriano, F., Caltagirone, C., and Spalletta, G. (2012). Hippocampal volume reduction in first-episode and chronic schizophrenia: a review and meta-analysis. Neuroscientist 18: 180–200.

Allan, S.M., Hodgekins, J., Beazley, P., and Oduola, S. (2020). Pathways to care in at-risk mental states: a systematic review. Early Interv. Psychiatry, https://doi.org/10.1111/eip.13053.

Althwanay, A., AlZamil, N.A., Almukhadhib, O.Y., Alkhunaizi, S., and Althwanay, R. (2020). Risks and protective factors of the prodromal stage of psychosis: a literature review. Cureus 12.

Appiah-Kusi, E., Wilson, R., Colizzi, M., Foglia, E., Klamerus, E., Caldwell, A., and Bhattacharyya, S. (2019). Childhood trauma and being at-risk for psychosis are associated with higher peripheral endocannabinoids. Psychol. Med. 1–10.

Arseneault, L., Cannon, M., Witton, J., and Murray, R.M. (2004). Causal association between cannabis and psychosis: examination of the evidence. Br. J. Psychiatry 184: 110–117.

Augustin, S.M. and Lovinger, D.M. (2018). Functional relevance of endocannabinoid-dependent synaptic plasticity in the central nervous system. ACS Chem. Neurosci. 9: 2146–2161.

Baumeister, D., Akhtar, R., Ciufolini, S., Pariante, C.M., and Mondelli, V. (2016). Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. Mol. Psychiatry 21: 642–649.

Beyer, C.E., Dwyer, J.M., Piesla, M.J., Platt, B.J., Shen, R., Rahman, Z., and Bingham, B. (2010). Depression-like phenotype following chronic CB1 receptor antagonism. Neurobiol. Dis. 39: 148–155.

Bioque, M., Cabrera, B., García-Bueno, B., Mac-Dowell, K.S., Torrent, C., Saiz, P.A., and Bernardo, M. (2016). Dysregulated peripheral endocannabinoid system signaling is associated with cognitive deficits in first-episode psychosis. J. Psychiatr. Res. 75: 14–21.

Bioque, M., García-Bueno, B., MacDowell, K.S., Meseguer, A., Saiz, P.A., Parellada, M., and Bernardo, M. (2013). Peripheral endocannabinoid system dysregulation in first-episode psychosis. Neuropsychopharmacology 38: 2568–2577.

Bloomfield, M.A., Morgan, C.J., Egerton, A., Kapur, S., Curran, H.V., and Howes, O.D. (2014). Dopaminergic function in cannabis users and its relationship to cannabis-induced psychotic symptoms. Biol. Psychiatry 75: 470–478.

Borgan, F., Laurikainen, H., Veronese, M., Marques, T.R., Haaparanta-Solin, M., Solin, O., and Di Forti, M. (2019). *In vivo* availability of cannabinoid 1 receptor levels in patients with first-episode psychosis. JAMA Psychiatry 76: 1074–1084.

Breitborde, N.J., Srihari, V.H., and Woods, S.W. (2009). Review of the operational definition for first-episode psychosis. Early Interv. Psychiatry 3: 259–265.

Brewin, C.R., Cloitre, M., Hyland, P., Shevlin, M., Maercker, A., Bryant, R.A., and Somasundaram, D. (2017). A review of current evidence regarding the ICD-11 proposals for diagnosing PTSD and complex PTSD. Clin. Psychol. Rev. 58: 1–15.

Cameron, C., Watson, D., and Robinson, J. (2014). Use of a synthetic cannabinoid in a correctional population for posttraumatic stress disorder–related insomnia and nightmares, chronic pain, harm reduction, and other indications: a retrospective evaluation. J. Clin. Psychopharmacol. 34: 559.

Carr, V., Halpin, S., Lau, N., O'Brien, S., Beckmann, J., and Lewin, T. (2000). A risk factor screening and assessment protocol for schizophrenia and related psychosis. Aust. N. Z. J. Psychiatry 34: 170–180.

Ceccarini, J., De Hert, M., Van Winkel, R., Peuskens, J., Bormans, G., Kranaster, L., and Van Laere, K. (2013). Increased ventral striatal CB1 receptor binding is related to negative symptoms in drug-free patients with schizophrenia. Neuroimage 79: 304–312.

Cerqueira, J.J., Almeida, O.F., and Sousa, N. (2008). The stressed prefrontal cortex. Left? Right! Brain. Behav. Immun. 22: 630–638.

Chiang, K.P., Gerber, A.L., Sipe, J.C., and Cravatt, B.F. (2004). Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. Hum. Mol. Genet. 13: 2113–2119.

Choukèr, A., Kaufmann, I., Kreth, S., Hauer, D., Feuerecker, M., Thieme, D., and Schelling, G. (2010). Motion sickness, stress and the endocannabinoid system. PLoS One 5, https://doi.org/10.1371/journal.pone.0010752.

Cota, D. (2007). CB1 receptors: emerging evidence for central and peripheral mechanisms that regulate energy balance, metabolism, and cardiovascular health. Diabetes Metab. Res. Rev. 23: 507–517.

Cullen, A.E., Zunszain, P.A., Dickson, H., Roberts, R.E., Fisher, H.L., Pariante, C.M., and Laurens, K.R. (2014). Cortisol awakening response and diurnal cortisol among children at elevated risk for schizophrenia: relationship to psychosocial stress and cognition. Psychoneuroendocrinology 46: 1–13.

Da Silva, G.E. and Takahashi, R.N. (2002). SR 141716A prevents Δ 9-tetrahydrocannabinol-induced spatial learning deficit in a Morris-type water maze in mice. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 26: 321–325.

De Marchi, N., De Petrocellis, L., Orlando, P., Daniele, F., Fezza, F., and Di Marzo, V. (2003). Endocannabinoid signalling in the blood of patients with schizophrenia. Lipids Health Dis. 2: 5.

de Melo Rodrigues, L.C., Conti, C.L., and Nakamura-Palacios, E.M. (2011). Clozapine and SCH 23390 prevent the spatial working memory disruption induced by Δ 9-THC administration into the medial prefrontal cortex. Brain Res. 1382: 230–237.

Devane, W.A., Dysarz, F.3., Johnson, M.R., Melvin, L.S., and Howlett, A.C. (1988). Determination and characterization of a cannabinoid receptor in rat brain. Mol. Pharmacol. 34: 605–613.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., and Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258: 1946–1949.

Di Marzo, V., and Després, J.P. (2009). CB1 antagonists for obesity—what lessons have we learned from rimonabant? Nat. Rev. Endocrinol. 5: 633.

Dlugos, A., Childs, E., Stuhr, K.L., Hillard, C.J., and De Wit, H. (2012). Acute stress increases circulating anandamide and other N-acylethanolamines in healthy humans. Neuropsychopharmacology 37: 2416–2427.

Eggan, S.M., Hashimoto, T., and Lewis, D.A. (2008). Reduced cortical cannabinoid1 receptor messenger RNA and protein expression in schizophrenia. Arch. Gen. Psychiatry 65: 772–784.

Eggan, S.M., Lazarus, M.S., Stoyak, S.R., Volk, D.W., Glausier, J.R., Huang, Z.J., and Lewis, D.A. (2012). Cortical glutamic acid decarboxylase 67 deficiency results in lower cannabinoid 1 receptor messenger RNA expression: implications for schizophrenia. Biol. Psychiatry 71: 114–119.

Elms, L., Shannon, S., Hughes, S., and Lewis, N. (2019). Cannabidiol in the treatment of post-traumatic stress disorder: a case series. J. Altern. Complement. Med. 25: 392–397.

Enman, N.M., Arthur, K., Ward, S.J., Perrine, S.A., and Unterwald, E.M. (2015). Anhedonia, reduced cocaine reward, and dopamine dysfunction in a rat model of posttraumatic stress disorder. Biol. Psychiatry 78: 871–879.

Ferrarelli, F., and Mathalon, D. (2020). The prodromal phase: time to broaden the scope beyond transition to psychosis? Schizophr. Res. 216: 5–6.

Fowler, I.L., Carr, V.J., Carter, N.T., and Lewin, T.J. (1998). Patterns of current and lifetime substance use in schizophrenia. Schizophr. Bull. 24: 443–455.

Fusar-Poli, P. (2017). The clinical high-risk state for psychosis (CHR-P), version II. Schizophr. Bull. 43: 44–7.

Gaebel, W., and Zielasek, J. (2015). Focus on psychosis. Dialogues Clin. Neurosci. 17: 9.

Ganon-Elazar, E. and Akirav, I. (2009). Cannabinoid receptor activation in the basolateral amygdala blocks the effects of stress on the conditioning and extinction of inhibitory avoidance. J. Neurosci. 29: 11078–11088.

Gao, W., Walther, A., Wekenborg, M., Penz, M., and Kirschbaum, C. (2020). Determination of endocannabinoids and N-acylethanolamines in human hair with LC-MS/MS and their relation to symptoms of depression, burnout, and anxiety. Talanta 217: 121006.

Gibson, C.L., Nia, A.B., Spriggs, S.A., DeFrancisco, D., Swift, A., Perkel, C., and Kim-Schulze, S. (2020). Cannabinoid use in psychotic patients impacts inflammatory levels and their association with psychosis severity. Psychiatry Res 293: 113380.

Gilpin, N.W., Herman, M.A., and Roberto, M. (2015). The central amygdala as an integrative hub for anxiety and alcohol use disorders. Biol. Psychiatry 77: 859–869.

Giuffrida, A., Leweke, F.M., Gerth, C.W., Schreiber, D., Koethe, D., Faulhaber, J., and Piomelli, D. (2004). Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology 29: 2108–2114.

Glass, M. and Felder, C.C. (1997). Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. J. Neurosci. 17: 5327–5333.

Haller, J., Matyas, F., Soproni, K., Varga, B., Barsy, B., Nemeth, B., and Hajos, N. (2007). Correlated species differences in the effects of cannabinoid ligands on anxiety and on GABAergic and glutamatergic synaptic transmission. Eur. J. Neurosci. 25: 2445–2456.

Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Costa, B.R., and Rice, K.C. (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J. Neurosci. 11: 563–583.

Hill, M.N. and Tasker, J.G. (2012). Endocannabinoid signaling, glucocorticoid-mediated negative feedback, and regulation of the hypothalamic-pituitary-adrenal axis. Neuroscience 204: 5–16.

Hill, M.N., Bierer, L.M., Makotkine, I., Golier, J.A., Galea, S., McEwen, B.S., and Yehuda, R. (2013). Reductions in circulating endocannabinoid levels in individuals with post-traumatic stress disorder following exposure to the World Trade Center attacks. Psychoneuroendocrinology 38: 2952–2961.

Hill, M.N., Hillard, C.J., and McEwen, B.S. (2011). Alterations in corticolimbic dendritic morphology and emotional behavior in cannabinoid CB1 receptor–deficient mice parallel the effects of chronic stress. Cereb. Cortex 21: 2056–2064.

Hill, M.N., McLaughlin, R.J., Bingham, B., Shrestha, L., Lee, T.T., Gray, J.M., and Viau, V. (2010). Endogenous cannabinoid signaling is essential for stress adaptation. Proc. Natl. Acad. Sci. U. S. A. 107: 9406–9411.

Hill, M.N., Miller, G.E., Carrier, E.J., Gorzalka, B.B., and Hillard, C.J. (2009). Circulating endocannabinoids and N-acyl ethanolamines are differentially regulated in major depression and following exposure to social stress. Psychoneuroendocrinology 34: 1257–1262.

Hillard, C.J. (2000). Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. Prostag. Other Lipid Mediat. 61: 3–18.

Hillard, C.J. (2018). Circulating endocannabinoids: from whence do they come and where are they going? Neuropsychopharmacology 43: 155–172.

Hindocha, C., Freeman, T.P., Schafer, G., Gardner, C., Bloomfield, M.A., Bramon, E., and Curran, H.V. (2020). Acute effects of cannabinoids on addiction endophenotypes are moderated by genes encoding the CB1 receptor and FAAH enzyme. Addiction Biol. 25: 12762.

Hirvonen, J., Goodwin, R.S., Li, C.T., Terry, G.E., Zoghbi, S.S., Morse, C., and Innis, R.B. (2012). Reversible and regionally selective downregulation of brain cannabinoid CB 1 receptors in chronic daily cannabis smokers. Mol. Psychiatry 17: 642–649.

Holman, E.A., Guijarro, A., Lim, J., and Piomelli, D. (2014). Effects of acute stress on cardiac endocannabinoids, lipogenesis, and inflammation in rats. Psychosom. Med. 76: 20.

Jean-Gilles, L., Braitch, M., Latif, M.L., Aram, J., Fahey, A.J., Edwards, L.J., and Showe, L.C. (2015). Effects of pro-inflammatory cytokines on cannabinoid CB 1 and CB 2 receptors in immune cells. Acta Physiol. 214: 63–74.

Jiang, B., Xiong, Z., Yang, J., Wang, W., Wang, Y., Hu, Z.L., and Chen, J.G. (2012). Antidepressant-like effects of ginsenoside Rg1 are due to activation of the BDNF signalling pathway and neurogenesis in the hippocampus. Br. J. Pharmacol. 166: 1872–1887.

Joëls, M., Sarabdjitsingh, R.A., and Karst, H. (2012). Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes. Pharmacol. Rev. 64: 901–938.

Kathuria, S., Gaetani, S., Fegley, D., Valiño, F., Duranti, A., Tontini, A., and Giustino, A. (2003). Modulation of anxiety through blockade of anandamide hydrolysis. Nat. Med. 9: 76–81.

Keen, L., II, Pereira, D., and Latimer, W. (2014). Self-reported lifetime marijuana use and interleukin-6 levels in middle-aged African Americans. Drug Alcohol Depend. 140: 156–160.

Keller, J., Gomez, R., Williams, G., Lembke, A., Lazzeroni, L., Murphy, G.M., and Schatzberg, A.F. (2017). HPA axis in major depression: cortisol, clinical symptomatology and genetic variation predict cognition. Mol. Psychiatry 22: 527–536.

Knipscheer, J., Sleijpen, M., Frank, L., de Graaf, R., Kleber, R., ten Have, M., and Dückers, M. (2020). Prevalence of potentially traumatic events, other life events and subsequent reactions indicative for posttraumatic stress disorder in The Netherlands: a general population study based on the trauma screening questionnaire. Int. J. Environ. Res. Publ. Health 17: 172.

Koethe, D., Giuffrida, A., Schreiber, D., Hellmich, M., Schultze-Lutter, F., Ruhrmann, S., and Leweke, F.M. (2009). Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis. Br. J. Psychiatry 194: 371–372.

Koethe, D., Pahlisch, F., Hellmich, M., Rohleder, C., Mueller, J.K., Meyer-Lindenberg, A., and Leweke, F.M. (2019). Familial abnormalities of endocannabinoid signaling in schizophrenia. World J. Psychiatry 20: 117–125.

Kühn, S., Musso, F., Mobascher, A., Warbrick, T., Winterer, G., and Gallinat, J. (2012). Hippocampal subfields predict positive symptoms in schizophrenia: first evidence from brain morphometry. Transl. Psychiatry 2: 127.

Laprairie, R.B., Bagher, A.M., Kelly, M.E.M., and Denovan-Wright, E.M. (2015). Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. Br. J. Pharmacol. 172: 4790–4805.

Leweke, F.M., Piomelli, D., Pahlisch, F., Muhl, D., Gerth, C.W., Hoyer, C., and Koethe, D. (2012). Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl. Psychiatry 2: e94.

Lin, C.C., Chen, T.Y., Cheng, P.Y., and Liu, Y.P. (2020). Early life social experience affects adulthood fear extinction deficit and associated dopamine profile abnormalities in a rat model of PTSD. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 109914, https://doi.org/10.1016/j.pnpbp.2020.109914.

Lin, C.C., Tung, C.S., and Liu, Y.P. (2016). Escitalopram reversed the traumatic stress-induced depressed and anxiety-like symptoms but not the deficits of fear memory. Psychopharmacology 233: 1135–1146.

Lisboa, S.F., Resstel, L.B., Aguiar, D.C., and Guimarães, F.S. (2008). Activation of cannabinoid CB1 receptors in the dorsolateral periaqueductal gray induces anxiolytic effects in rats submitted to the Vogel conflict test. Eur. J. Pharmacol. 593: 73–78.

Lisboa, S.F., Vila-Verde, C., Rosa, J., Uliana, D.L., Stern, C.A.J., Bertoglio, L.J., and Guimaraes, F.S. (2019). Tempering aversive/traumatic memories with cannabinoids: a review of evidence from animal and human studies. Psychopharmacology 236: 201–226.

Luchicchi, A., and Pistis, M. (2012). Anandamide and 2-arachidonoylglycerol: pharmacological properties, functional features, and emerging specificities of the two major endocannabinoids. Mol. Neurobiol. 46: 374–392.

Maccarrone, M., Valverde, O., Barbaccia, M.L., Castañé, A., Maldonado, R., Ledent, C., and Finazzi-Agrò, A. (2002). Age-related changes of anandamide metabolism in CB1 cannabinoid receptor knockout mice: correlation with behaviour. Eur. J. Neurosci. 15: 1178–1186.

MacLean, K.I., and Littleton, J.M. (1977). Environmental stress as a factor in the response of rat brain catecholamine metabolism to Δ 8-tetrahydrocannabinol. Eur. J. Pharmacol. 41: 171–182.

Madsen, H.B., Guerin, A.A., and Kim, J.H. (2017). Investigating the role of dopamine receptor-and parvalbumin-expressing cells in extinction of conditioned fear. Neurobiol. Learn. Mem. 145: 7–17.

Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., and Di Marzo, V. (2002). The endogenous cannabinoid system controls extinction of aversive memories. Nature 418: 530–534.

Martin, M., Ledent, C., Parmentier, M., Maldonado, R., and Valverde, O. (2002). Involvement of CB1 cannabinoid receptors in emotional behaviour. Psychopharmacology 159: 379–387.

Mathers, D.C. and Ghodse, A.H. (1992). Cannabis and psychotic illness. Br. J. Psychiatry Psychiatry 161: 648–653.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., and Bonner, T.I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346: 561–564.

McFarlane, A.C., Barton, C.A., Yehuda, R., and Wittert, G. (2011). Cortisol response to acute trauma and risk of posttraumatic stress disorder. Psychoneuroendocrinology 36: 720–727.

McGuire, P., Robson, P., Cubala, W.J., Vasile, D., Morrison, P.D., Barron, R., and Wright, S. (2018). Cannabidiol (CBD) as an adjunctive therapy in schizophrenia: a multicenter randomized controlled trial. Am. J. Psychiatry 175: 225–231.

Mehta, N.D., Stevens, J.S., Li, Z., Gillespie, C.F., Fani, N., Michopoulos, V., and Felger, J.C. (2020). Inflammation, reward circuitry and symptoms of anhedonia and PTSD in trauma-exposed women. Soc. Cognit. Affect Neurosci. 5: 1046–1055.

Melis, M., Pistis, M., Perra, S., Muntoni, A.L., Pillolla, G., and Gessa, G.L. (2004). Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. J. Neurosci. 24: 53–62.

Minichino, A., Senior, M., Brondino, N., Zhang, S.H., Godwlewska, B.R., Burnet, P.W., and Lennox, B.R. (2019). Measuring disturbance of the endocannabinoid system in psychosis: a systematic review and meta-analysis. JAMA Psychiatry 76: 914–923.

Mishima, K., Egashira, N., Hirosawa, N., Fujii, M., Matsumoto, Y., Iwasaki, K., and Fujiwara, M. (2001). Characteristics of learning and memory impairment induced by ⊿9-tetrahydrocannabinol in rats. Jpn. J. Pharmacol. 87: 297–308.

Misiak, B., Krefft, M., Bielawski, T., Moustafa, A.A., Sąsiadek, M.M., and Frydecka, D. (2017). Toward a unified theory of childhood trauma and psychosis: a comprehensive review of epidemiological, clinical, neuropsychological and biological findings. Neurosci. Biobehav. Rev. 75: 393–406.

Mizrahi, R. (2016). Social stress and psychosis risk: common neurochemical substrates? Neuropsychopharmacology 41: 666–674.

Mizrahi, R., Kenk, M., Suridjan, I., Boileau, I., George, T.P., McKenzie, K., and Rusjan, P. (2014). Stress-induced dopamine response in subjects at clinical high risk for schizophrenia with and without concurrent cannabis use. Neuropsychopharmacology 39: 1479–1489.

Mondelli, V., Pariante, C.M., Navari, S., Aas, M., D'Albenzio, A., Di Forti, M., and Papadopoulos, A.S. (2010). Higher cortisol levels are associated with smaller left hippocampal volume in first-episode psychosis. Schizophr. Res. 119: 75–78.

Moreira, F.A., and Wotjak, C.T. (2009). Cannabinoids and anxiety. Behavioral neurobiology of anxiety and its treatment. Curr Top. Behav. Neurosci. 429–450, https://doi.org/10.1007/7854_2009_16.

Murillo-Rodriguez, E., Désarnaud, F., and Prospéro-García, O. (2006). Diurnal variation of arachidonoylethanolamine, palmitoylethanolamide and oleoylethanolamide in the brain of the rat. Life Sci. 79: 30–37.

Myin-Germeys, I., Marcelis, M., Krabbendam, L., Delespaul, P., and van Os, J. (2005). Subtle fluctuations in psychotic phenomena as functional states of abnormal dopamine reactivity in individuals at risk. Biol. Psychiatry 58: 105–110.

Naderi, N., Haghparast, A., Saber-Tehrani, A., Rezaii, N., Alizadeh, A.M., Khani, A., and Motamedi, F. (2008). Interaction between cannabinoid compounds and diazepam on anxiety-like behaviour of mice. Pharmacol. Biochem. Behav. 89: 64–75.

Newell, K.A., Deng, C., and Huang, X.F. (2006). Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. Exp. Brain Res. 172: 556–560.

Niederhoffer, N., Hansen, H.H., Fernandez-Ruiz, J.J., and Szabo, B. (2001). Effects of cannabinoids on adrenaline release from adrenal medullary cells. Br. J. Pharmacol. 134: 1319–1327.

Nissen, W., Szabo, A., Somogyi, J., Somogyi, P., and Lamsa, K.P. (2010). Cell type-specific long-term plasticity at glutamatergic synapses onto hippocampal interneurons expressing either parvalbumin or CB1 cannabinoid receptor. J. Neurosci. 30: 1337–1347.

Norman, R.M., and Malla, A.K. (1993). Stressful life events and schizophrenia: I: a review of the research. Br. J. Psychiatry 162: 161–166.

Pagotto, U., Marsicano, G., Fezza, F., Theodoropoulou, M., Grubler, Y., Stalla, J., and Lutz, B. (2001). Normal human pituitary gland and pituitary adenomas express cannabinoid receptor type 1 and synthesize endogenous cannabinoids: first evidence for a direct role of cannabinoids on hormone modulation at the human pituitary level. J. Clin. Endocrinol. Metab. 86: 2687–2696.

Pan, B., Wang, W., Zhong, P., Blankman, J.L., Cravatt, B.F., and Liu, Q.S. (2011). Alterations of endocannabinoid signaling, synaptic plasticity, learning, and memory in monoacylglycerol lipase knock-out mice. J. Neurosci. 31: 13420–13430.

Parolaro, D., Realini, N., Vigano, D., Guidali, C., and Rubino, T. (2010). The endocannabinoid system and psychiatric disorders. Exp. Neurol. 224: 3–14.

Passos, I.C., Vasconcelos-Moreno, M.P., Costa, L.G., Kunz, M., Brietzke, E., Quevedo, J., and Kauer-Sant'Anna, M. (2015). Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression. Lancet Psychiatry 2: 1002–1012.

Pruessner, M., Bechard-Evans, L., Pira, S., Joober, R., Collins, D.L., Pruessner, J.C., and Malla, A.K. (2017). Interplay of hippocampal volume and hypothalamus-pituitary-adrenal axis function as markers of stress vulnerability in men at ultra-high risk for psychosis. Psychol. Med. 47: 471–483.

Pruessner, M., Lepage, M., Collins, D.L., Pruessner, J.C., Joober, R., and Malla, A.K. (2015). Reduced hippocampal volume and hypothalamus–pituitary–adrenal axis function in first episode psychosis: evidence for sex differences. NeuroImage Clin. 7: 195–202.

Quinn, H.R., Matsumoto, I., Callaghan, P.D., Long, L.E., Arnold, J.C., Gunasekaran, N., and Matsuda-Matsumoto, H. (2008). Adolescent rats find repeated Δ 9-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. Neuropsychopharmacology 33: 1113–1126.

Radhakrishnan, R., Wilkinson, S.T., and D'Souza, D.C. (2014). Gone to pot–a review of the association between cannabis and psychosis. Front. Psychiatry 5: 54.

Ranganathan, M., Cortes-Briones, J., Radhakrishnan, R., Thurnauer, H., Planeta, B., Skosnik, P., and Surti, T. (2016). Reduced brain cannabinoid receptor availability in schizophrenia. Biol. Psychiatry 79: 997–1005.

Raymundi, A.M., da Silva, T.R., Sohn, J.M., Bertoglio, L.J., and Stern, C.A. (2020). Effects of Δ 9-tetrahydrocannabinol on aversive memories and anxiety: a review from human studies. BMC Psychiatry 20: 1–17.

Reggio, P.H. and Traore, H. (2000). Conformational requirements for endocannabinoid interaction with the cannabinoid receptors, the anandamide transporter and fatty acid amidohydrolase. Chem. Phys. Lipids 108: 15–35.

Reuter, A.R., Bumb, J.M., Mueller, J.K., Rohleder, C., Pahlisch, F., Hanke, F., and Schwarz, E. (2017). Association of anandamide with altered binocular depth inversion illusion in schizophrenia. World J. Psychiatry 18: 483–488.

Richardson, K.A., Hester, A.K., and McLemore, G.L. (2016). Prenatal cannabis exposure-The "first hit" to the endocannabinoid system. Neurotoxicol. Teratol. 58: 5–14.

Rivera, P., del Mar Fernández-Arjona, M., Silva-Peña, D., Blanco, E., Vargas, A., López-Ávalos, M.D., and Suárez, J. (2018). Pharmacological blockade of fatty acid amide hydrolase (FAAH) by URB597 improves memory and changes the phenotype of hippocampal microglia despite ethanol exposure. Biochem. Pharmacol. 157: 244–257.

Roitman, P., Mechoulam, R., Cooper-Kazaz, R., and Shalev, A. (2014). Preliminary, open-label, pilot study of add-on oral Δ 9-tetrahydrocannabinol in chronic post-traumatic stress disorder. Clin. Drug Invest. 34: 587–591.

Rosen, J.L., Miller, T.J., D'Andrea, J.T., McGlashan, T.H., and Woods, S.W. (2006). Comorbid diagnoses in patients meeting criteria for the schizophrenia prodrome. Schizophr. Res. 85: 124–131.

Sapolsky, R.M., Romero, L.M., and Munck, A.U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21: 55–89.

Sbarski, B. and Akirav, I. (2020). Cannabinoids as therapeutics for PTSD. Pharmacol. Ther. 107551.

Scheller, A. and Kirchhoff, F. (2016). Endocannabinoids and heterogeneity of glial cells in brain function. Front. Integr. Neurosci. 10: 24.

Schoenfeld, T.J., Rhee, D., Martin, L., Smith, J.A., Sonti, A.N., Padmanaban, V., and Cameron, H.A. (2019). New neurons restore structural and behavioral abnormalities in a rat model of PTSD. Hippocampus 29: 848–861.

Seeman, P. (2006). Targeting the dopamine D2 receptor in schizophrenia. Expert Opin. Ther. Targets 10: 515–531.

Seif, T., Makriyannis, A., Kunos, G., Bonci, A., and Hopf, F.W. (2011). The endocannabinoid 2-arachidonoylglycerol mediates D1 and D2 receptor cooperative enhancement of rat nucleus accumbens core neuron firing. Neuroscience 193: 21–33.

Shonesy, B.C., Bluett, R.J., Ramikie, T.S., Báldi, R., Hermanson, D.J., Kingsley, P.J., and Patel, S. (2014). Genetic disruption of 2-arachidonoylglycerol synthesis reveals a key role for endocannabinoid signaling in anxiety modulation. Cell Rep. 9: 1644–1653.

Si, P., Liu, S., Tong, D., Cheng, M., Wang, L., and Cheng, X. (2018). Association of polymorphisms of NAPE-PLD and FAAH genes with schizophrenia in Chinese Han population. Zhonghua yi xue yi chuan xue za zhi [Chin. J. Med. Genet.] 35: 215–218.

Sloan, M.E., Grant, C.W., Gowin, J.L., Ramchandani, V.A., and Le Foll, B. (2019). Endocannabinoid signaling in psychiatric disorders: a review of positron emission tomography studies. Acta Pharmacol. Sin. 40: 342–350.

Stalder, T. and Kirschbaum, C. (2012). Analysis of cortisol in hair–state of the art and future directions. Brain Behav. Immun. 26: 1019–1029.

Steiner, M.A. and Wotjak, C.T. (2008). Role of the endocannabinoid system in regulation of the hypothalamic-pituitary-adrenocortical axis. Prog. Brain Res. 170: 397–432.

Stella, N., Schweitzer, P., and Piomelli, D. (1997). A second endogenous cannabinoid that modulates long-term potentiation. Nature 388: 773–778.

Steudte-Schmiedgen, S., Stalder, T., Schönfeld, S., Wittchen, H.U., Trautmann, S., Alexander, N., and Kirschbaum, C. (2015). Hair cortisol concentrations and cortisol stress reactivity predict PTSD symptom increase after trauma exposure during military deployment. Psychoneuroendocrinology 59: 123–133.

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., and Waku, K. (1995). 2-Arachidonoylgylcerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem. Biophys. Res. Commun. 215: 89–97.

Szeszko, P.R., Lehrner, A., and Yehuda, R. (2018). Glucocorticoids and hippocampal structure and function in PTSD. Harv. Rev. Psychiatry 26: 142–157.

Teicher, M.H., Andersen, S.L., Polcari, A., Anderson, C.M., Navalta, C.P., and Kim, D.M. (2003). The neurobiological consequences of early stress and childhood maltreatment Neurosci. Biobehav. Rev. 27: 33–44.

Thornicroft, G. (1990). Cannabis and psychosis. Br. J. Psychiatry 157: 25–33.

Torrisi, S.A., Leggio, G.M., Drago, F., and Salomone, S. (2019). Therapeutic challenges of post-traumatic stress disorder: focus on the dopaminergic system. Front. Pharmacol. 10: 404.

Urigüen, L., Pérez-Rial, S., Ledent, C., Palomo, T., and Manzanares, J. (2004). Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. Neuropharmacology 46: 966–973.

van Os, J. and Guloksuz, S. (2017). A critique of the "ultra-high risk" and "transition" paradigm. World Psychiatry 16: 200–206.

Varvel, S.A., Cravatt, B.F., Engram, A.E., and Lichtman, A.H. (2006). Fatty acid amide hydrolase (–/–) mice exhibit an increased sensitivity to the disruptive effects of anandamide or oleamide in a working memory water maze task. J. Pharmacol. Exp. Ther. 317: 251–257.

Varvel, S., Hamm, R., Martin, B., and Lichtman, A. (2001). Differential effects of Δ 9-THC on spatial reference and working memory in mice. Psychopharmacology 157: 142–150.

Velakoulis, D., Pantelis, C., McGorry, P.D., Dudgeon, P., Brewer, W., Cook, M., and Singh, B. (1999). Hippocampal volume in first-episode psychoses and chronic schizophrenia: a high-resolution magnetic resonance imaging study. Arch. Gen. Psychiatry 56: 133–141.

Vigano, D., Guidali, C., Petrosino, S., Realini, N., Rubino, T., Di Marzo, V., and Parolaro, D. (2009). Involvement of the endocannabinoid system in phencyclidine-induced cognitive deficits modelling schizophrenia. Int. J. Neuropsychopharmacol. 12: 599–614.

Walder, D.J., Walker, E.F., and Lewine, R.J. (2000). Cognitive functioning, cortisol release, and symptom severity in patients with schizophrenia. Biol. Psychiatry 48: 1121–1132.

Walker, E.F., Trotman, H.D., Pearce, B.D., Addington, J., Cadenhead, K.S., Cornblatt, B.A., and Tsuang, M.T. (2013). Cortisol levels and risk for psychosis: initial findings from the North American prodrome longitudinal study. Biol. Psychiatry 74: 410–417.

Wamsteeker, J.I., Kuzmiski, J.B., and Bains, J.S. (2010). Repeated stress impairs endocannabinoid signaling in the paraventricular nucleus of the hypothalamus. J. Neurosci. 30: 11188–11196.

Watts, J.J., Jacobson, M.R., Lalang, N., Boileau, I., Tyndale, R.F., Kiang, M., and Mizrahi, R. (2020). Imaging brain fatty acid amide hydrolase in untreated patients with psychosis. Biol. Psychiatry.

Wilker, S., Pfeiffer, A., Elbert, T., Ovuga, E., Karabatsiakis, A., Krumbholz, A., and Kolassa, I.T. (2016). Endocannabinoid concentrations in hair are associated with PTSD symptom severity. Psychoneuroendocrinology 67: 198–206.

Wise, L.E., Thorpe, A.J., and Lichtman, A.H. (2009). Hippocampal CB 1 receptors mediate the memory impairing effects of Δ 9-tetrahydrocannabinol. Neuropsychopharmacology 34: 2072–2080.

Wiskerke, J., Irimia, C., Cravatt, B.F., De Vries, T.J., Schoffelmeer, A.N., Pattij, T., and Parsons, L.H. (2012). Characterization of the effects of reuptake and hydrolysis inhibition on interstitial endocannabinoid levels in the brain: an in vivo microdialysis study. ACS Chem. Neurosci. 3: 407–417.

Wittmann, G., Deli, L., Kalló, I., Hrabovszky, E., Watanabe, M., Liposits, Z., and Fekete, C. (2007). Distribution of type 1 cannabinoid receptor (CB1)-immunoreactive axons in the mouse hypothalamus. J. Comp. Neurol. 503: 270–279.

Wolkowitz, O.M., Burke, H., Epel, E.S., and Reus, V.I. (2009). Mood, memory, and mechanisms. Ann. NY Acad. Sci. 1179: 19–40.

Wong, D.F., Kuwabara, H., Horti, A.G., Raymont, V., Brasic, J., Guevara, M., and Rahmim, A. (2010). Quantification of cerebral cannabinoid receptors subtype 1 (CB1) in healthy subjects and schizophrenia by the novel PET radioligand [11C] OMAR. Neuroimage 52: 1505–1513.

Xu, H., Perez, S., Cornil, A., Detraux, B., Prokin, I., Cui, Y., and Venance, L. (2018). Dopamine–endocannabinoid interactions mediate spike-timing-dependent potentiation in the striatum. Nat. Commun. 9: 1–18.

Yung, A.R., Killackey, E., Hetrick, S.E., Parker, A.G., Schultze-Lutter, F., Klosterkoetter, J., and McGorry, P.D. (2007). The prevention of schizophrenia. Int. Rev. Psychiatry 19: 633–646.

Zanettini, C., Panlilio, L.V., Alicki, M., Goldberg, S.R., Haller, J., and Yasar, S. (2011). Effects of endocannabinoid system modulation on cognitive and emotional behavior. Front. Behav. Neurosci. 5: 57.

Zavitsanou, K., Garrick, T., and Huang, X.F. (2004). Selective antagonist [3H] SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 28: 355–360.

Zhuang, J., Yang, D.P., Nikas, S.P., Zhao, J., Guo, J., and Makriyannis, A. (2013). The interaction of fatty acid amide hydrolase (FAAH) inhibitors with an anandamide carrier protein using ^{19}F-NMR. AAPS J. 15: 477–482.

Ziegler, C.G., Mohn, C., Lamounier-Zepter, V., Rettori, V., Bornstein, S.R., Krug, A.W., and Ehrhart-Bornstein, M. (2010). Expression and function of endocannabinoid receptors in the human adrenal cortex. Horm. Metab. Res. 42: 88–92.

frontiers | Frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 17 May 2022
doi: 10.3389/fnbeh.2022.903100

Trauma Disrupts Reinforcement Learning in Rats—A Novel Animal Model of Chronic Stress Exposure

Tomasz Bielawski[1], Jarosław Drapała[2], Paweł Krowicki[3], Bartłomiej Stańczykiewicz[1] and Dorota Frydecka[1]*

[1] Department of Psychiatry, Wrocław Medical University, Wrocław, Poland, [2] Department of Computer Science and Systems Engineering, Faculty of Information and Communication Technology, Wrocław University of Science and Technology, Wrocław, Poland, [3] Department of Laser Technologies, Automation and Production Management, Faculty of Mechanical Engineering, Wrocław University of Science and Technology, Wrocław, Poland

Trauma, as well as chronic stress that characterizes a modern fast-paced lifestyle, contributes to numerous psychopathologies and psychological problems. Psychiatric patients with traumas, as well as healthy individuals who experienced traumas in the past, are often characterized by diminished cognitive abilities. In our protocol, we used an animal model to explore the influence of chronic trauma on cognitive abilities and behavior in the group of 20 rats (Rattus norvegicus). The experimental group was introduced to chronic (12 consecutive days) exposure to predator odor (bobcat urine). We measured the reinforcement learning of each individual before and after the exposition *via* the Probabilistic Selection Task (PST) and we used Social Interaction Test (SIT) to assess the behavioral changes of each individual before and after the trauma. In the experimental group, there was a significant decrease in reinforcement learning after exposure to a single trauma (Wilcoxon Test, $p = 0.034$) as well as after 11 days of chronic trauma (Wilcoxon-test, $p = 0.01$) in comparison to pre-trauma performance. The control group, which was not exposed to predator odor but underwent the same testing protocol, did not present significant deterioration in reinforcement learning. In cross-group comparisons, there was no difference between the experimental and control group in PST before odor protocol (U Mann-Whitney two-sided, $p = 0.909$). After exposure to chronic trauma, the experimental group deteriorated in PST performance compared to control (U Mann-Whitney Two-sided, $p = 0.0005$). In SIT, the experimental group spent less time in an Interaction Zone with an unfamiliar rat after trauma protocol (Wilcoxon two-sided test, $p = 0.019$). Major strengths of our models are: (1) protocol allows investigating reinforcement learning before and after exposition to chronic trauma, with the same group of rats, (2) translational scope, as the PST is displayed on touchscreen, similarly to human studies, (3) protocol delivers chronic trauma that impairs reward learning, but behaviorally does not induce full-blown anhedonia, thus rats performed voluntarily throughout all the procedures.

Keywords: reinforcement learning, trauma, PTSD, predator odor, chronic stress

OPEN ACCESS

Edited by:
Adebobola Imeh-Nathaniel, North Greenville University, United States

Reviewed by:
Ayodeji O. Ipinmoroti, Alabama State University, United States
Akane Nagano, RIKEN Center for Brain Science (CBS), Japan

****Correspondence:***
Tomasz Bielawski
tomaszbielawski90@gmail.com

Specialty section:
This article was submitted to Learning and Memory, a section of the journal Frontiers in Behavioral Neuroscience

Received: *23 March 2022*
Accepted: *25 April 2022*
Published: *17 May 2022*

Citation:
Bielawski T, Drapała J, Krowicki P, Stańczykiewicz B and Frydecka D (2022) Trauma Disrupts Reinforcement Learning in Rats—A Novel Animal Model of Chronic Stress Exposure. Front. Behav. Neurosci. 16:903100. doi: 10.3389/fnbeh.2022.903100

INTRODUCTION

Throughout life, the environment puts numerous stressors on every living organism. In humans, extreme stress (trauma) captures a range of severe adverse experiences, such as physical, sexual, or emotional abuse, neglect, parental death, bullying, or omission by caregiver during childhood. Trauma contributes to the development of numerous mental disorders such as posttraumatic stress disorder (PTSD), anxiety disorders, schizophrenia, personality disorders, mood disorders (Jansen et al., 2016; Misiak et al., 2017). It is estimated that prevalence of PTSD reaches 7% in general population (McLaughlin et al., 2015), while in subgroups exposed to severe psychological trauma numbers are even more prominent, for example, 10% of US veterans meet criteria of PTSD (Mota et al., 2016) as well as 60% minor refugees in Germany that sought general medical treatment (Veeser et al., 2021). In the general population, only a small proportion of individuals with a positive history of traumatic events develop full-blown PTSD (Breslau, 2009). Trauma affects cognitive abilities (Petkus et al., 2018; Aas et al., 2019), disrupts the immune system (Mehta et al., 2020), causes structural changes in the brain (Assogna et al., 2020), affects the severity of symptoms among those with mental disorders (Duhig et al., 2015; Ay and Erbay, 2018; Bailey et al., 2018). Chronic stress, defined as an exposition to a series of stressful or potentially traumatic events, characterizes a modern, fast-paced western lifestyle (Matosin et al., 2017). Chronic stress turns out to be closely related to numerous health issues: obesity, diabetes, mental disorders, psychological deficits, substance dependence (Sinha, 2008; Farag and Gaballa, 2011; Misiak et al., 2017; Bielawski et al., 2019). All are major epidemiological health concerns that generate enormous public cost (Simon et al., 2006; Farag and Gaballa, 2011; Laramée et al., 2013; Masodkar et al., 2016). The purpose of this study is to present a novel protocol to examine cognitive impairment in reinforcement learning as chronic trauma progresses. We use a simplified Probabilistic Selection Task (PST) to approximate our model to human studies. In humans, experimental studies of PTSD, chronic stress, and trauma are limited. Therefore, our research is to explore the translational scope of PTSD studies in rodents. We want to test whether rats will perform voluntarily while exposed to chronic trauma. If so, our aim is to study rats' ability to learn the PST protocol, as well as their ability to adapt to a system, where interaction with a touchscreen is related to reward collection. Our procedure examines reward learning before and after exposure to chronic trauma, with the same group of rats. This approach allows us to measure cognitive disruptions as the trauma progresses. We hypothesize that rats exposed to trauma will perform poorer in PST, in comparison to their performance before exposure to chronic trauma. Moreover, we want to explore whether a single exposure to trauma will affect cognitive functioning. Furthermore, we hypothesize that traumatized individuals will be less socially oriented during Social Interaction Test (SIT), compared to the control.

MATERIALS AND METHODS

Theoretical Background

Trauma, Cognition, and Chronic Stress Rationale

Medically oriented understanding of psychological trauma is strictly related with PTSD diagnosis (Yehuda, 1998), while in psychoanalytic approach trauma is a powerful stimulus, that breaches one's psychological defense mechanisms, and induces experience of helplessness (Rothgeb, 1971). In both definitions trauma is an extreme stress, that is beyond one's ability to cope with. An abundant literature presents negative impact of trauma on cognitive functions in patients with psychosis (Lysaker et al., 2001; Schenkel et al., 2005; Shannon et al., 2011) and among healthy individuals who experienced trauma in the past (Majer et al., 2010; Vasilevski and Tucker, 2016; Petkus et al., 2018). Trauma and prolonged (chronic) stress activate the hypothalamic-pituitary-adrenal (HPA) axis *via* the rise of corticosteroids, activate the endocannabinoid system, and indirectly affect dopamine bursts in the striatum and medial prefrontal cortex (Joëls et al., 2012; Bielawski et al., 2019). Different regions of the brain (for example hippocampus, amygdala, medial prefrontal cortex, hypothalamus) involved in stimulus recognition, memory, and learning are affected by increased detrimental corticosteroids rise during chronic or acute stress (Pruessner et al., 2017; Bielawski et al., 2019). The neurobiology of trauma and its impact on cognitive abilities is complex, and studies in human subjects have certain limitations. Thus, several animal models have been developed to assess symptoms associated with exposure to trauma and the development of PTSD (Whitaker et al., 2014; Harro, 2018; Planchez et al., 2019). The Diagnostic and Statistical Manual of Mental Disorders version 5 (DSM-V) delivered by the American Psychiatric Association (APA) presents four clusters of symptoms of PTSD: intrusive recollection of the original traumatic event, avoidance of trauma-related reminders, negative changes in cognition and mood, and alterations in arousal or reactivity, each of which must start or be significantly exacerbated after exposure to the traumatic event (Roehr, 2013). The variety of animal models put its focus on different aspects of PTSD symptomatology, such as contextual avoidance (Albrechet-Souza et al., 2020), changes in arousal and reactivity (Knox et al., 2012), and behavior alterations (Krishnan et al., 2007). These models measure different parameters after the exposition to stress. Our approach is to measure cognitive and behavioral parameters as chronic trauma progresses. That way, an animal model gives us an opportunity to expose rats to chronic stress, as we measure their cognitive functions simultaneously. Chronic stress lacks a clear definition, but most authors agree that it is an exposition to a series of intense, potentially traumatic experiences or involvement in prolonged stress situations that leads to psychopathologies and/or adverse medical conditions (Matosin et al., 2017). Chronic stress is widely used in animal models of anxiety disorders, depression, and PTSD (Saavedra-Rodríguez and Feig, 2013; Reber et al., 2016; Wang et al., 2021). In humans, prolonged stress is an important factor in etiopathology of different mental disorders (Matosin et al., 2017; McEwen, 2017;

Ross et al., 2017), for example chronic stress can induce mild PTSD symptoms in humans (Davidson and Baum, 1986). Stress influences the ability to learn from rewards among those with a familial predisposition to psychosis and individuals with major depressive disorder (Reinen et al., 2021). Furthermore, chronic stress induces hyper inflammation, thus being discussed to enhance susceptibility to infectious diseases such as COVID-19 (Lamontagne et al., 2021), or mental diseases linked to immune system dysregulations (Dennison et al., 2012). Chronic exposure to trauma is particularly harmful; many individuals repeatedly exposed to traumatic events carry a heavy burden of psychopathologies (Sharhabani-Arzy et al., 2003; Éthier et al., 2004; Salcioglu et al., 2017). In our experiment, we expose male Wistar Rats to chronic trauma for 12 consecutive days. In the literature, there are animal models of PTSD that reveal alteration in cognitive performance, although they often apply a single prolonged stress procedure (George et al., 2015). Indeed, single exposure to predator odor is sufficient to induce trauma (Albrechet-Souza and Gilpin, 2019), but our goal is to mimic chronic stress, thus our protocol's prolonged exposure to stressful stimulus with parallel cognitive examination.

Probabilistic Selection Task and Social Interaction Test

In humans, the Probabilistic Selection Task (PST) was shown to be associated with dopaminergic effects on learning (Frank et al., 2007). Positron emission tomography and functional magnetic resonance imaging studies showed that reinforcement-based decisions are associated with signaling in the striatum and prefrontal cortex (Jocham et al., 2011; Kasanova et al., 2018). Furthermore, PST was used to assess learning deficits among those with PTSD (Myers et al., 2013). During PST, participants are presented stimulus pairs and learn to choose one of them. After each choice, probabilistic feedback follows the choice to

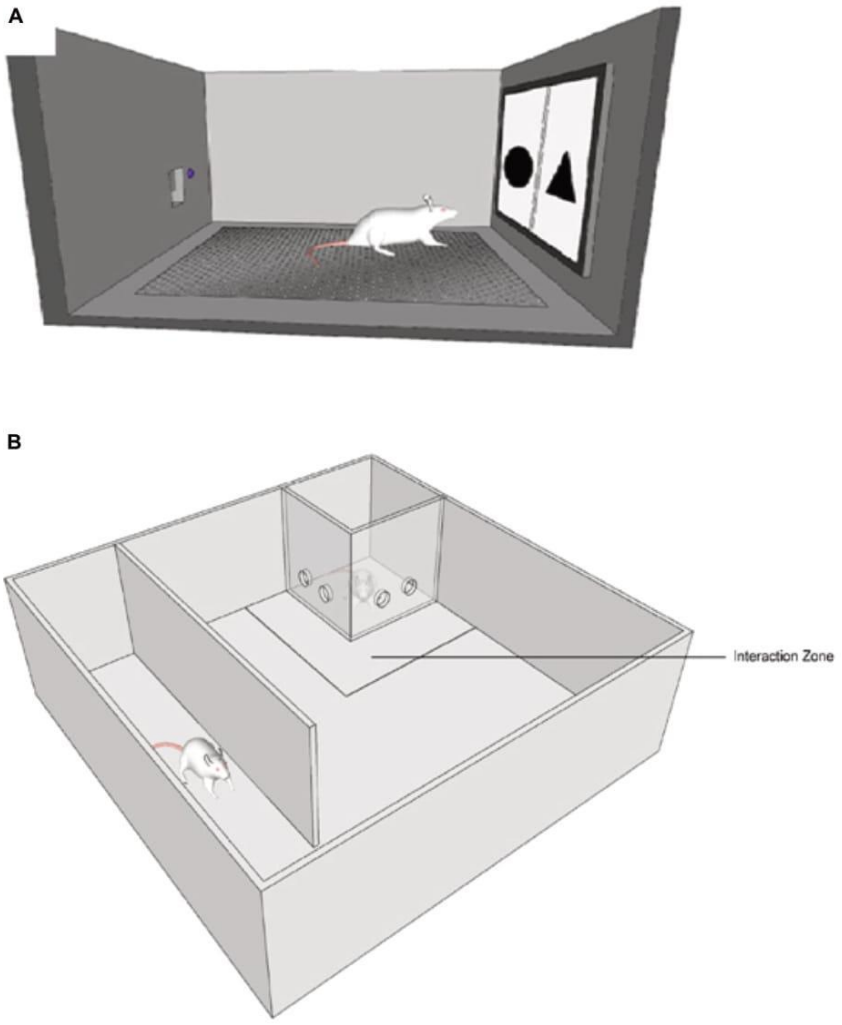


FIGURE 1 | (A) Probabilistic Selection Task testing chamber. **(B)** Social Interaction Test testing chamber.

indicate whether it was correct or incorrect. PST (and its different variants) are widely used in animal studies—in rodents stimulus selection is most often recorded *via* nose poke in aperture (Amitai et al., 2014) or by pressing the lever (George et al., 2015; Seib et al., 2020), while in humans selection is usually done *via* tap on a touchscreen or pressing a button on a keyboard (Frank et al., 2004).

The Social Interaction Test (SIT) is a popular method to assess levels of anxiety, social interaction, locomotor activity, and arousal in rodents (File and Seth, 2003). In our experiment, an examined rat is introduced into the test box with a tunnel, open field arena, and Interaction Zone with unfamiliar rat. Examined rat behavior is monitored; time spent in different parts of the test arena, number of droppings, or freezing behavior. Our model explores cognitive changes among Wistar Rats through the PST, as well as anxiety level and social interaction through the Social Interaction Test. We used SIT procedure similar to the one in social defeat experiments (Golden et al., 2011; Toyoda, 2017).

Predator Odor as Traumatizing Factor

In our study, we use an animal model with predator odor exposure that produces behavioral, physiological, and molecular alterations that recapitulate many of the same alterations observed in PTSD patients (Cohen et al., 2012). We use bobcat urine as a stressor, it is a well-established model used in a series of studies done by Gilpin and colleagues (Albrechet-Souza and Gilpin, 2019). Bobcat urine contains the biogenic amine 2-phenylethylamine, which activates specific receptors within the rodent olfactory cortex, the trace amine-associated receptor 4 (TAART4), and can induce avoidance behavior in rats and mice (Ferrero et al., 2011). Furthermore, bobcat urine activates the amygdala-piriform transition area, which is responsible for increases in circulating stress hormones (Kondoh et al., 2016). In 1993, Yehuda and Antelman developed 5 criteria that animal models must meet, to parallel PTSD-related phenotypes: (1) Even a brief stressor should be able to induce biological and behavioral sequelae of PTSD, (2) The stressor should be able to produce PTSD-like sequelae in a dose-dependent manner, (3) Stressors should produce biological alterations that persist over time or become more pronounced with passage of time, (4) The stressor should induce biobehavioral alterations that have the potential for bidirectional expression, (5) Interindividual variability in response to a stressor should be present either as a function of experience, genetics, or an interaction of the two (Yehuda and Antelman, 1993). Studies done with bobcat urine meet most of those criteria (Albrechet-Souza and Gilpin, 2019), and are well discussed in the context of animal PTSD model (Albrechet-Souza et al., 2020, 2021). Taking the literature mentioned above, we feel confident using this type of traumatizing stimulus in our protocol.

Subjects

In our procedure, we used male Wistar Rats (Animal Research Center, Wrocław Medical University, PL) in a total number of 26 individuals ($n = 26$), although 20 individuals were included in our experiment ($n = 20$). Rats arrived at the age of 39–42 days, weighing 210–245 g at the day of arrival, were submitted to a handling period (7 days), and then entered P0. Six individuals did not meet the criteria to enter the P1, and were excluded during P0. Excluded animals either: (1) did not learn the tapping procedure throughout phase 0 or (2) presented freezing behavior during 3 consecutive days. Due to housing conditions and experimental procedure, the exclusion of a rat resulted in the exclusion of its cotenant. Therefore, even though $n = 3$ rats met the exclusion criteria, the total sum of $n = 6$ individuals was excluded.

A random group of rats ($n = 10$) participated as a control group, the second group ($n = 10$) participated as an experimental group ($n = 10$). Rats were pair housed on a non-reversed 12 h/12 h light/dark cycle (lights off at 7 p.m.). All behavioral tests were constructed during the light period. Rats had *ad libitum* access to food (dry pellets) and water.

The experiment was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All procedures were approved by the Local Ethics Committee for Animal Experiments, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland.

Testing Chambers

The PST chamber was part of the device built by our team to measure PST in rats. It had a perforated metal floor that allowed animals to move freely and comfortably. Under the perforated floor there was a compartment where a sponge with odor could have been placed. The walls and floor of the chamber were easy to sanitize and safe for the animals to explore. The front wall had a hole, where a touchscreen apparatus displayed stimuli. Opposite the front wall, there was a feeder and a diode. Feeder was the place where rewards was delivered, a diode signaled when reward was about to be delivered (see **Figure 1A**).

SIT chamber was constructed from polyvinyl chloride (PCV) and Plexiglas. The main structure was a square 90 × 90 × 40 cm (length × width × height). Inside, there was a PCV wall 70 × 30 cm (length × height) that formed a tunnel. Furthermore, two additional transparent Plexiglas walls (20 × 30 cm) formed a closed space in one of the corners, where a new and unfamiliar rat was trapped (see **Figure 1B**). The 25 cm from plexiglas walls was marked as an "interaction Zone."

Procedure

PST- one pair of stimuli is presented in random order arrangement (left of right side of the screen) (see **Figure 2B**). Rats learned to choose one pair. Feedback was probabilistic; it means that in BC trials, a choice of stimulus B results in 90% positive feedback (10% negative feedback), while choice of stimulus C results in 90% negative feedback (10% positive feedback). Feedback follows the choice to indicate if it was correct (reward) or incorrect (punishment). The correct choice resulted in reward—a drop of sweet protein shake (Strawberry Nutridrink Protein, NUTRICIA, Poland). Incorrect choice resulted in punishment—lack of reward. The touchscreen was 26.5 cm width × 17 cm height and "tappable"—nose poke, strike, or touch with paw resulted with stimulus selection. When the stimulus was selected, the touchscreen went black for 8 s and a reward was delivered to the feeder, simultaneously with a light signal.

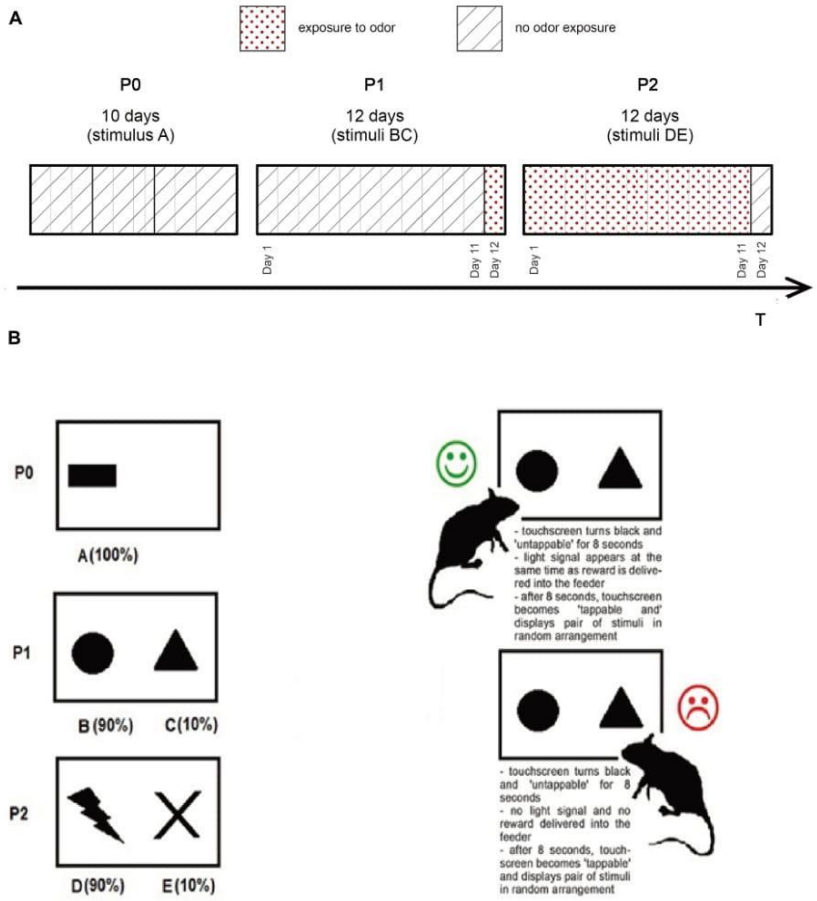


FIGURE 2 | (A) Experiment time schedule. **(B)** Probabilistic Selection Task procedure. P0, Phase 0; P1, Phase 1; P2, Phase 2.

After 8 s, the touchscreen displayed a randomly arranged pair of stimuli again.

SIT was performed twice throughout the experiment, P1 Day 6 and P2 Day 6. Examined individual was placed at the beginning of the tunnel. The session lasted 10 min and was videotaped.

Experimental Design

The rats were subjected to 1 week of handling before the phase 0. During handling sessions, rats were exposed to a sweet liquid, to adapt with sweet reward and feeder mouthpiece. An experiment consisted of 3 phases: phase 0 (P0), phase 1 (P1), and phase 2 (P2) (see **Figure 2A**). Each rat was examined *via* PST in the testing chamber once every day. Our protocol is a variation of the autoshaping task described by Horner et al. (2013).

Phase 0

P0 lasted 10 days and was designed to teach each animal the experimental procedure. During Day 1–3, the paired rats (according to the pair housing) were placed in the testing chamber to accommodate. The rats were able to explore the chamber for 20 min and collect rewards. During the first 6 days, the touchscreen displayed one visual stimulus on the left or right side (see **Figure 2B**). During the first 6 days, the rest of the touchscreen was "untappable"—there was no selection when tap occurred outside the stimulus sector. From 4 to Day 10, the rats were placed in the testing chamber separately, 10 min each.

Throughout Day 7–10 the stimulus was randomly displayed on the left or right side of the touchscreen, although the whole surface of the touchscreen was tappable. Tap delivered within the sector outside of the stimulus resulted in punishment—touchscreen went black for 8 s, no reward was delivered into the feeder. After 8 s, the touchscreen displayed the stimulus again randomly (left or right).

Phase 1

P1 lasted 12 days. Throughout P1, a pair of stimuli (B and C) was used in PST (see **Figure 2B**). Each animal was placed in the testing chamber for 20 min or until the session was completed. After each session, the testing chamber was thoroughly cleaned with disinfectant. The last day (P1 day 12) animals were exposed to predator odor, a sponge soaked with 3 ml of bobcat urine (Lynx rufus; Maine Outdoor Solutions, Hermon, ME, United States)

was placed on the testing chamber floor. In the control group, sponges were not soaked with bobcat urine.

Phase 2

P2 lasted 12 days. Throughout P2, a pair of stimuli (D and E) was used in PST (see **Figure 2B**). Each animal was placed in the testing chamber for 20 min or until the session was completed. After each session, the testing chamber was thoroughly cleaned with disinfectant. Throughout P2, a sponge soaked with bobcat urine (Lynx rufus; Maine Outdoor Solutions, Hermon, ME, United States) was placed under the testing chamber floor. The Last day (P2 Day 12) the animals were not exposed to predator odor. Control rats are treated identically to rats exposed to odors, but the sponges were not soaked with bobcat urine.

Data Collection

During the experiment, the rats performed PST once a day. Each session had 20 trials, the sessions ended when the last trial was completed or when 20 min passed. P1 and P2 lasted 12 days; we measured performance of each rat during 1, 11, and Day 12 (see **Figure 2A**). During those days, we recorded the number of wins (rewards delivered) and loses (punishment received).

In the experimental group, Day 1 was the day when a novel pair of stimuli was presented for the first time. Day 12 was the last day with a pair of known stimuli, but with changed environmental factors (odor or no odor exposure). Thus, P1 Day 1 was the first day when stimuli BC were displayed during PST, without exposure to odor. Day 11 of P1 was the day when stimuli BC were displayed without odor for the last time. P1 Day 12 was the day when BC stimuli were displayed for the last time, but this time with odor exposure. Accordingly, Day 1 ofP2 was the first day when stimuli DE were displayed during PST sessions, with odor exposure. P2 Day 11 was the day when DE stimuli were displayed with odor for the last time. P2 Day 12 was the day when stimuli DE were displayed for the last time, but this time without exposure to odor (see **Figure 2A**).

Behavioral Analysis

Video records were scored by the independent observer, who used stopwatch to measure the time spent in the Interaction Zone of each rat. Interaction Zone was outlined on the SIT floor. Crossing the line with hind limbs was considered as entry into the Interaction Zone.

Statistical Analysis

Analysis and interpretation of behavioral data acquired *via* PST is commonly aided by different variants of theoretical Q learning models (Frank et al., 2004; Frank, 2006; Frank and Claus, 2006; Brown et al., 2018; Kane et al., 2019; Metha et al., 2020). In this way, the research hypothesis is expressed as a set of mathematical equations that govern the analysis of the data. However, the theoretical model introduces its own assumptions and requires advanced routines to adjust the model to the dataset, which may bias the results in an unpredictable manner. Since our study involves a small amount of data, we decided to rely only on directly measurable variables, making the analysis model independent; thus, we present our data without a computational framework.

The test score of each individual was calculated during Days 1, 11, and 12—ratio of the gained rewards to all trials taken that day

$$T\ score_{Dx} = \frac{N\ rewards_{Dx}}{N\ rewards_{Dx} + N\ losses_{Dx}},$$

where $N\ rewards_{Dx}$ is the total number of rewards received during day X (Dx) and $N\ losses_{Dx}$ is the total number of punishment received during day X (Dx).

Then, we calculated the WinRatio of each individual for P1 and P2. WinRatio was a difference between Test score Day 11 and Test Score Day 1:

$$WinRatio = \left[\frac{N\ rewards_{D11}}{N\ rewards_{D11} + N\ losses_{D11}}\right] - \left[\frac{N\ rewards_{D1}}{N\ rewards_{D1} + N\ losses_{D1}}\right]$$

Day 1 and Day 11 test scores (used to calculate individual WinRatios) are presented in **Figure 3A**. Each rat's P1 WinRatio and P2 WinRatio is presented numerically in **Figure 3B**. Days 11 and 12 test scores are presented in **Figure 4**.

Due to a low number of rats and possibly non-normal distribution of variables, we used non-parametric statistical tests. To compare the performance of PST during P1 and P2 of the same rat, we used the Wilcoxon two-sided test. In cross-group comparisons, the U-Mann-Whitney two-sided test was used. Behavioral results were analyzed using the Wilcoxon two-sided test to compare times each rat spent in an Interaction Zone before and after the trauma, U-Mann-Whitney two-sided test was applied for cross-group comparisons. The statistical significance level was established at $p < 0.05$.

Statistical analysis was performed using the scipy.stats library belonging to the Python programming language ecosystem.[1]

RESULTS

With each individual's WinRatio for P1 (no odor) and P2 (with odor), we compared reinforcement learning before (P1) and after (P2) exposure to trauma in the experimental group, as well as reinforcement learning in the control group (see **Figure 3B**). In the experimental group, WinRatio during P1 was significantly greater than during P2 (Wilcoxon test, $p = 0.01$). In the control group, there was no significant difference in WinRatio between P1 and P2 (Wilcoxon Two-Sided Test, $p = 0.73$). In cross-group comparisons, the control group had a higher P2 WinRatio than experimental group P2 WinRatio (U Mann-Whitney Two-sided, $p = 0.0005$). There was no significant difference between the experimental P1 WinRatio and the control P1 WinRatio (two-sided Mann-Whitney U, $p = 0.909$). In general, both groups WinRatios are presented in **Figure 5A**.

The test score was calculated for Day 12 in P1 and P2 (see **Figure 4**). In the experimental group, the P1 Day 12 Test score

[1]https://docs.scipy.org/doc/scipy/reference/stats.html

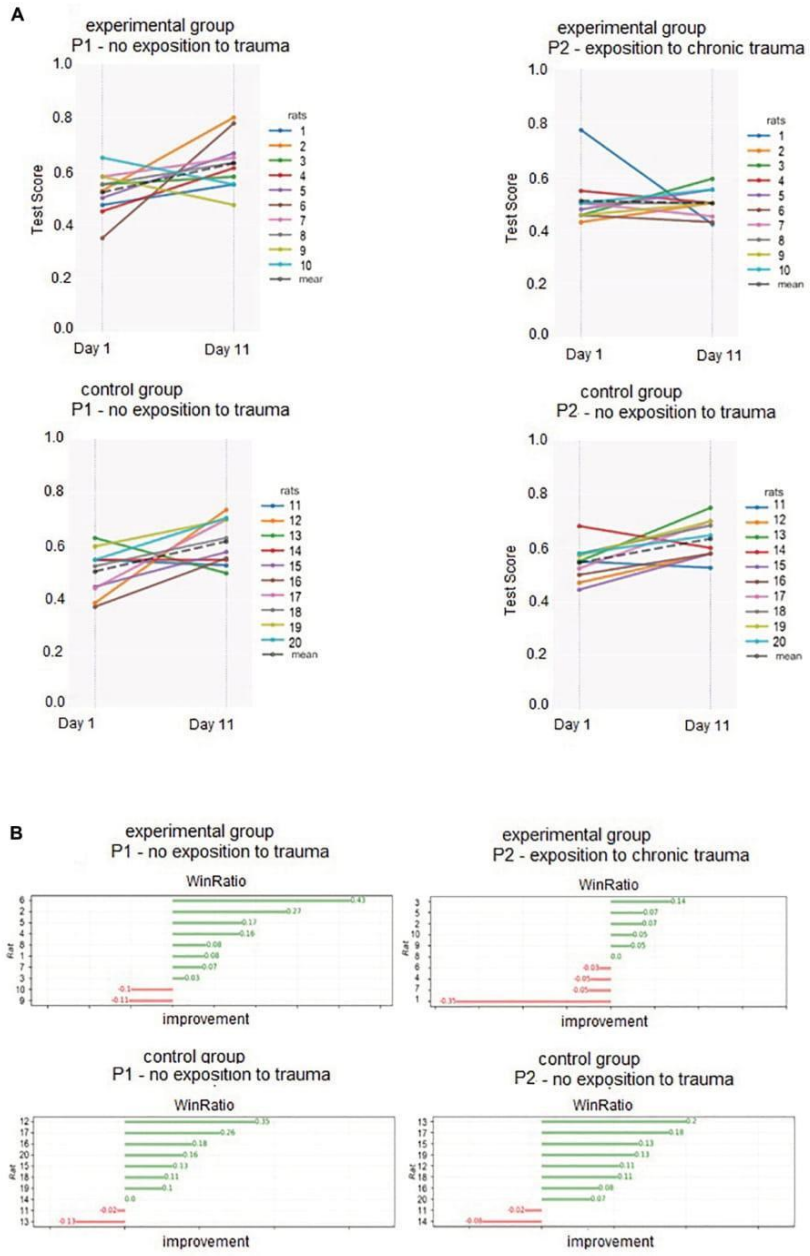


FIGURE 3 | (A) Test scores of each individual (obtained in Days 1 and 11) used to calculate P1 and P2 WinRatios. **(B)** Numerical representation of the overall WinRatio of each individual in P1 and P2.

was significantly worse than the P1 Day 11 test score (Wilcoxon Test, $p = 0.034$). In the control group, the P1 Day 12 Test score was similar to the P1 Day 11 test score (Wilcoxon two-sided, $p = 0.0557$). In the experimental group, day 12 P1 and day 12 P2 Day 12 did not differ (Wilcoxon, two sides, $p = 1.0$). In the control group, Day 12 P1 and Day 12 P2 Day 12 did not differ significantly (Wilcoxon two-sided, $p = 0.314$). In cross-group comparisons, the experimental group P1 Day 12 test score was significantly lower than in the control group (U Mann-Whitney, $p = 0.003$).

Figure 5B present differences in the time spent in an Interaction Zone of SIT in P1 and P2.The experimental group spent significantly more time in the Interaction Zone before trauma (P1) compared to time spent in Interaction Zone after predator odor (P2) (Wilcoxon two-sided test, $p = 0.019$). In the control group, there were no significant differences in the

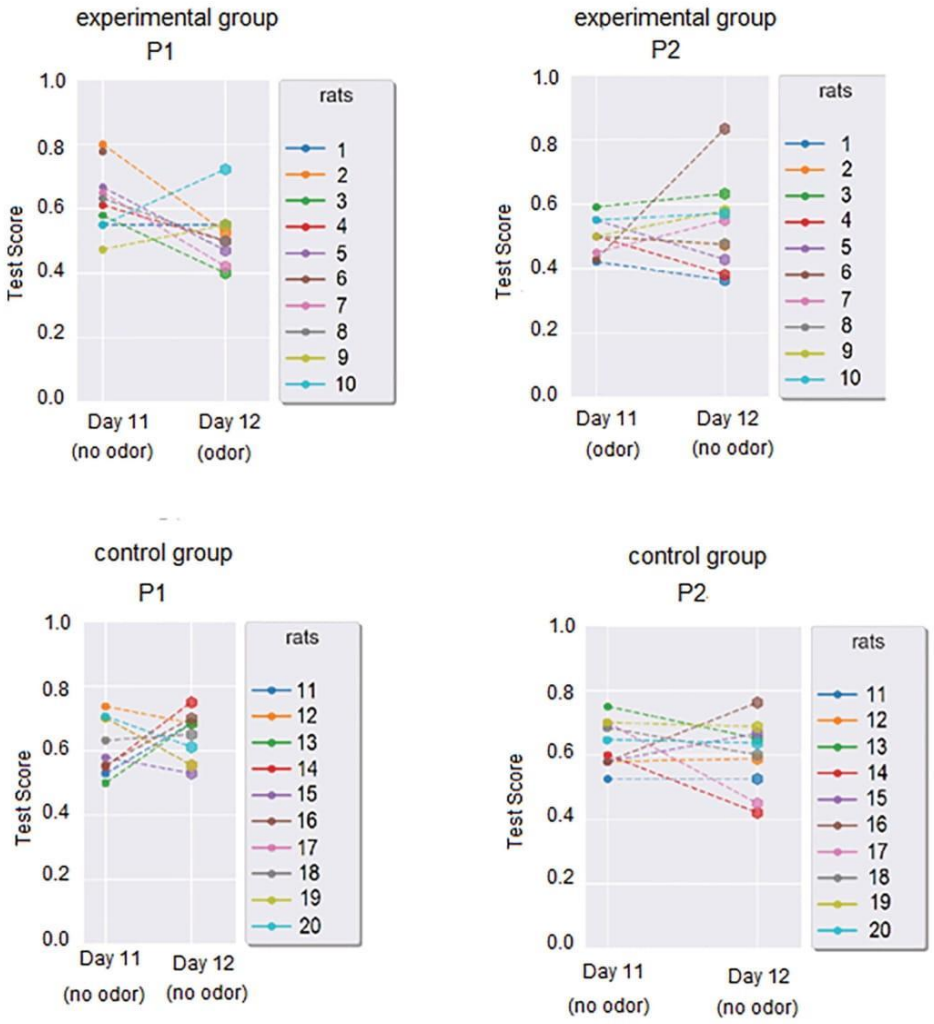


FIGURE 4 | Test scores gained during PST in the last 2 days of each phase. For the experimental group, P1 Day 11 was the day with known stimuli in PST and no odor, but the P1 Day 12 was the first exposure to odor, with stimuli known from previous days. Inversely, P2 Day 11 was the day with known stimuli in PST with odor, while P2 Day 12 was the day with known stimuli in PST, but without odor exposure.

time spent in an Interaction Zone during P1 and P2 (Wilcoxon two-sided test, $p = 0.43$). During P1, the experimental group spent similar time in an Interaction Zone to the control group (Wilcoxon two-sided test, $p = 0.038$). Similarly, cross-group comparisons did not reveal differences between both groups in time spent in an Interaction Zone during P2 (Wilcoxon two-sided test, $p = 0.91$) (see **Figure 5B**).

DISCUSSION

In our study, we examined reinforcement learning (through PST) before and after trauma and compared obtained results with the untraumatized control group. In the experimental group, exposure to chronic trauma (which occurred every day for 12 consecutive days) significantly reduced the ability to perform on PST. The decline in cognitive ability was significant immediately after the first exposure to trauma, although this result is not surprising. Previous findings indicate that single exposure to predator odor is sufficient to induce a behavioral and physiological response such as avoidance (Albrechet-Souza and Gilpin, 2019) or an increase in alcohol intake (Edwards et al., 2013). To our knowledge, we are the first to report a decline in reinforcement learning immediately after exposure to predator odor. We did not find a significant improvement in PST performance 1 day after the odor removal. This result stays in line with studies reporting that the consequences of odor exposure persist weeks after initial exposure (Albrechet-Souza and Gilpin, 2019; Schreiber et al., 2019). To the best of our knowledge, our study is the first to examine rodent cognitive abilities *via*

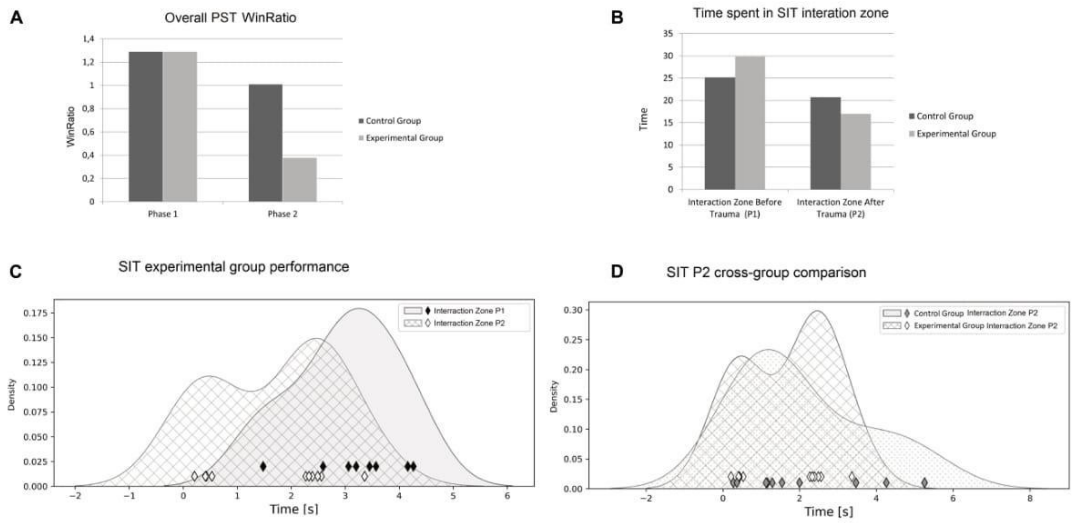


FIGURE 5 | (A) Rewards collected in PST throughout the entire experiment. **(B)** Time spent in an Interaction Zone in SIT, comparison between two groups of rats. **(C)** Time spent in an Interaction Zone during SIT (experimental group). Raw measurements data are drawn as diamonds. For easy visual groups comparison we provided kernel density estimates of Probability Density Functions (Silverman, 2018). The experimental group spent significantly more time in an Interaction Zone before trauma exposure (P1). Furthermore, exposure to trauma (P2) induced a bimodal data distribution that has not occurred in P1. **(D)** The experimental group P2 times compared to the control group P2 times. There is no significant differences in group comparisons ($p = 0.91$), but this may be due to bimodality that characterizes post stress-performance of the experimental group.

PST before and after exposure to predator odor. Moreover, our study confirmed bobcat urine utility as a traumatizing factor, as it significantly affected cognitive abilities, and influenced social behavior among rats exposed to odor.

Overall, the control group performed significantly better in P2 of the experiment. During that period of time, the experimental group was chronically exposed to predator odor. This enforced vigilance and anxiety among rats, which resulted in significant deterioration in PST performance, even though neither punishment nor the physical threat was ever delivered. There are numerous animal models with severe physical punishments, for example foot shock, underwater trauma, restrained stress (Whitaker et al., 2014). Our model is not one of them; the punishment was the lack of the reward. In humans, there are protocols that expose subjects to the possibility of punishment that is never delivered. These studies confirm that anticipation stress reduces reward sensitivity, reward responsiveness (Bogdan and Pizzagalli, 2006; Berghorst et al., 2013) and generally impairs reinforcement learning (Cavanagh et al., 2011). Interestingly, it is hypothesized that stress-susceptible individuals may be more vulnerable to punishment than reward collection (Berghorst et al., 2013). In that case, our protocol (which did not present tangible punishment) may have been less perceptive to those subjects. On the other hand, literature implies that individuals who are less stress-susceptible may be more vulnerable to reward collection than to punishment deliverance (Cavanagh et al., 2011), an observation that validates our approach. This distinction in susceptibility is discussed to be related to striatal dopamine levels, which are known to guide decision making in relation to learning from positive and negative stimuli. Patients with pharmacologically elevated dopamine levels learn better from rewards in PST, compared to those with reduced dopamine levels, who learn better to avoid punishment in PST (Frank et al., 2004). Thus, we hypothesize that the experimental group performed in PST poorer in P2, due to disrupted dopamine levels in the striatum. This implies decline in PST was related to the disruption in reward learning circuits. In humans, exposure to chronic stressors results in blunted ventral striatal (VS) neural activity during reward processing in healthy individuals (Nikolova et al., 2012), as well as in those with PTSD (Mehta et al., 2020). The prominent function of dopaminergic VS neurotransmission in reinforcement learning was confirmed in human positron emission tomography studies that mark right caudate and VS as motivational centers of engagement in activity that brings profit (Kasanova et al., 2017). Stress-related blunted dopaminergic neurotransmission results in overall worse performance in PST, a phenomenon that was observed among individuals with a familial risk of psychosis. Thus, disruption in VS is often symptomatically related to anhedonia, depression, and motivation deficits in both humans and animals (Malone et al., 2009; Roesch et al., 2009; Corral-Frías et al., 2015). We hypothesize that chronic trauma, induced in the experimental group, reduced dopamine level in VS that decreased the performance of experimental rats in PST P2. Our protocol delivered chronic trauma that compromised reward learning, but behaviorally did not induce full-blown anhedonia. We believe that is an important advantage of our model—rats perform voluntarily, which facilitates measurement of cognitive and behavioral deficits in rodents.

Our results are in agreement with studies that indicate deterioration in cognitive abilities among those exposed to

trauma. Schizophrenia patients with a history of trauma exhibit poorer cognitive functioning in terms of memory, executive functions, attention, concentration, and mental speed (Misiak et al., 2017). Computational studies present altered reinforcement learning in veterans with diagnosed PTSD, indicating alteration in reward and punishment perception and valuation (Myers et al., 2013; Brown et al., 2018). Moreover, individuals with PTSD have increased sensitivity to an unexpected outcome during PST (Brown et al., 2018). To our knowledge, this phenomenon has not been validated in animal models, although we believe our protocol may be in use in further research of this topic. If this mechanism of overreaction to an unexpected outcome occurs in the rodent model of trauma, it could have explained the deterioration in learning during P2. We hypothesize that our traumatized subjects were more susceptible to unexpected punishment in P2—as feedback was probabilistic, rewarding stimulus rarely delivered punishment. To test this hypothesis in the future, our protocol needs to be recreated using a computational model.

In SIT, the experimental group proved to be less socially oriented in P2, in comparison to P1—after trauma, rats spent less time in an Interaction Zone with an unfamiliar rat. In humans, chronic trauma influences social interactions, especially in children. Youngsters exposed to chronic traumatic stress present substantial difficulties in constructing relationships. They have troubles in interactions with other children as they often display avoidant symptoms, present inadequately sensitive flight/fight responses, respond to minor stressors by freezing (Streeck-Fischer and van der Kolk, 2000). In another study, adults with PTSD after 2-years of military deployments presented avoidance behavior, social withdrawal, had less positive engagement in relation with their families during post-deployment reengagement (Brockman et al., 2016). In rodent, chronic social defeat model reveals significant decreases in interpersonal interactions after exposure to trauma (Venzala et al., 2012). We believe results obtained during our experiment stays in line with these reports. We hypothesize that it may be related to dopamine disruption, since social behavior in rodents has been shown to be strongly dependent on neural activity in the ventral tegmental area (VTA) of the brain (Chaudhury et al., 2013). Dopamine neurons in VTA project signals to different structures in the striatum (for example, nucleus accumbens) as the well as amygdala or medial prefrontal cortex. Manipulation in neural projection dynamics of VTA influences social interactions in rodents (Gunaydin et al., 2014); therefore, we hypothesize that our trauma protocol disrupts dopamine levels in the midbrain, which results in reduced social behavior after exposition. In the control group, there was no significant difference in SIT performance in P1 and P2, as rats were not exposed to trauma. Similarly, there was no significant difference in the performance of the experimental group P1 and the control group P1 in SIT, as none of the subjects was exposed to predator odor. Although the experimental group spent significantly more time in an Interaction Zone during P1 in comparison with P2, statistical analysis does not reveal differences in time spent in an Interaction Zone between experimental group and the control during P2. This result is inconclusive—two factors have to be taken into consideration. First, a performance difference was observed (see **Figure 5B**), but we cannot support this with statistical verification, probably due to the small number of rats tested. Second, the distribution of the time spent in an Interaction Zone among rats exposed to trauma was bimodal (see **Figure 5C**). This makes the verification of this particular result ambiguous, as a control group did not present this tendency (see **Figure 5D**). This may be a random result, as the group was small in number, but it may also be hypothesized that exposition to trauma divided the experimental group into two subgroups; individuals more susceptible to chronic trauma (less time in an Interaction Zone) and those more resilient (more time in an Interaction Zone). This requires further verification with a larger group, but if confirmed, that would imply that SIT shows individual variability in reactivity to stress induced by predator odor.

Limitations

There are components of our research that should be expanded. As discussed earlier, a categorization is often applied in human studies of the subject, where individuals are characterized as stress-susceptible or resilient. We believe that our protocol could benefit if such a distinction was applied. A viable possibility may be the Avoiders/Non-Avoiders distinction proposed by Albrechet-Souza and Gilpin (2019) in their animal model of PTSD, or a hypothesized distinction delivered by SIT, as we discussed in paragraph above. While rats were in PST chambers, we did not videotape their activity. This is why we could not provide behavioral data from that time-period, that might have been interesting. Our conclusions regarding dopamine-related VTA and VS activity need further verification by molecular studies in animal models. Furthermore, there are interesting reports on striatal activity heavily influenced by increased inflammatory biomarkers, in the context of trauma (Mehta et al., 2020). We believe that our protocol could be of use in further exploration of these topics.

We believe further studies with our protocol should apply an additional group of rats exposed to non-predator odor. This could validate our approach with bobcat urine as a stressor, and deliver much needed comparative context. Changes in rodent behavior could be explored in exposure to different odors, for example alpha-pinene or green leaf odor that are known to have stress-alleviating effects (Akutsu et al., 2003). Studies that use different odors to examine behavioral and cognitive changes are sparse, thus we hypothesize our protocol could be of use to study this subject. We believe this comparative context would deliver interesting results in the wide issue of rodents behavioral and cognitive performance analysis.

CONCLUSION

We present our protocol that may be useful in assessing cognitive abilities in rodents. Rats performed PST voluntarily, when exposed to chronic trauma induced by predator odor. Performance in PST was measured before and after trauma in the same group of rats. Subjects obtained better results in PST before exposure to predator odor. Overall, the experimental group

scored lower in PST compared to not-traumatized control. After exposure to chronic trauma, rats were less socially oriented in SIT, compared to the results obtained before the trauma protocol. Moreover, traumatized rats presented a bimodal tendency in time spent in an Interaction Zone with unknown rat, but due to a small number of animals tested, this result needs further verification.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the Local Ethics Committee for Animal Experiments, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland.

AUTHOR CONTRIBUTIONS

TB designed the research, carried out laboratory experiments, and wrote the manuscript with input from all authors. PK designed and constructed Testing Chambers. JD performed mathematical calculations and analyzed obtained data. BS delivered theoretical framework. DF provided critical feedback and helped shape the research.

FUNDING

This study was sponsored by the Wrocław Medical University (Grant no. STM.c230.18.037).

REFERENCES

Aas, M., Pizzagalli, D. A., Laskemoen, J. F., Reponen, E. J., Ueland, T., Melle, I., et al. (2019). Elevated hair cortisol is associated with childhood maltreatment and cognitive impairment in schizophrenia and in bipolar disorders. *Schizophrenia Res.* 213, 65–71. doi: 10.1016/j.schres.2019.01.011

Akutsu, H., Kikusui, T., Takeuchi, Y., and Mori, Y. (2003). Effects of alpha-pinene odor in different concentrations on stress-induced hyperthermia in rats. *J. Vet. Med. Sci.* 65, 1023–1025. doi: 10.1292/jvms.65.1023

Albrechet-Souza, L., and Gilpin, N. W. (2019). The predator odor avoidance model of post-traumatic stress disorder in rats. *Behav. Pharmacol.* 30, 105–114. doi: 10.1097/FBP.0000000000000460

Albrechet-Souza, L., Nastase, A. S., Hill, M. N., and Gilpin, N. W. (2021). Amygdalar endocannabinoids are affected by predator odor stress in a sex-specific manner and modulate acoustic startle reactivity in female rats. *Neurobiol. Stress* 15:100387. doi: 10.1016/j.ynstr.2021.100387

Albrechet-Souza, L., Schratz, C. L., and Gilpin, N. W. (2020). Sex differences in traumatic stress reactivity in rats with and without a history of alcohol drinking. *Biol. Sex Differ.* 11:27. doi: 10.1186/s13293-020-00303-w

Amitai, N., Young, J. W., Higa, K., Sharp, R. F., Geyer, M. A., and Powell, S. B. (2014). Isolation rearing effects on probabilistic learning and cognitive flexibility in rats. *Cogn. Affect. Behav. Neurosci.* 14, 388–406. doi: 10.3758/s13415-013-0204-4

Assogna, F., Piras, F., and Spalletta, G. (2020). "Neurobiological Basis of Childhood Trauma and the Risk for Neurological Deficits Later in Life," in *Childhood Trauma in Mental Disorders: A Comprehensive Approach*, eds G. Spalletta, D. Janiri, F. Piras, and G. Sani (Cham: Springer International Publishing), 385–410. doi: 10.1007/978-3-030-49414-8_18

Ay, R., and Erbay, L. G. (2018). Relationship between childhood trauma and suicide probability in obsessive-compulsive disorder. *Psychiatr. Res.* 261, 132–136. doi: 10.1016/j.psychres.2017.12.054

Bailey, T., Alvarez-Jimenez, M., Garcia-Sanchez, A. M., Hulbert, C., Barlow, E., and Bendall, S. (2018). Childhood Trauma Is Associated With Severity of Hallucinations and Delusions in Psychotic Disorders: A Systematic Review and Meta-Analysis. *Schizophrenia Bull.* 44, 1111–1122. doi: 10.1093/schbul/sbx161

Berghorst, L., Bogdan, R., Frank, M., and Pizzagalli, D. (2013). Acute stress selectively reduces reward sensitivity. *Front. Hum. Neurosci.* 7:133 doi: 10.3389/fnhum.2013.00133

Bielawski, T., Misiak, B., Moustafa, A., and Frydecka, D. (2019). Epigenetic mechanisms, trauma, and psychopathology: targeting chromatin remodeling complexes. *Rev.Neurosci.* 30, 595–604. doi: 10.1515/revneuro-2018-0055

Bogdan, R., and Pizzagalli, D. A. (2006). Acute Stress Reduces Reward Responsiveness: Implications for Depression. *Biol. Psychiatr.* 60, 1147–1154. doi: 10.1016/j.biopsych.2006.03.037

Breslau, N. (2009). The Epidemiology of Trauma. PTSD, and Other Posttrauma Disorders. *Trauma Violence Abuse* 10, 198–210. doi: 10.1177/1524838009334448

Brockman, C., Snyder, J., Gewirtz, A., Gird, S. R., Quattlebaum, J., Schmidt, N., et al. (2016). Relationship of Service Members' Deployment Trauma, PTSD Symptoms and Experiential Avoidance to Postdeployment Family Reengagement. *J. Fam. Psychol.* 30, 52–62. doi: 10.1037/fam0000152

Brown, V. M., Zhu, L., Wang, J. M., Frueh, B. C., King-Casas, B., and Chiu, P. H. (2018). Associability-modulated loss learning is increased in posttraumatic stress disorder. *eLife* 7:e30150. doi: 10.7554/eLife.30150

Cavanagh, J. F., Frank, M. J., and Allen, J. J. B. (2011). Social stress reactivity alters reward and punishment learning. *Soc. Cogn. Affect. Neurosci.* 6, 311–320. doi: 10.1093/scan/nsq041

Chaudhury, D., Walsh, J. J., Friedman, A. K., Juarez, B., Ku, S. M., Koo, J. W., et al. (2013). Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. *Nature* 493, 532–536. doi: 10.1038/nature11713

Cohen, H., Kozlovsky, N., Alona, C., Matar, M. A., and Joseph, Z. (2012). Animal model for PTSD: From clinical concept to translational research. *Neuropharmacology* 62, 715–724. doi: 10.1016/j.neuropharm.2011.04.023

Corral-Frías, N. S., Nikolova, Y. S., Michalski, L. J., Baranger, D. A. A., Hariri, A. R., and Bogdan, R. (2015). Stress-related anhedonia is associated with ventral striatum reactivity to reward and transdiagnostic psychiatric symptomatology. *Psychol. Med.* 45, 2605–2617. doi: 10.1017/S0033291715000525

Davidson, L. M., and Baum, A. (1986). Chronic stress and posttraumatic stress disorders. *J. Consult. Clin. Psychol.* 54, 303–308. doi: 10.1037/0022-006X.54.3.303

Dennison, U., McKernan, D., Cryan, J., and Dinan, T. (2012). Schizophrenia patients with a history of childhood trauma have a pro-inflammatory phenotype. *Psychol. Med.* 42, 1865–1871. doi: 10.1017/S0033291712000074

Duhig, M., Patterson, S., Connell, M., Foley, S., Capra, C., Dark, F., et al. (2015). The prevalence and correlates of childhood trauma in patients with early psychosis. *Aust. N.Z.J. Psychiatr.* 49, 651–659. doi: 10.1177/0004867415575379

Edwards, S., Baynes, B. B., Carmichael, C. Y., Zamora-Martinez, E. R., Barrus, M., Koob, G. F., et al. (2013). Traumatic stress reactivity promotes excessive alcohol drinking and alters the balance of prefrontal cortex-amygdala activity. *Transl. Psychiatr.* 3, e296–e296. doi: 10.1038/tp.2013.70

Éthier, L. S., Lemelin, J.-P., and Lacharité, C. (2004). A longitudinal study of the effects of chronic maltreatment on children's behavioral and emotional problems. *Child Abuse Neglect* 28, 1265–1278. doi: 10.1016/j.chiabu.2004.07.006

Farag, Y. M. K., and Gaballa, M. R. (2011). Diabesity: an overview of a rising epidemic. *Nephrol. Dialysis Transplant.* 26, 28–35. doi: 10.1093/ndt/gfq576

Ferrero, D. M., Lemon, J. K., Fluegge, D., Pashkovski, S. L., Korzan, W. J., Datta, S. R., et al. (2011). Detection and avoidance of a carnivore odor by prey. *Proc.Natl. Acad. Sci.* 108, 11235–11240. doi: 10.1073/pnas.1103317108

File, S. E., and Seth, P. (2003). A review of 25 years of the social interaction test. *Eur. J. Pharmacol.* 463, 35–53. doi: 10.1016/S0014-2999(03)01273-1

Frank, M. J. (2006). Hold your horses: A dynamic computational role for the subthalamic nucleus in decision making. *Neural. Networks* 19, 1120–1136. doi: 10.1016/j.neunet.2006.03.006

Frank, M. J., and Claus, E. D. (2006). Anatomy of a decision: Striato-orbitofrontal interactions in reinforcement learning, decision making, and reversal. *Psychol. Rev.* 113, 300–326. doi: 10.1037/0033-295X.113.2.300

Frank, M. J., Moustafa, A. A., Haughey, H. M., Curran, T., and Hutchison, K. E. (2007). Genetic triple dissociation reveals multiple roles for dopamine in reinforcement learning. *Proc.Natl. Acad. Sci.* 104, 16311–16316. doi: 10.1073/pnas.0706111104

Frank, M. J., Seeberger, L. C., and O'reilly, R. C. (2004). By carrot or by stick: cognitive reinforcement learning in parkinsonism. *Science* 306, 1940–1943. doi: 10.1126/science.1102941

George, S. A., Rodriguez-Santiago, M., Riley, J., Abelson, J. L., Floresco, S. B., and Liberzon, I. (2015). Alterations in cognitive flexibility in a rat model of post-traumatic stress disorder. *Behav. Brain Res.* 286, 256–264. doi: 10.1016/j.bbr.2015.02.051

Golden, S. A., Covington, H. E., Berton, O., and Russo, S. J. (2011). A standardized protocol for repeated social defeat stress in mice. *Nat. Protoc.* 6, 1183–1191. doi: 10.1038/nprot.2011.361

Gunaydin, L. A., Grosenick, L., Finkelstein, J. C., Kauvar, I. V., Fenno, L. E., Adhikari, A., et al. (2014). Natural Neural Projection Dynamics Underlying Social Behavior. *Cell* 157, 1535–1551. doi: 10.1016/j.cell.2014.05.017

Harro, J. (2018). Animals, anxiety, and anxiety disorders: How to measure anxiety in rodents and why. *Behav. Brain Res.* 352, 81–93. doi: 10.1016/j.bbr.2017.10.016

Horner, A. E., Heath, C. J., Hvoslef-Eide, M., Kent, B. A., Kim, C. H., Nilsson, S. R., et al. (2013). The touchscreen operant platform for testing learning and memory in rats and mice. *Nat. Protoc.* 8, 1961–1984.

Jansen, K., Cardoso, T. A., Fries, G. R., Branco, J. C., Silva, R. A., Kauer-Sant'Anna, M., et al. (2016). Childhood trauma, family history, and their association with mood disorders in early adulthood. *Acta Psychiatr. Scand.* 134, 281–286. doi: 10.1111/a.12551

Jocham, G., Klein, T. A., and Ullsperger, M. (2011). Dopamine-Mediated Reinforcement Learning Signals in the Striatum and Ventromedial Prefrontal Cortex Underlie Value-Based Choices. *J. Neurosci.* 31, 1606–1613. doi: 10.1523/JNEUROSCI.3904-10.2011

Joëls, M., Sarabdjitsingh, R. A., and Karst, H. (2012). Unraveling the Time Domains of Corticosteroid Hormone Influences on Brain Activity: Rapid. Slow, and Chronic Modes. *Pharmacol. Rev.* 64, 901–938. doi: 10.1124/pr.112.005892

Kane, G. A., Bornstein, A. M., Shenhav, A., Wilson, R. C., Daw, N. D., and Cohen, J. D. (2019). Rats exhibit similar biases in foraging and intertemporal choice tasks. *eLife* 8:e48429. doi: 10.7554/eLife.48429

Kasanova, Z., Ceccarini, J., Frank, M. J., Amelsvoort, T., van, Booij, J., et al. (2017). Striatal dopaminergic modulation of reinforcement learning predicts reward—oriented behavior in daily life. *Biol. Psychol.* 127, 1–9. doi: 10.1016/j.biopsycho.2017.04.014

Kasanova, Z., Ceccarini, J., Frank, M. J., van Amelsvoort, T., Booij, J., Heinzel, A., et al. (2018). Daily-life stress differentially impacts ventral striatal dopaminergic modulation of reward processing in first-degree relatives of individuals with psychosis. *Eur. Neuropsychopharmacol.* 28, 1314–1324. doi: 10.1016/j.euroneuro.2018.10.002

Knox, D., George, S. A., Fitzpatrick, C. J., Rabinak, C. A., Maren, S., and Liberzon, I. (2012). Single prolonged stress disrupts retention of extinguished fear in rats. *Learn. Mem.* 19, 43–49. doi: 10.1101/lm.024356.111

Kondoh, K., Lu, Z., Ye, X., Olson, D. P., Lowell, B. B., and Buck, L. B. (2016). A specific area of olfactory cortex involved in stress hormone responses to predator odours. *Nature* 532, 103–106. doi: 10.1038/nature17156

Krishnan, V., Han, M.-H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., et al. (2007). Molecular Adaptations Underlying Susceptibility and Resistance to Social Defeat in Brain Reward Regions. *Cell* 131, 391–404. doi: 10.1016/j.cell.2007.09.018

Lamontagne, S. J., Pizzagalli, D. A., and Olmstead, M. C. (2021). Does inflammation link stress to poor COVID-19 outcome? *Stress Health* 37, 401–414. doi: 10.1002/smi.3017

Laramée, P., Kusel, J., Leonard, S., Aubin, H.-J., François, C., and Daeppen, J.-B. (2013). The Economic Burden of Alcohol Dependence in Europe. *Alcohol Alcohol.* 48, 259–269. doi: 10.1093/alcalc/agt004

Lysaker, P., Meyer, P., Evans, J., and Marks, K. (2001). Neurocognitive and Symptom Correlates of Self-Reported Childhood Sexual Abuse in Schizophrenia Spectrum Disorders. *Ann. Clin. Psychiatr.* 13, 89–92. doi: 10.3109/10401230109148953

Majer, M., Nater, U. M., Lin, J.-M. S., Capuron, L., and Reeves, W. C. (2010). Association of childhood trauma with cognitive function in healthy adults: a pilot study. *BMC Neurol.* 10:61. doi: 10.1186/1471-2377-10-61

Malone, D. A., Dougherty, D. D., Rezai, A. R., Carpenter, L. L., Friehs, G. M., Eskandar, E. N., et al. (2009). Deep Brain Stimulation of the Ventral Capsule/Ventral Striatum for Treatment-Resistant Depression. *Biol. Psychiatr.* 65, 267–275. doi: 10.1016/j.biopsych.2008.08.029

Masodkar, K., Johnson, J., and Peterson, M. J. (2016). A Review of Posttraumatic Stress Disorder and Obesity: Exploring the Link. *Prim. Care Companion CNS Dis.* 18:10.4088/PCC.15r01848 doi: 10.4088/PCC.15r01848

Matosin, N., Cruceanu, C., and Binder, E. B. (2017). Preclinical and Clinical Evidence of DNA Methylation Changes in Response to Trauma and Chronic Stress. *Chronic. Stress* 1:2470547017710764. doi: 10.1177/2470547017710764

McEwen, B. S. (2017). Neurobiological and Systemic Effects of Chronic Stress. *Chronic Stress* 1:2470547017692328. doi: 10.1177/2470547017692328

McLaughlin, K. A., Koenen, K. C., Friedman, M. J., Ruscio, A. M., Karam, E. G., Shahly, V., et al. (2015). Sub-threshold Post Traumatic Stress Disorder in the WHO World Mental Health Surveys. *Biol. Psychiatr.* 77, 375–384. doi: 10.1016/j.biopsych.2014.03.028

Mehta, N. D., Stevens, J. S., Li, Z., Gillespie, C. F., Fani, N., Michopoulos, V., et al. (2020). Inflammation, reward circuitry and symptoms of anhedonia and PTSD in trauma-exposed women. *Soc. Cogn. Affect. Neurosci.* 15, 1046–1055. doi: 10.1093/scan/nsz100

Metha, J. A., Brian, M. L., Oberrauch, S., Barnes, S. A., Featherby, T. J., Bossaerts, P., et al. (2020). Separating Probability and Reversal Learning in a Novel Probabilistic Reversal Learning Task for Mice. *Front. Behav. Neurosci.* 13:270. doi: 10.3389/fnbeh.2019.00270

Misiak, B., Krefft, M., Bielawski, T., Moustafa, A. A., Sąsiadek, M. M., and Frydecka, D. (2017). Toward a unified theory of childhood trauma and psychosis: A comprehensive review of epidemiological, clinical, neuropsychological and biological findings. *Neurosci. Biobehav. Rev.* 75, 393–406. doi: 10.1016/j.neubiorev.2017.02.015

Mota, N., Tsai, J., Kirwin, P. D., Harpaz-Rotem, I., Krystal, J. H., Southwick, S. M., et al. (2016). Late-Life Exacerbation of PTSD Symptoms in US Veterans: Results From the National Health and Resilience in Veterans Study. *J. Clin. Psychiatr.*77:10211. doi: 10.4088/JCP.15m10101

Myers, C. E., Moustafa, A. A., Sheynin, J., VanMeenen, K. M., Gilbertson, M. W., Orr, S. P., et al. (2013). Learning to Obtain Reward, but Not Avoid Punishment, Is Affected by Presence of PTSD Symptoms in Male Veterans: Empirical Data and Computational Model. *PLoS One* 8:e72508. doi: 10.1371/journal.pone.0072508

Nikolova, Y., Bogdan, R., and Pizzagalli, D. A. (2012). Perception of a Naturalistic Stressor Interacts with 5-HTTLPR/rs25531 Genotype and Gender to Impact Reward Responsiveness. *Neuropsychobiology* 65, 45–54. doi: 10.1159/000329105

Petkus, A. J., Lenze, E. J., Butters, M. A., Twamley, E. W., and Wetherell, J. L. (2018). Childhood Trauma is Associated with Poorer Cognitive Performance in Older Adults. *J. Clin. Psychiatr.*79:16m11021. doi: 10.4088/JCP.16m11021

Planchez, B., Surget, A., and Belzung, C. (2019). Animal models of major depression: drawbacks and challenges. *J. Neural. Transm.* 126, 1383–1408. doi: 10.1007/s00702-019-02084-y

Pruessner, M., Bechard-Evans, L., Pira, S., Joober, R., Collins, D. L., Pruessner, J. C., et al. (2017). Interplay of hippocampal volume and hypothalamus-pituitary-adrenal axis function as markers of stress vulnerability in men at ultra-high risk for psychosis. *Psychol. Med.* 47, 471–483. doi: 10.1017/S0033291716002658

Reber, S. O., Langgartner, D., Foertsch, S., Postolache, T. T., Brenner, L. A., Guendel, H., et al. (2016). Chronic subordinate colony housing paradigm: A mouse model for mechanisms of PTSD vulnerability, targeted prevention, and treatment—2016 Curt Richter Award Paper. *Psychoneuroendocrinol.* 74, 221–230. doi: 10.1016/j.psyneuen.2016.08.031

Reinen, J. M., Whitton, A. E., Pizzagalli, D. A., Slifstein, M., Abi-Dargham, A., McGrath, P. J., et al. (2021). Differential reinforcement learning responses to positive and negative information in unmedicated individuals with depression. *Eur. Neuropsychopharmacol.* 53, 89–100. doi: 10.1016/j.euroneuro.2021.08.002

Roesch, M. R., Singh, T., Brown, P. L., Mullins, S. E., and Schoenbaum, G. (2009). Ventral Striatal Neurons Encode the Value of the Chosen Action in Rats Deciding between Differently Delayed or Sized Rewards. *J. Neurosci.* 29, 13365–13376. doi: 10.1523/JNEUROSCI.2572-09.2009

Roehr, B. (2013). American psychiatric association explains DSM-5. *BMJ* 346:f3591. doi: 10.1136/bmj.f3591

Ross, R. A., Foster, S. L., and Ionescu, D. F. (2017). The Role of Chronic Stress in Anxious Depression. *Chronic Stress* 1:247054701668947. doi: 10.1177/2470547016689472

Rothgeb, C. L. (1971). *Standard Edition of the Complete Psychological Works of Sigmund Freud*. Available online at: https://eric.ed.gov/?id=ED062645. (accessed February 25, 2022).

Saavedra-Rodríguez, L., and Feig, L. A. (2013). Chronic Social Instability Induces Anxiety and Defective Social Interactions Across Generations. *Biol. Psychiatr.* 73, 44–53. doi: 10.1016/j.biopsych.2012.06.035

Salcioglu, E., Urhan, S., Pirinccioglu, T., and Aydin, S. (2017). Anticipatory fear and helplessness predict PTSD and depression in domestic violence survivors. *Psychol. Trauma* 9, 117–125. doi: 10.1037/tra0000200

Schenkel, L. S., Spaulding, W. D., DiLillo, D., and Silverstein, S. M. (2005). Histories of childhood maltreatment in schizophrenia: Relationships with premorbid functioning, symptomatology, and cognitive deficits. *Schizophrenia Res.* 76, 273–286. doi: 10.1016/j.schres.2005.03.003

Schreiber, A. L., McGinn, M. A., Edwards, S., and Gilpin, N. W. (2019). Predator Odor Stress Blunts Alcohol Conditioned Aversion. *Neuropharmacology* 144, 82–90. doi: 10.1016/j.neuropharm.2018.10.019

Seib, D. R., Espinueva, D. F., Floresco, S. B., and Snyder, J. S. (2020). A role for neurogenesis in probabilistic reward learning. *Behav. Neurosci.* 134, 283–295. doi: 10.1037/bne0000370

Shannon, C., Douse, K., McCusker, C., Feeney, L., Barrett, S., and Mulholland, C. (2011). The Association Between Childhood Trauma and Memory Functioning in Schizophrenia. *Schizophrenia Bull.* 37, 531–537. doi: 10.1093/schbul/sbp096

Sharhabani-Arzy, R., Amir, M., Kotler, M., and Liran, R. (2003). The Toll of Domestic Violence: PTSD among Battered Women in an Israeli Sample. *J. Interpers. Violence* 18, 1335–1346. doi: 10.1177/0886260503256842

Silverman, B. W. (2018). *Density Estimation for Statistics and Data Analysis*. New York, NY: Routledge.

Simon, G. E., Von Korff, M., Saunders, K., Miglioretti, D. L., Crane, P. K., van Belle, G., et al. (2006). Association Between Obesity and Psychiatric Disorders in the US Adult Population. *Arch. Gen. Psychiatr.* 63, 824–830. doi: 10.1001/archpsyc.63.7.824

Sinha, R. (2008). Chronic Stress, Drug Use, and Vulnerability to Addiction. *Ann.N.Y Acad. Sci.* 1141, 105–130. doi: 10.1196/annals.1441.030

Streeck-Fischer, A., and van der Kolk, B. A. (2000). Down will come baby, cradle and all: diagnostic and therapeutic implications of chronic trauma on child development. *Aus. N.Z. J. Psychiatr.* 34, 903–918. doi: 10.1080/000486700265

Toyoda, A. (2017). Social defeat models in animal science: What we have learned from rodent models. *Anim. Sci. J.* 88, 944–952 doi: 10.1111/asj.12809

Vasilevski, V., and Tucker, A. (2016). Wide-ranging cognitive deficits in adolescents following early life maltreatment. *Neuropsychology* 30, 239–246. doi: 10.1037/neu0000215

Veeser, J., Barkmann, C., Schumacher, L., Zindler, A., Schön, G., and Barthel, D. (2021). Post-traumatic stress disorder in refugee minors in an outpatient care center: prevalence and associated factors. *Eur. Child Adolesc. Psychiatr.* [Epub ahead of print]. doi: 10.1007/s00787-021-01866-8

Venzala, E., García-García, A. L., Elizalde, N., Delagrange, P., and Tordera, R. M. (2012). Chronic social defeat stress model: behavioral features, antidepressant action, and interaction with biological risk factors. *Psychopharmacology* 224, 313–325. doi: 10.1007/s00213-012-2754-5

Wang, W., Liu, W., Duan, D., Bai, H., Wang, Z., and Xing, Y. (2021). Chronic social defeat stress mouse model: Current view on its behavioral deficits and modifications. *Behav. Neurosci.* 135, 326–335. doi: 10.1037/bne0000418

Whitaker, A. M., Gilpin, N. W., and Edwards, S. (2014). Animal Models of Post-Traumatic Stress Disorder and Recent Neurobiological Insights. *Behav. Pharmacol.* 25, 398–409. doi: 10.1097/FBP.0000000000000069

Yehuda, R. (1998). *Psychological Trauma*.(Washington, D.C: American Psychiatric Pub)

Yehuda, R., and Antelman, S. M. (1993). Criteria for Rationally Evaluating Animal Models of Posttraumatic Stress Disorder. *Biol. Psychiatr.* 8, 479–86. doi: 10.1016/0006-3223(93)90001-t

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Podsumowanie wyników

Część pierwsza:

Bielawski T., Misiak B., Moustafa A., Frydecka D.: Epigenetic Mechanisms, trauma, and psychopathology: targeting chromatin remodeling complexes. *Reviews in the Neurosciences* 2019; 30: 595–604

W pracy przeglądowej przedstawiono epigenetyczne zmiany zachodzące pod wpływem ekspozycji na traumę. Wykazano, w jaki sposób, pod wpływem ekspozycji na stres, ATP-zależne kompleksy re-modulujące chromatynę sterują ekspresją genów, oddziałując na funkcje poznawcze jednostki. Opisano geny i białka budujące ATP-zależne kompleksy, które w trakcie pojawienia się traumy psychicznej przyczyniają się do powstania objawów wycofania społecznego (białko BAZ1A), determinują uczenie się na podstawie kar i nagród (białko BAZ1B, geny z grupy *SMARCA*) oraz odpowiadają za konsolidowanie szlaków pamięciowych (kompleksy białkowe nBAF). Praca poglądowa pozwoliła stwierdzić, iż badania z wykorzystaniem modeli zwierzęcych, z chroniczną ekspozycją na stres, wywołują zmiany epigenetyczne, wpływające na funkcje poznawcze badanych osobników. W pracy odniesiono się także do potencjalnych ograniczeń opisywanych współcześnie modeli zwierzęcych, które proponują zbyt krótkie okno czasowe na badanie zmian zachodzących pod wpływem procesów epigenetycznych.

Część druga:

Bielawski T., Albrechet-Souza L., Frydecka D.: Endocannabinoid system in trauma and psychosis: distant guardian of mental stability.*Reviews in the Neurosciences* 2021. 32(7) 707-722.

W pracy zaprezentowano teoretyczny model, w którym chroniczna ekspozycja na stres i traumy powoduje upośledzenie funkcji ochronnej układu endokannabinoidowego, co przyczynia się do powstawania licznych psychopatologii. W wyczerpujący sposób omówiono fizjologię działania układu endokannabinoidowego, który moduluje aktywność osi stresu HPA, chroni, między innymi hipokamp oraz przyśrodkową część kory przedczołowej przed działaniem kortykosteroidów, tym samym wpływając na funkcje poznawcze jednostki. Wskazano funkcjonalny obszar współoddziaływania układu endokannabinoidowego, osi HPA oraz szlaku wydzielniczego dopaminy (pole brzuszne nakrywki), który może być kluczowy dla odzyskiwania potraumatycznej homeostazy. Omówione wyniki badań wskazują dominującą rolę układu endokannabinoidowego w nadzorowaniu "*extinction learning*", czyli uczenia, które polega na wygaszaniu dawniej uwarunkowanych reakcji i zastępowaniu jej nowymi szlakami pamięciowymi, co może okazać się kluczowym do radzenia sobie z objawami PTSD, takimi jak flashbacki lub intruzywne myśli. Praca wykazała, iż 2-AG (*2-Arachidonoylglycerol*), SEA(*N-stearoylethanolamine*), PEA (*Palmitoylethanolamide*), OEA (*Oleoylethanolamide*), mogą pełnić funkcję biomarkerów przeżytych traum.

Część trzecia:

Bielawski T., Drapała J., Krowicki P., Stańczykiewicz B., Frydecka D.: Trauma disrupts reinforcement learning in rats - a novel animal model of chronic stress exposure. *Frontiers In Behavioral Neuroscience* 2022; 185

Zaprojektowana i zbudowana maszyna pozwoliła na przeniesienie procedury badawczej PST z modelu ludzkiego na model zwierzęcy, z użyciem ekranu dotykowego, wyświetlającego nagradzające i karzące bodźce. Co więcej, udowodniono, że nowo opracowana procedura badania umożliwia śledzenie zmian funkcji poznawczych (uczenia się na podstawie kar i nagród) podczas procesu ekspozycji na traumę, nie wywołując pełnoobjawowej anhedonii lub katatonii, która uniemożliwiłaby dalsze badania laboratoryjne. Pod względem wyników osiąganych w PST grupa eksperymentalna nie różniła się od grupy kontrolnej w trakcie pierwszego etapu. W drugim etapie grupa eksperymentalna (traumatyzowana) cechowała się istotnie gorszymi wynikami PST w porównaniu do grupy kontrolnej (nie-traumatyzowanej), oraz w porównaniu do wyników PST, osiąganych przez te same osobniki w poprzednim etapie. Już pierwszy dzień ekspozycji na traumę spowodował gwałtowne pogorszenie uczenia się ze wzmocnień osobników z grupy eksperymentalnej (mierzonymi za pomocą PST), ta tendencja utrzymywała się przez kolejne 11 dni chronicznej ekspozycji na stres. W behawioralnym badaniu SIT osobniki z grupy eksperymentalnej osiągały istotnie gorsze wyniki niż na etapie przed-ekspozycyjnym. Analiza wyników SIT wykazała bimodalność w grupie traumatyzowanej, jednak tendencja do wyodrębnienia się podgrupy osobników bardziej odpornych na traumatyzację (mniej izolujących się) oraz osobników mniej odpornych na traumatyzację (bardziej izolujących się), nie osiągnęła istotności statystycznej.

Wnioski

A. Opisana w badaniu procedura pozwala na:

- badanie zmian w uczeniu się z nagród i kar zachodzących podczas doświadczenia traumy w skutek ekspozycji na chroniczny stres, z wykorzystaniem modelu zwierzęcego imitującego model ludzki,

-obserwację zmian behawioralnych zachodzących podczas rozwoju traumy, wskutek ekspozycji na chroniczny stres,

- wykorzystanie PST do oceny uczenia się na podstawie nagród i kar, w formie identycznej do tej, stosowanej w badaniach modelu ludzkiego (ekran dotykowy na którym wyświetlane są symbole).

B. Otrzymane wyniki prezentują:

- wyraźne pogorszenie uczenia się ze wzmocnień pod wpływem pierwszej ekspozycji na traumatyzujący bodziec w porównaniu z wynikami PST sprzed traumatyzacji oraz w porównaniu z wynikami PST grupy kontrolnej,

- utrzymujący się stan obniżonego uczenia się z nagród i kar po 11 dniach chronicznej ekspozycji w porównaniu z wynikami PST sprzed traumatyzacji, oraz w porównaniu z wynikami PST grupy kontrolnej,

-brak poprawy w uczeniu się ze wzmocnień po 24 godzinach od ostatniej ekspozycji na doświadczenie traumatyczne,

- skuteczność i użyteczność zapachu drapieżnika (predator odor*)* jako silnego stresora, wywołującego wyraźne zmiany w uczeniu się na podstawie nagród i kar, nie wywołującego jednak pełnoobjawowej anhedonii i katatonii (freezing behavior) u zwierząt, co uniemożliwiłoby dalsze badania,

- wpływ chronicznego stresu na preferencję do izolacji i wycofania z interakcji społecznych widoczny w badaniu SIT,w porównaniu do zachowania prezentowanego przed traumatyzacją, w grupie tych samych osobników,

- specyficzną, wyciszającą funkcję układu endokannabinoidowego względem osi HPA, podczas ekspozycji na silny stres i traumę (wniosek pracy teoretycznej),

- potencjalne biomarkery przeżytych traum będące ligandami receptorów kannabinoidowych (wniosekpracy teoretycznej),

- rolę ATP-zależych kompleksów re-modulujące chromatynę w powstawaniu objawów wycofania, zubożenia funkcji poznawczych, pogorszenia pamięci w trakcie ekspozycji na silny stres i traumę (wniosek pracy teoretycznej)

C. Opisana w badaniu procedura może:

- zostać wykorzystana do wyodrębnienia osobników wykazujących większą podatność *(*susceptible) oraz tych wykazujących większą odporność na doświadczenie traumatyczne (resilient), oraz do opisania i zbadania biologicznego podłoża tego zjawiska z uwzględnieniem endokannabinoidów, jako markerów przeżytych traum,

- zostać wykorzystana do badań epigenetycznych zmian zachodzących w strukturach mózgu, odpowiedzialnych za proces uczenia się na podstawie kar i nagród pod wpływem ekspozycji na chroniczny stres.

Literatura:

1. Yehuda R. *Psychological Trauma*. American Psychiatric Pub, 1998.

2. Rothgeb CL. *Abstracts of the Standard Edition of the Complete Psychological Works of Sigmund Freud*. 1971https://eric.ed.gov/?id=ED062645 (accessed 10 Apr2022).

3. Ferdowsian H, Merskin D. Parallels in Sources of Trauma, Pain, Distress, and Suffering in Humans and Nonhuman Animals. *Journal of Trauma & Dissociation* 2012; **13**: 448–468.

4. Jansen K, Cardoso TA, Fries GR, Branco JC, Silva RA, Kauer-Sant'Anna M *et al.* Childhood trauma, family history, and their association with mood disorders in early adulthood. *Acta PsychiatrScand* 2016; **134**: 281–286.

5. Misiak B, Krefft M, Bielawski T, Moustafa AA, Sąsiadek MM, Frydecka D. Toward a unified theory of childhood trauma and psychosis: A comprehensive review of epidemiological, clinical, neuropsychological and biological findings. *Neuroscience & Biobehavioral Reviews* 2017; **75**: 393–406.

6. Bailey T, Alvarez-Jimenez M, Garcia-Sanchez AM, Hulbert C, Barlow E, Bendall S. Childhood Trauma Is Associated With Severity of Hallucinations and Delusions in Psychotic Disorders: A Systematic Review and Meta-Analysis. *Schizophrenia Bulletin* 2018; **44**: 1111–1122.

7. Duhig M, Patterson S, Connell M, Foley S, Capra C, Dark F *et al.* The prevalence and correlates of childhood trauma in patients with early psychosis. *Aust N Z J Psychiatry* 2015; **49**: 651–659.

8. Petkus AJ, Lenze EJ, Butters MA, Twamley EW, Wetherell JL. Childhood Trauma is Associated with Poorer Cognitive Performance in Older Adults. *J Clin Psychiatry* 2018; **79**: 16m11021.

9. Mehta ND, Stevens JS, Li Z, Gillespie CF, Fani N, Michopoulos V *et al.* Inflammation, reward circuitry and symptoms of anhedonia and PTSD in trauma-exposed women. *Soc Cogn Affect Neurosci* 2020; **15**: 1046–1055.

10. Assogna F, Piras F, Spalletta G. Neurobiological Basis of Childhood Trauma and the Risk for Neurological Deficits Later in Life. In: Spalletta G, Janiri D, Piras F, Sani G (eds). *Childhood Trauma in Mental Disorders: A Comprehensive Approach*. Springer International Publishing: Cham, 2020, pp 385–410.

11. Streeck-Fischer A, van der Kolk BA. Down will come baby, cradle and all: diagnostic and therapeutic implications of chronic trauma on child development. *Australian and New Zealand Journal of Psychiatry* 2000; **34**: 903–918.

12. Davidson LM, Baum A. Chronic stress and posttraumatic stress disorders. *Journal of Consulting and Clinical Psychology* 1986; **54**: 303–308.

13. Farag YMK, Gaballa MR. Diabesity: an overview of a rising epidemic. *Nephrology Dialysis Transplantation* 2011; **26**: 28–35.

14. Laramée P, Kusel J, Leonard S, Aubin H-J, François C, Daeppen J-B. The Economic Burden of Alcohol Dependence in Europe. *Alcohol and Alcoholism* 2013; **48**: 259–269.

15. Masodkar K, Johnson J, Peterson MJ. A Review of Posttraumatic Stress Disorder and Obesity: Exploring the Link. *Prim Care Companion CNS Disord* 2016; **18**: 10.4088/PCC.15r01848.

16. Matosin N, Cruceanu C, Binder EB. Preclinical and Clinical Evidence of DNA Methylation Changes in Response to Trauma and Chronic Stress. *Chronic Stress* 2017; **1**: 2470547017710764.

17. Sinha R. Chronic Stress, Drug Use, and Vulnerability to Addiction. *Annals of the New York Academy of Sciences* 2008; **1141**: 105–130.

18. Breslau N. The Epidemiology of Trauma, PTSD, and Other Posttrauma Disorders. *Trauma, Violence, & Abuse* 2009; **10**: 198–210.

19. Knipscheer J, Sleijpen M, Frank L, de Graaf R, Kleber R, ten Have M *et al.*Prevalence of Potentially Traumatic Events, Other Life Events and Subsequent Reactions Indicative for Posttraumatic Stress Disorder in the Netherlands: A General Population Study Based on the Trauma Screening Questionnaire. *IJERPH* 2020; **17**: 1725.

20. McLaughlin KA, Koenen KC, Friedman MJ, Ruscio AM, Karam EG, Shahly V *et al.* Subthreshold Post Traumatic Stress Disorder in the WHO World Mental Health Surveys. *Biol Psychiatry* 2015; **77**: 375–384.

21. Mota N, Tsai J, Kirwin PD, Harpaz-Rotem I, Krystal JH, Southwick SM *et al.* Late-Life Exacerbation of PTSD Symptoms in US Veterans: Results From the National Health and Resilience in Veterans Study. *J Clin Psychiatry* 2016; **77**: 10211.

22. Veeser J, Barkmann C, Schumacher L, Zindler A, Schön G, Barthel D. Post-traumatic stress disorder in refugee minors in an outpatient care center: prevalence and associated factors. *Eur Child Adolesc Psychiatry* 2021. doi:10.1007/s00787-021-01866-8.

23. Varese F, Smeets F, Drukker M, Lieverse R, Lataster T, Viechtbauer W *et al.* Childhood Adversities Increase the Risk of Psychosis: A Meta-analysis of Patient-Control, Prospective- and Cross-sectional Cohort Studies. *Schizophrenia Bulletin* 2012; **38**: 661–671.

24. Porter C, Palmier-Claus J, Branitsky A, Mansell W, Warwick H, Varese F. Childhood adversity and borderline personality disorder: a meta-analysis. *Acta Psychiatrica Scandinavica* 2020; **141**: 6–20.

25. Vasilevski V, Tucker A. Wide-ranging cognitive deficits in adolescents following early life maltreatment. *Neuropsychology* 2016; **30**: 239–246.

26. Lysaker P, Meyer P, Evans J, Marks K. Neurocognitive and Symptom Correlates of Self-Reported Childhood Sexual Abuse in Schizophrenia Spectrum Disorders. *Ann of Clinical Psychiatry* 2001; **13**: 89–92.

27. Shannon C, Douse K, McCusker C, Feeney L, Barrett S, Mulholland C. The Association Between Childhood Trauma and Memory Functioning in Schizophrenia. *Schizophrenia Bulletin* 2011; **37**: 531–537.

28. Majer M, Nater UM, Lin J-MS, Capuron L, Reeves WC. Association of childhood trauma with cognitive function in healthy adults: a pilot study. *BMC Neurol* 2010; **10**: 61.

29. Joëls M, Sarabdjitsingh RA, Karst H. Unraveling the Time Domains of Corticosteroid Hormone Influences on Brain Activity: Rapid, Slow, and Chronic Modes. *Pharmacol Rev* 2012; **64**: 901–938.

30. Schoenfeld TJ, Rhee D, Martin L, Smith JA, Sonti AN, Padmanaban V *et al.* New neurons restore structural and behavioral abnormalities in a rat model of PTSD. *Hippocampus* 2019; **29**: 848–861.

31. Cerqueira JJ, Almeida OFX, Sousa N. The stressed prefrontal cortex. Left? Right! *Brain, Behavior, and Immunity* 2008; **22**: 630–638.

32. Sapolsky RM, Romero LM, Munck AU. How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions. 2000; **21**: 35.

33. Pruessner M, Lepage M, Collins DL, Pruessner JC, Joober R, Malla AK. Reduced hippocampal volume and hypothalamus–pituitary–adrenal axis function in first episode psychosis: Evidence for sex differences. *NeuroImage: Clinical* 2015; **7**: 195–202.

34. Aas M, Pizzagalli DA, Laskemoen JF, Reponen EJ, Ueland T, Melle I *et al.*Elevated hair cortisol is associated with childhood maltreatment and cognitive impairment in schizophrenia and in bipolar disorders. *Schizophrenia research* 2019; **213**: 65–71.

35. Sbarski B, Akirav I. Cannabinoids as therapeutics for PTSD. *Pharmacology & Therapeutics* 2020; **211**: 107551.

36. Ptashne M. On the use of the word 'epigenetic'. *Current Biology* 2007; **17**: R233–R236.

37. Tsankova N, Renthal W, Kumar A, Nestler EJ. Epigenetic regulation in psychiatric disorders. *Nat Rev Neurosci* 2007; **8**: 355–367.

38. Banerjee N, Polushina T, Bettella F, Giddaluru S, Steen VM, Andreassen OA *et al.* Recently evolved human-specific methylated regions are enriched in schizophrenia signals. *BMC Evol Biol* 2018; **18**: 63.

39. Peter CJ, Akbarian S. Balancing histone methylation activities in psychiatric disorders. *Trends in Molecular Medicine* 2011; **17**: 372–379.

40. Pries L-K, Gülöksüz S, Kenis G. DNA Methylation in Schizophrenia. In: Delgado-Morales R (ed). *Neuroepigenomics in Aging and Disease*. Springer International Publishing: Cham, 2017, pp 211–236.

41. Golden SA, Covington HE, Berton O, Russo SJ. A standardized protocol for repeated social defeat stress in mice. *Nat Protoc* 2011; **6**: 1183–1191.

42. Sun H, Damez–Werno DM, Scobie KN, Shao N, Dias C, Rabkin J *et al.* ACF chromatin remodeling complex mediates stress–induced depressive–like behavior. *Nat Med* 2015; **21**: 1146–1153.

43. Vogel-Ciernia A, Matheos DP, Barrett RM, Kramár E, Azzawi S, Chen Y *et al.* The Neuron-specific Chromatin Regulatory Subunit BAF53b is Necessary for Synaptic Plasticity and Memory. *Nat Neurosci* 2013; **16**: 552–561.

44. Mistry M, Gillis J, Pavlidis P. Genome-wide expression profiling of schizophrenia using a large combined cohort. *Mol Psychiatry* 2013; **18**: 215–225.

45. Chaudhury D, Walsh JJ, Friedman AK, Juarez B, Ku SM, Koo JW *et al.* Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. *Nature* 2013; **493**: 532–536.

46. Yoo M, Choi K-Y, Kim J, Kim M, Shim J, Choi J-H *et al.* BAF53b, a Neuron-Specific Nucleosome Remodeling Factor, Is Induced after Learning and Facilitates Long-Term Memory Consolidation. *J Neurosci* 2017; **37**: 3686–3697.

47. Vignali M, Hassan AH, Neely KE, Workman JL. ATP-Dependent Chromatin-Remodeling Complexes. *Mol Cell Biol* 2000; **20**: 1899–1910.

48. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K *et al.* 2-Arachidonoylgylcerol: A Possible Endogenous Cannabinoid Receptor Ligand in Brain. *Biochemical and Biophysical Research Communications* 1995; **215**: 89–97.

49. Augustin SM, Lovinger DM. Functional Relevance of Endocannabinoid-Dependent Synaptic Plasticity in the Central Nervous System. *ACS Chem Neurosci* 2018; **9**: 2146–2161.

50. Hill MN, Hillard CJ, McEwen BS. Alterations in Corticolimbic Dendritic Morphology and Emotional Behavior in Cannabinoid CB1 Receptor–Deficient Mice Parallel the Effects of Chronic Stress. *Cerebral Cortex* 2011; **21**: 2056–2064.

51. Appiah-Kusi E, Wilson R, Colizzi M, Foglia E, Klamerus E, Caldwell A *et al.* Childhood trauma and being at-risk for psychosis are associated with higher peripheral endocannabinoids. *Psychol Med* 2020; **50**: 1862–1871.

52. Koethe D, Pahlisch F, Hellmich M, Rohleder C, Mueller JK, Meyer-Lindenberg A *et al.*Familial abnormalities of endocannabinoid signaling in schizophrenia. *The World Journal of Biological Psychiatry* 2019; **20**: 117–125.

53. Frank MJ, Seeberger LC, O'Reilly RC. By Carrot or by Stick: Cognitive Reinforcement Learning in Parkinsonism. *Science* 2004; **306**: 1940–1943.

54. Jocham G, Klein TA, Ullsperger M. Dopamine-Mediated Reinforcement Learning Signals in the Striatum and Ventromedial Prefrontal Cortex Underlie Value-Based Choices. *J Neurosci* 2011; **31**: 1606–1613.

55. Kasanova Z, Ceccarini J, Frank MJ, van Amelsvoort T, Booij J, Heinzel A *et al.* Daily-life stress differentially impacts ventral striatal dopaminergic modulation of reward processing in first-degree relatives of individuals with psychosis. *European Neuropsychopharmacology* 2018; **28**: 1314–1324.

56. Brown VM, Zhu L, Wang JM, Frueh BC, King-Casas B, Chiu PH. Associability-modulated loss learning is increased in posttraumatic stress disorder. *eLife* 2018; **7**: e30150.

57. Myers CE, Moustafa AA, Sheynin J, VanMeenen KM, Gilbertson MW, Orr SP *et al.* Learning to Obtain Reward, but Not Avoid Punishment, Is Affected by Presence of PTSD Symptoms in Male Veterans: Empirical Data and Computational Model. *PLOS ONE* 2013; **8**: e72508.

58. Frydecka D, Piotrowski P, Bielawski T, Pawlak E, Kłosińska E, Krefft M *et al.*Confirmation Bias in the Course of Instructed Reinforcement Learning in Schizophrenia-Spectrum Disorders. *Brain Sciences* 2022; **12**: 90.

59. Frydecka D, Misiak B, Piotrowski P, Bielawski T, Pawlak E, Kłosińska E *et al.* The Role of Dopaminergic Genes in Probabilistic Reinforcement Learning in Schizophrenia Spectrum Disorders. *Brain Sciences* 2022; **12**: 7.

60. George SA, Rodriguez-Santiago M, Riley J, Abelson JL, Floresco SB, Liberzon I. Alterations in cognitive flexibility in a rat model of post-traumatic stress disorder. *Behav Brain Res* 2015; **286**: 256–264.

61. Amitai N, Young JW, Higa K, Sharp RF, Geyer MA, Powell SB. Isolation rearing effects on probabilistic learning and cognitive flexibility in rats. *Cogn Affect BehavNeurosci* 2014; **14**: 388–406.

62. File SE, Seth P. A review of 25 years of the social interaction test. *European Journal of Pharmacology* 2003; **463**: 35–53.

63. Frank MJ, Claus ED. Anatomy of a decision: Striato-orbitofrontal interactions in reinforcement learning, decision making, and reversal. *Psychological Review* 2006; **113**: 300–326.

64. Kane GA, Bornstein AM, Shenhav A, Wilson RC, Daw ND, Cohen JD. Rats exhibit similar biases in foraging and intertemporal choice tasks. *eLife* 2019; **8**: e48429.

Załączniki

1. Informacja o źródłach finansowania badań:

Grant UMW Młodzi Naukowcy, STM.c230.18.037

2. Oświadczenia współautorów prac:

LSU Health
NEW ORLEANS

School of Medicine
Department of Cell Biology and Anatomy

May 6th, 2022

To whom it may concern

I hereby confirm that I provided feedback on the manuscript titled "Endocannabinoid system in trauma and psychosis: Distant guardian of mental stability" (doi:10.1515/revneuro-2020-0102) authored by Bielawski T, Albrechet-Souza L and Frydecka D, and published in the Reviews in the Neurosciences 2021. In addition, I helped to shape the final form of the manuscript and was involved in formulating responses for the reviewers' comments.

Sincerely,

Lucas Albrechet-Souza, Ph.D.

Dept. of Cell Biology & Anatomy
LSUHSC School of Medicine
1901 Perdido St., Rm 6144
New Orleans, LA 70112
(504) 568-5643

Wrocław, 17.05.2022

dr inż. Jarosław Drapała
Katedra Informatyki i Inżynierii Systemów
Wydział Informatyki i Telekomunikacji
Politechnika Wrocławska

OŚWIADCZENIE

Oświadczam, że w pracy: *Bielawski T., Drapała J., Krowicki P., Stańczykiewicz B., Frydecka D., (2022) "Trauma disrupts reinforcement learning in rats - a novel animal model of chronic stress exposure.", Frontiers In Behavioral Neuroscience* mój udział polegał na matematycznej analizie zebranych danych, statystycznym opracowaniu uzyskanych wyników, współtworzeniu rycin prezentujących wyniki badań oraz na przygotowaniu korekt manuskryptu pracy.

Podpis

Drapała Jarosław

Wrocław, 27.04.2022

Prof. dr hab. n. med. Dorota Frydecka
Katedra Psychiatrii
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy: *Bielawski, T., Misiak, B., Moustafa, A., & Frydecka, D. (2019). Epigenetic mechanisms, trauma, and psychopathology: targeting chromatin remodeling complexes. Reviews in the Neurosciences, 30(6), 595-604.*, brałam udział w kwerendzie literatury tematu oraz w przygotowaniu korekt gotowego manuskryptu.

Podpis

Wrocław, 17.05.2022

Prof. dr hab. n. med. Dorota Frydecka
Katedra Psychiatrii
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy: *Bielawski T, Albrechet-Souza L, Frydecka D. Endocannabinoid system in trauma and psychosis: distant guardian of mental stability. Reviews in the Neurosciences 2021. doi:10.1515/revneuro-2020-0102,* mój udział polegał na przygotowaniu korekt manuskryptu oraz wprowadzeniu zmian do manuskryptu na podstawie otrzymanych recenzji z czasopisma.

Podpis

Wrocław, 28.04.2022

Prof. dr hab. n. med. Dorota Frydecka
Katedra Psychiatrii
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy: *Bielawski T., Drapała J., Krowicki P., Stańczykiewicz B., Frydecka D., (2022) "Trauma disrupts reinforcement learning in rats - a novel animal model of chronic stress exposure.", Frontiers In Behavioral Neuroscience* mój udział polegał na: opiece merytorycznej w trakcie przygotowywania wniosku o grant który umożliwił realizację badania, opiece merytorycznej w trakcie opracowywania procedur doświadczeń laboratoryjnych z użyciem modelu zwierzęcego wykorzystanego w pracy, przygotowaniu korekt manuskryptu.

Podpis

Wrocław, 17.05.2022

prof. dr hab. Błażej Misiak
Zakład Psychiatrii Konsultacyjnej i Badań Neurobiologicznych
Katedra Psychiatrii
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy: *Bielawski, T., Misiak, B., Moustafa, A., & Frydecka, D. (2019). Epigenetic mechanisms, trauma, and psychopathology: targeting chromatin remodeling complexes. Reviews in the Neurosciences, 30(6), 595-604.*, mój udział polegał na korekcie manuskryptu pod względem merytorycznym oraz językowym, oraz na wprowadzaniu zmian do manuskryptu na podstawie otrzymanych recenzji z czasopisma.

Podpis

Wrocław, 27.04.2022

dr n. med. Bartłomiej Stańczykiewicz prof. UMW
Zakład Psychiatrii Konsultacyjnej i Badań Neurobiologicznych
Katedra Psychiatrii
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy: *Bielawski T., Drapała J., Krowicki P., Stańczykiewicz B., Frydecka D., (2022) "Trauma disrupts reinforcement learning in rats - a novel animal model of chronic stress exposure", Frontiers In Behavioral Neuroscience,* brałem udział w opiece merytorycznej dotyczącej planowania i przeprowadzenia procedur doświadczeń laboratoryjnych z użyciem modelu zwierzęcego, korekcie manuskryptu oraz wprowadzeniu zmian do manuskryptu na podstawie otrzymanych recenzji z czasopisma.

Uniwersytet Medyczny we Wrocławiu
Katedra Psychiatrii
ZAKŁAD PSYCHIATRII KONSULTACYJNEJ
I BADAŃ NEUROBIOLOGICZNYCH
Bartłomiej Stańczykiewicz, profesor uczelni

Wrocław, 27.04.2022

Tomasz Bielawski
doktorant Katedry Psychiatrii
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam że w pracy: *Bielawski T., Drapała J., Krowicki P., Stańczykiewicz B., Frydecka D., (2022) "Trauma disruptsreinforcement learning in rats - a novelanimal model of chronicstress exposure", Frontiers In BehavioralNeuroscience,* byłem odpowiedzialny za opracowanie protokołu do badań, współtworzenie projektu urządzenia do badań funkcji poznawczych, przeprowadzenie badań laboratoryjnych, napisanie manuskryptu wraz z przedstawieniem graficznym części wyników, przygotowanie rycin zawartych w manuskrypcie oraz przygotowanie odpowiedzi na uwagi recenzentów.

Wrocław, 27.04.2022

Tomasz Bielawski
doktorant Katedry Psychiatrii
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

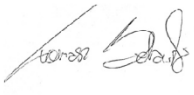
Oświadczam, że w pracy: *Bielawski T., Misiak B., Moustafa A., Frydecka D.: Epigenetic mechanisms, trauma, and psychopathology: targeting chromatin remodeling complexes. Reviews in the Neurosciences 2019; 30: 595–604* byłem odpowiedzialny za kwerendę literatury, napisanie manuskryptu pracy, przygotowanie rycin i tabel oraz przygotowanie odpowiedzi na uwagi recenzentów.

Wrocław, 27.04.2022

Tomasz Bielawski
doktorant Katedry Psychiatrii
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy: *Bielawski T., Albrechet-Souza L., Frydecka D.: Endocannabinoid system in trauma and psychosis: distant guardian of mental stability. Reviews in the Neurosciences 2021. 32(7)707-722*, byłem odpowiedzialny za kwerendę literatury, napisanie manuskryptu pracy, przygotowanie rycin i tabel oraz przygotowanie odpowiedzi na uwagi recenzentów.



Wrocław, 17.05.2022

dr inż. Paweł Krowicki
Katedra Technologii Laserowych, Automatyzacji i Organizacji Produkcji
Wydział Mechaniczny
Politechnika Wrocławska

OŚWIADCZENIE

Oświadczam, że w pracy *Bielawski T., Drapała J., Krowicki P., Stańczykiewicz B., Frydecka D., (2022) "Trauma disrupts reinforcement learning in rats - a novel animal model of chronic stress exposure.", Frontiers In Behavioral Neuroscience* mój udział polegał na współtworzeniu projektu maszyny wykorzystanej w trakcie badań laboratoryjnych, zbudowaniu dwóch maszyn które umożliwiły badanie zdolności poznawczych w zwierzęcym modelu traumy, przeprowadzeniu testów maszyn oraz wprowadzaniu poprawek oprogramowania służącego do badania i gromadzenia danych oraz w przygotowaniu korekt manuskryptu pracy.

Podpis
Paweł Krowicki

17.05.2022

To whom it may concern

I hereby confirm that I provided feedback on the manuscript titled: *Epigenetic mechanisms, trauma, and psychopathology: targeting chromatin remodeling complexes.*. authored by *Bielawski, T., Misiak, B., Moustafa, A., & Frydecka, D.* and published in the *Reviews in the Neurosciences, 30(6), 595-604 (2019).* In addition I helped to shape the final form of the manuscript and delivered language corrections.

Sincerely,

Ahmed Moustafa

prof. Ahmed Moustafa

School of Psychology,
Faculty of Society and Design,
Bond University, Queensland,
Gold Coast, Australia

3. Nota biograficzna

Tomasz Bielawski urodził się 22 lutego 1990 r. we Wrocławiu. Jest absolwentem Uniwersytetu Wrocławskiego, w 2012 otrzymał licencjat z mikrobiologii na Katedrze Mikrobiologii i Genetyki UWr, a w 2017 tytuł magistra Nauk Biologicznych. W 2018 otrzymał dyplom magistra psychologii Uniwersytetu Wrocławskiego. Od 2017 jest doktorantem w Katedrze i Klinice Psychiatrii Uniwersytetu Medycznego we Wrocławiu. W czasie studiów doktoranckich odbywał szkolenia między innymi na Uniwersytecie Sophia Antipolis w Nicei, odbył także staż w Szkole Medycznej Uniwersytetu Stanowego w Luizjanie. Tomasz Bielawski jest psychoterapeutą psychodynamicznym certyfikującym się w Krakowskiej Szkole Systemowej, prowadzonej przez prof. Bogdana DeBarbaro, oraz członkiem Polskiego Towarzystwa Psychiatrycznego. Jest autorem i współautorem publikacji o sumarycznym wskaźniku cytowań 33,68 Impact Factor oraz twórcą urządzenia służącego do badania funkcji poznawczych, zgłoszonego jako Wynalazek do Urzędu Patentowego Rzeczypospolitej Polskiej.

4. Wykaz Publikacji Autora

1. Misiak B, Krefft M, Bielawski T, Moustafa AA, Sąsiadek MM, Frydecka D. Toward a unified theory of childhood trauma and psychosis: A comprehensive review of epidemiological, clinical, neuropsychological and biological findings. Neuroscience & Biobehavioral Reviews 2017; 75: 393–406.
2. Żelaźniewicz A, Bielawski T, Nowak J, Pawłowski B. Body symmetry and reproductive hormone levels in women. Women & Health 2019; 59: 391–405.
3. Bielawski T, Misiak B, Moustafa A, Frydecka D. Epigenetic mechanisms, trauma, and psychopathology: targeting chromatin remodeling complexes. Reviews in the Neurosciences 2019; 30: 595–604.
4. Bielawski T, Albrechet-Souza L, Frydecka D. Endocannabinoid system in trauma and psychosis: distant guardian of mental stability. Reviews in the Neurosciences 2021; 32: 707–722.
5. Frydecka D, Piotrowski P, Bielawski T, Pawlak E, Kłosińska E, Krefft M et al. Confirmation Bias in the Course of Instructed Reinforcement Learning in Schizophrenia-Spectrum Disorders. Brain Sciences 2022; 12: 90.
6. Frydecka D, Misiak B, Piotrowski P, Bielawski T, Pawlak E, Kłosińska E et al. The Role of Dopaminergic Genes in Probabilistic Reinforcement Learning in Schizophrenia Spectrum Disorders. Brain Sciences 2022; 12: 7.
7. Kowalski K, Bogudzińska B, Stańczykiewicz B, Piotrowski P, Bielawski T, Samochowiec J et al. The Deficit Schizophrenia Subtype Is Associated with Low Adherence to the Mediterranean Diet: Findings from a Case–Control Study. Journal of Clinical Medicine 2022; 11: 568.
8. Bielawski T, Drapała J, Krowicki P, Stańczykiewicz B, Frydecka D. Trauma Disrupts Reinforcement Learning in Rats—A Novel Animal Model of Chronic Stress Exposure. Frontiers in Behavioral Neuroscience 2022; 16

Sumaryczny Impact Factor: 33,68
Sumarycznaliczba pkt. MNiSW/KBN: 850

5. Zgoda komisji etycznej

UCHWAŁA NR 14/2019

z dnia 20.03.2019

Lokalnej Komisji Etycznej do spraw doświadczeń na zwierzętach we Wrocławiu

§ 1

Na podstawie art. 48 ust. 1 pkt. 1 / art. 48 ust. 1 pkt. 2[1] ustawy z dnia 15 stycznia 2015r. o ochronie zwierząt wykorzystywanych do celów naukowych lub edukacyjnych (Dz. U. poz. 266), zwanej dalej „ustawą" po rozpatrzeniu wniosku pt.: "Chroniczny stres a funkcjonowanie poznawcze jednostki w zwierzęcym modelu traumy " z dnia 11.03.2019, złożonego przez Wydział Lekarski Uniwersytetu Medycznego We Wrocławiu adres ul. Mikulicza-Radeckiego 5, 50-345 Wrocław,[2] zaplanowanego przez dr Bartłomieja Stańczykiewicza[3]

Lokalna Komisja Etyczna:

WYRAŻA ZGODĘ[4]

Na przeprowadzenie doświadczeń na zwierzętach w zakresie wniosku.

§ 2

W wyniku rozpatrzenia wniosku o którym mowa w § , Lokalna Komisja Etyczna ustaliła, że:

1. Wniosek należy przypisać do kategorii: Badania podstawowe (Etologia lub zachowanie zwierząt lub biologia zwierząt)
2. Najwyższy stopień dotkliwości proponowanych procedur to: umiarkowany
3. Doświadczenia będą przeprowadzane na gatunkach lub grupach gatunków[5]: 40, mysz domowa C57BL/6J, 7/8 tygodni
4. Doświadczenia będą przeprowadzane przez: dr Stańczykiewicz Bartłomiej, mgr Bielawski Tomasz, mgr Klyta Magdalena, inż Czyszczoń Kamila
5. Doświadczenie będzie przeprowadzane w terminie[6] od 01.04.2019 do 31.12.2020
6. Doświadczenie będzie przeprowadzone w ośrodku[7]: nie dotyczy
7. Doświadczenie będzie przeprowadzone poza ośrodkiem w : nie dotyczy

---

[1] Niewłaściwy zapis usunąć

[2]imię i nazwisko oraz adres i miejsce zamieszkania albo nazwę oraz adres i siedzibę użytkownika, który przeprowadzi to doświadczenie, z tym że w przypadku gdy użytkownikiem jest osoba fizyczna wykonująca działalność gospodarczą, zamiast adresu i miejsca zamieszkania tej osoby – adres i miejsce wykonywania działalności, jeżeli są inne niż adres i miejsce zamieszkania tej osoby;

[3]imię i nazwisko osoby, która zaplanowała i jest odpowiedzialna za przeprowadzenie doświadczenia

[4] Niewłaściwy zapis usunąć

[5] Podać liczbę, szczep/stado, wiek/stadium rozwoju

[6] Nie dłużej niż 5 lat

[7] Podać jeśli jest to inny ośrodek niż użytkownik

8. Użyte do procedur zwierzęta dzikie zostaną odłowione przez ....., w sposób: nie dotyczy
9. Doświadczenie zostanie[8] poddane ocenie retrospektywnej w terminie do 3 miesięcy od dnia przekazania przez użytkownika dokumentacji, mającej stanowić podstawę dokonania oceny retrospektywnej. Użytkownik jest zobowiązany do przekazania ww. dokumentacji niezwłocznie, tj. w terminie, o którym mowa w art. 52 ust. 2 ustawy.

§ 3

Uzasadnienie:

Osoba do kontaktu Pan Tomasz Bielawski wezwany na posiedzenie wyjaśnił, że:

- zdolności poznawcze zwierząt to szeroki obszar badań. Badanie ma na celu poznanie zmian w zachowaniu przed i po wystąpieniu traumy u zwierząt. Zapach moczu kota wykorzystany w badaniu jest wystarczająco dużym czynnikiem stresogennym (zapach drapieżnika).
- Wyniki badania mają posłużyć rozwojowi nauki i odejściu od bardziej drastycznych metod wywoływania stresu u zwierząt jak np. basen Morrisa (labirynt wodny Morrisa).
- Nowe urządzenie oraz aplikacja mają służyć uczeniu się a nie zapamiętywaniu procedury. W tym celu będzie stosowana zamiana symboli.

Zalecenia Komisji:

Projekt wyższego ryzyka związany z opracowaniem lub wytworzeniem nowego urządzenia w związku z czym zostanie poddany ocenie retrospektywnej.

Wniosek został przyjęty przez Komisję (6 osób głosowało za, 1 osoba wstrzymała się, 1 osoba przeciw) oraz wyznaczony do oceny retrospektywnej (7 osób głosowało za wyznaczeniem wniosku, 1 osoba wstrzymała się).

§ 4

Integralną część niniejszej uchwały stanowi kopia wniosku, o którym mowa w § 1.

LOKALNA KOMISJA ETYCZNA
DS. DOŚWIADCZEŃ NA ZWIERZĘTACH
WE WROCŁAWIU
Instytut Immunologii i Terapii Doświadczalnej PAN
53-114 Wrocław, ul Rudolfa Weigla 12
tel (71) 337 11 72 wew. 181, (71) 370 99 30 wew 181

(Pieczęć lokalnej komisji etycznej)

dr hab. Joanna Wietrzyk
Podpis przewodniczącego komisji

Przewodnicząca
Lokalnej Komisji Etycznej
ds. Doświadczeń na Zwierzętach
we Wrocławiu

Pouczenie:

Zgodnie z art. 33 ust. 3 i art. 40 ustawy w zw. z art. 127 § 1 i 2 oraz 129 § 2 ustawy z dnia z dnia 14 czerwca 1960 r. Kodeks postępowania administracyjnego (Dz. U. 2017, poz. 1257 – t.j.; dalej KPA) od

[8] Niewłaściwy zapis usunąć

uchwały Lokalnej Komisji Etycznej strona może wnieść, za jej pośrednictwem, odwołanie do Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w terminie 14 od dnia doręczenia uchwały.

Na podstawie art. 127a KPA w trakcie biegu terminu do wniesienia odwołania strona może zrzec się prawa do jego wniesienia, co należy uczynić wobec Lokalnej Komisji Etycznej, która wydała uchwałę. Z dniem doręczenia Lokalnej Komisji Etycznej oświadczenia o zrzeczeniu się prawa do wniesienia odwołania przez ostatnią ze stron postępowania, decyzja staje się ostateczna i prawomocna.

Otrzymuje:

1) Użytkownik,
2) Organizacja społeczna dopuszczona do udziału w postępowaniu (jeśli dotyczy)
3) a/a

Użytkownik kopie przekazuje:

- Osoba planująca doświadczenie
- Zespół ds. dobrostanu

UCHWAŁA NR 006/2020

z dnia 15.01.2020

Lokalnej Komisji Etycznej do spraw doświadczeń na zwierzętach we Wrocławiu

§ 1

Lokalna komisja etyczna po rozpatrzeniu wniosku o wydanie zgody na wykorzystanie dodatkowych zwierząt w procedurach pt.: „Chroniczny stres a funkcjonowanie poznawcze jednostki w zwierzęcym modelu traumy." z dnia 07.01.2020, złożonego Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu, Wydział Lekarski, adres ul. J. Mikulicza Radeckiego 5, 50-345 Wrocław.,[1] zaplanowanego przez dr n. med. Bartłomieja Stańczykiewicza[2]a dotyczącego:

dodatkowej liczby/gatunków zwierząt

w ramach wydanej przez komisję zgody uchwałą nr 014/2019 w dn. 11.03.2019

WYRAŻA ZGODĘ[3]

na dokonanie zmian w zakresie określonym poniżej.

§ 2

1. Najwyższy stopień dotkliwości proponowanych procedur po zatwierdzonych zmianach to: umiarkowany (wprowadzone zmiany nie wpłynęły na zmianę kategorii dotkliwości)
2. Zespół prowadzący doświadczenia rozszerza się o następujące osoby (nazwisko i imię, nazwa użytkownika): nie dotyczy
3. Doświadczenie będzie przeprowadzane w terminie[4] nie dotyczy
4. W planowanych doświadczenia użytych zostanie ~~dodatkowo~~: **zamiennie** 30 samców szczura wędrownego (rattus norvegicus) szczepu Wistar Rats

---

[1]imię i nazwisko oraz adres i miejsce zamieszkania albo nazwę oraz adres i siedzibę użytkownika, który przeprowadzi to doświadczenie, z tym że w przypadku gdy użytkownikiem jest osoba fizyczna wykonująca działalność gospodarczą, zamiast adresu i miejsca zamieszkania tej osoby – adres i miejsce wykonywania działalności, jeżeli są inne niż adres i miejsce zamieszkania tej osoby;

[2]imię i nazwisko osoby, która zaplanowała i jest odpowiedzialna za przeprowadzenie doświadczenia

[3] Niewłaściwy zapis usunąć

[4] Nie dłużej niż 5 lat

§ 3

Uzasadnienie:

Zaproszony pan Tomasz Bielawski wyjaśnił, iż na podstawie zdobytych kwalifikacji i wiedzy, oraz posiadanych dzisiaj kompetencji (których nie posiadał w dniu składania wniosku 014/2019) do przeprowadzenia badań zaplanowanych we wniosku, na który LKE wydała zgodę nr 014/2019 w dniu 11.03.2019 postanowił zmienić model badawczy z myszy na szczury, dzięki czemu również zmniejszy liczbę wykorzystanych zwierząt z 40 do 30. Pan Bielawski wyjaśnił, iż badania przeprowadzone na szczurach pozwolą na szybsze przeprowadzenie badań, w krótszym czasie uzyskać te same, lub nawet bardziej wartościowe wyniki wykorzystując mniejszą liczbę zwierząt. W trakcie przeprowadzonych konsultacji statystycznych ustalono, iż grupa kontrolna może składać się z 10 osobników, nie ma konieczności wykorzystywania 20 szczurów w tej grupie. Natomiast w grupie badanej zaplanowano zużycie 20 zwierząt, chociaż wystarczyłoby 15 osobników. Ale 5 zwierząt zaplanowano na wypadek, gdyby któreś ze szczurów nie chciały się uczyć i zaistniałaby potrzeba uzupełnienia grupy o osobniki uczące się chętniej.

Po zakończonych badaniach szczury zostaną oddane do adopcji.

Pan Tomasz Bielawski zadeklarował, że od zaplanowanej daty rozpoczęcia badań w kwietniu 2019 roku nie wykorzystano żadnych myszy i nie zostaną wykorzystane żadne z 40 myszy z uchwały 014/2019.

Wniosek uzyskał zgodę na realizację (8 osób głosowało za udzieleniem zgody, 1 osoba głosowała przeciw).

§ 4

Integralną część niniejszej uchwały stanowi kopia wniosku, o którym mowa w § 1

(Pieczęć lokalnej komisji etycznej)

LOKALNA KOMISJA ETYCZNA
DS. DOŚWIADCZEŃ NA ZWIERZĘTACH
WE WROCŁAWIU
Instytut Immunologii i Terapii Doświadczalnej PAN
53-114 Wrocław, ul Rudolfa Weigla 12
tel (71) 337 11 72 wew. 181, (71) 370 99 30 wew 181

Otrzymuje Użytkownik

Podpisy przewodniczącegokomisji

dr hab. Joanna Wietrzyk

Przewodnicząca
Lokalnej Komisji Etycznej
ds. Doświadczeń na Zwierzętach
we Wrocławiu

Pouczenie:

Od decyzji komisji można wnieść odwołanie do Krajowej Komisji Etycznej w terminie 14 od dnia otrzymania uchwały.

Użytkownik kopie przekazuje:

- Osoba planująca doświadczenie
- Zespół ds. dobrostanu