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Badanie roli tendencyjności poznawczej w indywidualnej podatności na przejście od kontrolowanego używania do niekontrolowanego nadużywania alkoholu w modelu zwierzęcym

Investigating the role of cognitive bias in individual susceptibility to transition from controlled use to uncontrolled alcohol abuse in an animal model

Praca Doktorska wykonana w Pracowni Neurobiologii Emocji i Procesów Poznawczych

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„Pomiędzy bodźcem i reakcją jest przestrzeń – w tej przestrzeni leży wolność i moc wyboru naszej odpowiedzi.” – Viktor E. Frankl

Spis treści

Spis artykułów naukowych wchodzących w skład pracy doktorskiej

1. Cieslik-Starkiewicz A, Noworyta K, Solich J, Korlatowicz A, Faron-Górecka A, Rygula R. (2024) Trait sensitivity to positive feedback is a predisposing factor for several aspects of compulsive alcohol drinking in male rats: behavioural, physiological, and molecular correlates. Psychopharmacology. 241, 33-47. doi: 10.1007/s00213-023-06460-1.

   IF_{2023} = 3.5

   MEiN = 140

2. Cieslik A, Noworyta K, Rygula R. (2022) Trait sensitivity to negative feedback determines the intensity of compulsive alcohol seeking and taking in male rats. Journal of Psychiatry and Neuroscience. 47, E252-E262. doi: 10.1503/jpn.210220.

   IF_{2022} = 4.3

   MEiN = 140

3. Cieslik-Starkiewicz A, Noworyta K, Solich J, Korlatowicz A, Faron-Górecka A, Rygula R. (2024) Identification of genes regulated by trait sensitivity to negative feedback and prolonged alcohol consumption in rats. Pharmacological Reports. 76, 207-215. doi: 10.1007/s43440-023-00563-4.

   IF_{2023} = 3.6

   MEiN = 100

4. Cieslik-Starkiewicz A, Piksa M, Noworyta K, Solich J, Pabian P, Latocha K, Faron-Górecka A, Rygula R. (2024) Unveiling the power of optimism: exploring behavioral and neuromolecular correlates of alcohol seeking and drinking in rats with biased judgment. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 135, 111124. doi: 10.1016/j.pnpbp.2024.111124. Zaakceptowano, w druku.

   IF_{2023} = 5.3

   MEiN = 100

Wykaz najważniejszych skrótów

ACC - kora zakrętu obręczy (ang. A*nterior Cingulate Cortex*)

ACI – test interpretacji bodźca niejednoznacznego (ang. *Ambiguous-Cue Interpretation*)

ACTH - hormon adrenokortykotropowy (ang. *adrenocorticotropic hormone)*

ADH1 – dehydrogenaza alkoholowa 1 (ang. *alcohol dehydrogenase 1*)

AUD – zaburzenie używania alkoholu (ang. *Alcohol Use Disorder*)

Amy - ciało migdałowate (ang. A*mygdala*)

DA – dopamina (ang. *dopamine*)

GABA – kwas γ-aminomasłowy (ang. *γ-aminobutyric acid*)

L-DOPA – 3,4-dihydroksy-L-fenyloalanina (ang. *L-3,4-dihydroxyphenylalanine*)

MAO-A – monoaminooksydaza A (ang. *monoaminoxidase A*)

MAO-B – monoaminooksydaza B (ang. *monoaminoxidase B*)

mPFC - środkowa kora przedczołowa (ang. *Medial Prefrontal Cortex*)

NAc – jądro półleżące (ang. *Nucleus Accumbens*)

OFC – kora oczodołowo-czołowa (ang. *Orbitofrontal Cortex*)

PFC – kora przedczołowa (ang. *Prefrontal Cortex*)

PRL – test probabilistycznego przeuczania (ang. *Probabilistic Reversal Learning*)

SERT – transporter serotoniny (ang. *serotonin transporter*)

SSRI – selektywny inhibitor wychwytu zwrotnego serotoniny (ang. *selective serotonin reuptake inhibitor*)

Trp – tryptofan (ang. *tryptophan)*

2BC – test wyboru między dwiema butelkami z dostępem przerywanym (ang. *intermittent access two-bottle choice paradigm*)

5-HT – serotonina (5-hydroksytryptamina, ang. *5-hydroxytryptamine*)

Streszczenie

Zaburzenie używania alkoholu (AUD) jest powszechnym zaburzeniem psychicznym, charakteryzującym się stopniowym nasilaniem konsumpcji alkoholu oraz cyklami remisji i nawrotów. Chociaż wiele badań koncentruje się na etiologii AUD, nadal brakuje pełnego zrozumienia czynników poznawczych, które mogą predysponować do rozwoju tego zaburzenia. W ostatnich dekadach coraz więcej uwagi poświęca się roli tendencyjności poznawczej jako istotnemu czynnikowi wpływającemu na podatność na różne zaburzenia psychiczne, takie jak depresja i lęk, będących często jednym z powodów sięgania po alkohol. W przypadku AUD wykazano, że tendencyjność poznawcza wpływa na sposób, w jaki osoby uzależnione od alkoholu postrzegają i interpretują bodźce związane z alkoholem. Badanie związku przyczynowo-skutkowego między tendencyjnością poznawczą a AUD jest jednak utrudnione, zwłaszcza przed wystąpieniem uzależnienia.

Aby lepiej zrozumieć tę zależność, w mojej pracy zastosowałam model zwierzęcy, który pozwala na ocenę tendencyjności poznawczej u szczurów przed długotrwałą ekspozycją na alkohol. Celem badań było określenie roli różnych aspektów tendencyjności poznawczej, takich jak wrażliwość na pozytywne i negatywne informacje zwrotne oraz tendencyjność oceny, definiowana jako optymizm i pesymizm, w indywidualnej podatności na przejście od kontrolowanego używania do niekontrolowanego nadużywania alkoholu.

W pierwszych badaniach sprawdziłam, czy wrażliwość na pozytywne i negatywne informacje zwrotne, mierzone jako stabilne cechy poznawcze, wpływają na nabywanie i utrzymywanie zachowań związanych z poszukiwaniem i spożywaniem alkoholu u szczurów Wrażliwość na pozytywne i negatywne informacje zwrotne oceniłam za pomocą serii testów probabilistycznego przeuczania. Eskalację konsumpcji alkoholu u szczurów osiągnęłam poprzez zastosowanie przerywanego swobodnego dostępu do alkoholu. Następnie zbadałam wpływ wrażliwości na informacje zwrotne na rozwój kompulsywnego poszukiwania alkoholu w sytuacji, gdy było ono karane. Mierzyłam także motywację do poszukiwania oraz szybkość wygaszania reakcji instrumentalnej i przywracania zachowań poszukiwawczych alkoholu po okresie abstynencji. Dodatkowo, wraz ze współpracownikami z Pracowni Farmakologii Biochemicznej IF PAN zmierzyliśmy poziom mRNA w wybranych regionach mózgu oraz poziom

kortykosteronu i hormonu adrenokortykotropowego (ACTH) we krwi, aby powiązać wyniki behawioralne z mechanizmami biologicznymi.

Wykazałam, że niższa wrażliwość na pozytywne informacje zwrotne u szczurów była związana z większą motywacją do poszukiwania alkoholu po negatywnych doświadczeniach z nim związanych. Szczury niewrażliwe na pozytywne informacje zwrotne były bardziej skłonne do ponownego poszukiwania alkoholu po okresie abstynencji i miały wyższy poziom hormonów stresu we krwi, w porównaniu do zwierząt wrażliwych na ten typ informacji. Z kolei wyższa wrażliwość na negatywne informacje zwrotne związana była z mniejszą podatnością na rozwój kompulsywnego poszukiwania alkoholu i tendencją do szybszego wygaszania zachowań poszukiwawczych, gdy alkohol przestał być dostępny, w porównaniu do zwierząt niewrażliwych. Efekty behawioralne były powiązane ze zmianami w ekspresji genów związanych z funkcjonowaniem różnych układów neurotransmisyjnymi mózgu i metabolizmem etanolu.

Następnie przeanalizowałam, jak optymizm i pesymizm mierzone jako stabilne i trwałe cechy poznawcze, wpływają na zachowania związane ze spożywaniem alkoholu u szczurów. Zwierzęta poddałam testom interpretacji bodźca niejednoznacznego, aby ocenić ich skłonności do optymizmu lub pesymizmu. Stosując paradygmat swobodnego dostępu i paradygmaty instrumentalne, zbadałam u zwierząt zachowania związane z piciem i poszukiwaniem alkoholu. Wraz ze współpracownikami z Pracowni Farmakologii Biochemicznej IF PAN przeprowadziliśmy także analizę ekspresji genów w wybranych strukturach mózgu oraz określiliśmy gęstość receptorów 5-HT_{1A}, 5-HT_{2A} i D_2 za pomocą analizy autoradiograficznej. Na podstawie przeprowadzonych eksperymentów, wykazaliśmy, że w warunkach swobodnego dostępu szczury „pesymistyczne" spożywały więcej alkoholu niż „optymistyczne", co wiązało się ze zmianami w ekspresji genów i gęstości receptorów 5-HT_{2A} w jądrze półleżącym.

Uzyskane wyniki sugerują, że różne aspekty tendencyjności poznawczej, takie jak wrażliwość na pozytywne i negatywne informacje zwrotne oraz skłonność do optymizmu lub pesymizmu, mają istotny wpływ na indywidualną podatność na przejście od kontrolowanego do niekontrolowanego nadużywania alkoholu. Te odkrycia mogą pomóc w lepszym zrozumieniu mechanizmów prowadzących do AUD i przyczynić się do opracowania nowych strategii terapeutycznych.

Abstract

Alcohol Use Disorder (AUD) is a common mental disorder characterized by the gradual escalation of alcohol consumption and cycles of remission and relapse. Although much research focuses on the etiology of AUD, a comprehensive understanding of the cognitive factors that may predispose individuals to develop this disorder is still lacking. In recent decades, increasing attention has been given to the role of cognitive bias as a significant factor influencing vulnerability to various mental disorders, such as depression and anxiety, which are often among the reasons for turning to alcohol. In the case of AUD, cognitive bias has been shown to affect how individuals with alcohol dependence perceive and interpret alcohol-related cues. However, studying the cause-and-effect relationship between cognitive bias and AUD is challenging, particularly before the onset of addiction.

To better understand this relationship, I utilized an animal model, which allows for the assessment of cognitive bias in rats before long-term alcohol exposure. The study aimed to determine the role of various aspects of biased cognition, such as sensitivity to positive and negative feedback, as well as judgement bias, defined as optimism and pessimism, in individual susceptibility to transitioning from controlled alcohol use to uncontrolled alcohol abuse.

In the initial studies, I investigated whether sensitivity to positive and negative feedback, measured as stable cognitive traits, affects the acquisition and maintenance of alcohol-seeking and consumption behaviors in rats. Sensitivity to positive and negative feedback was assessed using a series of probabilistic reversal learning tests. I induced alcohol consumption escalation in rats through intermittent free access to alcohol. I then examined the interaction of feedback sensitivity and propensity to the development of compulsive alcohol seeking when it was punished. Additionally, I measured motivation to seek alcohol, the rate of extinction of instrumental responses, and the reinstatement of alcohol-seeking behaviors after a period of abstinence. Furthermore, together with colleagues from the Laboratory of Biochemical Pharmacology at the Institute of Pharmacology PAS, we measured mRNA levels in selected brain regions and levels of corticosterone and adrenocorticotropic hormone in the blood to correlate behavioral results with biological mechanisms.

I showed that lower sensitivity to positive feedback in rats was associated with an increased motivation to seek alcohol after negative experiences related to it. Rats insensitive to positive feedback were more likely to reinstate alcohol-seeking after a period of abstinence and had higher levels of stress hormones in their blood compared to rats sensitive to positive feedback. Conversely, higher sensitivity to negative feedback reduced susceptibility to compulsive alcohol-seeking and accelerated the extinction of such behaviors when alcohol was no longer available. These behavioral effects were associated with changes in gene expression related to various neurotransmitter systems in the brain and ethanol metabolism.

I then analyzed how optimism and pessimism, measured as stable and enduring behavioral traits, interact with alcohol-related behaviors in rats. The animals underwent ambiguous-cue interpretation tests to assess their tendencies towards optimism or pessimism. Using free access and instrumental paradigms, I examined their alcohol-seeking and consumption behaviors. We also conducted gene expression analysis in selected brain structures and determined the density of 5-HT_{1A}, 5-HT_{2A}, and D_2 receptors using autoradiographic analysis. We found that under free access conditions, "pessimistic" rats consumed more alcohol than "optimistic" rats, which was associated with changes in gene expression and 5-HT_{2A} receptor density in the nucleus accumbens.

The results suggest that various aspects of biased cognition, such as sensitivity to positive and negative feedback and tendencies towards optimism or pessimism, significantly interact with individual susceptibility to transitioning from controlled to uncontrolled alcohol abuse. These findings may enhance our understanding of the mechanisms underlying AUD and contribute to the development of new therapeutic strategies.

Wprowadzenie

Alkohol odgrywał znaczącą rolę w historii człowieka, będąc nie tylko źródłem rozrywki, ale także istotnym elementem kulturowym i społecznym. Już około 4000 lat p.n.e., kiedy to starożytne cywilizacje Mezopotamii i Egiptu warzyły piwo i wino, napoje alkoholowe były wykorzystywane w ceremoniach religijnych i świętach. Alkohol miał zastosowanie również w praktyce – pozwalał konserwować żywność, był wykorzystywany w przemyśle, a nawet medycynie. Chociaż prawie wszyscy dorośli ludzie odczuli konsekwencje spożycia alkoholu przynajmniej raz w swoim życiu, większość osób nie uzależnia się od tej substancji (Grant *et al.*, 2015). Pomimo to, zaburzenie używania alkoholu (ang. *Alcohol Use Disorder*, AUD) stanowi jedną z najczęstszych i najbardziej śmiertelnych chorób psychicznych, przyczyniając się na świecie do około 3 milionów zgonów rocznie (World Health Organization, 2024). AUD to choroba przewlekła, o złożonej symptomatologii, charakteryzująca się postępującą w czasie eskalacją od umiarkowanego do nadmiernego spożycia alkoholu. Często opiera się na cyklach remisji i nawrotów, co pokazuje trudności związane z osiągnięciem i utrzymaniem długotrwałej abstynencji (Schuckit, 2009). AUD ma wpływ na niemal każdy aspekt życia jednostki, prowadząc do negatywnych konsekwencji zdrowotnych i społecznych, takich jak trudności w relacjach interpersonalnych czy stygmatyzacja w społeczeństwie, ale także zawodowych (Room, 2005). Chroniczne spożywanie alkoholu może prowadzić do chorób wątroby, układu sercowo-naczyniowego i zaburzeń neurologicznych (Rehm *et al.*, 2009). Wiąże się także ze zwiększonym ryzykiem zaburzeń psychicznych, emocjonalnych, deficytów poznawczych i zaburzeń oceny sytuacji (Schmidt *et al.*, 2016). Te ostatnie mogą skutkować tendencją do przeceniania pozytywnych efektów spożywania alkoholu i bagatelizowania jego negatywnych konsekwencji, co z kolei może prowadzić do utrwalania nawyków związanych z nadużywaniem alkoholu.

W badania nad przyczynami zachorowalności na AUD zaangażowanych jest wiele dyscyplin naukowych, takich jak medycyna, psychologia, socjologia, farmakologia czy neuropsychologia. Współczesne modele AUD uznają wzajemne oddziaływanie czynników biologicznych, społecznych i psychologicznych w rozwoju i utrzymywaniu się tego zaburzenia. Wśród czynników biologicznych kluczową rolę stanowią predyspozycje genetyczne, które mogą być zarówno specyficzne dla etanolu (np. związane z jego metabolizmem), jak i niespecyficzne (np. związane z impulsywnością czy samoregulacją) (Schuckit, 2009; Carvalho *et al.*, 2019).

Społeczny aspekt AUD uwzględnia wpływ czynników środowiskowych, w tym dynamiki rodziny i presji rówieśniczej. Dostępność alkoholu, status społeczno-ekonomiczny i kulturowe podejście do spożywania alkoholu również odgrywają istotną rolę w kształtowaniu indywidualnych wzorców picia (Collins, 2016). Co więcej, prenatalna ekspozycja na alkohol czy negatywne doświadczenia z dzieciństwa mogą wpływać na nadużywanie alkoholu w dorosłym życiu (Dube *et al.*, 2002; Goldschmidt *et al.*, 2019). Zależność między osobowością i podatnością na rozwój AUD jest złożona. Badania prowadzone w ciągu ostatnich lat nie pozwoliły wyodrębnić konkretnego zestawu cech osobowości, który umożliwiałby jednoznacznie determinować podatność na uzależnienie od alkoholu. Jednakże różne aspekty osobowości, jak np. wysoki poziom impulsywności, zostały powiązane zarówno z rozwojem, jak i nawrotem AUD. Teorie poznawcze podkreślają rolę nieadaptacyjnych wzorców myślenia, takich jak tendencyjność poznawcza, w utrzymywaniu się zachowań związanych z piciem. Wrażliwość na wzmocnienie wpływaj na powody, dla których ludzie po sięgają po alkohol, takie jak redukcja stresu, radzenie sobie z negatywnymi emocjami lub wzmocnienie pozytywnego nastroju. Może więc stanowić istotny czynnik w rozwoju i utrzymywaniu się AUD.

Tendencyjność poznawcza

Umysł przekształca fizyczne właściwości świata zewnętrznego na użyteczne jednostki informacji, tzw. wewnętrzne reprezentacje (Lindsay & Norman, 2013). Proces ten może zostać zakłócony m.in. przez fizjologiczne ograniczenia percepcji (Kellogg, 2003), różnice indywidualne, w tym przeszłe doświadczenia (Kellogg, 2003), a także tendencyjność aparatu poznawczego, sprawiając, że wewnętrzne reprezentacje nie są idealnym odzwierciedleniem zewnętrznego świata (Kellogg, 2003). W mojej pracy skupiłam się na tendencyjności poznawczej. Pojęcie to zyskało znaczną uwagę dzięki noblowskiej pracy psychologów Amosa Tversky'ego i Daniela Kahnemana w latach 70. ubiegłego wieku. Badacze ci wykazali, że ludzkie osądy często znacznie odbiegają od tego, co jest zgodne z teorią prawdopodobieństwa lub logiką (Tversky & Kahneman, 1974). Tendencyjność poznawcza odnosi się do odchyleń od racjonalnej oceny, kiedy jednostki tworzą subiektywne reprezentacje wewnętrzne w oparciu o informacje zewnętrze. Zniekształcenia te często wynikają z przetwarzania informacji za pomocą heurystyk – uproszczonych reguł wnioskowania, które mogą prowadzić do poważnych błędów w ocenie, zniekształceń percepcji i, w rezultacie, do niepoprawnych wniosków (Kahneman & Tversky, 1972). Pomimo tego, że takie przetwarzanie informacji mogłoby się

wydawać dezadaptacyjne, jest wręcz przeciwnie. Ludzki umysł zdolny jest do utrzymania uwagi przez krótki czas, co czyni automatyzmy (heurystyki) idealnym rozwiązaniem, by sprawnie funkcjonować stosunkowo niskim kosztem. Niemniej jednak, w przypadkach, gdy problem do rozwiązania jest bardziej złożony, to takie szybkie bezrefleksyjne wnioskowanie heurystyczne obarczone jest błędami.

Wrażliwość na informacje zwrotne

Konsekwencje podjętych decyzji kształtują zachowanie zarówno ludzi, jak i zwierząt. Wszystkie zachowania ukierunkowane na cel składają się z dwóch kluczowych komponentów. Pierwszym jest proces decyzyjny, który rozpoczyna się od przedstawienia dostępnych opcji i prowadzi do wyboru tej o najwyższej oczekiwanej wartości. Drugi to proces uczenia się, w którym kluczową rolę odgrywa wrażliwość na wzmocnienie i informacje zwrotne (Shuell, 1986). Zdolność do prawidłowego interpretowania reakcji środowiska na wykonywane działania ma istotne znaczenie w kontekście zwiększenia zysków i unikania strat. Bogata literatura dotycząca warunkowania instrumentalnego pokazuje, że pozytywne i negatywne wzmocnienie skutecznie zmieniają siłę i kierunek zachowania, wzmacniając lub osłabiając określone reakcje (Kirsch *et al.*, 2004). Informacje zwrotne dostarczają niezbędnych wskazówek, które pomagają jednostkom zrozumieć konsekwencje swoich działań, formując relację między bodźcem a reakcją. W efekcie, umiejętność reagowania na wzmocnienia w postaci informacji zwrotnych jest kluczowa dla rozwoju efektywnych strategii przetrwania i funkcjonowania w złożonym świecie.

Zdolność do prawidłowej interpretacji bodźców ze środowiska jest często obarczona błędami za sprawą dysproporcji pomiędzy wrażliwością na pozytywne i negatywne wzmocnienie. W efekcie osoba może być nadwrażliwa na informacje o popełnionych błędach, stronnicza w interpretacji niejednoznaczności czy cechować się zawyżonymi lub zaniżonymi oczekiwaniami odnośnie przyszłości. Liczne teorie podkreślają kluczową rolę tendencyjności poznawczej w etiologii i nawrotach zaburzeń psychicznych. Na przykład według Aarona Becka tendencyjność poznawcza stanowi jeden z najważniejszych czynników determinujących podatność na wystąpienie i prawdopodobieństwo nawrotu depresji (Beck, 2002). Jej rola została potwierdzona przez liczne badania, w których testowano przetwarzanie i wrażliwość na informacje zwrotne w depresji (Ingram *et al.*, 1983; Elliott *et al.*, 1997; Gollan *et al.*, 2008; Taylor Tavares *et al.*, 2008). Zaburzenia przetwarzania informacji zaobserwowano także u pacjentów

cierpiących na zaburzenia lękowe, schizofrenię i chorobę afektywną dwubiegunową (Pizzagalli *et al.*, 2008a; Culbreth *et al.*, 2016). Co ważne, również u pacjentów z AUD występuje zaburzone przetwarzanie informacji, zwłaszcza związanych z alkoholem, co szerzej opisuję w dalszej części rozprawy.

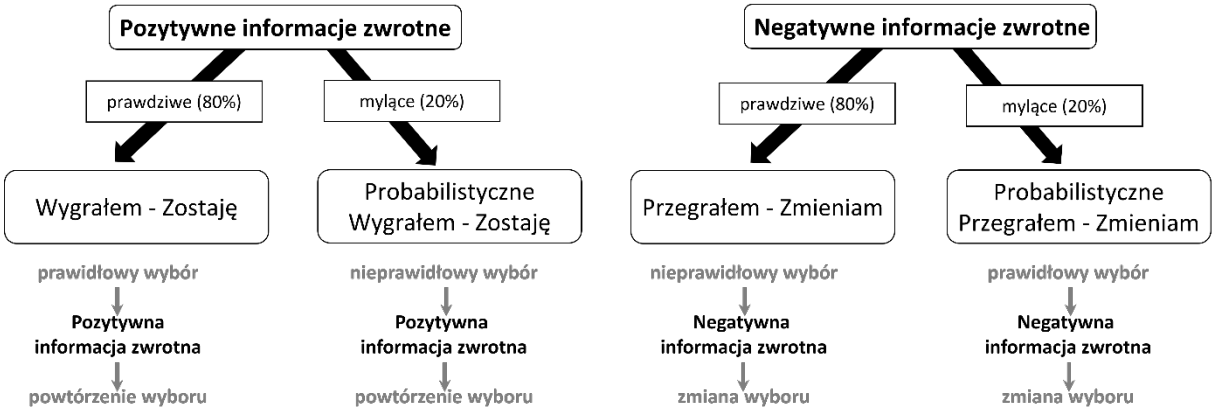
Gdy ludzie wykorzystują informację zwrotną do kierowania swoim zachowaniem, poznawczemu komponentowi tego procesu towarzyszy wzajemne oddziaływanie pozytywnych i negatywnych reakcji afektywnych, które mogą albo poprawić, albo zakłócić wykonanie zadania. Pozytywna reakcja emocjonalna może działać motywująco do osiągnięcia sukcesu. Z kolei negatywna reakcja afektywna wynika z nadwrażliwości na negatywne informacje zwrotne i często skutkuje obniżeniem zdolności do wykonania zadania.

Eksperymentalny pomiar wrażliwości na wzmocnienia

Skuteczną i powszechnie stosowaną metodą do pomiaru wrażliwości na pozytywne i negatywne informacje zwrotne, a więc wrażliwości na wzmocnienie, jest ocena zachowań „Wygrałem-Zostaję" (ang. *Win-Stay*) i „Przegrałem-Zmieniam" (ang. *Lose-Shift*) w teście Probabilistycznego Przeuczania (ang. *Probabilistic Reversal Learning,* PRL) (Ryc. 1). Wprowadzenie przedklinicznej wersji tego testu w 2010 roku umożliwiło badanie wrażliwości na informacje zwrotne także u zwierząt (Bari *et al.*, 2010). Podczas testu, w każdej próbie prezentowane są dwie dźwignie, a zwierzęta, metodą prób i błędów, uczą się wybierać bodziec prawidłowy (nagradzany w większości (np. 80%) lub karany/nienagradzany w mniejszości (np. 20%) prób) i unikać nieprawidłowego (karanego/nienagradzanego w większości (np. 80%)) i nagradzanego w mniejszości (np. 20%) prób). Nagroda ma zazwyczaj formę pokarmu, a karą może być delikatny szok elektryczny. Badane zwierzęta wykonują kilka kolejnych serii testów, a każda z nich składa się z określonej liczby prób. Wartość bodźców co jakiś czas ulega odwróceniu (zwykle po kilku poprawnych reakcjach), to znaczy, że bodziec, który w większości przypadków był nagradzany, staje się zazwyczaj karany i *vice versa*. Zmiany te wymagają od zwierząt dostosowywania reakcji w celu zmaksymalizowania liczby uzyskanych nagród i unikania kar. Zarówno po dokonaniu prawidłowego, jak i nieprawidłowego wyboru, w niewielkiej liczbie prób (np. 20%), każdy bodziec skutkuje otrzymaniem, odpowiednio, fałszywie negatywnej i fałszywie pozytywnej informacji zwrotnej (kary/nagrody). Wprowadzenie wzmocnienia probabilistycznego zwiększa złożoność zadania, ponieważ informacje otrzymane po uprzednio dokonanym wyborze są niewystarczające, aby kierować

zachowaniem. Badane zwierzęta muszą zatem angażować własne funkcje poznawcze i ich tendencyjność, aby śledzić historię otrzymanych nagród i kar, i wybierać ten bodziec, który jest bardziej korzystny. Pozwala to wnioskować o ich indywidualnej wrażliwości na wzmocnienia. Domyślnie zwierzęta muszą nauczyć się ignorować sporadyczne wprowadzające w błąd fałszywie negatywne i fałszywie pozytywne informacje, które wynikają z probabilistycznego charakteru testu. Ostatnie badania z naszego laboratorium wykazały, że u gryzoni wrażliwość na negatywne i pozytywne informacje zwrotne stanowią stabilne i trwałe cechy behawioralne. Co ważne, cechy te są od siebie niezależne, a wrażliwość na pozytywne informacje zwrotne jest powiązana

z elastycznością poznawczą (Noworyta-Sokolowska *et al.*, 2019).



Rycina 1 Parametry behawioralne mierzone w teście probabilistycznego przeuczania (PRL). Analiza zachowań „Wygrałem-Zostaję" i „Przegrałem-Zmieniam" oparta jest na analizie decyzji podejmowanych na podstawie wyników poprzednich prób. Uwzględniane są cztery typy zachowań:
1) prawidłowy wybór skutkujący pozytywną informacją zwrotną, który jest powtarzany w kolejnej próbie,
2) nieprawidłowy wybór skutkujący pozytywną informacją zwrotną, który jest powtarzany w kolejnej próbie,
3) nieprawidłowy wybór skutkujący negatywną informacją zwrotną, który jest zmieniany w kolejnej próbie,
4) prawidłowy wybór skutkujący negatywną informacją zwrotną, który jest zmieniany w kolejnej próbie. Proporcja zachowań „Wygrałem-Zostaję" stanowi bezpośrednią miarę wrażliwości na pozytywne informacje zwrotne i może być traktowana jako wskaźnik wrażliwości na pozytywne wzmocnienie. Wrażliwość na negatywne informacje zwrotne ocenia się na podstawie proporcji probabilistycznych zachowań „Przegrałem-Zmieniam" w stosunku do wszystkich nienagradzanych wyborów dla danego bodźca. Na podstawie (Rygula *et al.*, 2018).

Neurochemiczne podłoża wrażliwości na wzmocnienia

Neurochemiczne mechanizmy determinujące wrażliwość na pozytywne i negatywne informacje zwrotne nie są dobrze poznane, jednak podejrzewa się, że kluczowe w nich są geny (i ich produkty białkowe) zaangażowane w funkcjonowanie różnych układów neurotransmisyjnych, odgrywające istotną rolę w regulacji emocji, motywacji i uczenia się (Wise, 2004; Fischer & Ullsperger, 2017; Wolf *et al.*, 2018). Jednym z najczęściej badanych neuroprzekaźników w kontekście wrażliwości na informacje zwrotne jest dopamina (DA). W 2004 roku wykazano, że zmniejszona dostępność DA, którą można zaobserwować np. u pacjentów z chorobą Parkinsona, wiąże się z lepszym uczeniem na podstawie negatywnych informacji zwrotnych, w porównaniu do pozytywnych informacji zwrotnych (Frank *et al.*, 2004). Co ważne, farmakologiczne nasilenie transmisji DA odwracało tą tendencję, co zwiększało wrażliwość pacjentów na pozytywne informacje zwrotne (Frank *et al.*, 2004). Inne badania farmakologiczne wykazały, że aktywacja receptorów dopaminergicznych D_2 stymuluje uczenie się jedynie na podstawie pozytywnych, a nie negatywnych informacji zwrotnych, co dodatkowo sugeruje zaangażowanie układu DA w uczenie się na podstawie nagrody (Pessiglione *et al.*, 2006; Pizzagalli *et al.*, 2008b; Eisenegger *et al.*, 2014). Wykazano również, że uczenie się na podstawie nieoczekiwanych nagród i kar zależne jest od poziomu DA w prążkowiu (Cools *et al.*, 2009).

Coraz większa liczba badań wskazuje na rolę serotoniny (5-HT) w kształtowaniu wrażliwości na informacje zwrotne. Dotychczas opublikowane wyniki sugerują, że farmakologiczna interwencja w neurotransmisję 5-HT, zarówno u ludzi, jak i zwierząt, prowadzi do zmian we wrażliwości na negatywne informacje zwrotne (Chamberlain *et al.*, 2006; Cools *et al.*, 2008; Bari *et al.*, 2010). Wykazano, że jednorazowe podanie niskiej dawki citalopramu, należącego do grupy selektywnych inhibitorów wychwytu zwrotnego 5-HT (ang. *selective serotonin reuptake inhibitors*, SSRI), aktywuje presynaptyczne receptory 5-HT_{1A} zwiększając wrażliwość na fałszywie negatywne informacje zwrotne w teście PRL zarówno u ludzi, jak i zwierząt (Chamberlain *et al.*, 2006; Bari *et al.*, 2010). Co ciekawe, podanie wyższej dawki leku wywołuje efekt odwrotny. Inne badanie również wykazało, że u zwierząt zmniejszenie wychwytu zwrotnego 5-HT, poprzez podanie innego leku z grupy SSRI, escitalopramu, także powoduje zmniejszenie wrażliwości na negatywne informacje zwrotne (Ineichen *et al.*, 2012). Z kolei podwyższenie wrażliwości na negatywne informacje zwrotne odnotowano po obniżeniu

poziomu 5-HT przez deplecję jej prekursona – tryptofanu (Trp). (Evers *et al.*, 2005; Cools *et al.*, 2008). Wyniki wspomnianych badań wskazują na rolę układów DA i 5-HT w kształtowaniu wrażliwości na informacje zwrotne.

Rola wrażliwości na informacje zwrotne w zaburzeniu używania alkoholu

Ponieważ spożywanie alkoholu ma działanie nagradzające (Everitt & Robbins, 2005), zwłaszcza w początkowym okresie picia, liczne badania potwierdziły związek między wrażliwością na pozytywne wzmocnienie a wyższym spożyciem alkoholu, zwłaszcza w formie intensywnego picia (ang. *binge drinking*) (Loxton & Dawe, 2001; Franken & Muris, 2006; Feil & Hasking, 2008). Osoby o wysokiej wrażliwości na pozytywne wzmocnienie doświadczały znacznie silniejszego pragnienia picia alkoholu podczas ekspozycji na bodźce z nim związane niż osoby o niskiej wrażliwości (Franken, 2002). Z kolei niska wrażliwość na wzmocnienie pozytywne związana była z negatywnym stanem emocjonalnym, który prowadził do prób samoleczenia alkoholem ze względu na jego właściwości anksjolityczne (Heinz et al., 2009; Veilleux et al., 2014). Pochodną wrażliwości na wzmocnienie jest rodzaj motywacji do spożywania alkoholu, zwany piciem dla nagrody (ang. *reward drinking*) lub ulgi (ang. *relief drinking*). Picie dla nagrody polega na spożywaniu alkoholu jako formy dostarczania sobie przyjemności. Z kolei picie dla ulgi odnosi się do konsumpcji alkoholu jako środka łagodzącego stres, lęk lub napięcie emocjonalne. Osoby angażujące się w ten rodzaj picia używają alkohol jako sposób radzenia sobie z negatywnymi emocjami i sytuacjami stresowymi. Używanie alkoholu dla nagrody lub ulgi jest zgodne z allostatycznym modelem uzależnienia. Model ten zakłada, że na początku uzależnienia alkohol jest spożywany dla jego nagradzających właściwości, natomiast w późniejszych etapach uzależnienia picie motywowane jest głównie uniknięciem stresu i złagodzeniem efektów odstawienia (Koob & Moal, 1997). Podejście to może mieć implikacje kliniczne, ponieważ niedawne badania wykazały, że osoby, u których picie jest motywowane nagrodą, odnoszą większe korzyści z farmakoterapii naltreksonem, natomiast u osób pijących dla ulgi skuteczniejszy jest akamprozat (Mann *et al.*, 2018; Witkiewitz *et al.*, 2019).

Zakłada się, że osoby cierpiące na AUD są mniej wrażliwe na negatywne konsekwencje podejmowanych działań i mają mniejsze zdolności do wykorzystywania negatywnych informacji zwrotnych do kierowania i dostosowywania swojego zachowania do zmieniających się warunków otoczenia, co sugeruje deficyty w przetwarzaniu informacji (Bechara *et al.*, 2002).

Jednak pomimo licznych doniesień łączących AUD z osłabieniem zdolności decyzyjnych, znacznie mniej wiadomo na temat zaburzonego przetwarzania informacji zwrotnych przed rozwojem alkoholizmu. Jak dotąd brakuje danych na temat wpływu zmienionej (zwiększonej/zmniejszonej) wrażliwości na informacje zwrotne na przejście od rekreacyjnego używania do kompulsywnego nadużywania alkoholu. Głównie dlatego, że trudno jest uzyskać informacje o wrażliwości na informacje zwrotne u ludzi przed rozwojem uzależnienia. Pomocne w tym przypadku są modele zwierzęce, które pozwalają na zmierzenie poziomu wrażliwości na pozytywne i negatywne informacje zwrotne przed długotrwałą ekspozycją na alkohol.

Tendencyjność oceny (optymizm/pesymizm)

Zainteresowanie tendencyjnością oceny w kontekście zaburzeń psychicznych ma swoje korzenie w dwóch kluczowych odkryciach: po pierwsze, zdrowe osoby są zazwyczaj optymistycznie nastawione do przyszłości (Scheier & Carver, 1985); po drugie, istnieją istotne różnice w walencji tendencyjności oceny pomiędzy osobami zdrowymi a cierpiącymi na zaburzenia afektywne (Blanchette & Richards, 2010). Tendencyjność oceny można definiować jako względną reakcję na niejednoznaczny bodziec, wyrażającą się interpretacją tego bodźca i oczekiwaniem na konsekwencje tej reakcji (Boleij *et al.*, 2012; Bateson & Nettle, 2015). Osoby, które reagują na bodźce niejednoznaczne podobnie jak na bodziec pozytywny, oczekują pozytywnych konsekwencji i są określane jako optymistyczne. Natomiast osoby, które na bodźce niejednoznaczne reagują podobnie jak na bodźce negatywne, oczekują negatywnych konsekwencji i określane są jako pesymistyczne. Pomiar tendencyjności oceny w podejmowaniu decyzji na podstawie sygnałów niejednoznacznych stanowi stosunkowo nowe i obiecujące podejście do oceny afektu u ludzi. Choć powszechnie tendencyjność oceny (optymizm/pesymizm) mierzy się za pomocą kwestionariuszy, to eksperymentalny pomiar reakcji na bodziec niejednoznaczny stanowi bardziej rzetelne źródło informacji, ze względu brak subiektywnego charakteru odpowiedzi u osób badanych. Wykazano, że reakcje na niejednoznaczność korelują ze stanem afektywnym (pozytywnym lub negatywnym) (Iigaya *et al.*, 2016). Na przykład osoby w negatywnych stanach emocjonalnych częściej dokonują negatywnych („pesymistycznych") ocen na temat niejednoznacznych wydarzeń lub bodźców niż osoby doświadczające pozytywnych emocji.

Eksperymentalne paradygmaty służące do oceny optymizmu/pesymizmu

Związek między tendencyjnością oceny a stanem afektywnym u ludzi zainspirował badaczy zainteresowanych identyfikacją poznawczych mechanizmów leżących u podłoża stanu afektywnego, do opracowania paradygmatów służących ocenie optymizmu i pesymizmu u zwierząt. Przełomowym badaniem okazała się praca Harding i współpracowników z 2004 roku, w której wykazano, że tendencyjność oceny występuje także u zwierząt (Harding *et al.*, 2004). Opracowany wówczas test interpretacji bodźca niejednoznacznego (ang. *Ambiguous-Cue Interpretation,* ACI) stosuje się obecnie do pomiaru tendencyjności oceny u wielu różnych gatunków zwierząt, od szympansów, przez owce, psy, aż po pszczoły (Bateson *et al.*, 2011; Burman *et al.*, 2011; Doyle *et al.*, 2011; Bateson & Nettle, 2015). Podstawowa wersja tego testu opiera się na treningu zwierząt tak, aby wykonywały reakcję na „pozytywny" bodziec (ton o określonej częstotliwości) w celu osiągnięcia pozytywnego wyniku (np. nagrody w postaci jedzenia) i powstrzymywały się od reakcji na bodziec „negatywny" (ton o innej częstotliwości), aby uniknąć kary (np. lekkiego szoku elektrycznego). Gdy zwierzęta prawidłowo rozróżniają sygnały „pozytywne" i „negatywne", wprowadzany jest dodatkowy bodziec niejednoznaczny (ton o pośredniej częstotliwości). Tendencyjność oceny jest określana przez sposób, w jaki zwierzęta interpretują ten bodziec. Zwierzęta, których interpretacja bodźca niejednoznacznego wskazuje na oczekiwanie pozytywnych konsekwencji są określane jako „optymistyczne", a te, które reagują w sposób sugerujący oczekiwanie negatywnych konsekwencji, jako „pesymistyczne" (Bateson, 2016).

Neurochemiczne podłoża tendencyjności oceny (optymizmu/pesymizmu)

Przy pomocy testu ACI w ostatnich latach wykazano, że tendencyjnością oceny zwierząt można manipulować za pomocą interwencji behawioralnej i farmakologicznej. Szczury, u których wywołano pozytywne emocje poprzez somatosensoryczną stymulację, tj. łaskotanie (ang. *tickling*) wykazywały optymistyczną tendencyjność oceny (Rygula *et al.*, 2012). Natomiast negatywne emocje spowodowane utratą statusu społecznego przyczyniały się do wystąpienia pesymizmu (Papciak *et al.*, 2013). Inne badania wykazały, że nieselektywna stymulacja układu DA za pomocą D-amfetaminy wywoływała u szczurów „optymistyczną" tendencyjność oceny (Rygula *et al.*, 2014; Hales *et al.*, 2017). Późniejsze badania z wykorzystaniem prekursora dopaminy 3,4-dihydroksy-L-fenyloalaniny (L-DOPA), przeprowadzone w naszym laboratorium,

wykazały, że wpływ DA na modulację tendencyjności oceny zależny jest od wyjściowego poziomu optymizmu/pesymizmu (Golebiowska & Rygula, 2017).

Inne badania przedkliniczne wykazały, że farmakologiczna modulacja układu 5-HT poprzez podawanie leków z grupy SSRI może modyfikować interpretację bodźców niejednoznacznych u zwierząt. Udowodniono, że jednorazowe podanie niskiej dawki citalopramu istotnie zwiększało tendencję do negatywnej interpretacji w paradygmacie ACI. Co ciekawe, podanie wyższych dawek tego leku wywoływało tendencyjność optymistyczną (Rygula *et al.*, 2014). Aby wyjaśnić te przeciwstawne efekty, zaproponowano, że tendencyjność pesymistyczna obserwowana po podaniu małej dawki citalopramu wynikała z tymczasowego wyciszenia aktywności układu 5-HT poprzez stymulację autoreceptorów serotoniny 5-HT_{1A} w jądrach szwu (Sprouse & Aghajanian, 1987; Sharp & Foster, 1989). Zgodnie z tą hipotezą podawanie wyższych dawek citalopramu znosi ten mechanizm i umożliwia przesunięcie tendencyjności oceny w stronę optymizmu. Podobny wzrost poziomu optymizmu zaobserwowano w przypadku chronicznych podań innego leku z grupy SSRI – fluoksetyny (Anderson *et al.*, 2013). Badanie przeprowadzone przez Doyle i wsp. wykazało, że podanie p-chlorofenyloalaniny, inhibitora hydroksylazy Trp, enzymu odpowiedzialnego za syntezę 5-HT, wywołuje u owiec pesymistyczną tendencyjność oceny (Doyle *et al.*, 2011). Podobny efekt zaobserwowano po deplecji 5-HT w paradygmacie ACI u świń (Stracke *et al.*, 2017).

Chociaż początkowo tendencyjność oceny postrzegano głownie jako pochodną aktualnego stanu afektywnego, podatnego na wpływ środowiska, ostatnie badania wykazały, że optymizm i pesymizm można uznać za względnie stabilne i trwałe cechy poznawcze (Rygula *et al.*, 2013; Drozd *et al.*, 2016; Curzytek *et al.*, 2018). Pomimo że podłoże tych cech wciąż pozostaje słabo poznane, niedawne badania sugerują, że indywidualne różnice w tendencyjności oceny są przynajmniej częściowo zależne od ekspresji genów w mózgu (Fox *et al.*, 2009; Kloke *et al.*, 2014; Krakenberg *et al.*, 2019; Boddington *et al.*, 2020). Analiza ekspresji genów zaangażowanych w funkcjonowanie układów DA i 5-HT w korę przedczołową (ang. *Prefrontal Cortex*, PFC) kur wykazała, że wyższy poziom optymizmu u tych zwierząt związany był ze zwiększoną ekspresją receptorów dopaminy D_1, natomiast niższy poziom powiązany był z podwyższoną ekspresją receptorów 5-HT_{2A} (Boddington *et al.*, 2020). Dotychczas wykazano także, że nosiciele krótkiego allelu genu *Sert*, kodującego transporter serotoniny (SERT), charakteryzują się negatywną tendencyjnością oceny (Beevers *et al.*, 2009;

Fox *et al.*, 2009). Odkrycia te sugerują udział wspomnianych układów neurotransmisyjnych w kształtowaniu tendencyjności oceny.

Rola tendencyjności oceny w zaburzeniu używania alkoholu

Tendencyjność oceny odgrywa rolę w zaburzeniu używania alkoholu, wpływając na sposób, w jaki chorzy postrzegają i interpretują sygnały związane z tą substancją. Na przykład, osoby cierpiące na AUD mogą wykazywać tendencję do przeceniania pozytywnych efektów spożywania alkoholu, takich jak poprawa nastroju czy redukcja stresu, jednocześnie bagatelizując negatywne konsekwencje, takie jak problemy zdrowotne czy społeczne (Noworyta *et al.*, 2022). Tego rodzaju selektywna uwaga i pamięć mogą prowadzić do błędnych przekonań i decyzji, które utrwalają nawyki związane z alkoholem. W rezultacie tendencyjność poznawcza może wzmacniać mechanizmy leżące u podstaw uzależnienia, utrudniając podejmowanie racjonalnych decyzji dotyczących ograniczenia lub zaprzestania picia.

Indywidualne różnice we wrażliwości na wzmocnienia mogą wpływać na nabywanie i utrzymywanie pozytywnych i negatywnych oczekiwań dotyczących skutków spożywania alkoholu. Oczekiwania te mogą być definiowane jako wyolbrzymione przekonania o pozytywnym lub negatywnym wpływie alkoholu na zachowanie, nastrój i emocje (Leigh, 1989) i odgrywają kluczową rolę w podejmowaniu decyzji dotyczących picia alkoholu oraz w trajektoriach jego używania (Jones et al., 2001). Rola optymistycznej i pesymistycznej tendencyjności oceny w AUD była postulowana od dawna (Brown *et al.*, 1980; Christiansen & Goldman, 1983; Blume *et al.*, 2003; Blume & Blume, 2014).Tendencyjność oceny wpływa na sposób, w jaki jednostki postrzegają i reagują na ryzyko oraz nagrodę, potencjalnie zakłócając procesy podejmowania decyzji, faworyzując krótkoterminowe nagradzające skutki spożycia alkoholu, pomimo znanych negatywnych konsekwencji.

Osoby optymistyczne mogą zaniżać ryzyko związane z nadmiernym spożywaniem alkoholu, jednocześnie przeceniając swoją zdolność do samokontroli przy jego wysokiej dostępności (Fromme & D'Amico, 2000; Blume *et al.*, 2003). Z drugiej strony, pesymizm, objawiający się przez zwiększone skupienie na negatywnych bodźcach i emocjach, może podtrzymywać używanie alkoholu jako strategię radzenia sobie z problemami (Veilleux *et al.*, 2014). Badania na modelach zwierzęcych dostarczyły dodatkowych dowodów na rolę optymistycznej i pesymistycznej tendencyjności oceny w zaburzeniach psychicznych. Niedawne badania przeprowadzone w naszym laboratorium wykazały, że u szczurów poziom optymizmu

przewiduje podatność zwierząt na anhedonię (Rygula *et al.*, 2013) i deficyty motywacyjne (Drozd *et al.*, 2017) wywołane stresem. Inne badania pokazały, że tendencyjność oceny jest związana z różnicami w motywacji do zdobywania nagrody (Rygula *et al.*, 2015), wrażliwością na negatywne informacje zwrotne (Rygula & Popik, 2016), zachowaniami ryzykownymi (Drozd *et al.*, 2016) oraz profilem immunologicznym (Curzytek *et al.*, 2018) – wszystkie te czynniki mogą być związane z różnymi aspektami AUD.

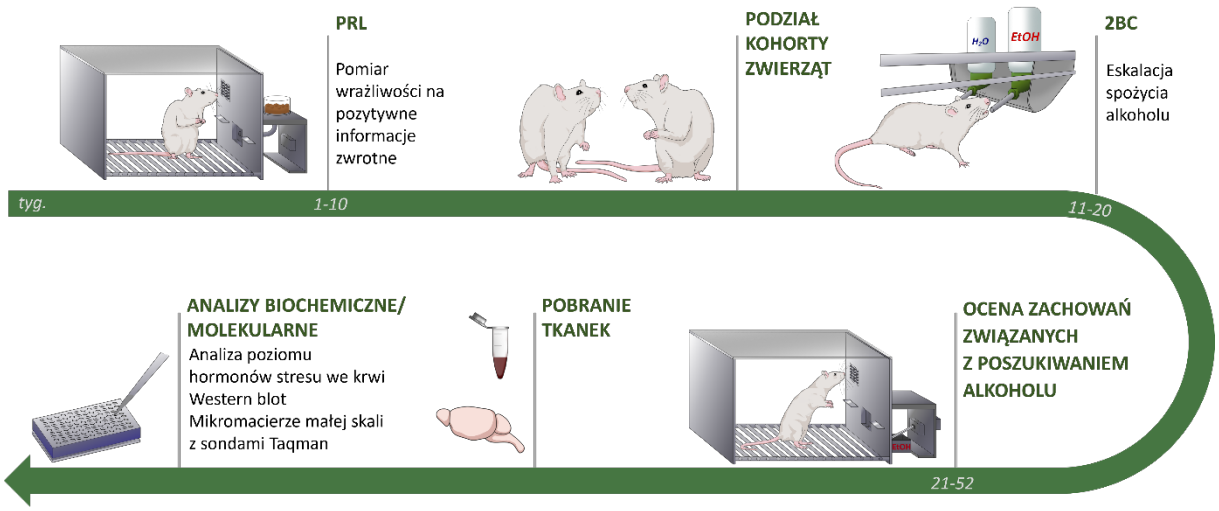
Zrozumienie roli opisanych aspektów tendencyjności poznawczej dostarcza istotnych wskazówek wyjaśniających, dlaczego niektóre jednostki rozwijają i utrzymują AUD, a także zidentyfikować potencjalne nowe kierunki terapeutyczne.

Cel badań

Zaburzenia związane z używaniem alkoholu stanowią jedno z głównych wyzwań zdrowia publicznego w XXI wieku, mając istotne konsekwencje społeczne. Nadużywanie alkoholu prowadzi do licznych problemów zdrowotnych, zarówno fizycznych, jak i psychicznych, aktów przemocy oraz zachowań ryzykownych, co dodatkowo obciąża systemy opieki zdrowotnej. Z uwagi na złożoną etiologię AUD, zrozumienie czynników odpowiedzialnych za rozwój, utrzymywanie i nawracanie tego zaburzenia jest niezbędne do opracowania skutecznych strategii prewencji i terapii. W kontekście coraz częściej postulowanej roli indywidualnych cech w podatności na nadużywanie alkoholu, niniejsza praca miała na celu zgłębienie roli wybranych aspektów tendencyjności poznawczej w przejściu od kontrolowanego używania do niekontrolowanego nadużywania alkoholu w modelu zwierzęcym. Poprzez analizę aspektów zarówno behawioralnych, jak i biochemicznych związanych z tendencyjnością poznawczą i spożywaniem alkoholu u szczurów, przedstawione badania dążyły do lepszego zrozumienia tej złożonej problematyki.

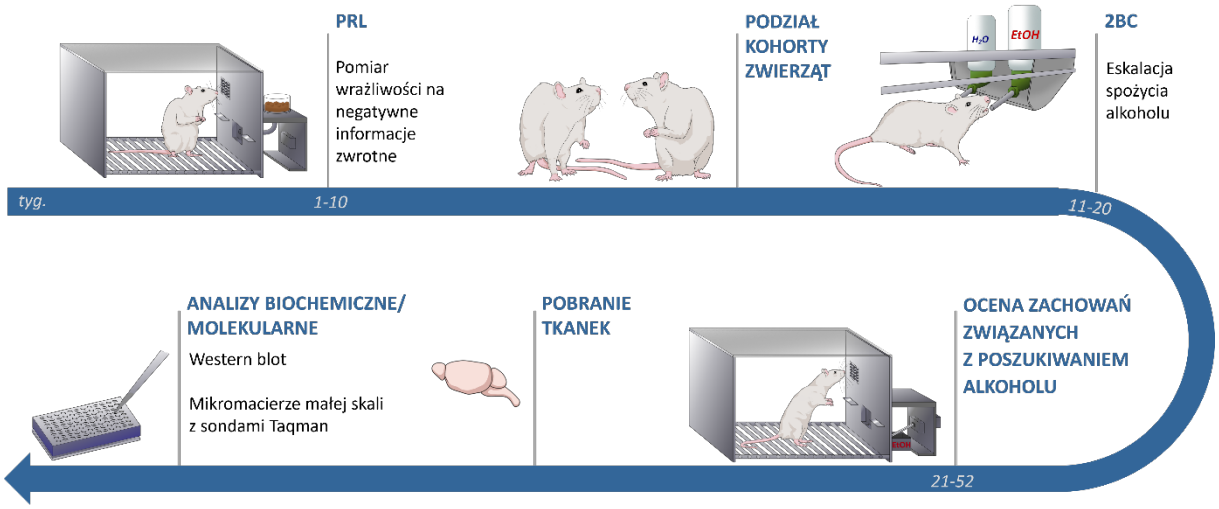
Szczegółowe zadania badawcze obejmowały:

1) Określenie wpływu wrażliwości na pozytywne informacje zwrotne, jako stabilnej i trwałej cechy behawioralnej, na zachowania związane z konsumpcją alkoholu, jego kompulsywnym poszukiwaniem, motywacją do spożywania alkoholu oraz wygaszaniem i przywracaniem zachowań poszukiwawczych u szczurów (publikacja 1; Ryc. 2).



Rycina 2 Schemat eksperymentu. Zwierzęta były trenowane i testowane w serii testów probabilistycznego przeuczania (PRL) w celu określenia wrażliwości na pozytywne informacje zwrotne. Na jej podstawie dokonano podziału kohorty zwierząt na grupę pijąca alkohol lub wodę (kontrolną). W procedurze swobodnego wyboru między dwoma butelkami (2BC), z dostępem przerywanym, zmierzono spożycie płynów u zwierząt. Ocena zachowań związanych z poszukiwaniem alkoholu obejmowała trening na dźwignię „nagradzającą" (ang. *taking lever)* i „poszukiwawczą" (ang. *seeking lever*), pomiar motywacji, pomiar zachowań kompulsywnego poszukiwania w obliczu kary, a następnie wygaszanie zachowań poszukiwawczych alkoholu i ich przywrócenie po 30 dniach abstynencji. Po zakończeniu badań behawioralnych pobrano tkankę mózgu oraz krew i wykonano analizy biochemiczne.

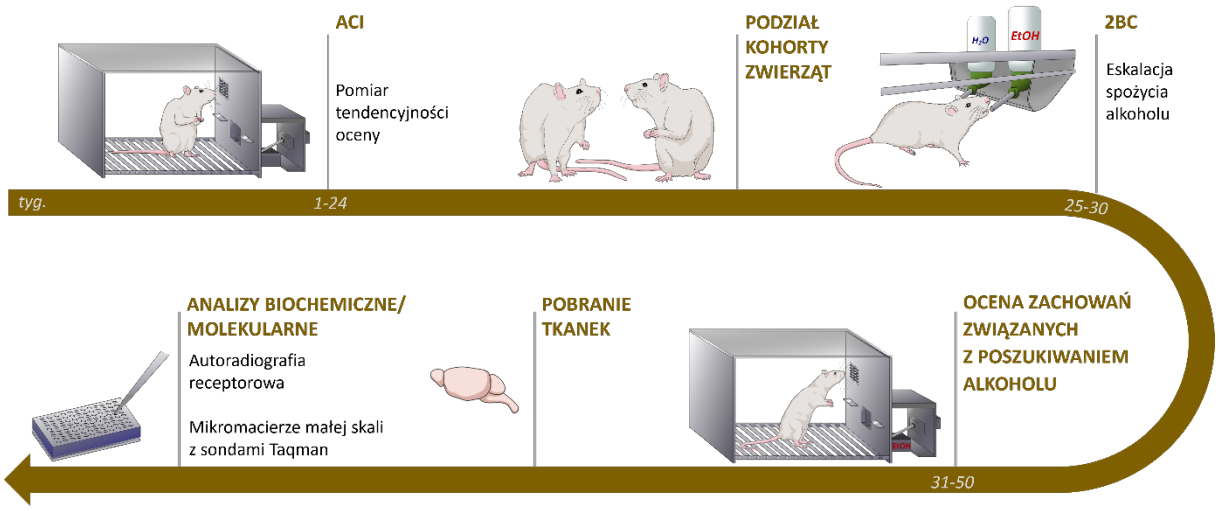
2) Badanie roli wrażliwości na negatywne informacje zwrotne, jako stabilnej i trwałej cechy behawioralnej, w rozwoju kompulsywnego poszukiwania alkoholu u szczurów, eskalacji spożycia alkoholu, motywacji do jego poszukiwania oraz wygaszania i przywracania zachowań związanym z poszukiwaniem alkoholu u szczurów (publikacja 2 i 3; Ryc. 3).



Rycina 3 Schemat eksperymentu. Zwierzęta były trenowane i testowane w serii testów probabilistycznego przeuczania (PRL) aby ocenić ich wrażliwość na negatywne informacje zwrotne. Podziału kohorty zwierząt na grupę

pijąca alkohol lub wodę (kontrolną) dokonano w oparciu o zmierzoną wrażliwość na negatywne informacje zwrotne. W procedurze swobodnego wyboru między dwoma butelkami (2BC), z dostępem przerywanym, zmierzono spożycie płynów u zwierząt. Testy mierzące zachowania poszukiwawcze alkoholu obejmowały trening na dźwignię „nagradzającą" (ang. *taking lever*) i „poszukiwawczą" (ang. *seeking lever*), pomiar motywacji, ocenę zachowań kompulsywnego poszukiwania w obliczu kary, a następnie wygaszanie zachowań związanych z poszukiwaniem alkoholu i ich przywrócenie po 30 dniach abstynencji. Po zakończeniu badań behawioralnych pobrano tkankę mózgową do pomiaru poziomu mRNA i białka w wybranych strukturach mózgu szczurów.

3) Badanie relacji pomiędzy optymistyczną i pesymistyczną tendencyjnością oceny, a zachowaniami związanymi ze spożywaniem alkoholu u szczurów, a także rozwojem zachowań związanych z jego poszukiwaniem. Zachowania te obejmowały motywację do poszukiwania alkoholu, jego kompulsywne poszukiwanie w obliczu kary oraz wygaszanie i przywracanie tego zachowania po okresie abstynencji (publikacja 4; Ryc. 4).



Rycina 4 Schemat eksperymentu. Zwierzęta trenowano i testowano w serii testów interpretacji bodźca niejednoznacznego (ACI) w celu zbadania tendencyjności oceny. Na podstawie oceny poziomu optymizmu i pesymizmu dokonano podziału kohorty zwierząt na grupę pijąca alkohol lub wodę (kontrolną). Następnie u zwierząt zmierzono spożycie płynów w procedurze swobodnego wyboru między dwoma butelkami (2BC), z dostępem przerywanym. Testy mierzące zachowania poszukiwawcze alkoholu obejmowały trening na dźwignię „nagradzającą" (ang. *taking lever*) i „poszukiwawczą" (ang. *seeking lever*), pomiar motywacji, ocenę zachowań kompulsywnego poszukiwania w obliczu kary, a następnie wygaszanie zachowań poszukiwawczych alkoholu i ich przywrócenie po 30 dniach abstynencji. Po zakończeniu badań behawioralnych pobrano tkankę do analizy poziomu mRNA i gęstości receptorów w wybranych strukturach mózgu szczurów.

Przeprowadzone badania

Najnowsze badania z ostatniej dekady podkreślają znaczenie badania korelatów ludzkich cech osobowości w modelach zwierzęcych jako wartościowej metody identyfikacji markerów poznawczych związanych z różnymi zaburzeniami psychicznymi (Anderson *et al.*, 2013; Hales *et al.*, 2017; Stracke *et al.*, 2017; Rygula *et al.*, 2018). Badania stanowiące podstawę niniejszej pracy doktorskiej skupione były na zrozumieniu roli wybranych cech behawioralnych w indywidualnej podatności na powstawanie, utrzymywanie się i nawracanie zaburzeń psychicznych, takich jak zaburzenie używania alkoholu. Trudności w gromadzeniu danych na temat tendencyjności poznawczej u pacjentów przed zdiagnozowaniem AUD w praktyce uniemożliwiają prowadzenie badań podłużnych na ludziach. Badania opisane w mojej rozprawie doktorskiej, wykorzystujące model zwierzęcy, pozwoliły wnioskować o związku przyczynowo-skutkowym między wybranymi aspektami tendencyjności poznawczej, takimi jak wrażliwość na pozytywne i negatywne informacje zwrotne oraz tendencyjność oceny, definiowana jako optymizm i pesymizm, mierzonymi jako stabilne cechy poznawcze, a różnymi aspektami uzależnienia od alkoholu. Zastosowanie zaawansowanych testów behawioralnych pozwoliło określić kierunek i intensywność tendencyjności poznawczej u szczurów. Następnie, korzystając z kompleksowego zestawu testów do badania zachowań związanych ze spożywaniem i poszukiwaniem alkoholu, oceniono, jak wpływa ona na przejście od kontrolowanego do kompulsywnego picia alkoholu u szczurów. Ponieważ otrzymane wyniki zostały już obszernie przedyskutowane w załączonych publikacjach, poniżej przedstawiłam najważniejsze wnioski płynące z przeprowadzonych eksperymentów, w celu podsumowania badań składających się na niniejszą pracę doktorską.

Wpływ wrażliwości na pozytywne informacje zwrotne na zachowania związane z piciem alkoholu

Badania opublikowane w publikacji pt. *„Trait sensitivity to positive feedback is a predisposing factor for several aspects of compulsive alcohol drinking in male rats: behavioural, physiological, and molecular correlates”* autorstwa Cieslik-Starkiewicz A, Noworyta K, Solich J, Korlatowicz A, Faron-Górecka A, Rygula R. opublikowanej w *Psychopharmacology (2024b)* skupione były na określeniu, w jaki sposób wrażliwość na pozytywne informacje zwrotne, mierzona jako stabilna cecha poznawcza, wpływa na poziom picia alkoholu,

motywację do spożywania alkoholu, ale także rozwój zachowań związanych z jego kompulsywnym poszukiwaniem, a w końcu wygaszanie i przywracanie tych zachowań. Wykazałam, że u szczurów szczepu *Sprague Dawley,* w warunkach swobodnego dostępu, następowała istotna eskalacja ilości spożywanego alkoholu. Wzrost ten jednak nie był zależny od wrażliwości na pozytywne informacje zwrotne. W instrumentalnych paradygmatach poszukiwania i spożywania alkoholu wykazałam, że u szczurów niska wrażliwość na pozytywne informacje wiązała się z podwyższonym poziomem motywacji do poszukiwania alkoholu po doświadczeniu negatywnych konsekwencji tego zachowania, to znaczy po serii testów, w których poszukiwanie alkoholu było karane szokiem elektrycznym. Otrzymane wyniki ujawniły również, że zwierzęta niewrażliwe na pozytywne informacje zwrotne szybciej przywracały zachowania związane z poszukiwaniem alkoholu po okresie wymuszonej abstynencji, tzn. potrzebowały mniej testów, aby przywrócić instrumentalną odpowiedź związaną z poszukiwaniem alkoholu do poziomu bazalnego.

Jak już wspominano powyżej, duża zmienność AUD u ludzi często związana jest z różnicami w motywacji do sięgania po alkohol. Na podstawie przedstawionych badań można wnioskować, że picie w celu uzyskania nagrody oraz picie dla ulgi, również, do pewnego stopnia, obserwowane jest u zwierząt laboratoryjnych. Wyniki zaprezentowane w publikacji Cieslik-Starkiewicz i wsp. (2024b) sugerują, że u szczurów niewrażliwych na pozytywne informacje zwrotne wyższa motywacja do poszukiwania alkoholu po nieprzyjemnym doświadczeniu kary w odpowiedzi na poszukiwanie alkoholu, może być interpretowana jako picie dla ulgi. Podobnie, awersyjny okres abstynencji mógł nasilić negatywny afekt u szczurów. Aby go zniwelować, zwierzęta niewrażliwe intensywniej poszukiwały alkoholu, w porównaniu do zwierząt wrażliwych. Ponadto, u zwierząt niewrażliwych na pozytywne informacje zwrotne zaobserwowaliśmy podwyższony poziom hormonów stresu, tzn. hormonu adrenokortykotropowego (ACTH) i kortykosteronu, we krwi po zakończeniu badań behawioralnych , w porównaniu do szczurów, które spożywały wodę, sugerując wyższy poziom stresu w tej grupie. Warto wspomnieć, że rosnąca liczba badań łączy zaburzone przetwarzanie informacji z symptomatologią zaburzeń psychicznych związanych ze stresem (Robinson *et al.*, 2012; Homan *et al.*, 2019; Roberts *et al.*, 2022), do których, jak potwierdziłam w przedstawionych badaniach, może należeć AUD.

Efekty behawioralne związane wrażliwością na pozytywne informacje zwrotne i piciem alkoholu u szczurów były powiązane także z różnicami w ekspresji genów i białek w regionach korowych i podkorowych mózgu. U zwierząt niewrażliwych na pozytywne informacje zwrotne, w jądrze półleżącym (ang. *Nucleus Accumbens,* NAc), wykazaliśmy wyższy poziom ekspresji genów związanych z *Htr1a*, *Gabbr2*, *Grm2* i *Slc6a3*, a także *Npy*, który w przypadku Gabbr2 został zweryfikowany również na poziomie białka $GABA_BR_2$. Dla *Htr2a* i *Slc6a4* w NAc poziom mRNA był wyższy u zwierząt z grupy kontrolnej, niewrażliwych na pozytywne informacje zwrotne, w porównaniu do szczurów niewrażliwych pijących alkohol. Dla *Htr2a* różnice wykazaliśmy także na poziomie białka $5\text{-}HT_{2A}$. Z kolei w korze zakrętu obręczy (ang. *Anterior Cingulate Cortex*, ACC) poziom ekspresji był wyższy u szczurów niewrażliwych na pozytywne informacje zwrotne w porównaniu do ich wrażliwych odpowiedników dla *Drd1*, *Gria1* i *Htr3a*, w środkowej korze przedczołowej (ang. *Medial Prefrontal Cortex,* mPFC) dla *Cat*, a w ciele migdałowatym (ang. *Amygdala*, Amy) dla *Maob*. W przypadku *Maob* różnice zaobserwowaliśmy również na poziomie białka monoaminooksydazy B (MAO-B). Wykazaliśmy także, że poziom *Adh1* w mPFC i *Gabbr1* w Amy był wyższy w grupie szczurów pijących alkohol i niewrażliwych na pozytywne informacje zwrotne w porównaniu ze szczurami niewrażliwymi z grupy kontrolnej i wrażliwymi na pozytywne informacje zwrotne z grupy pijącej alkohol. Analiza ujawniła również istotne interakcje między wpływem wrażliwości na pozytywne informacje zwrotne i piciem alkoholu w przypadku *Tph2* i *Drd2* w Amy oraz *Adh1* w korze oczodołowo-czołowej (ang. O*rbitofrontal Cortex*, OFC). Ponadto wykazaliśmy istotny wpływ długotrwałej ekspozycji na alkohol na ekspresję różnych genów, przy czym poziom mRNA był wyższy w grupie pijącej alkohol w porównaniu z grupą pijącą wodę, w przypadku *Comt* i *Maoa* w ACC, *Adh1*, *Cat* i *Comt* w mPFC, *Gad1* i *Drd2* w Amy, dla *Cat*, *Gad2* i *Htr1a* w OFC oraz dla *Adh1* w NAc. W przypadku *Slc6a4* w NAc poziom mRNA był niższy u szczurów pijących alkohol w porównaniu do szczurów pijących wodę. Ponadto dla dehydrogenazy alkoholowej 1 (ADH1) zaobserwowaliśmy istotne różnice w mPFC i NAc między grupami pijącymi alkohol i wodę.

Wpływ wrażliwości na negatywne informacje zwrotne na zachowania związane z piciem alkoholu

Wyniki opisane w pracy pt. „*Trait sensitivity to negative feedback determines the intensity of compulsive alcohol seeking and taking in male rats*" autorstwa Cieslik A, Noworyta

K, Rygula R. opublikowanej w *Journal of Psychiatry and Neuroscience* (2022) również potwierdziły, że u szczurów szczepu *Sprague Dawley* następuje wzrost ilości alkoholu, spożywanego w warunkach swobodnego dostępu. Równocześnie stwierdziłam brak istotnych różnic w konsumpcji alkoholu między zwierzętami zaklasyfikowanymi jako mniej i bardziej wrażliwe na negatywne informacje zwrotne. Wykazałam także, że zwiększona wrażliwość na negatywne informacje zwrotne obniżała podatność szczurów na rozwój kompulsywnego poszukiwania alkoholu w sytuacji, gdy zachowanie to było karane. Przeprowadzone przeze mnie badania ujawniły również szybsze wygaszenie zachowań związanych z poszukiwaniem alkoholu, po zaprzestaniu jego dostępności, u zwierząt bardziej wrażliwych na negatywne informacje zwrotne. Na podstawie wspomnianej wcześniej teorii wyjaśniającej motywację do spożywania alkoholu, u szczurów o wyższej wrażliwości na negatywne informacje zwrotne można by się spodziewać zwiększonego spożywania alkoholu w celu zniwelowania negatywnych stanów afektywnych. Przeciwnie, w przeprowadzonych badaniach zwiększona wrażliwość na negatywne informacje zwrotne mogła mieć działanie protekcyjne wobec rozwoju zachowań związanych z niekontrolowanym spożywaniem alkoholu. Faktycznie, zwiększona wrażliwość na negatywne konsekwencje podejmowanych działań, takich jak kara podczas poszukiwania alkoholu, powodowała łatwiejsze zaniechanie zachowań poszukiwawczych. Wynik ten dodatkowo podkreśla wykazaną wcześniej niezależność cech wrażliwości na pozytywne i negatywne informacje zwrotne (Noworyta-Sokolowska *et al.*, 2019).

Ponadto w publikacji pt. "*Identification of genes regulated by trait sensitivity to negative feedback and prolonged alcohol consumption in rats*" autorstwa Cieslik-Starkiewicz A, Noworyta K, Solich J, Korlatowicz A, Faron-Górecka A, Rygula R opublikowanej w *Pharmacological Reports* (2024a) wykazałam, że opisane powyżej efekty behawioralne związane były z różnicami w ekspresji genów związanych z przekaźnictwem DA, 5-HT, glutaminianergicznym czy GABAergicznym (kwas γ-amiomasłowy). Wykazaliśmy, że w ACC szczury, które były bardziej wrażliwe na negatywne informacje zwrotne, miały niższy poziom ekspresji *Gad2* oraz wyższy poziom ekspresji *Drd2* i *Slc6a4*, w porównaniu do zwierząt mniej wrażliwych. Podobnie w mPFC i OFC, szczury bardziej wrażliwe na negatywne informacje zwrotne miały niższy poziom ekspresji *Maoa* niż mniej wrażliwe. Co więcej, w OFC poziom ekspresji *Gria1* i *Htr3a*, był niższy u szczurów bardziej wrażliwych na negatywne informacje zwrotne. Potwierdziliśmy również, że długotrwała ekspozycja na alkohol prowadzi do istotnych

zmian w ekspresji genów w różnych zbadanych obszarach mózgu szczurów. W ACC szczury pijące alkohol miały istotnie wyższe poziomy ekspresji *Comt* i *Maoa* w porównaniu do szczurów niepijących. W mPFC poziomy *Comt* i *Htr2b* były wyższe u zwierząt pijących alkohol w porównaniu z grupą kontrolną. Szczury poddane długotrwałej ekspozycji na alkohol miały w NAc znacząco wyższy poziom *Adh1*, i niższy poziom ekspresji *Slc6a4*. W OFC szczurów pijących alkohol wykazaliśmy wyższy poziom ekspresji *Gad2* i niższy poziom *Htr1a* w porównaniu ze szczurami kontrolnymi. Stwierdziliśmy także, że w ACC poziomy ekspresji *Gabra1*, *Gabbr2*, *Grin2a*, *Grin2b* i *Grm3* oraz Grin2a w OFC są istotnie zależne zarówno od wrażliwości na negatywne informacje zwrotne, jak i długotrwałej ekspozycji na alkohol. Dla enzymów monoaminooksydazy A (MAO-A) w mPFC i ADH1 w mPFC i NAc, różnice udało się wykazać także na poziomie białka.

Wyniki badań opublikowanych w publikacjach 1 i 2 pozwalają na lepsze zrozumienie mechanizmów związanych z konsumpcją alkoholu dla nagrody i dla ulgi (Cieslik *et al.*, 2022; Cieslik-Starkiewicz *et al.*, 2024b). Odkrycia sugerują, że ocena wrażliwości na pozytywne i negatywne informacje zwrotne może stanowić kluczowy element w opracowywaniu przedklinicznych modeli odpowiadającym dwóm wyżej wymienionym wzorcom picia alkoholu. Te z kolei mogłyby być następnie wykorzystane do planowania nowych strategii terapeutycznych lub prewencyjnych do walki z uzależnieniem od alkoholu.

Wpływ tendencyjności oceny na kształtowanie zachowań związanych z piciem alkoholu u szczurów

Jedną z cech osobowości mogących modulować zachowania związane ze spożywaniem alkoholu jest tendencyjność oceny, a zatem optymizm i pesymizm. Przykładowo, u ludzi optymizm może upośledzać zdolność do dokładnej oceny ryzyka i korzyści, prowadząc do przeceniania nagradzających efektów alkoholu i niedoceniania jego negatywnych konsekwencji (Blume i in. 2003; Leeman i in. 2009). Jednakże do tej pory nie było wiadomo czy tendencyjność oceny, jako stabilna i trwała cecha poznawcza, może determinować indywidualną podatność na przejście od rekreacyjnego do kompulsywnego picia. W publikacji pt. „*Unveiling the power of optimism: exploring behavioral and neuromolecular correlates of alcohol seeking and drinking in rats with biased judgment*" Cieslik-Starkiewicz A, Piksa M, Noworyta K, Solich J, Pabian P, Latocha K, Faron-Górecka A, Rygula R zaakceptowanej do publikacji w *Progress in Neuro-Psychopharmacology & Biological Psychiatry* (2024c), stanowiącej ostatni filar niniejszej

pracy doktorskiej, wykazałam że szczury scharakteryzowane jako „optymistyczne" piły znacząco mniej alkoholu niż ich „pesymistyczni" odpowiednicy. To zwiększone spożycie alkoholu u pesymistów widoczne było wyłącznie w paradygmacie swobodnego dostępu do alkoholu i nie przekładało się na istotne różnice w rozwoju kompulsywnego poszukiwania alkoholu i picia alkoholu, szybkości wygaszania reakcji instrumentalnej ani nawrotu zachowań poszukiwawczych alkoholu po okresie abstynencji.

Biorąc pod uwagę, że tendencyjność oceny może odzwierciedlać stan emocjonalny jednostki, gdzie osoby w negatywnym stanie afektywnym mają skłonność do pesymizmu, a osoby w pozytywnym stanie afektywnym do optymizmu, zwiększone spożycie alkoholu zaobserwowane u szczurów „pesymistycznych" może odzwierciedlać jeden ze wspomnianych wcześniej wzorców picia u ludzi, mianowicie „picia dla ulgi" (Grodin *et al.*, 2024). Ten wzorzec spożycia alkoholu jest charakterystyczny dla osób, które piją, aby złagodzić negatywne emocje lub stres. Analogicznie u „pesymistycznych" szczurów, zwiększone spożycie alkoholu mogło być związane z próbą łagodzenia negatywnego stanu afektywnego. Opierając się na tym założeniu, brak zauważalnego wpływu tendencyjności oceny w bardziej zaawansowanym paradygmacie poszukiwania i picia alkoholu można przypisać do wspomnianego negatywnego stanu afektywnego, który prawdopodobnie skutkował obniżoną motywacją u szczurów do wykonywania instrumentalnej reakcji naciskania na dźwignie. Reasumując, efekty pesymizmu były najbardziej widoczne podczas spontanicznych sesji picia, ponieważ wymagały one od szczurów mniejszego zaangażowania poznawczego i konieczności podejmowania decyzji. Taki scenariusz sugeruje, że w warunkach obniżonego wysiłku poznawczego, wrodzona pesymistyczna tendencyjność oceny ma bardziej wyraźny wpływ na zachowanie związane z piciem alkoholu u szczurów. Na poziomie molekularnym niższe spożycie alkoholu obserwowane u „optymistycznych" szczurów było związane ze zmianami w ekspresji genów zaangażowanych w przekaźnictwo 5-HT, glutaminianergiczne, DA, GABAergiczne i w metabolizm alkoholu. U zwierząt pijących alkohol zaobserwowaliśmy wyższą ekspresję genów *Grin2a* i *Slc1a2* w mPFC oraz *Drd2* w OFC, w porównaniu ze zwierzętami pijącymi wodę. Natomiast ekspresja takich genów jak *Grm2* w ACC i Amy oraz *Grin2b* w NAc była niższa w grupie pijącej alkohol, w porównaniu do grupy kontrolnej. Ponadto wykazaliśmy, że poziom ekspresji *Maoa*, był wyższy w mPFC szczurów „pesymistycznych" w porównaniu do ich „optymistycznych" odpowiedników. Zaobserwowaliśmy także, że poziom ekspresji *Htr2b* w OFC

oraz *Grm2* w Amy był niższy u szczurów „pesymistycznych" w porównaniu do zwierząt zaklasyfikowanych jako „optymistyczne" Istotne interakcje między wpływem tendencyjności oceny i długotrwałej ekspozycji na alkohol zaobserwowaliśmy w ekspresji *Slc6a4*, *Gabbr2* i *Adh1* w mPFC; *Slc1a2* i *Slc6a4* w NAc; *Maoa* w OFC; i *Grm3* w Amy. Co więcej, ilościowa autoradiografia receptorów ujawniła zmniejszoną gęstość receptorów serotoninowych 5-HT_{2A} u „pesymistycznych" szczurów pijących alkohol, w porównaniu do „optymistycznych" osobników z tej samej grupy oraz zwierząt „pesymistycznych" z grupy kontrolnej, w skorupie i rdzeniu NAc (Cieslik-Starkiewicz *et al.*, 2024c).

Molekularne podłoże relacji między tendencyjnością poznawczą a zachowaniami związanymi z piciem alkoholu u szczurów

Aby zrozumieć molekularne podstawy złożonych interakcji między tendencyjnością poznawczą a zachowaniami związanymi z poszukiwaniem i spożywaniem alkoholu, w przeprowadzonych eksperymentach, wraz ze współpracownikami z Pracowni Farmakologii Biochemicznej, przeanalizowałam poziom mRNA wybranych genów w pięciu obszarach mózgu: trzech regionów korowych (mPFC, ACC i OFC) oraz dwóch podkorowych (NAc i Amy). Na podstawie dokładnej analizy literatury, wybraliśmy około 30 genów, podzielonych na pięć grup:

1) geny związane z funkcjonowaniem i regulacją układu 5-HT
2) geny kodujące białka związane z neurotransmisją DA
3) geny związane z układem glutaminanergicznym i
4) GABAergicznym
5) geny kodujące białka uczestniczące w metabolizmie alkoholu

Wyniki przedstawione w publikacjach Cieślik i wsp. (2022) oraz Cieślik-Starkiewicz i wsp. (2024a i c) ujawniły istotne różnice w ekspresji genów oraz, w niektórych przypadkach białek, w różnych obszarach mózgu, które mogą wpływać na indywidualną podatność na rozwój zachowań związanych z poszukiwaniem i spożywaniem alkoholu. Analiza poziomu mRNA miała charakter przesiewowy, mający na celu wyodrębnienie genów zaangażowanych w kształtowanie tendencyjności poznawczej i/lub podatności na przejście od kontrolowanego używania do niekontrolowanego nadużywania alkoholu. W rzeczywistości, spośród licznych

zbadanych genów, udało się wyodrębnić jeden, który łączy wszystkie trzy badane aspekty tendencyjności poznawczej z podatnością na zwiększoną konsumpcję alkoholu i stanowi potencjalny wspólny element molekularnego mechanizmu tych zależności.

Różnice w ekspresji genu *Slc6a4*, kodującego SERT, zaobserwowałam w NAc zarówno w doświadczeniach dotyczących wrażliwości na pozytywne informacje zwrotne, negatywne informacje zwrotne, jak i tendencyjności oceny. Dla *Slc6a4* w NAc wśród zwierząt niewrażliwych na pozytywne informacje zwrotne poziom mRNA był wyższy u w grupie kontrolnej w porównaniu do szczurów pijących alkohol. Z kolei na poziomie białka zaobserwowaliśmy, że poziom SERT był wyższy u zwierząt niewrażliwych na pozytywne informacje zwrotne, w porównaniu do zwierząt wrażliwych. Wykazaliśmy także, że u zwierząt bardziej wrażliwych na negatywne informacje zwrotne poziom ekspresji był wyższy w porównaniu ze zwierzętami mniej wrażliwymi, niezależnie od historii spożycia alkoholu. Ponadto, u zwierząt „pesymistycznych" z grupy kontrolnej poziom mRNA Slc6a4 był wyższy w porównaniu ze szczurami „optymistycznymi" w tej samej grupy i z „pesymistycznymi" pijącymi alkohol. Jednak najbardziej intrygujące różnice w ekspresji tego genu zaobserwowaliśmy między zwierzętami „optymistycznymi" i „pesymistycznymi" w mPFC. Poziom ekspresji był wyższy u szczurów „optymistycznych" z grupy kontrolnej w porównaniu do zwierząt „pesymistycznych". Z kolei w grupie pijącej alkohol było odwrotnie – poziom ekspresji był wyższy u szczurów „pesymistycznych" niż u „optymistycznych". Ponadto zwierzęta „optymistyczne" z grupy kontrolnej miały wyższy poziom ekspresji w porównaniu do ich odpowiedników z grupy pijącej alkohol. Sugeruje to, że wpływ długotrwałego spożycia alkoholu na ekspresję genu *Slc6a4* jest zależny od wyjściowego poziomu optymizmu i pesymizmu.

Nasze wyniki sugerują, że zarówno gen *Slc6a4*, jak i białko SERT mogą stanowić molekularną podstawę mechanizmu łączącego tendencyjność poznawczą z zachowaniami związanymi z piciem alkoholu u zwierząt. Wpływ alkoholu na poziom mRNA SERT został potwierdzony w różnych zwierzęcych modelach spożycia alkoholu i uzależnienia. Ekspozycja na alkohol powodowała podwyższony poziom ekspresji SERT w regionach związanych z układem nagrody, takich jak NAc, czy przetwarzaniem informacji, takich jak mPFC (Chen *et al.*, 2023; Diehl & Redish, 2023). Ponadto kilka badań wykazało, że genetyczne lub farmakologicznie obniżona ekspresja SERT prowadzi do niższej wrażliwości na negatywne informacje zwrotne,

cechy skorelowanej z pesymistyczną tendencyjnością oceny (Ineichen *et al.*, 2012; den Ouden *et al.*, 2013; Rygula & Popik, 2016).

Postuluje się, że niski poziom zewnątrzkomórkowej 5-HT wiąże się ze zwiększonym spożyciem alkoholu, a zatem zwiększenie poziomu 5-HT może zmniejszać spożycie alkoholu (Sellers *et al.*, 1992). Faktycznie, badania wykazały, że picie alkoholu i zachowania związane z jego spożywaniem można zmniejszyć poprzez usunięcie genu kodującego SERT. Myszy pozbawione genu kodującego SERT (ang. *SERT knockout*) spożywały istotnie mniej alkoholu w warunkach swobodnego wyboru w porównaniu do zwierząt typu dzikiego (Boyce-Rustay *et al.*, 2006; Lamb & Daws, 2013). Inne badania wykazały, że obniżenie zewnątrzkomórkowego poziomu 5-HT, poprzez usunięcie genu *Tph2* kodującego hydroksylazę Trp 2, enzymu odpowiedzialnego za syntezę 5-HT, skutkowało zwiększonym spożyciem alkoholu u myszy (Zaniewska *et al.*, 2022). Ponadto poziom zewnątrzkomórkowej 5-HT można zwiększyć farmakologicznie, blokując SERT za pomocą SSRI. Wykazano, że chroniczne podania SSRI zmniejszają ilość spożywanego alkoholu (Murphy *et al.*, 1988; Gardell *et al.*, 1997; Lamb & Järbe, 2001). Otrzymane przeze mnie wyniki również potwierdziły istotną rolę układu 5-HT w kształtowaniu zachowań związanych z piciem alkoholu. Co najważniejsze, po raz pierwszy pokazały, że układ ten zaangażowany jest w molekularny mechanizm łączący badane aspekty tendencyjności poznawczej z indywidualną podatnością na niekontrolowane spożywanie alkoholu.

Podsumowując, otrzymane w ramach pracy doktorskiej wyniki sugerują, że różne aspekty tendencyjności poznawczej mogą modulować podatność na rozwój i utrzymywanie się uzależnienia od alkoholu. Choć obszar ten wymaga dalszych badań, udało się wskazać potencjalne molekularne cele, które mogą być zaangażowane w kształtowanie tej zależności.

Podsumowanie

Badania przeprowadzone w ramach mojej rozprawy doktorskiej wykazały, że różne aspekty tendencyjności poznawczej, takie jak wrażliwość na pozytywne i negatywne informacje zwrotne oraz tendencyjność oceny, definiowana jako optymizm i pesymizm, wpływają na podatność zwierząt na przejście od kontrolowanego używania do niekontrolowanego nadużywania alkoholu. Wykazałam, że obniżona wrażliwość na pozytywne informacje zwrotne zwiększa motywację do poszukiwania alkoholu po doświadczeniu negatywnych konsekwencji związanych z tym zachowaniem. Przyspiesza ona również nawrót zachowań związanych z poszukiwaniem alkoholu po okresie wymuszonej abstynencji. Ponadto wyniki moich badań ujawniły, że u szczurów zwiększona wrażliwość na negatywne informacje zwrotne zmniejszała ich podatność na rozwój kompulsywnego poszukiwania alkoholu, utrzymywanego pomimo ryzyka kary. Cecha ta była również związana z szybszym wygaszaniem poszukiwania alkoholu, gdy ten nie był już dostępny. W ostatnim badaniu wykazałam, że szczury „pesymistyczne" spożywały znacznie więcej alkoholu niż zwierzęta sklasyfikowane jako „optymistyczne". Jednak różnice te widoczne były tylko w sytuacji swobodnego dostępu do alkoholu, a zanikały w zadaniach wymagających większego zaangażowania poznawczego (tj. podejmowania decyzji). Uzyskane wyniki sugerują, że badane aspekty tendencyjności poznawczej mogą przyczynić się do ustanowienia nowych poznawczych markerów podatności na rozwój uzależnienia od alkoholu.

Wnioski płynące z badań stanowiących podstawę niniejszej pracy doktorskiej ograniczone są przez fakt, że wszystkie opisane wyniki zostały uzyskane na podstawie doświadczeń przeprowadzonych jedynie na samcach szczurów. U ludzi, różnice płciowe we wzorcach spożywania alkoholu, takich jak częstotliwość, ilość czy motywacja do picia, ale też różnice związane z samym metabolizmem etanolu, są szeroko udokumentowane (Ashley *et al.*, 1977; King *et al.*, 2003; Diehl *et al.*, 2007; Hartwell & Ray, 2013; Erol & Karpyak, 2015). Istniejące badania wskazują na występowanie różnic zależnych od płci także w modelu zwierzęcym, m.in. wykazano, że samice spożywają więcej alkoholu niż samce (Cailhol & Mormède, 2001; Maldonado-Devincci *et al.*, 2010), a płeć może być także czynnikiem determinującym skuteczność stosowanej farmakoterapii (Moore & Lynch, 2015; Matzeu *et al.*, 2018). Dlatego też określenie, czy różne aspekty tendencyjności poznawczej kształtują zachowania związane

ze spożywaniem alkoholu także u samic oraz czy wzorce tych interakcji są zależne od płci, wydaje się wysoce istotnym zadaniem, które należy uwzględnić w dalszych badaniach.

Jako że analiza poziomu mRNA miała charakter przesiewowy, nie pozwoliła precyzyjnie wskazać molekularnych mechanizmów, które łączą tendencyjność poznawczą z indywidualną podatnością na przejście od kontrolowanego używania do niekontrolowanego nadużywania alkoholu. Ponadto sama obserwacja zmian w ekspresji genów nie dostarcza informacji na temat dynamiki tych procesów ani o tym, jak różne mechanizmy współpracują ze sobą w czasie, prowadząc do określonych zachowań. Chociaż część różnic w ekspresji genów udało się zweryfikować na poziomie białka (Cieslik-Starkiewicz *et al.*, 2024a; b), nie było to możliwe w przypadku wszystkich transkryptów z uwagi na niedostępność specyficznych przeciwciał skierowanym przeciwko badanym białkom. Dlatego, choć przedstawione powyżej wyniki stanowią drogowskaz do dalszego poszukiwania molekularnych markerów indywidualnej podatności na AUD, potrzebne są dalsze badania, aby w pełni zrozumieć mechanizmy leżące u podstaw interakcji cech osobowości z rozwojem uzależnienia. Takie dalsze, bardziej szczegółowe badania mogą już być skoncentrowane na konkretnych szlakach biologicznych i wykorzystywać np. genetyczne modele zwierzęce pozbawione zidentyfikowanych przez nas genów lub pomiar poziomu konkretnych neuroprzekaźników we wskazanych regionach mózgu.

Bibliografia

Anderson, M.H., Munafò, M.R. & Robinson, E.S. (2013) Investigating the psychopharmacology of cognitive affective bias in rats using an affective tone discrimination task. *Psychopharmacology (Berl)*, **226**, 601-613.

Ashley, M.J., Olin, J.S., le Riche, W.H., Kornaczewski, A., Schmidt, W. & Rankin, J.G. (1977) Morbidity in alcoholics: evidence for accelerated development of physical disease in women. *Archives of Internal Medicine*, **137**, 883-887.

Bari, A., Theobald, D.E., Caprioli, D., Mar, A.C., Aidoo-Micah, A., Dalley, J.W. & Robbins, T.W. (2010) Serotonin modulates sensitivity to reward and negative feedback in a probabilistic reversal learning task in rats. *Neuropsychopharmacology*, **35**, 1290-1301.

Bateson, M. (2016) Optimistic and pessimistic biases: a primer for behavioural ecologists. *Current Opinion in Behavioral Sciences*, **12**, 115-121.

Bateson, M., Desire, S., Gartside, Sarah E. & Wright, Geraldine A. (2011) Agitated Honeybees Exhibit Pessimistic Cognitive Biases. *Current Biology*, **21**, 1070-1073.

Bateson, M. & Nettle, D. (2015) Development of a cognitive bias methodology for measuring low mood in chimpanzees. *PeerJ*, **3**, e998.

Bechara, A., Dolan, S. & Hindes, A. (2002) Decision-making and addiction (part II): myopia for the future or hypersensitivity to reward? *Neuropsychologia*, **40**, 1690-1705.

Beck, A.T. (2002) Cognitive models of depression. *Clinical advances in cognitive psychotherapy: Theory and application*, **14**, 29-61.

Beevers, C.G., Wells, T.T., Ellis, A.J. & McGeary, J.E. (2009) Association of the serotonin transporter gene promoter region (5-HTTLPR) polymorphism with biased attention for emotional stimuli. *Journal of Abnormal Psychology*, **118**, 670.

Blanchette, I. & Richards, A. (2010) The influence of affect on higher level cognition: A review of research on interpretation, judgement, decision making and reasoning *Cognition and Emotion*, pp. 561 — 595.

Blume, A.W. & Blume, A.K. (2014) Alcohol outcome expectancies and consequences: Do people think themselves into and out of consequences. *Psychology Applications & Developments*, **1**, 17-23.

Blume, A.W., Lostutter, T.W., Schmaling, K.B. & Marlatt, G.A. (2003) Beliefs about drinking behavior predict drinking consequences. *Journal of Psychoactive Drugs*, **35**, 395-399.

Boddington, R., Gómez Dunlop, C.A., Garnham, L.C., Ryding, S., Abbey-Lee, R.N., Kreshchenko, A. & Løvlie, H. (2020) The relationship between monoaminergic gene expression, learning, and optimism in red junglefowl chicks. *Animal Cognition*, **23**, 901-911.

Boleij, H., Klooster, J.v.t., Lavrijsen, M., Kirchhoff, S., Arndt, S.S. & Ohl, F. (2012) A test to identify judgement bias in mice. *Behavioural Brain Research*, **233**, 45-54.

Boyce-Rustay, J.M., Wiedholz, L.M., Millstein, R.A., Carroll, J., Murphy, D.L., Daws, L.C. & Holmes, A. (2006) Ethanol-Related Behaviors in Serotonin Transporter Knockout Mice. *Alcoholism: Clinical and Experimental Research*, **30**, 1957-1965.

Brown, S.A., Goldman, M.S., Inn, A. & Anderson, L.R. (1980) Expectations of reinforcement from alcohol: their domain and relation to drinking patterns. *Journal of consulting and Clinical Psychology*, **48**, 419.

Burman, O., McGowan, R., Mendl, M., Norling, Y., Paul, E., Rehn, T. & Keeling, L. (2011) Using judgement bias to measure positive affective state in dogs. *Applied Animal Behaviour Science*, **132**, 160-168.

Cailhol, S. & Mormède, P. (2001) Sex and strain differences in ethanol drinking: effects of gonadectomy. *Alcoholism: Clinical and Experimental Research*, **25**, 594-599.

Carvalho, A.F., Heilig, M., Perez, A., Probst, C. & Rehm, J. (2019) Alcohol use disorders. *The Lancet*, **394**, 781-792.

Chamberlain, S.R., Müller, U., Blackwell, A.D., Clark, L., Robbins, T.W. & Sahakian, B.J. (2006) Neurochemical modulation of response inhibition and probabilistic learning in humans. *Science*, **311**, 861-863.

Chen, G., Lai, S., Bao, G., Ke, J., Meng, X., Lu, S., Wu, X., Xu, H., Wu, F., Xu, Y., Xu, F., Bi, G.Q., Peng, G., Zhou, K. & Zhu, Y. (2023) Distinct reward processing by subregions of the nucleus accumbens. *Cell Rep*, **42**, 112069.

Christiansen, B.A. & Goldman, M.S. (1983) Alcohol-related expectancies versus demographic/background variables in the prediction of adolescent drinking. *J Consult Clin Psychol*, **51**, 249-257.

Cieslik, A., Noworyta, K. & Rygula, R. (2022) Trait sensitivity to negative feedback determines the intensity of compulsive alcohol seeking and taking in male rats. *J Psychiatry Neurosci*, **47**, E252-E262.

Cieslik-Starkiewicz, A., Noworyta, K., Solich, J., Korlatowicz, A., Faron-Górecka, A. & Rygula, R. (2024a) Identification of genes regulated by trait sensitivity to negative feedback and prolonged alcohol consumption in rats. *Pharmacol Rep*, **76**, 207-215.

Cieslik-Starkiewicz, A., Noworyta, K., Solich, J., Korlatowicz, A., Faron-Górecka, A. & Rygula, R. (2024b) Trait sensitivity to positive feedback is a predisposing factor for several aspects of compulsive alcohol drinking in male rats: behavioural, physiological, and molecular correlates. *Psychopharmacology (Berl)*, **241**, 33-47.

Cieslik-Starkiewicz, A., Piksa, M., Noworyta, K., Solich, J., Pabian, P., Latocha, K., Faron-Górecka, A. & Rygula, R. (2024c) Unveiling the power of optimism: Exploring behavioral and neuromolecular correlates of alcohol seeking and drinking in rats with biased judgement. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, **135**, 111124.

Collins, S.E. (2016) Associations Between Socioeconomic Factors and Alcohol Outcomes. *Alcohol Res*, **38**, 83-94.

Cools, R., Frank, M.J., Gibbs, S.E., Miyakawa, A., Jagust, W. & D'Esposito, M. (2009) Striatal dopamine predicts outcome-specific reversal learning and its sensitivity to dopaminergic drug administration. *J Neurosci*, **29**, 1538-1543.

Cools, R., Robinson, O.J. & Sahakian, B. (2008) Acute Tryptophan Depletion in Healthy Volunteers Enhances Punishment Prediction but Does not Affect Reward Prediction. *Neuropsychopharmacology*, **33**, 2291-2299.

Culbreth, A.J., Gold, J.M., Cools, R. & Barch, D.M. (2016) Impaired Activation in Cognitive Control Regions Predicts Reversal Learning in Schizophrenia. *Schizophr Bull*, **42**, 484-493.

Curzytek, K., Kubera, M., Trojan, E., Wójcik, K., Basta-Kaim, A., Detka, J., Maes, M. & Rygula, R. (2018) The effects of pessimism on cell-mediated immunity in rats. *Prog Neuropsychopharmacol Biol Psychiatry*, **80**, 295-303.

den Ouden, H.E., Daw, N.D., Fernandez, G., Elshout, J.A., Rijpkema, M., Hoogman, M., Franke, B. & Cools, R. (2013) Dissociable effects of dopamine and serotonin on reversal learning. *Neuron*, **80**, 1090-1100.

Diehl, A., Croissant, B., Batra, A., Mundle, G., Nakovics, H. & Mann, K. (2007) Alcoholism in women: is it different in onset and outcome compared to men? *European archives of psychiatry and clinical neuroscience*, **257**, 344-351.

Diehl, G.W. & Redish, A.D. (2023) Differential processing of decision information in subregions of rodent medial prefrontal cortex. *Elife*, **12**.

Doyle, R.E., Lee, C., Deiss, V., Fisher, A.D., Hinch, G.N. & Boissy, A. (2011) Measuring judgement bias and emotional reactivity in sheep following long-term exposure to unpredictable and aversive events. *Physiology & Behavior*, **102**, 503-510.

Drozd, R., Cieslak, P.E., Rychlik, M., Rodriguez Parkitna, J. & Rygula, R. (2016) Cognitive Judgment Bias Interacts with Risk Based Decision Making and Sensitivity to Dopaminergic Challenge in Male Rats. *Front Behav Neurosci*, **10**, 163.

Drozd, R., Rojek-Sito, K. & Rygula, R. (2017) The trait 'pessimism' does not interact with cognitive flexibility but makes rats more vulnerable to stress-induced motivational deficits: Results from the attentional set-shifting task. *Behav Brain Res*, **335**, 199-207.

Dube, S.R., Anda, R.F., Felitti, V.J., Edwards, V.J. & Croft, J.B. (2002) Adverse childhood experiences and personal alcohol abuse as an adult. *Addictive Behaviors*, **27**, 713-725.

Eisenegger, C., Naef, M., Linssen, A., Clark, L., Gandamaneni, P.K., Müller, U. & Robbins, T.W. (2014) Role of Dopamine D2 Receptors in Human Reinforcement Learning. *Neuropsychopharmacology*, **39**, 2366-2375.

Elliott, R., Sahakian Bj Fau - Herrod, J.J., Herrod Jj Fau - Robbins, T.W., Robbins Tw Fau - Paykel, E.S. & Paykel, E.S. (1997) Abnormal response to negative feedback in unipolar depression: evidence for a diagnosis specific impairment. *Journal of Neurology, Neurosurgery, and Psychiatry*, **63**, 74-82.

Erol, A. & Karpyak, V.M. (2015) Sex and gender-related differences in alcohol use and its consequences: Contemporary knowledge and future research considerations. *Drug and alcohol dependence*, **156**, 1-13.

Everitt, B.J. & Robbins, T.W. (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature neuroscience*, **8**, 1481-1489.

Evers, E.A.T., Cools, R., Clark, L., van der Veen, F.M., Jolles, J., Sahakian, B.J. & Robbins, T.W. (2005) Serotonergic Modulation of Prefrontal Cortex during Negative Feedback in Probabilistic Reversal Learning. *Neuropsychopharmacology*, **30**, 1138-1147.

Feil, J. & Hasking, P. (2008) The relationship between personality, coping strategies and alcohol use. *Addiction Research & Theory*, **16**, 526-537.

Fischer, A.G. & Ullsperger, M. (2017) An Update on the Role of Serotonin and its Interplay with Dopamine for Reward. *Frontiers in Human Neuroscience*, **11**.

Fox, E., Ridgewell, A. & Ashwin, C. (2009) Looking on the bright side: biased attention and the human serotonin transporter gene. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 1747-1751.

Frank, M.J., Seeberger, L.C. & O'Reilly, R.C. (2004) By Carrot or by Stick: Cognitive Reinforcement Learning in Parkinsonism. *Science*, **306**, 1940-1943.

Franken, I.H. & Muris, P. (2006) BIS/BAS personality characteristics and college students' substance use. *Personality and Individual Differences*, **40**, 1497-1503.

Fromme, K. & D'Amico, E.J. (2000) Measuring adolescent alcohol outcome expectancies. *Psychol Addict Behav*, **14**, 206-212.

Gardell, L.R., Whalen, C.A., Chattophadyay, S., Cavallaro, C.A., Hubbell, C.L. & Reid, L.D. (1997) Combination of Naltrexone and Fluoxetine on Rats'Propensity to Take Alcoholic Beverage. *Alcoholism: Clinical and Experimental Research*, **21**, 1435-1439.

Goldschmidt, L., Richardson, G.A., De Genna, N.M., Cornelius, M.D. & Day, N.L. (2019) Prenatal alcohol exposure and offspring alcohol use and misuse at 22 years of age: A prospective longitudinal study. *Neurotoxicology and Teratology*, **71**, 1-5.

Golebiowska, J. & Rygula, R. (2017) Effects of acute dopaminergic and serotonergic manipulations in the ACI paradigm depend on the basal valence of cognitive judgement bias in rats. *Behav Brain Res*, **327**, 133-143.

Gollan, J.K., Pane, H.T., McCloskey, M.S. & Coccaro, E.F. (2008) Identifying differences in biased affective information processing in major depression. *Psychiatry Research*, **159**, 18-24.

Grant, B.F., Goldstein, R.B., Saha, T.D., Chou, S.P., Jung, J., Zhang, H., Pickering, R.P., Ruan, W.J., Smith, S.M., Huang, B. & Hasin, D.S. (2015) Epidemiology of DSM-5 Alcohol Use Disorder: Results From the National Epidemiologic Survey on Alcohol and Related Conditions III. *JAMA Psychiatry*, **72**, 757-766.

Grodin, E.N., Baskerville, W.A., Meredith, L.R., Nieto, S. & Ray, L.A. (2024) Reward, relief, and habit drinking profiles in treatment seeking individuals with an AUD. *Alcohol Alcohol*, **59**.

Hales, C.A., Houghton, C.J. & Robinson, E.S.J. (2017) Behavioural and computational methods reveal differential effects for how delayed and rapid onset antidepressants effect decision making in rats. *Eur Neuropsychopharmacol*, **27**, 1268-1280.

Harding, E.J., Paul, E.S. & Mendl, M. (2004) Cognitive bias and affective state. *Nature*, **427**, 312-312.

Hartwell, E.E. & Ray, L.A. (2013) Sex moderates stress reactivity in heavy drinkers. *Addictive Behaviors*, **38**, 2643-2646.

Heinz, A.J., Veilleux, J.C. & Kassel, J.D. (2009) The role of cognitive structure in college student problem drinking. *Addictive Behaviors*, **34**, 212-218.

Homan, P., Levy, I., Feltham, E., Gordon, C., Hu, J., Li, J., Pietrzak, R.H., Southwick, S., Krystal, J.H., Harpaz-Rotem, I. & Schiller, D. (2019) Neural computations of threat in the aftermath of combat trauma. *Nature Neuroscience*, **22**, 470-476.

Iigaya, K., Jolivald, A., Jitkrittum, W., Gilchrist, I.D., Dayan, P., Paul, E. & Mendl, M. (2016) Cognitive Bias in Ambiguity Judgements: Using Computational Models to Dissect the Effects of Mild Mood Manipulation in Humans. *PLOS ONE*, **11**, e0165840.

Ineichen, C., Sigrist, H., Spinelli, S., Lesch, K.-P., Sautter, E., Seifritz, E. & Pryce, C.R. (2012) Establishing a probabilistic reversal learning test in mice: Evidence for the processes mediating reward-stay and punishment-shift behaviour and for their modulation by serotonin. *Neuropharmacology*, **63**, 1012-1021.

Ingram, R.E., Smith, T.W. & Brehm, S.S. (1983) Depression and information processing: Self-schemata and the encoding of self-referent information. *Journal of Personality and Social Psychology*, **45**, 412-420.

Jones, B.T., Corbin, W. & Fromme, K. (2001) A review of expectancy theory and alcohol consumption. *Addiction*, **96**, 57-72.

Kahneman, D. & Tversky, A. (1972) Subjective probability: A judgment of representativeness. *Cognitive Psychology*, **3**, 430-454.

Kellogg, R.T. (2003) *Cognitive psychology, 2nd ed*. Sage Publications, Inc, Thousand Oaks, CA, US.

King, A.C., Bernardy, N.C. & Hauner, K. (2003) Stressful events, personality, and mood disturbance:: Gender differences in alcoholics and problem drinkers. *Addictive behaviors*, **28**, 171-187.

Kirsch, I., Lynn, S.J., Vigorito, M. & Miller, R.R. (2004) The role of cognition in classical and operant conditioning. *Journal of clinical psychology*, **60**, 369-392.

Kloke, V., Schreiber, R.S., Bodden, C., Möllers, J., Ruhmann, H., Kaiser, S., Lesch, K.-P., Sachser, N. & Lewejohann, L. (2014) Hope for the best or prepare for the worst? Towards a spatial cognitive bias test for mice. *PLoS One*, **9**, e105431.

Koob, G.F. & Moal, M.L. (1997) Drug abuse: hedonic homeostatic dysregulation. *Science*, **278**, 52-58.

Krakenberg, V., Von Kortzfleisch, V.T., Kaiser, S., Sachser, N. & Richter, S.H. (2019) Differential effects of serotonin transporter genotype on anxiety-like behavior and cognitive judgment bias in mice. *Frontiers in behavioral neuroscience*, **13**, 263.

Lamb, R.J. & Daws, L.C. (2013) Ethanol self-administration in serotonin transporter knockout mice: unconstrained demand and elasticity. *Genes, Brain and Behavior*, **12**, 741-747.

Lamb, R.J. & Järbe, T.U.C. (2001) Effects of Fluvoxamine on Ethanol-Reinforced Behavior in the Rat. *Journal of Pharmacology and Experimental Therapeutics*, **297**, 1001.

Leigh, B.C. (1989) In search of the Seven Dwarves: issues of measurement and meaning in alcohol expectancy research. *Psychological bulletin*, **105**, 361.

Lindsay, P.H. & Norman, D.A. (2013) *Human information processing: An introduction to psychology*. Academic press.

Loxton, N.J. & Dawe, S. (2001) Alcohol abuse and dysfunctional eating in adolescent girls: The influence of individual differences in sensitivity to reward and punishment. *International Journal of Eating Disorders*, **29**, 455-462.

Maldonado-Devincci, A.M., Alipour, K.K., Michael, L.A. & Kirstein, C.L. (2010) Repeated binge ethanol administration during adolescence enhances voluntary sweetened ethanol intake in young adulthood in male and female rats. *Pharmacology Biochemistry and Behavior*, **96**, 476-487.

Mann, K., Roos, C.R., Hoffmann, S., Nakovics, H., Leménager, T., Heinz, A. & Witkiewitz, K. (2018) Precision Medicine in Alcohol Dependence: A Controlled Trial Testing Pharmacotherapy Response Among Reward and Relief Drinking Phenotypes. *Neuropsychopharmacology*, **43**, 891-899.

Matzeu, A., Terenius, L. & Martin-Fardon, R. (2018) Exploring Sex Differences in the Attenuation of Ethanol Drinking by Naltrexone in Dependent Rats During Early and Protracted Abstinence. *Alcoholism: Clinical and Experimental Research*, **42**, 2466-2478.

Moore, C.F. & Lynch, W.J. (2015) Alcohol preferring (P) rats as a model for examining sex differences in alcohol use disorder and its treatment. *Pharmacology Biochemistry and Behavior*, **132**, 1-9.

Murphy, J.M., Waller, M.B., Gatto, G.J., McBride, W.J., Lumeng, L. & Li, T.K. (1988) Effects of fluoxetine on the intragastric self-administration of ethanol in the alcohol preferring P line of rats. *Alcohol*, **5**, 283-286.

Noworyta, K., Cieslik, A. & Rygula, R. (2022) Reinforcement-based cognitive biases as vulnerability factors in alcohol addiction: From humans to animal models. *Br J Pharmacol*, **179**, 4265-4280.

Noworyta-Sokolowska, K., Kozub, A., Jablonska, J., Rodriguez Parkitna, J., Drozd, R. & Rygula, R. (2019) Sensitivity to negative and positive feedback as a stable and enduring behavioural trait in rats. *Psychopharmacology (Berl)*, **236**, 2389-2403.

Papciak, J., Popik, P., Fuchs, E. & Rygula, R. (2013) Chronic psychosocial stress makes rats more 'pessimistic'in the ambiguous-cue interpretation paradigm. *Behavioural brain research*, **256**, 305-310.

Pessiglione, M., Seymour, B., Flandin, G., Dolan, R.J. & Frith, C.D. (2006) Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. *Nature*, **442**, 1042-1045.

Pizzagalli, D.A., Goetz E Fau - Ostacher, M., Ostacher M Fau - Iosifescu, D.V., Iosifescu Dv Fau - Perlis, R.H. & Perlis, R.H. (2008a) Euthymic patients with bipolar disorder show decreased reward learning in a probabilistic reward task. *Biological Psychiatry*, **64**, 162-168.

Pizzagalli, D.A., Iosifescu, D., Hallett, L.A., Ratner, K.G. & Fava, M. (2008b) Reduced hedonic capacity in major depressive disorder: Evidence from a probabilistic reward task. *Journal of Psychiatric Research*, **43**, 76-87.

Rehm, J., Mathers, C., Popova, S., Thavorncharoensap, M., Teerawattananon, Y. & Patra, J. (2009) Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *The Lancet*, **373**, 2223-2233.

Roberts, C., Apergis-Schoute, A.M., Bruhl, A., Nowak, M., Baldwin, D.S., Sahakian, B.J. & Robbins, T.W. (2022) Threat reversal learning and avoidance habits in generalised anxiety disorder. *Translational Psychiatry*, **12**, 216.

Robinson, O.J., Cools, R., Carlisi, C.O., Sahakian, B.J. & Drevets, W.C. (2012) Ventral Striatum Response During Reward and Punishment Reversal Learning in Unmedicated Major Depressive Disorder. *American Journal of Psychiatry*, **169**, 152-159.

Room, R. (2005) Stigma, social inequality and alcohol and drug use. *Drug and Alcohol Review*, **24**, 143-155.

Rygula, R., Golebiowska, J., Kregiel, J., Kubik, J. & Popik, P. (2015) Effects of optimism on motivation in rats. *Front Behav Neurosci*, **9**, 32.

Rygula, R., Noworyta-Sokolowska, K., Drozd, R. & Kozub, A. (2018) Using rodents to model abnormal sensitivity to feedback in depression. *Neuroscience & Biobehavioral Reviews*, **95**, 336-346.

Rygula, R., Papciak, J. & Popik, P. (2013) Trait pessimism predicts vulnerability to stress-induced anhedonia in rats. *Neuropsychopharmacology*, **38**, 2188-2196.

Rygula, R., Papciak, J. & Popik, P. (2014) The effects of acute pharmacological stimulation of the 5-HT, NA and DA systems on the cognitive judgement bias of rats in the ambiguous-cue interpretation paradigm. *Eur Neuropsychopharmacol*, **24**, 1103-1111.

Rygula, R., Pluta, H. & Popik, P. (2012) Laughing rats are optimistic. *PLoS One*, **7**, e51959.

Rygula, R. & Popik, P. (2016) Trait "pessimism" is associated with increased sensitivity to negative feedback in rats. *Cogn Affect Behav Neurosci*, **16**, 516-526.

Scheier, M.F. & Carver, C.S. (1985) Optimism, coping, and health: assessment and implications of generalized outcome expectancies. *Health psychology*, **4**, 219.

Schmidt, T., Roser, P., Juckel, G., Brüne, M., Suchan, B. & Thoma, P. (2016) Social cognition and social problem solving abilities in individuals with alcohol use disorder. *Journal of Clinical and Experimental Neuropsychology*, **38**, 974-990.

Schuckit, M.A. (2009) Alcohol-use disorders. *The Lancet*, **373**, 492-501.

Sellers, E.M., Higgins, G.A. & Sobell, M.B. (1992) 5-HT and alcohol abuse. *Trends in Pharmacological Sciences*, **13**, 69-75.

Sharp, T. & Foster, G. (1989) In vivo measurement using microdialysis of the release and metabolism of 5-hydroxytryptamine in raphe neurones grafted to the rat hippocampus. *Journal of neurochemistry*, **53**, 303-306.

Shuell, T.J. (1986) Cognitive conceptions of learning. *Review of educational research*, **56**, 411-436.

Sprouse, J.S. & Aghajanian, G.K. (1987) Electrophysiological responses of serotoninergic dorsal raphe neurons to 5-HT1A and 5-HT1B agonists. *Synapse*, **1**, 3-9.

Stracke, J., Otten, W., Tuchscherer, A., Puppe, B. & Düpjan, S. (2017) Serotonin depletion induces pessimistic-like behavior in a cognitive bias paradigm in pigs. *Physiol Behav*, **174**, 18-26.

Taylor Tavares, J.V., Clark, L., Furey, M.L., Williams, G.B., Sahakian, B.J. & Drevets, W.C. (2008) Neural basis of abnormal response to negative feedback in unmedicated mood disorders. *Neuroimage*, **42**, 1118-1126.

Tversky, A. & Kahneman, D. (1974) Judgment under Uncertainty: Heuristics and Biases. *Science*, **185**, 1124-1131.

Veilleux, J.C., Skinner, K.D., Reese, E.D. & Shaver, J.A. (2014) Negative affect intensity influences drinking to cope through facets of emotion dysregulation. *Personality and Individual Differences*, **59**, 96-101.

Wise, R.A. (2004) Dopamine, learning and motivation. *Nature Reviews Neuroscience*, **5**, 483-494.

Witkiewitz, K., Roos, C.R., Mann, K. & Kranzler, H.R. (2019) Advancing Precision Medicine for Alcohol Use Disorder: Replication and Extension of Reward Drinking as a Predictor of Naltrexone Response. *Alcoholism: Clinical and Experimental Research*, **43**, 2395-2405.

Wolf, D., Klasen, M., Eisner, P., Zepf, F.D., Zvyagintsev, M., Palomero-Gallagher, N., Weber, R., Eisert, A. & Mathiak, K. (2018) Central serotonin modulates neural responses to virtual violent actions in emotion regulation networks. *Brain structure and function*, **223**, 3327-3345.

World Health Organization (2024) Global status report on alcohol and health and treatment of substance use disorders.

Zaniewska, M., Mosienko, V., Bader, M. & Alenina, N. (2022) Tph2 Gene Expression Defines Ethanol Drinking Behavior in Mice *Cells*, **11**, 874.

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OŚWIADCZENIE

Oświadczam, że w pracach:

1. *Cieslik-Starkiewicz A, Noworyta K, Solich J, Korlatowicz A,* ***Faron-Górecka A****, Rygula R. (2024) Trait sensitivity to positive feedback is a predisposing factor for several aspects of compulsive alcohol drinking in male rats: behavioural, physiological, and molecular correlates. Psychopharmacology. 241, 33-47. doi: 10.1007/s00213-023-06460-1.*
2. *Cieslik-Starkiewicz A, Noworyta K, Solich J, Korlatowicz A,* ***Faron-Górecka A****, Rygula R. (2024) Identification of genes regulated by trait sensitivity to negative feedback and prolonged alcohol consumption in rats. Pharmacological Reports. 76, 207-215. doi: 10.1007/s43440-023-00563-4.*
3. *Cieslik-Starkiewicz A, Piksa M, Noworyta K, Solich J, Pabian P, Latocha K,* ***Faron-Górecka A****, Rygula R. (2024) Unveiling the power of optimism: exploring behavioral and neuromolecular correlates of alcohol seeking and drinking in rats with biased judgment. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 135, 111124. doi: 10.1016/j.pnpbp.2024.111124. Zaakceptowano, w druku.*

mój udział polegał na konceptualizacji badań, opracowaniu metodologii, przeprowadzeniu części eksperymentów biochemicznych/molekularnych i analizie danych, redagowaniu odpowiedzi na recenzje oraz pracy nad ostateczną wersją manuskryptów.

Wyrażam zgodę na wykorzystanie publikacji w postępowaniu doktorskim Agaty Cieślik-Starkiewicz oraz oświadczam, że powyższe wyniki nie zostaną ponownie wykorzystane w innych postępowaniach o nadanie stopnia doktora lub doktora habilitowanego.

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mój udział polegał na przeprowadzeniu pomiaru ekspresji wybranych genów z wykorzystaniem mikromacierzy małej skali z sondami TaqMan, analizie danych oraz pracy nad ostateczną wersją manuskryptów.

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mój udział polegał na przeprowadzeniu części pomiarów ilości białka metodą western blot, analizie danych oraz pracy nad ostateczną wersją manuksryptów.

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mój udział polegał na pomocy przy wykonaniu testu wyboru między dwiema butelkami z dostępem przerywanym oraz pracy nad ostateczną wersją manuskryptu.

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mój udział polegał na współprowadzeniu części eksperymentów behawioralnych, pomocy przy redagowaniu odpowiedzi na recenzje oraz pracy nad ostateczną wersją manuskryptów.

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mój udział polegał na zarządzaniu projektem, zdobyciu funduszy, konceptualizacji badań, opracowaniu metodologii, analizie danych, korekcie pierwszych wersji manuskryptów, redagowaniu odpowiedzi na recenzje oraz pracy nad ostateczną wersją manuskryptów.

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mój udział polegał na wykonaniu eksperymentów behawioralnych, współpracy z zespołem wykonującym analizy biochemiczne/molekularne, pomocy przy oczyszczaniu mRNA i białka (1 i 3), wykonaniu części pomiarów ilości białka metodą western blot (1 i 3), analizie danych, interpretacji uzyskanych wyników, wykonaniu rycin do manuskryptów, pisaniu pierwszych wersji manuskryptów, redagowaniu odpowiedzi na recenzje oraz pracy nad ostateczną wersją manuskryptów.

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ORIGINAL INVESTIGATION

Trait sensitivity to positive feedback is a predisposing factor for several aspects of compulsive alcohol drinking in male rats: behavioural, physiological, and molecular correlates

Agata Cieslik-Starkiewicz[1] · Karolina Noworyta[1] · Joanna Solich[2] · Agata Korlatowicz[2] · Agata Faron-Górecka[2] · Rafal Rygula[1]

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Abstract

Introduction Alcohol use disorder (AUD) is one of the most common psychiatric disorders and a leading cause of mortality worldwide. While the pathophysiology underlying AUD is relatively well known, the cognitive mechanisms of an individual's susceptibility to the development of alcohol dependence remain poorly understood. In this study, we investigated the theoretical claim that sensitivity to positive feedback (PF), as a stable and enduring behavioural trait, can predict individual susceptibility to the acquisition and maintenance of alcohol-seeking behaviour in rats.

Methods Trait sensitivity to PF was assessed using a series of probabilistic reversal learning tests. The escalation of alcohol intake in rats was achieved by applying a mix of intermittent free access and instrumental paradigms of alcohol drinking. The next steps included testing the influence of sensitivity to PF on the acquisition of compulsive alcohol-seeking behaviour in the seeking-taking punishment task, measuring motivation to seek alcohol, and comparing the speed of extinction and reinstatement of alcohol-seeking after a period of abstinence between rats expressing trait insensitivity and sensitivity to PF. Finally, trait differences in the level of stress hormones and in the expression of genes and proteins in several brain regions of interest were measured to identify potential physiological and neuromolecular mechanisms of the observed interactions.

Results We showed that trait sensitivity to PF in rats determines the level of motivation to seek alcohol following the experience of its negative consequences. They also revealed significant differences between animals classified as insensitive and sensitive to PF in their propensity to reinstate alcohol-seeking behaviours after a period of forced abstinence. The abovementioned effects were accompanied by differences in blood levels of stress hormones and differences in the cortical and subcortical expression of genes and proteins related to dopaminergic, serotonergic, and GABAergic neurotransmission.

Conclusion Trait sensitivity to PF can determine the trajectory of alcohol addiction in rats. This effect is, at least partially, mediated via distributed physiological and molecular changes within cortical and subcortical regions of the brain.

Keywords Feedback Sensitivity · Animal model · Alcohol · Rat

Introduction

Alcohol use disorder (AUD) is a chronic disease that is characterized by gradual escalation of alcohol consumption over time and a compulsive alcohol-seeking behaviour persisting despite negative consequences. While the pathophysiology underlying AUD is relatively well known, the cognitive mechanisms of an individual's susceptibility to the development of alcohol dependence remain poorly understood. One of the proposed cognitive phenotypes that is intermediate to AUD is aberrant sensitivity to reinforcement. Indeed, several studies have demonstrated a relationship between positive reinforcement sensitivity, particularly in relation to

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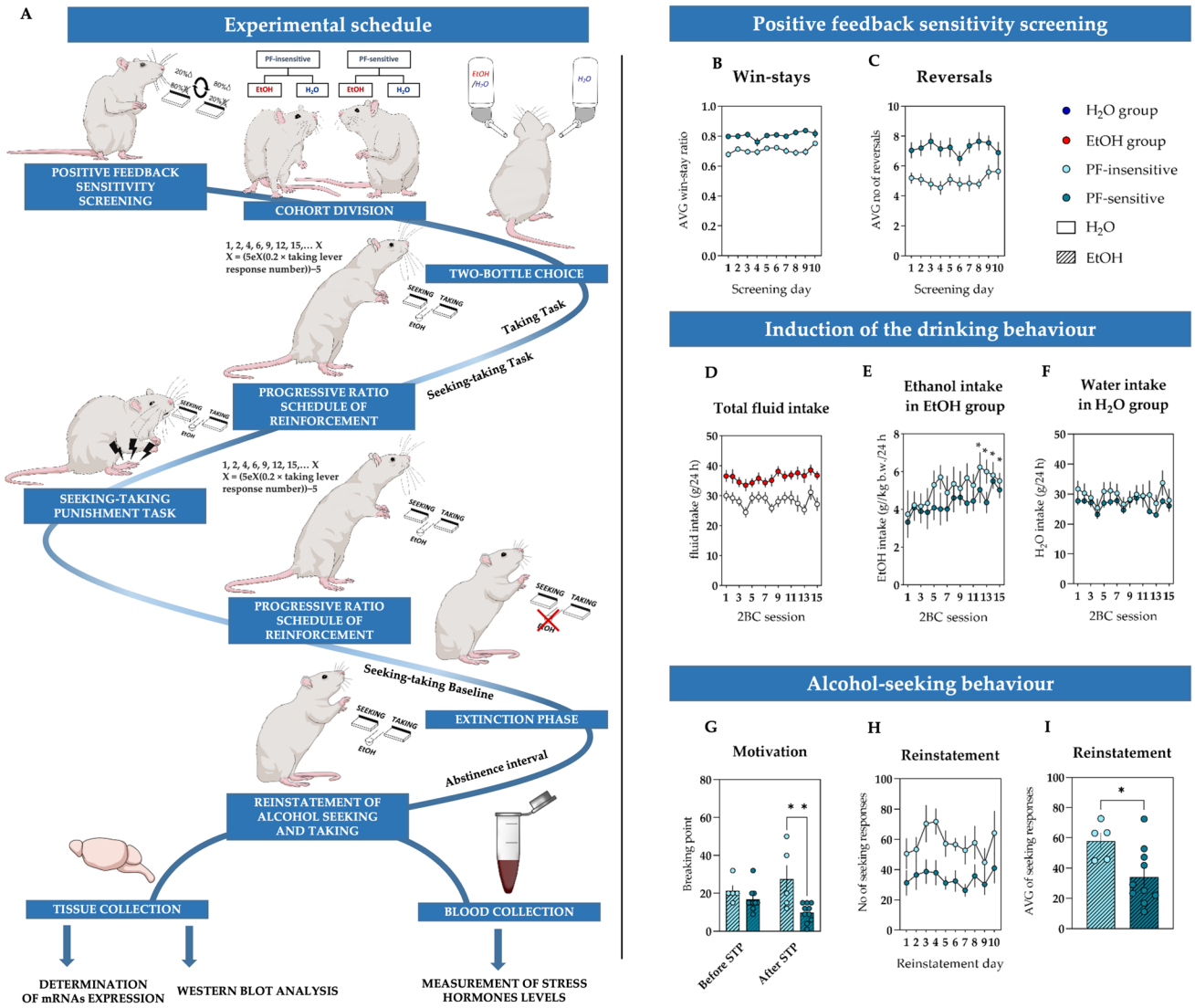

fun-seeking, and higher alcohol intake (Feil and Hasking 2008; Franken and Muris 2006; Loxton and Dawe 2001). On the other hand, decreased sensitivity to positive reinforcement was associated with the presence of negative affectivity, resulting in alcohol self-medicating as a way to alleviate negative emotional states (Heinz et al. 2009; Stewart et al. 2011; Veilleux et al. 2014). While many different approaches have been used to probe sensitivity to positive reinforcement, very few of them have tested it in the complex cognitive context, and even fewer have allowed for translational comparisons between humans and animal models.

One of the most effective, ecologically valid, and fully translational methods of measuring an individual's sensitivity to positive reinforcement is the assessment of "win-stay" behaviour in a probabilistic reversal learning (PRL) task (Cools et al. 2002; Paulus et al. 2002, 2003). The PRL involves adapting behaviour to changing stimulus-reward and stimulus-punishment contingencies to maximize reward and minimize punishment under conditions of uncertainty (Rygula et al. 2018). This behavioural paradigm has been successfully applied in research focused on neurochemical and neuroanatomical correlates of reinforcement sensitivity in healthy subjects as well as detecting cognitive deficits in a wide array of pathological states and animal models (Rygula et al. 2018). Recent studies from our laboratory demonstrated that in rodents, sensitivity to positive feedback (PF) is a stable and enduring behavioural trait (Noworyta-Sokolowska et al. 2019) that can affect the sensitivity of rats to the effects of pharmacological treatment (Noworyta and Rygula 2021). Another study revealed that sensitivity to negative feedback can determine the propensity of rats to compulsively drink alcohol (Cieslik et al. 2022).

In the current study, we combined this advanced behavioural technique allowing the determination of sensitivity to PF in rats as a stable and enduring trait, with the examination of the impact of this trait on individual susceptibility to the

◂**Fig. 1** The experimental schedule and parameters measured in animals classified as insensitive and sensitive to positive feedback (PF) and in water- (H_2O) and alcohol-drinking (EtOH) groups during feedback sensitivity screening, intermittent access two-bottle choice (2BC) sessions, and tests examining alcohol-seeking and taking behaviours. **A** To determine the effects of insensitivity/sensitivity to PF on the transition from controlled use to uncontrollable alcohol abuse, a cohort of rats was trained and tested in a series of PRL tests, and based on this "PF sensitivity screening", each rat was classified as insensitive or sensitive to PF. The cohort was further divided into alcohol (EtOH) and water (H_2O) drinking groups. To induce alcohol drinking behaviour and to measure progression in the amount of consumed alcohol, the rats were tested in the 2BC intermittent access paradigm. Subsequently, following the initial training in the taking and seeking-taking (ST) tasks, the rats' motivation to seek alcohol was measured using the progressive ratio schedule of the reinforcement (PRSR) paradigm. In the next steps, the influence of insensitivity/sensitivity to PF on alcohol-seeking behaviour was measured in the instrumental seeking-taking punishment (STP) task, following which the animals' motivation to seek alcohol was evaluated again using PRSR. Following rebaseline measurements of the seeking-taking behaviour, the effects of trait insensitivity/sensitivity to PF on alcohol-seeking behaviour were evaluated following termination of alcohol availability (extinction phase) and following 1 month of abstinence (reinstatement). At the end of the experiment, the animals were sacrificed, and the effects of prolonged alcohol consumption and its withdrawal on gene expression, protein levels, and blood levels of stress hormones were compared between PF-insensitive and PF-sensitive animals. **B** Average proportion of win-stay behaviours following a reward in rats classified as PF-insensitive (light blue circles) and PF-sensitive (dark blue circles) across all 10 screening Probabilistic Reversal Learning (PRL) tests; **C** Average number of reversals made by animals classified as PF-insensitive (light blue circles) and PF-sensitive (dark blue circles) during the 10 screening PRL tests; **D** Average daily fluid intake during all 15 2BC sessions in H_2O (white circles) and EtOH (red circles) groups; **E** Average alcohol intake (g/kg b.w./24 h) during all 15 2BC sessions in PF-insensitive (light blue circles) and PF-sensitive (dark blue circles) rats from the EtOH group. An asterisk indicates a significant ($p < 0.05$) difference in average (for all rats in the EtOH group) alcohol consumption on a given 2BC session compared to the first 2BC session; **F** Average water intake (g/24 h) during all 15 2BC sessions in PF-insensitive (light blue circles) and PF-sensitive (dark blue circles) rats from H_2O group; **G** the effects of PF sensitivity on motivation to seek alcohol. The break point in the PRSR tests conducted before and after the STP sessions in rats classified as PF-insensitive (light blue dashed bars) and PF-sensitive (dark blue dashed bars). A double asterisk indicates a significant ($p < 0.01$) difference between the PF-insensitive and PF-sensitive groups; **H**, **I** daily and averaged number of seeking responses during 10 ST tests following a 30-day abstinence interval. A single asterisk indicates a significant ($p < 0.05$) difference between the PF-insensitive and PF-sensitive groups. Data are presented as the mean ± SEM

acquisition and maintenance of alcohol-seeking behaviour. The escalation of ethanol intake in rats was achieved by applying a mix of intermittent free access and instrumental paradigms of alcohol drinking, such as the intermittent access two-bottle choice (2BC) (Cieslik et al. 2022) and seeking-taking (ST) tasks (Giuliano et al. 2018). The next steps included testing the influence of sensitivity to PF on the acquisition of compulsive alcohol-seeking behaviour in the seeking-taking punishment (STP) task and measuring motivation to seek alcohol in the progressive ratio schedule of reinforcement (PRSR) task. Finally, we measured how trait sensitivity to PF affected the extinction and reinstatement of alcohol-seeking after a period of abstinence. To identify potential physiological and neuromolecular mechanisms of the observed interactions between trait sensitivity to PF and the acquisition of compulsive alcohol drinking, we measured trait differences in the levels of stress hormones and the expression of genes and proteins in several brain regions of interest.

Materials and methods

Ethical statement

All experiments were conducted in accordance with the European Union guidelines for the care and use of laboratory animals (2010/63/EU). Experimental protocols were reviewed and approved by the 2nd Local Institutional Animal Care and Use Committee, Institute of Pharmacology Polish Academy of Sciences in Krakow. The authors attest that all efforts were made to minimize the number of animals used and their suffering.

Subjects and housing

We used 40 male Sprague−Dawley rats (Charles River, Germany) weighing 176–200 g upon arrival. Rats were group-housed (four animals per cage) in an enriched environment (plastic pipes 25 cm long and wooden blocks) under controlled temperature (21 ± 1 °C) and humidity (40–50%) under a 12-h light/dark cycle (lights on at 7:00 AM). Throughout the experiment, rats were mildly food restricted to 85% of their free-feeding weight (according to the normal growth curve recommended by the laboratory rodent supplier—Charles River Research Models and Services Catalogue) by providing 15 g of food pellet/rat/day (standard laboratory chow). Water was always available ad libitum. All behavioural procedures were performed during the light phase of the light/dark cycle.

Experimental apparatus

The PRL tests were conducted in operant conditioning chambers (Med Associates; St Albans, Vermont, USA) enclosed within a sound-attenuating box. Each chamber was equipped with a fan, house light, speaker, a food dispenser set to deliver a sucrose pellet (Dustless Precision Pellets, 45 mg; Bio-Serv, New Jersey, USA), fluid receptacle, and two retractable levers located at the sides of the feeder.

Tests examining alcohol-seeking and taking behaviours were conducted in the same operant chambers, except that

the levers were located on the wall opposite to the liquid dispenser, to create a new experimental environment that would not interfere with habits the animals acquired during sensitivity screening.

Experimental schedule

The experimental schedule is summarized in Fig. 1A.

Measuring sensitivity to PF using the PRL test

Initial training

During the initial stage of training, one of the levers (left/right levers counterbalanced) was extended. Each press on this lever resulted in sugar pellet delivery, with a fixed ratio 1 (FR1) schedule of reinforcement. After each press, the lever retracted for 3 s (inter-trial interval (ITI)) before the next trial began. If the lever was not pressed within 10 s, it was considered an omission. The criterion of less than 20% omissions had to be met before progressing to the second stage of the training. There was no pre-determined limit on the number of trials, and each training session lasted for 30 min.

The second stage of training involved random presentations of either the left or right lever. The rats were required to press each lever at least 30 times within 30-min training session. To avoid side bias during the PRL task, animals had to respond with similar frequency on both levers. This was achieved by ensuring that they made less than 7.5% omissions on each lever (i.e., less than 15% total omissions but equally distributed between the levers) for 3 consecutive training days. Once this criterion was met, the animals were ready to be tested in the PRL procedure.

PRL training

Each training session consisted of 200 trials, of which each lasted for a maximum of 22 s. The start of a trial was signalled by the house light, which remained on until the end of the trial. Two seconds after the trial started, both levers were presented, and one of them was randomly assigned as the "correct" lever, which delivered a reward (one sucrose pellet) 80% of the times it was pressed. Pressing on this lever was followed by 5 s ITI. A press on the other, "incorrect" lever would result in a rewarding outcome only 20% of the times it was pressed. A failure to respond within 10 s triggered the 5 s ITI and was counted as an omission. During the ITI, both levers were retracted, and the house light was turned off. The same ITI directly followed a punishing outcome i.e. no reward on 20% of the "correct" and 80% of the "incorrect" lever presses. The use of probabilistic reinforcement increased the complexity of the task in such a way that the information from any given choice was insufficient to guide future behaviour, and subjects must engage cognitive functions to track the reward history for both stimuli to determine the stimulus that was more beneficial overall.

After every 8 consecutive "correct" lever presses (regardless of the outcome), the criterion for the reversal of the outcome probabilities was reached. The previously "correct" lever now became "incorrect" and vice versa. This pattern was followed until the end of the session. The PRL training phase was repeated daily until the individual animals achieved sufficient performance levels. The criteria to be met were a minimum of 3 reversals completed during 3 consecutive training sessions, with less than 15% omissions per session.

Parameters measured in the PRL test

To measure rats' sensitivity to PF, all rewarded outcomes (true and misleading) followed by a decision to stay with the lever that delivered them (win-stays) were counted jointly for the "correct" and "incorrect" levers and expressed as a ratio of all rewarded outcomes on that lever. Additionally, the number of reversals completed during the experimental session served as a measure of the general performance of the animals on the task and as a measure of cognitive flexibility.

PF sensitivity screening

After achieving a stable performance in the PRL (a minimum of 3 reversals and less than 15% omissions in three consecutive sessions), the rats were tested in 10 consecutive PRL tests conducted over 10 consecutive days. Based on this "PF sensitivity screening", the animals were divided into PF-insensitive and PF-sensitive groups using a median split. The division was made based on the average ratio of pressing the same lever (win-stays) following both true and misleading rewards across all 10 screening tests.

Intermittent access to alcohol in the 2BC paradigm

To induce drinking behaviour and to determine the level of alcohol consumption in the EtOH group, 15 sessions of the 2BC procedure were conducted every second day. During the 2BC tests, animals were separated into individual cages for 24 h, where they were presented with either one bottle of 10% EtOH (w/w) and one bottle of water or two bottles of water for the EtOH and H_2O groups, respectively. To avoid potential effects of side preferences in drinking, the position of the bottles was changed after 12 h. The bottles were weighed before and after each session to determine total fluid intake for both groups (g/24 h) and alcohol consumption in the EtOH group (g EtOH/kg of body weight (b.w.)/24 h). The volume of liquids consumed was calculated

as the difference in bottle weights at the beginning and the end of each session, subtracting the volume lost due to dripping from bottles on an empty cage.

Acquisition of alcohol-seeking behaviour: taking task

The rats were trained to associate the pressing of the taking lever with the alcohol or water (in the case of water drinking control groups) delivery under a FR1 schedule of reinforcement. Each trial started with the insertion of the randomly assigned taking lever and a house light on (left/right levers counterbalanced). Pressing on the lever resulted in dipper presentation on the opposite side of the box, delivery of 0.1 ml of 15% EtOH (w/w) or water for the EtOH and H_2O groups, respectively, and simultaneous retraction of the taking lever. No response in 10 s triggered the 10 s ITI and was counted as an omission. Regardless of the result, each trial was followed by an ITI during which the levers were retracted and alcohol/water was not available. Rats were limited to a maximum of 60 rewards for a 30-min training session. After achieving the performance criterion of a minimum of 20 taking responses in three consecutive sessions, the animals were shifted to the ST task.

Acquisition of alcohol-seeking behaviour: ST task

During this task, each trial started with the insertion of the seeking lever, opposite to the randomly assigned taking lever, which remained retracted. The seeking lever response started the randomly applied interval of 1 to 15 s (RI 1–15 s), after which the taking lever was extended. Pressing of the taking lever under FR1 resulted in the presentation of the dipper on the opposite side of the box, delivery of 0.1 ml of 15% EtOH (w/w), and simultaneous retraction of both levers. Each trial was followed by a 10-s ITI during which both levers were retracted and alcohol was not available. If the animal did not press the seeking lever, the lever remained extended until the end of the session. Rats were limited to a maximum of 100 rewards for 45 min sessions. When animals performed a minimum of 20 responses in 3 consecutive sessions, their motivation to seek alcohol was tested in the PRSR task.

Measuring motivation to seek alcohol using the PRSR

Each trial started with the extension of the seeking lever, pressing on which resulted in taking lever extension after RI 1–15 s. The number of seeking lever presses required to produce this effect increased progressively with each successive taking lever response and EtOH delivery. The steps of the exponential progression used in our study were the same as those previously developed by Roberts and Bennet (Roberts and Bennett 1993) and previously used by Rygula and colleagues (Rygula et al. 2015b) and were based on the following equation: response ratio = (5eX(0.2 × taking lever response number)) − 5, rounded to the nearest integer. Thus, the values of the steps were 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc. Each trial was followed by 10 s ITI when both levers were retracted. Sessions lasted 30 min. The maximum number of lever presses a subject was willing to exert to obtain a reward was referred to as break point and served as the measure of motivation for alcohol.

Acquisition of alcohol-seeking behaviour: STP task

After the PRSR test, we measured the persistence of seeking behaviour in the face of aversive consequences using the STP task. In this paradigm, each trial started as described for the ST task, with the insertion of the seeking lever. Seeking lever response resulted either in a 1 s electric shock (0.10–0.50 mA), administered through a grid floor, or the extension of the taking lever after a random interval (RI 1–15 s). During each session, rats were limited to a maximum of 25 trials, of which 17 (70%) were reinforced by EtOH delivery following the lever response, and 8 (30%) were punished with foot shock. The intensity of the shock increased in daily sessions according to the following pattern: 0.10, 0.20, 0.30, 0.30, 0.40, 0.40, 0.50, and 0.50 mA. Although punishment occurred randomly in each session, never more than two consecutive trials resulted in a foot shock, and the first trial of the session was always reinforced. Upon completion of the STP task, rats were challenged again in the PRSR test and rebaselined in 5 ST test sessions.

Extinction and reinstatement of alcohol-seeking and taking behaviours

After the rebaseline procedure, all animals underwent between 5 and 20 (AVG = 12) daily 15 min extinction sessions, during which the seeking lever response under RI 1–15 s resulted in taking lever extension; however, the taking lever presses had no programmed consequences, and alcohol was not available. After reaching the extinction criterion (less than 5 seeking responses in 3 consecutive sessions), the rats were alcohol deprived and not tested for the following 30 days. This abstinence interval was chosen to more naturally reflect condition of relapse in humans after a longer period of time (Moe et al. 2022).

After 30 days of abstinence, the rats underwent a series of ST tests to measure how quickly they reinstate their alcohol-seeking behaviour and bring their performance up to the basal level. The animals were tested until they reached the criterion of an average number of seeking responses from 5

tests equal to or higher than the average number of seeking responses from the 5 rebaseline ST tests.

Tissue collection

At the end of the behavioural part of the experiment, the sacrificed rats were decapitated, and 5 brain structures were collected for biochemical analyses: 3 cortical (medial prefrontal cortex (mPFC), anterior cingulate (ACC), and orbitofrontal cortex (OFC)) and 2 subcortical areas (nucleus accumbens (Nacc) and amygdala (Amy)). All the above-mentioned brain areas have been previously demonstrated to be involved in the mediation of sensitivity to feedback (Clarke et al. 2014; Cools et al. 2002, 2009; Cservenka 2016; Dalton et al. 2014; Golebiowska and Rygula 2017b). Tissue was taken based on the "Rat Brain Atlas" of Paxinos & Watson (Paxinos and Watson 1998) and according to Achterberg and colleagues (Achterberg et al. 2015). The structures were frozen on dry ice and stored at − 70° C for further analysis.

Gene selection

The effects of trait sensitivity to PF and alcohol drinking on gene expression within selected brain regions were assessed using TaqMan Low Density Arrays (TLDA, described below). The predesigned TLDA allowed for the screening of 32 genes (29 candidate genes, 2 reference genes, and 1 endogenous gene control), which were potentially involved in mediating the effects of PF on alcohol-seeking and drinking in rats. Based on an extensive literature search and analysis of the effects of various genetic and pharmacological manipulations on sensitivity to feedback, 4 groups of genes were chosen. (1) Genes involved in the functioning and regulation of the serotonin (5-HT) system (e.g., serotonin receptors: 5-HT1A, 5-HT2A, serotonin transporter (SERT) and tryptophan hydroxylase). Indeed, it has been demonstrated in humans (Chamberlain et al. 2006; Cools et al. 2008; den Ouden Hanneke et al. 2013), nonhuman primates (Rygula et al. 2015a) and rodents (Bari et al. 2010; Golebiowska and Rygula 2017a; Ineichen et al. 2012; Rygula et al. 2014) that acute and permanent manipulations of the activity of the 5-HT system affect sensitivity to feedback. (2) Because, along with 5-HT, dopamine (DA) is the second neurotransmitter critically implicated in learning from feedback (Cools et al. 2009; Klein et al. 2007; Pessiglione et al. 2006), the second group of screened genes was chosen among those involved in dopaminergic neurotransmission (e.g., dopamine receptors: D1, D2, D4, dopamine transporter (DAT), tyrosine hydroxylase, monoaminooxidase (MAO) A and B, and catechol-O-methyltransferase (COMT)). (3) Because changes in brain DA neurotransmission often result from secondary neuroadaptations in other neurotransmitter systems, such as glutamate (Kauer and Malenka 2007) and γ-aminobutyiric acid (GABA) (Volkow et al. 2010), genes associated with these 2 neurotransmitter systems, e.g., the ionotropic glutamate receptors NMDA and AMPA, the metabotropic glutamate receptors mGLU2, mGLU3, and mGLU5, glutamate decarboxylase (GAD), and GABAA and GABAB receptors, constituted the third analyzed group. (4) The fourth group included genes involved in EtOH metabolism, such as catalase and alcohol dehydrogenase (Hipolito et al. 2007). (5) Last but not least, ribosomal protein L32 (*Rpl32*) and peptidylprolyl isomerase A (*Ppia*) were used as reference genes as described previously (Gąska et al. 2012).

Isolation of RNA from the brain structures

Total RNA was isolated from collected tissues using the RNeasy Plus Mini Kit (Qiagen, Germantown, MD, US) according to the manufacturer's instructions. The samples (8–11 per group) were homogenized with 600 µl of RTL Plus buffer with β-mercaptoethanol for 4 min at 50 Hz with TissueLyser LT (Qiagen, Germantown, MD, US). Then, gDNA Eliminator spin columns were used. Then, 600 µl of 70% ethanol was added to each sample and transferred to the RNeasy spin column. After washing the column, 30 µl of RNase-free water was added to the column for RNA elution. The quality and quantity of the isolated total RNA were evaluated by a NanoDrop ND-1000 (Thermo Fisher Scientific) and an Experion microcapillary electrophoresis system (Bio-Rad, Hercules, California, USA). Samples that passed the quality threshold (RIN > 8.0) were used for further experiments.

Isolation of protein from the brain structures

During RNA isolation, the protein was obtained using the cold acetone precipitation method. For this, 800 µl of cold acetone was added to 100 µl of flow-through acquired after RNA binding to the RNeasy spin column. The protein was precipitated for 1.5 h at -20 °C and centrifuged for 15 min at 14,000 rpm at 4 °C. The pellet was dissolved in a buffer containing 7 M urea, 2 M thiourea, 40 mM Tris, 4% CHAPS, 65 mM DTT, and protease inhibitor cocktail (Thermo Fisher Scientific) and stored at − 20 °C for future analysis.

Determination of mRNA expression by TaqMan gene expression array cards

The isolated RNAs were used to synthesize cDNA transcripts according to the manufacturer's protocol of the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). The amount of RNA was equalized for all samples depending on the structure. The obtained cDNA was mixed with TaqMan Universal PCR Master Mix, No AmpErase UNG (Thermo Fisher Scientific) to perform the

RT-qPCRs. qPCRs were carried out simultaneously using Custom TaqMan Gene Expression Array Cards (Thermo Fisher Scientific). The 29 genes that are potentially involved in the mediation of the effects of feedback sensitivity and alcohol-seeking and drinking in rats were placed on one Array Card. One Array Card was used to examine the mRNA expression of four samples in triplicate. The RT-qPCRs were run on a QuantStudio 12 K Flex System (Applied Biosystems, Waltham, Massachusetts, US). Data were further analysed with QuantStudio 12 K Flex Software (Applied Biosystems). A Ct value above 34 was considered undetectable. The same threshold equal to 0.20 was set for all samples for comparison. Then, the data were analysed with qBasePLUS 3.1 software (Biogazelle, Zwijnaarde, Belgium) (Hellemans et al. 2007), which uses a generalized model of the delta-delta-Ct approach, thereby supporting the use of gene specific amplification efficiencies and normalization with multiple reference genes. Rpl32 and Ppia were selected for normalization.

Western blot analysis

The concentration of proteins was determined using the Bradford Reagent (Sigma−Aldrich, Saint Louis, MO, USA) following the manufacturer's protocol. Equal concentrations of proteins were mixed with 4X Bolt® LDS Sample Buffer (Invitrogen, Waltham, MA, USA) and 10X Bolt® Sample Reducing Agent (Invitrogen) and then denatured at 70 °C for 10 min. Samples were separated on Bolt™ 4–12% Bis–Tris Plus Gels (Invitrogen) under reducing conditions in 20X Bolt® MES SDS Running Buffer (Invitrogen), incubated in 20% ethanol for 10 min, and transferred to immunoblot nitrocellulose membranes (iBlot® 2 Transfer Stacks, nitrocellulose, Invitrogen, Waltham, MA, USA) in accordance with the manufacturer's protocol. Primary and secondary antibodies were suspended in an iBind™ Solution Kit followed by membrane incubation on iBind™ Cards using the iBind™ Western Device (SLF1000, Invitrogen, Waltham, MA, USA) for 2.5 h or overnight. The following concentrations of primary antibodies were used to determine protein levels: 1:200 for MAO-B (mouse, cat. number: sc-515354; Santa Cruz Biotechnology), 1:2000 for ADH1 (rabbit, cat. number PA5- 8730, Invitrogen), 1:1000 for DRD1 (rat, cat. number: D2944, Sigma−Aldrich), 1:50 for GABABR2 (mouse, cat. number: sc-393286, Santa Cruz Biotechnology), 1:2000 for 5-HT1A (rabbit, cat number: PA5-77,745 Invitrogen), 1:1000 for 5-HT2A (rabbit, cat. number: ab216959, Abcam), 1:1000 for 5-HT3A (rabbit, cat. number: bs-2126R Bioss antibodies), 1:200 for CAT (mouse, cat. number: sc-271803) and 1:2000 for SERT (rabbit, cat. number: PA5-80,032, Invitrogen). The secondary anti-mouse (cat. number: A9044, Sigma Aldrich) and anti-rabbit (cat. number: ab6721, Abcam) antibodies were used at concentrations of 1:20 000. Anti-rat antibodies were used at a concentration of 1:1000 (cat. number: HAF005, Biotechne). As a loading control, β-actin (monoclonal anti-β-actin antibody produced in mouse, A5441, Sigma−Aldrich, Saint Louis, MO, USA) was applied at a concentration of 1:20 000, and its corresponding secondary antibody (anti-mouse IgG, A9044, Sigma−Aldrich, Saint Louis, MO, USA) was applied at a concentration of 1:20 000. The electrophoretic bands were detected using the Clarity™ Western ECL Substrate (Bio-Rad, Hercules, CA, USA) and FUJIFILM LAS-4000 (Fujifilm Life Science, USA) device. Blot analysis was performed using ImageJ 1.53e software (Wayne Rusband and NIH, USA). Due to limited gel spots, a minimum of three samples from different groups were included in each blot.

Measurement of blood stress hormone levels

To assess whether trait sensitivity to PF interacts with the effects of prolonged alcohol consumption on the level of stress hormones, on the day after the last behavioural procedure (between 09:00 am and 12:00 pm), the rats from the EtOH and H_2O groups were sacrificed and tested for blood concentrations of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) using a Merck Rat Stress Hormone Magnetic Bead Panel. For all animals, the blood was collected, after clotting, centrifuged at 1500 × g at 4 °C for 10 min. The obtained serum was stored at − 80 °C and analysed for ACTH and corticosterone concentrations according to the manufacturer's instructions.

Statistics

The data were analysed using SPSS (version 25.0, SPSS Inc., Chicago, IL, USA). The normality of the sensitivity to feedback data was verified using the Kolmogorov–Smirnov test. Nonparametric data were normalized by square rooting and, where appropriate, removing outliers. The physiological and molecular data were analysed using 2-way ANOVA. When the data could not be normalized, the Kruskall Wallis test was used. The screening, 2BC, ST, STP, extinction, and reinstatement data were analysed using two-way repeated-measures ANOVAs with the within-subject factor of test day/session and the between-subject factor of sensitivity to PF.

The differences between the PF-insensitive and PF-sensitive groups of rats in the average quantity of alcohol consumed and the number of tests needed to achieve extinction and reinstatement criteria were analysed using t tests or, for nonparametric data, using Mann– Whitney *U* tests. For pairwise comparisons, we adjusted the values using Sidak's correction for multiple comparisons (Howell 1997). All tests of significance were performed at $\alpha = 0.05$. We tested

the homogeneity of variance using Levene's test, and for repeated-measures analyses, we confirmed sphericity using Mauchly's test. The data are presented as the mean ± SEM.

Results

PRL training and testing

All animals fulfilled the PRL training criteria and qualified for PRL screening. On average, the animals reached the criteria after 6.78 ± 0.42 PRL tests. The PF-insensitive/PF-sensitive rats did not differ significantly in the number of PRL tests needed to reach the criterion (Mann− Whitney: $p = 0.631$, Figure S1).

PF sensitivity screening

The average proportion of win-stay behaviours in the animals classified as PF-insensitive ($N = 20$) ranged from 0.662 to 0.738, with an average of 0.706 ± 0.005. The average proportion of win-stay behaviours in the animals classified as PF-sensitive ($N = 20$) ranged from 0.745 to 0.892, with an average of 0.806 ± 0.011. The difference in sensitivity to PF between both subgroups ($F_{(1,38)} = 70.90$, $p < 0.001$) was stable across the screening period (not significant effect of screening day ($F_{(9,342)} = 1.369$, $p = 0.201$) and not significant sensitivity × screening day interaction ($F_{(9,342)} = 1.021$, $p = 0.422$)). The average number of reversals made by the animals classified as PF-insensitive was significantly lower than that for animals classified as PF-sensitive ($F_{(1,38)} = 35.800$, $p < 0.001$). This difference in the reversal performance between both subgroups was stable across the screening period (not significant effect of screening day ($F_{(9,342)} = 0.555$, $p = 0.833$) and not significant sensitivity × screening day interaction ($F_{(9,342)} = 0.617$, $p = 0.782$)). Individual data (proportion of win-stay and reversal performance across all 10 screening PRL tests) of all 40 animals are presented in figures S2A and S2B respectively.

As only 15 out of the 20 EtOH rats achieved the criteria of taking and ST tests (described in the next sections), and 19 out of the 20 H2O rats (because of the mistake in the treatment) were analysed further, the screening data for these 34 animals were as follows:

The average proportion of win-stay behaviours in the animals classified as PF-insensitive ($N = 16$) ranged from 0.662 to 0.738, with an average of 0.705 ± 0.007. The average proportion of win- stay behaviours in the animals classified as PF-sensitive ($N = 18$) ranged from 0.745 to 0.892, with an average of 0.805 ± 0.012. The difference in sensitivity to PF between both subgroups ($F_{(1,32)} = 51.61$, $p < 0.001$; Fig. 1B) was stable across the screening period (not significant effect of screening day ($F_{(9,288)} = 1.449$, $p = 0.167$) and not significant sensitivity × screening day interaction ($F_{(9,288)} = 1.188$, $p = 0.302$)).

The average number of reversals made by the animals classified as PF-insensitive was significantly lower than that for animals classified as PF-sensitive ($F_{(1,32)} = 27.27$, $p < 0.001$; Fig. 1C). This difference in the reversal performance between both subgroups was stable across the screening period (not significant effect of screening day ($F_{(9,288)} = 0.494$, $p = 0.878$) and not significant sensitivity × screening day interaction ($F_{(9,288)} = 0.778$, $p = 0.637$)).

Cohort division

Based on PF sensitivity screening, the animals were classified into two groups: PF-insensitive ($N = 20$) and PF-sensitive ($N = 20$). Then, according to the applied treatment, they were further randomly divided into four subgroups: $EtOH_{PF\text{-}insensitive}$ ($N = 9$), $EtOH_{PF\text{-}sensitive}$ ($N = 11$), $H_2O_{PF\text{-}insensitive}$ ($N = 11$), and $H_2O_{PF\text{-}sensitive}$ ($N = 9$) animals.

Because, as mentioned above, only 34 out of 40 initially trained animals completed all experimental procedures, ultimately, the groups analyzed in the present experiment were as follows: $EtOH_{PF\text{-}insensitive}$ ($N = 5$), $EtOH_{PF\text{-}sensitive}$ ($N = 10$), $H2O_{PF\text{-}insensitive}$ ($N = 11$), and $H2O_{PF\text{-}sensitive}$ ($N = 8$) animals.

Induction of drinking behaviour

During the 15 2BC sessions, rats from the EtOH group consumed more fluids than their conspecifics from the H_2O group (significant main effect of treatment ($F_{(1, 32)} = 11.000$, $p = 0.002$); Fig. 1D). Moreover, they significantly ($p < 0.05$) increased their alcohol intake with an average from the first test of 3.47 ± 0.58, reaching an average of 5.20 ± 0.32 g/kg/24 h in the last session (significant main effect of session ($F_{(14,\ 182)} = 2.613$, $p = 0.002$, Fig. 1E). We did not observe significant differences in alcohol consumption between PF-insensitive/PF-sensitive animals (nonsignificant effect of sensitivity ($F_{(1,\ 13)} = 0.103$, $p = 0.329$), nonsignificant session × sensitivity interaction ($F_{(14,\ 182)} = 0.456$, $p = 0.953$, Fig. 1E). There was no significant difference in water consumption between PF-insensitive/PF-sensitive rats from the H_2O group (nonsignificant sensitivity effect ($F_{(1,\ 17)} = 0.573$, $p = 0.460$; no sensitivity × session interaction ($F_{(14,\ 238)} = 0.805$, $p = 0.663$, Fig. 1F)).

One rat from the control group (PF-sensitive) was removed from the analysis and further experiments due to a mistake in the applied treatment. As only 15 out of the 20 rats achieved the criteria of taking and ST tests described in the next section, only these 15 animals were analysed regarding their consumption of alcohol in 2BC sessions and subsequent experimental steps.

Acquisition of alcohol-seeking behaviour in rats

In the next step, the animals from the EtOH and H_2O groups were trained to associate the pressing of the taking lever with the alcohol or water delivery under FR1. As mentioned above, only 15 out of the 20 rats from the EtOH group achieved the criteria for taking and ST tests. None of the rats from the H_2O group met the criteria.

After reaching the ST criterion, the rats were tested in the STP task. As the shock intensity increased from 0.10 to 0.50 mA during consecutive sessions, all rats gradually decreased the number of trials completed compared to the initial session (main shock intensity effect ($F_{(7,91)}=5.990$, $p<0.001$, Figure S3A). We did not observe significant differences in the number of trials completed between the PF-insensitive and PF-sensitive groups of rats (nonsignificant effect of sensitivity to PF ($F_{(1, 13)}=0.011$, $p=0.919$) and nonsignificant sensitivity to PF × shock intensity interaction ($F_{(8, 104)}=0.471$, $p=0.853$)).

Motivation to seek alcohol before and after the introduction of punishment

Additionally, to measure the impact of punishment in the STP task on rats' motivation for alcohol-seeking, we conducted two PRSR tests. In the first one, executed before STP tests, the rats' average break point was 18.33 ± 1.66, while in the second one, performed after the STP test, the average break point was 15.67 ± 3.36.

PF sensitivity had no significant effect on the break point of rats tested before the STP. Interestingly, however, the animals classified as PF-insensitive reached a significantly higher break point during the PRSR test performed after the STP tests than their PF-sensitive conspecifics (significant sensitivity effect ($F_{(1, 13)}=8.532$, $p=0.012$) and significant sensitivity × test interaction ($F_{(1, 13)}=6.185$, $p=0.027$); Fig. 1G).

Extinction and reinstatement of alcohol-seeking behaviour

After the second PRSR test, all animals underwent 5 additional ST tests. Following the rebaseline, rats were tested under ST extinction conditions, during which alcohol was not available. The number of sessions needed to achieve the extinction criterion ranged from 5 to 20, with an average of 12.07 ± 1.29. Sensitivity to PF had no significant impact on the length of extinction (t test; $p=0.808$, Figure S3B).

The effects of PF sensitivity on the reinstatement of alcohol-seeking were assessed following 30 days of forced abstinence. Throughout 10 tests, most of the animals reinstated their preextinction level of seeking responses. PF-sensitive animals showed a significantly lower number of seeking responses, with an average of 34.18 ± 6.00, than PF-insensitive rats, with an average of 57.96 ± 5.44 (main effect of sensitivity ($F_{(1, 13)}=6.400$, $p=0.025$, Fig. 1H, I) and a nonsignificant sensitivity × test interaction ($F_{(9, 117)}=0.403$, $p=0.931$). There was no significant difference in the number of tests needed to achieve the criterion between the PF-insensitive and PF-sensitive groups (Mann−Whitney test, $p=0.445$). Two animals (PF-sensitive) did not meet the reinstatement criterion.

The effects of PF sensitivity and alcohol consumption on gene expression levels

Statistical analysis of the effects of trait sensitivity to PF on the expression of genes revealed statistically significant intergroup differences in all investigated regions of interest except the OFC. In the ACC, the mRNA level was higher in the PF-insensitive rats compared to their PF-sensitive conspecifics, for *Drd1* ($F_{(1, 29)}=4.556$, $p=0.041$), *Gria1* ($F_{(1, 30)}=4.809$, $p=0.036$), and *Htr3a* ($F_{(1, 30)}=5.855$, $p=0.022$) (Fig. 2A); in the mPFC for *Cat* ($F_{(1, 30)}=9.431$, $p=0.005$) (Fig. 2B) and in the Amy for *Maob* ($F_{(1, 28)}=5.804$, $p=0.023$) (Fig. 2C). In the Nacc, the mRNA level was higher in PF-insensitive animals for *Gabbr2* ($F_{(1, 29)}=6.557$, $p=0.016$), *Grm2* ($F_{(1, 29)}=4.863$, $p=0.036$), *Htr1a* ($F_{(1, 30)}=6.452$, $p=0.017$), *Htr2a* ($F_{(1, 30)}=4.367$, $p=0.045$), *Npy* ($F_{(1, 30)}=10.02$, $p=0.004$), and *Slc6a3* ($F_{(1, 30)}=5.166$, $p=0.030$) (Fig. 2E).

The analysis also revealed significant interactions between the effects of trait sensitivity to PF and alcohol drinking on the expression of *Adh1* (F (1, 29) $=5.048$, $p=0.032$) in mPFC and *Gabbr1* ($F_{(1, 27)}=9.466$, $p=0.005$) in the Amy with mRNA level higher in $EtOH_{PF\text{-}insensitive}$ group compared to $H_2O_{PF\text{-}insensitive}$ and to $EtOH_{PF\text{-}sensitive}$ groups (Fig. 2B, C). For *Tph2* ($F_{(1, 28)}=4.732$, $p=0.038$), and *Drd2* ($F_{(1, 28)}=6.200$, $p=0.019$) in the Amy, and for *Htr2a*, ($F_{(1, 30)}=4.606$, $p=0.040$), and *Slc6a4* ($F_{(1, 29)}=4.977$, $p=0.034$) in the Nacc,the mRNA level was higher in $H_2O_{PF\text{-}insensitive}$ animals compared to $H_2O_{PF\text{-}sensitive}$ and $EtOH_{PF\text{-}insensitive}$ groups (Fig. 2C, E). We also revealed significant intergroup differences in the *Adh1* expression within the OFC (Kruskal−Wallis test: $p=0.038$).

The significant effects of alcohol drinking (treatment) were observed, with mRNA level higher in the EtOH group compared to H_2O-drinking ones, for *Comt* ($F_{(1, 30)}=5.223$, $p=0.030$) and *Maoa* ($F_{(1, 29)}=4.732$, $p=0.038$) in the ACC (Fig. 2A), for *Adh1* ($F_{(1, 29)}=5.072$, $p=0.032$), *Cat* ($F_{(1, 30)}=7.312$, $p=0.011$) and *Comt* ($F_{(1, 30)}=18.320$, $p<0.001$) in the mPFC (Fig. 2B), for *Gad1* ($F_{(1, 28)}=4.338$, $p=0.047$) and *Drd2* ($F_{(1, 28)}=5.092$, $p=0.032$) in the *Amy* (Fig. 2C), for *Cat* ($F_{(1, 30)}=5.351$, $p=0.028$), *Gad2* ($F_{(1, 30)}=6.329$, $p=0.018$) and *Htr1a* ($F_{(1, 30)}=6.362$, $p=0.017$) in the OFC (Fig. 2D), and for

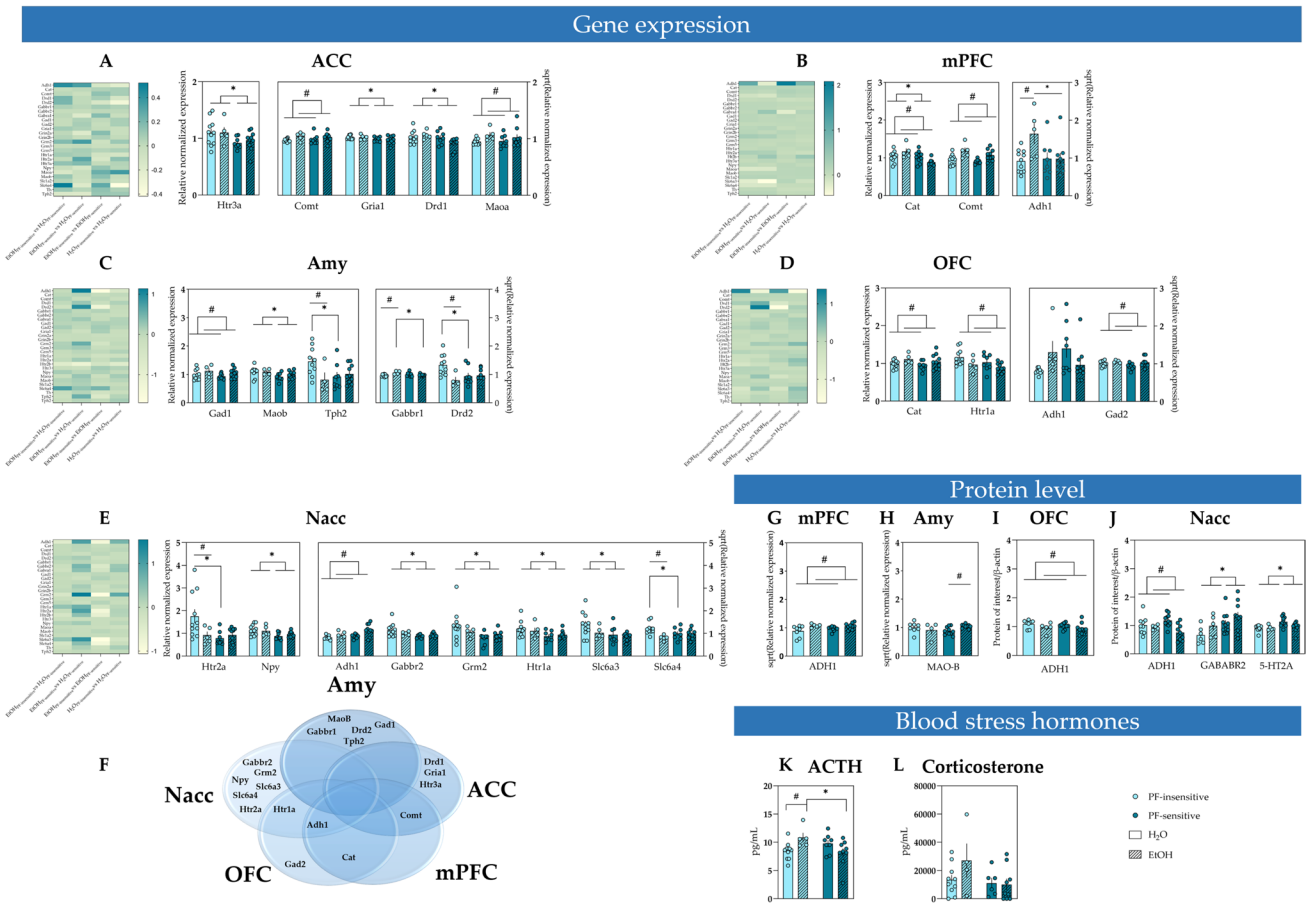

Fig. 2 Molecular and physiological differences associated with high and low sensitivity to positive feedback (PF) and alcohol drinking in rats. **A**–**E** Heatmaps and bar graphs demonstrating statistically significant differences in the relative normalized expression of the genes of interest in PF-insensitive (light blue bars) and PF-sensitive animals (dark blue bars) belonging to H_2O (open bars) and EtOH (dashed bars) drinking groups in **A** anterior cingulate cortex (ACC), **B** medial prefrontal cortex (mPFC), **C** amygdala (Amy), **D** orbitofrontal cortex (OFC), and **E** nucleus accumbens (Nacc). A single asterisk indicates significant ($p < 0.05$) difference between PF-insensitive and PF-sensitive groups. # indicates a significant ($p < 0.05$) difference between the EtOH and H_2O groups. **F** Venn diagram illustrating genes altered by sensitivity to PF and/or treatment in the brain structures studied using a TaqMan Array Card. **G**–**J** Protein to β-actin ratio for proteins selected based on the gene expression analysis in PF-insensitive (light blue bars) and PF-sensitive animals (dark blue bars) belonging to H_2O (open bars) and EtOH (dashed bars) groups in **G** mPFC, **H** Amy, **I** OFC, and **J** Nacc; A single asterisk indicates significant ($p < 0.05$) difference between PF-insensitive and PF-sensitive groups. A number sign indicates significant ($p < 0.05$) difference between PF-sensitive animals belonging to H_2O and EtOH groups. K) Blood ACTH concentration in PF-insensitive (light blue bars) and PF-sensitive (dark blue bars) animals in EtOH (dashed bars), and H_2O groups (open bars). A single asterisk indicates a significant ($p < 0.05$) difference between EtOH and H_2O groups. A number sign indicates a significant ($p < 0.05$) difference between the PF-insensitive and PF-sensitive groups. L) Blood corticosterone concentration in PF-insensitive (light blue bars) and PF-sensitive animals (dark blue bars) in the EtOH (dashed bars) and H_2O groups (open bars). Data are presented as the mean ± SEM

Adh1 ($F_{(1, 27)} = 8.590$, $p = 0.007$) in the Nacc (Fig. 2E). For *Slc6a4* ($F_{(1, 29)} = 6.895$, $p = 0.014$) in the Nacc the mRNA level was lower in the EtOH-drinking rats compared to the H_2O-drinking ones (Fig. 2E).

The results of statistical analysis of the effects of trait sensitivity to PF and its interactions with alcohol drinking on the expression of all investigated genes in all investigated brain regions are demonstrated in Table S1. The genes with significantly different expression in the investigated regions of interest are additionally presented in a Venn diagram (Fig. 2F).

To determine how various RNA expression levels affect protein levels, we performed Western blot analyses on protein products identified by TaqMan Gene Expression Array cards. Statistical analysis of the effects of trait sensitivity to PF on the protein levels revealed statistically significant intergroup differences, with the level of GABA-B receptor subunit 2 (GABABR2, gene: *Gabbr2*) ($F_{(1, 28)} = 5.422$, $p = 0.027$) and serotonin receptor 2A (5-HT2A, gene: *Htr2a*) ($F_{(1, 30)} = 6.689$, $p = 0.015$) higher in PF-sensitive rats compared to PF-insensitive group in the Nacc (Fig. 2J).

The analysis also revealed significant interactions between the effects of trait sensitivity to PF and alcohol drinking on the expression of monoamine oxidase B (MAO-B, gene: *Maob*) ($F_{(1,31)} = 7.650$, $p = 0.010$; Fig. 2H) in the Amy, with protein level higher in the $EtOH_{PF\text{-}sensitive}$ compared to $H_2O_{PF\text{-}sensitive}$ group.

The effect of alcohol consumption itself (treatment) was statistically significant for alcohol dehydrogenase 1 (ADH1, gene: *Adh1*) in the mPFC ($F_{(1, 29)} = 9.059$, $p = 0.005$; Fig. 2G), OFC ($F_{(1, 30)} = 4.753$, $p = 0.037$; Fig. 2I) and Nacc ($F_{(1, 30)} = 7.287$, $p = 0.011$; Fig. 2J) with the protein level higher in the EtOH group compared to the H_2O rats.

The table with the results of statistical analysis of the effects of trait sensitivity to PF and its interactions with alcohol drinking on levels of selected proteins, the expression of which was significantly affected by PF and/or alcohol drinking in all investigated brain regions (Table S2) and original western blot images used for quantification of protein levels are included in Supplemental material.

The effects of PF sensitivity and alcohol consumption on stress hormone levels

After the reinstatement of alcohol-seeking and taking, all animals were sacrificed and tested for stress hormone levels in the blood. Analysis of the ACTH level data revealed a significant treatment × PF sensitivity interaction ($F_{(1, 29)} = 9.132$, $p = 0.005$) with nonsignificant effects of treatment ($F_{(1, 29)} = 0.325$, $p = 0.573$) and sensitivity ($F_{(1, 29)} = 1.014$, $p = 0.322$; Fig. 2K). The ACTH level in the $H_2O_{PF\text{-}insensitive}$ group was significantly lower than that in the $EtOH_{PF\text{-}insensitive\ group}$ ($p = 0.044$). Additionally, in the EtOH group, the ACTH level in PF-sensitive rats was significantly lower than that in their PF-insensitive conspecifics ($p = 0.027$). There were no statistically significant differences ($p = 0.237$) between the PF-insensitive and PF-sensitive animals in the H_2O group.

Analysis of the corticosterone level data (Fig. 2L) revealed statistically nonsignificant but observable at the level of statistical trend, higher blood concentrations of corticosterone in PF-insensitive groups of animals compared to their PF-sensitive conspecifics (effect of PF sensitivity $F_{(1, 25)} = 3.378$, $p = 0.078$) regardless of the treatment (nonsignificant effect of treatment ($F_{(1, 25)} = 1.457$, $p = 0.239$) and nonsignificant treatment x sensitivity interaction ($F_{(1, 25)} = 1.929$, $p = 0.177$).

Discussion

The results of the present study showed that trait sensitivity to PF in rats determines the level of motivation to seek alcohol following the experience of its negative consequences. Our findings also revealed significant differences between animals classified as insensitive and sensitive to PF in their propensity to reinstate alcohol-seeking behaviours after the period of forced abstinence. The abovementioned effects were accompanied by differences in blood levels of stress hormones and differences in the cortical and subcortical expression of genes and proteins related to dopaminergic, serotonergic, and GABAergic neurotransmission.

Over the past decade, a growing number of studies have demonstrated that the assessment of cognitive correlates of human personality traits in animals could be very useful in searching for potential cognitive biomarkers of various psychiatric disorders. For instance, a study by Rygula and colleagues, using a rodent model, suggested that trait pessimism can serve as a cognitive biomarker of susceptibility to the development of stress-induced anhedonia – a core symptom of depression (Rygula et al. 2013). A few years later, studies by Noworyta and Rygula demonstrated that sensitivity to feedback, measured as a stable and enduring behavioural trait, can determine the effects of acute administration of antidepressant drugs (Noworyta-Sokolowska et al. 2019). In a recent study from our laboratory, Cieslik and colleagues showed that trait sensitivity to negative feedback predicts the vulnerability of rats to the acquisition of compulsive alcohol-seeking and consumption in a situation when these behaviours are being punished (Cieslik et al. 2022). They also showed significant differences between animals classified as less sensitive and more sensitive to negative feedback in their propensity to extinguish alcohol-seeking behaviours after the termination of alcohol availability. The effects of trait sensitivity to PF, described here, are in concert with and complement these recent observations, supporting at the same time the importance of the role that sensitivity to feedback plays in alcohol addiction.

One of the most influential types of classification of variability within AUD in humans is reward and relief drinking, or the extent to which individuals seek alcohol to enhance positive experiences (reward drinking) versus the extent to which individuals seek alcohol to relieve negative emotional and somatic states (relief drinking). Despite promising findings within this domain (linking reward/relief drinking phenotypes with responding to different pharmacological treatments), the lack of preclinical models of reward/relief drinking may hinder efforts to understand these phenomena on neurobiological, molecular and physiological levels. The pattern of results observed in our current study may help to implement such a model that could be based on measuring sensitivity to PF.

Indeed, insensitivity to PF might suggest decreased sensitivity to reward or even anhedonia, reflecting a negative affective state that can be relieved by drinking alcohol. Following this lead, the higher motivation to seek alcohol after the unpleasant and frustrating experience of punishment

observed in PF-insensitive rats (Fig. 1G) might also be interpreted as relief drinking. A similar interpretation can be applied to the increased alcohol-seeking observed in the animals insensitive to PF following a stressful and frustrating period of abstinence (Fig. 1H, I). This interpretation of behavioural patterns observed in the animals insensitive to PF is supported by the analysis of stress hormones in the blood, which demonstrated significantly higher level of ACTH (Fig. 2K) and nonsignificantly (statistical trend) elevated corticosterone (Fig. 2L) compared to the PF-sensitive animals, suggesting a higher level of stress in this group. Importantly, elevated level of ACTH was observed only in animals drinking alcohol and were absent in the control group. Additionally, the elevated level of stress hormones in the PF-insensitive animals, which resulted from alcohol-HPA axis interaction, could have also contributed per se to the enhanced motivation to seek and drink alcohol through activation of mesocorticolimbic reward circuitry (Piazza and Le Moal 1997). Indeed, several studies demonstrated that the administration of CORT increases alcohol consumption and adrenalectomy acts in the opposite way (Fahlke and Eriksson 2000; Fahlke et al. 1996).

One could also speculate that the protracted alcohol withdrawal applied in our study resulted in an elevation of reward threshold and increased negative affectivity in animals showing reduced hedonic capacity i.e. insensitivity to PF. Indeed, a stronger behavioural response to forced abstinence demonstrated by the PF-insensitive rats, which was manifested by the higher number of seeking lever presses in the ST task during reinstatement of the instrumental response, seems to support this claim. Since PF-insensitive animals are less sensitive to reward by their nature, alterations in reward threshold and sensitivity caused by prolonged alcohol consumption and withdrawal (Koob et al. 1998; Schulteis et al. 1995) were stronger and more evident in this group. It is worth mentioning that a growing number of studies link hyposensitivity to PF/altered processing of positively valanced information with the symptomatology of stress-triggered psychiatric and mood disorders (Robinson et al. 2012), one of which may be AUD.

The above-described differences at the behavioural and physiological levels were also associated with the differences in the expression of genes and proteins in several brain regions of interest. The main locus of differences between PF-insensitive and PF-sensitive rats was the Nacc, where differences in gene expression related to serotonergic (*Htr1a*, *Htr2a*, and *Slc6a4*), GABAergic (*Gabbr2*), glutamatergic (*Grm2*), and dopaminergic (*Slc6a3*) neurotransmission, as well as NPY neuromodulation, were revealed, and in some cases (5-HT2A and GABABR2) were also confirmed at the protein level. Considering the important role of serotonin in the mediation of impulsive actions observed in addiction, the differences in the components of the 5-HT system were not surprising. Indeed, preclinical research has shown that modulating activity at 5-HT2A receptors may block the expression of alcohol self-administration (Serra et al. 2022) and may also decrease the amount of alcohol intake (Berquist and Fantegrossi 2021). To our knowledge, however, this is the first study showing that differences in the expression of the 5-HT2A receptor can be associated with sensitivity to PF and, indirectly, with various aspects of alcohol addiction.

Similarly, the difference observed in the expression of the GABAB2 receptor, which in a number of previous studies was demonstrated to regulate alcohol sensitivity at the molecular and cellular levels, was not surprising (Farokhnia et al. 2018; Liang et al. 2006; Maccioni et al. 2010). Indeed, alterations in GABA signalling through pharmacological activation or deactivation of GABABRs were also shown to regulate behaviour and brain reward processes, as well as the reinforcing effects of drugs of abuse, including alcohol (Vlachou and Markou 2010). Analogous to 5-HT2A, the lower level of GABAB2 receptors in PF- insensitive animals suggests that decreased GABA signalling is linked to hyposensitivity to PF and stronger motivation to drink alcohol as well as proneness to reinstate drinking following a period of abstinence.

The second locus of the differences between the PF-insensitive and PF-sensitive rats was the Amy, where differences in the expression of genes related to dopaminergic (*Maob*) and GABAergic (*Gabbr1*) neurotransmission were revealed, and in the case of MAO-B, also confirmed at the protein level. Analyses of the intergroup differences in the level of MAO-B revealed a significant interaction between the effects of PF sensitivity and alcohol drinking. In animals sensitive to PF, the level of this enzyme was significantly higher in rats that consumed alcohol than in those that consumed water. Although MAO-B activity has been extensively investigated in alcoholism, there is a considerable inconsistency in the results. The finding of significantly higher MAO-B availability in PF-sensitive, alcohol-drinking animals is in line with some previous studies, which reported an increase in MAO-B levels and activity following chronic ethanol exposure (Ou et al. 2011; Zimatkin et al. 1997), but not with others, reporting no effects of alcohol consumption on MAO-B activity in rats (Della Corte et al. 1994; Sherif et al. 1993). Moreover, MAO-B has been believed to be involved in dopamine degradation, which supports the idea that the increased levels of this enzyme can be attributed to a decrease in extracellular dopamine concentration and enhanced sensitivity to rewarding feedback. However, the exact nature of the interaction between the level of MAO-B, PF sensitivity, and alcohol drinking should be unveiled by further studies.

Last but not least, significant, and confirmed at the protein level, differences in the expression of the *Adh1* gene were detected in the mPFC, and NAcc, where animals from the

alcohol drinking group demonstrated significantly higher levels of this enzyme compared to their water drinking conspecifics. This result seems to validate the applied alcohol drinking procedure at the molecular level. Indeed, mammalian Adhs play a key role in alcohol metabolism and in the interindividual differences it exerts on the body (Edenberg 2007). Chronic alcohol abuse has been demonstrated to lead to Adh induction, increasing alcohol metabolism; thus, elevated levels of this enzyme in EtOH drinking groups confirm efficient exposure to chronic alcohol in our animal model.

Conclusions and limitations

Based on the results of the present experiments, it seems that using rodent-based models, such as the preclinical PRL, can help to reveal neurobiological processes linked with reinforcement-based cognitive biases and their role in AUD. Although we hope this research has provided enough evidence to support the validity of the claim that sensitivity to PF can determine the trajectories of alcohol addiction, there are still a number of outstanding issues that future research will need to address. First, we still do not know the degree of the causal relationship between increased/decreased sensitivity to PF and vulnerability to AUD. Further development of translational preclinical tests of sensitivity to PF should help to elucidate this issue and may help to design personalized treatments based on these cognitive variables. Second, although we have demonstrated that there are distributed changes in physiological and molecular variables within multiple regions of the brain that occur over the course of alcohol use in rats and can persist into periods of abstinence, further studies looking at neurochemical correlates of altered feedback sensitivity in this context are needed. Although the WB method is one of the most reliable techniques for protein identification and quantification, its application is limited by the availability of high-quality specific primary antibodies against a given protein. For this reason, we were not able to confirm changes at the protein level in the expression of certain genes (Comt, Drd2, Gria1, Tph2, Grm2, and Npy), which could provide additional valuable insights into the changes induced by PF- sensitivity x alcohol interactions.

We also need more detailed pharmacological studies using drugs with known profiles in humans to understand the value of targeting PF sensitivity in AUD. It will be highly desirable to use voltammetry, optogenetics, or other biosensors and electrophysiological measures to characterize neuronal pathways and to elucidate the exact function and dynamic balance between cortical and subcortical regions involved in the interaction between PF sensitivity and AUD. Finally, further conceptual and empirical development is required to provide an integrated account of the role of PF sensitivity in the aetiology, development, and recurrence of AUD.

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Author contribution All authors have made a substantial contribution to the concept and design of the article and revised it critically for important intellectual content. All authors approved the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability The data that support the findings of this study are available from the corresponding author, [RR], upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

References

Achterberg EJ, van Kerkhof LW, Damsteegt R, Trezza V, Vanderschuren LJ (2015) Methylphenidate and atomoxetine inhibit social play behavior through prefrontal and subcortical limbic mechanisms in rats. J Neurosci 35:161–169

Bari A, Theobald DE, Caprioli D, Mar AC, Aidoo-Micah A, Dalley JW, Robbins TW (2010) Serotonin modulates sensitivity to reward and negative feedback in a probabilistic reversal learning task in rats. Neuropsychopharmacology 35:1290–1301

Berquist MD, Fantegrossi WE (2021) Effects of 5-HT2A receptor agonist 2,5-dimethoxy-4-iodoamphetamine on alcohol consumption in Long-Evans rats. Behav Pharmacol 32:382–391

Chamberlain SR, Müller U, Blackwell AD, Clark L, Robbins TW, Sahakian BJ (2006) Neurochemical modulation of response inhibition and probabilistic learning in humans. Science 311:861–863

Cieslik A, Noworyta K, Rygula R (2022) Trait sensitivity to negative feedback determines the intensity of compulsive alcohol seeking and taking in male rats. J Psychiatry Neurosci : JPN 47:E252-e262

Clarke HF, Cardinal RN, Rygula R, Hong YT, Fryer TD, Sawiak SJ, Ferrari V, Cockcroft G, Aigbirhio FI, Robbins TW, Roberts AC (2014) Orbitofrontal dopamine depletion upregulates caudate

dopamine and alters behavior via changes in reinforcement sensitivity. J Neurosci 34:7663–7676
Cools R, Clark L, Owen AM, Robbins TW (2002) Defining the neural mechanisms of probabilistic reversal learning using event-related functional magnetic resonance imaging. J Neurosci 22:4563–4567
Cools R, Roberts AC, Robbins TW (2008) Serotoninergic regulation of emotional and behavioural control processes. Trends Cogn Sci 12:31–40
Cools R, Frank MJ, Gibbs SE, Miyakawa A, Jagust W, D'Esposito M (2009) Striatal dopamine predicts outcome-specific reversal learning and its sensitivity to dopaminergic drug administration. J Neurosci 29:1538–1543
Cservenka A (2016) Neurobiological phenotypes associated with a family history of alcoholism. Drug Alcohol Depend 158:8–21
Dalton GL, Phillips AG, Floresco SB (2014) Preferential involvement by nucleus accumbens shell in mediating probabilistic learning and reversal shifts. J Neurosci 34:4618–4626
Della Corte L, Bianchi L, Colivicchi A, Kennedy NP, Tipton KF (1994) The effects of ethanol on rat brain monoamine oxidase activities. In: Tipton KF, Youdim MBH, Barwell CJ, Callingham BA, Lyles GA (eds) Amine Oxidases: function and dysfunction. Springer Vienna, Vienna, pp 75–81
den Ouden Hanneke EM, Daw Nathaniel D, Fernandez G, Elshout Joris A, Rijpkema M, Hoogman M, Franke B, Cools R (2013) Dissociable effects of dopamine and serotonin on reversal learning. Neuron 80:1090–1100
Edenberg HJ (2007) The Genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res Health 30:5–5
Fahlke C, Eriksson CJP (2000) Effect of adrenelectomy and exposure to corticosterone on alcohol-preferring and alcohol-avoiding rat lines. Alcohol Alcohol 35:139–144
Fahlke C, H»rd E, Hansen S (1996) Facilitation of ethanol consumption by intracerebroventricular infusions of corticosterone. Psychopharmacology 1996 127:1 127: 133–139
Farokhnia M, Deschaine SL, Sadighi A, Farinelli LA, Lee MR, Akhlaghi F, Leggio L (2018) A deeper insight into how GABA-B receptor agonism via baclofen may affect alcohol seeking and consumption: lessons learned from a human laboratory investigation. Molecular Psychiatry 26(2):545–555
Feil J, Hasking P (2008) The relationship between personality, coping strategies and alcohol use. Addict Res Theory 16:526–537
Franken IHA, Muris P (2006) BIS/BAS personality characteristics and college students' substance use. Personality Individ Differ 40:1497–1503
Gąska M, Kuśmider M, Solich J, Faron-Górecka A, Krawczyk MJ, Kułakowski K, Dziedzicka-Wasylewska M (2012) Analysis of region-specific changes in gene expression upon treatment with citalopram and desipramine reveals temporal dynamics in response to antidepressant drugs at the transcriptome level. Psychopharmacology 223:281–297
Giuliano C, Peña-Oliver Y, Goodlett CR, Cardinal RN, Robbins TW, Bullmore ET, Belin D, Everitt BJ (2018) Evidence for a long-lasting compulsive alcohol seeking phenotype in rats. Neuropsychopharmacology 43:728–738
Golebiowska J, Rygula R (2017a) Effects of acute dopaminergic and serotonergic manipulations in the ACI paradigm depend on the basal valence of cognitive judgement bias in rats. Behav Brain Res 327:133–143
Golebiowska J, Rygula R (2017b) Lesions of the orbitofrontal but not medial prefrontal cortex affect cognitive judgment bias in rats. Front Behav Neurosci 11:51
Heinz AJ, Veilleux JC, Kassel JD (2009) The role of cognitive structure in college student problem drinking. Addict Behav 34:212–218
Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J (2007) qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol 8:R19
Hipolito L, Sanchez MJ, Polache A, Granero L (2007) Brain metabolism of ethanol and alcoholism: an update. Curr Drug Metab 8:716–727
Howell DC (1997) Statistical methods for psychology, 4th edn. Wadsworth, Belmont, California
Ineichen C, Sigrist H, Spinelli S, Lesch KP, Sautter E, Seifritz E, Pryce CR (2012) Establishing a probabilistic reversal learning test in mice: evidence for the processes mediating reward-stay and punishment-shift behaviour and for their modulation by serotonin. Neuropharmacology 63:1012–1021
Kauer JA, Malenka RC (2007) Synaptic plasticity and addiction. Nat Rev Neurosci 8:844–858
Klein TA, Neumann J, Reuter M, Hennig J, von Cramon DY, Ullsperger M (2007) Genetically determined differences in learning from errors. Science 318:1642–1645
Koob GF, Roberts AJ, Schulteis G, Parsons LH, Heyser CJ, Hyytia P, Merlo-Pich E, Weiss F (1998) Neurocircuitry targets in ethanol reward and dependence. Alcohol Clin Exp Res 22:3–9
Liang JH, Chen F, Krstew E, Cowen MS, Carroll FY, Crawford D, Beart PM, Lawrence AJ (2006) The GABAB receptor allosteric modulator CGP7930, like baclofen, reduces operant self-administration of ethanol in alcohol-preferring rats. Neuropharmacology 50:632–639
Loxton NJ, Dawe S (2001) Alcohol abuse and dysfunctional eating in adolescent girls: The influence of individual differences in sensitivity to reward and punishment. Int J Eat Disord 29:455–462
Maccioni P, Thomas AW, Carai MAM, Gessa GL, Malherbe P, Colombo G (2010) The positive allosteric modulator of the GABAB receptor, rac-BHFF, suppresses alcohol self-administration. Drug Alcohol Depend 109:96–103
Moe FD, Moltu C, McKay JR, Nesvag S, Bjornestad J (2022) Is the relapse concept in studies of substance use disorders a "one size fits all" concept? A systematic review of relapse operationalisations. Drug Alcohol Rev 41:743–758
Noworyta K, Rygula R (2021) Phenotypes of reinforcement sensitivity as predictors of the response to acute antidepressant treatment in rats. Eur Neuropsychopharmacol 43:102–115
Noworyta-Sokolowska K, Kozub A, Jablonska J, Rodriguez Parkitna J, Drozd R, Rygula R (2019) Sensitivity to negative and positive feedback as a stable and enduring behavioural trait in rats. Psychopharmacology 236:2389–2403
Ou X-M, Johnson C, Lu D, Johnson S, Paul IA, Austin MC, Iyo AH, Miguel-Hidalgo JJ, Luo J, Bell RL, Grunewald M, Wang J, Sittman DB (2011) Ethanol increases TIEG2–MAO B cell death cascade in the prefrontal cortex of ethanol-preferring rats. Neurotox Res 19:511–518
Paulus MP, Hozack N, Frank L, Brown GG (2002) Error rate and outcome predictability affect neural activation in prefrontal cortex and anterior cingulate during decision-making. Neuroimage 15:836–846
Paulus MP, Hozack N, Frank L, Brown GG, Schuckit MA (2003) Decision making by methamphetamine-dependent subjects is associated with error-rate-independent decrease in prefrontal and parietal activation. Biol Psychiat 53:65–74
Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates
Pessiglione M, Seymour B, Flandin G, Dolan RJ, Frith CD (2006) Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. Nature 442:1042–1045
Piazza PV, Le Moal M (1997) Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. Brain Res Rev 25:359–372

Roberts DCS, Bennett SAL (1993) Heroin self-administration in rats under a progressive ratio schedule of reinforcement. Psychopharmacology 111:215–218

Robinson OJ, Cools R, Carlisi CO, Sahakian BJ, Drevets WC (2012) Ventral striatum response during reward and punishment reversal learning in unmedicated major depressive disorder. Am J Psychiatry 169:152–159

Rygula R, Papciak J, Popik P (2013) Trait pessimism predicts vulnerability to stress-induced anhedonia in rats. Neuropsychopharmacology 38:2188–2196

Rygula R, Papciak J, Popik P (2014) The effects of acute pharmacological stimulation of the 5-HT, NA and DA systems on the cognitive judgement bias of rats in the ambiguous-cue interpretation paradigm. Eur Neuropsychopharmacol 24:1103–1111

Rygula R, Clarke HF, Cardinal RN, Cockcroft GJ, Xia J, Dalley JW, Robbins TW, Roberts AC (2015a) Role of central serotonin in anticipation of rewarding and punishing outcomes: effects of selective amygdala or orbitofrontal 5-HT depletion. Cereb Cortex 25:3064–3076

Rygula R, Golebiowska J, Kregiel J, Kubik J, Popik P (2015b) Effects of optimism on motivation in rats. Front Behav Neurosci 9:32–32

Rygula R, Noworyta-Sokolowska K, Drozd R, Kozub A (2018) Using rodents to model abnormal sensitivity to feedback in depression. Neurosci Biobehav Rev 95:336–346

Schulteis G, Markou A, Cole M, Koob GF (1995) Decreased brain reward produced by ethanol withdrawal. Proc Natl Acad Sci U S A 92:5880–5884

Serra YA, Barros-Santos T, Anjos-Santos A, Kisaki ND, Jovita-Farias C, Leite JPC, Santana MCE, Coimbra J, de Jesus NMS, Sulima A, Barbosa PCR, Malpezzi-Marinho ELA, Rice KC, Oliveira-Lima AJ, Berro LF, Marinho EAV (2022) Role of 5-HT receptors in the effects of ayahuasca on ethanol self-administration using a two-bottle choice paradigm in male mice. Psychopharmacology 239:1679–1687

Sherif F, Wahlstöbm G, Oreland L (1993) Brain GABA-transaminase and monoamine oxidase after chronic ethanol treatment in rats. Alcoholism Clin Exp Res 17:1313–1318

Stewart SH, Sherry SB, Comeau MN, Mushquash CJ, Collins P, Van Wilgenburg H (2011) Hopelessness and excessive drinking among aboriginal adolescents: the mediating roles of depressive symptoms and drinking to cope. Depression Research and Treatment 2011

Veilleux JC, Skinner KD, Reese ED, Shaver JA (2014) Negative affect intensity influences drinking to cope through facets of emotion dysregulation. Personality Individ Differ 59:96–101

Vlachou S, Markou A (2010) GABAB receptors in reward processes. Adv Pharmacol 58:315–371

Volkow ND, Wang GJ, Fowler JS, Tomasi D, Telang F, Baler R (2010) Addiction: Decreased reward sensitivity and increased expectation sensitivity conspire to overwhelm the brain's control circuit. BioEssays 32:748–755

Zimatkin SM, Tsydik VF, Lelevich VV (1997) Alcohol consumption alters monoamine oxidase activities in brain structures: relation to ethanol craving. Casopis Lekaru Ceskych 136:666–669

Supplemental material

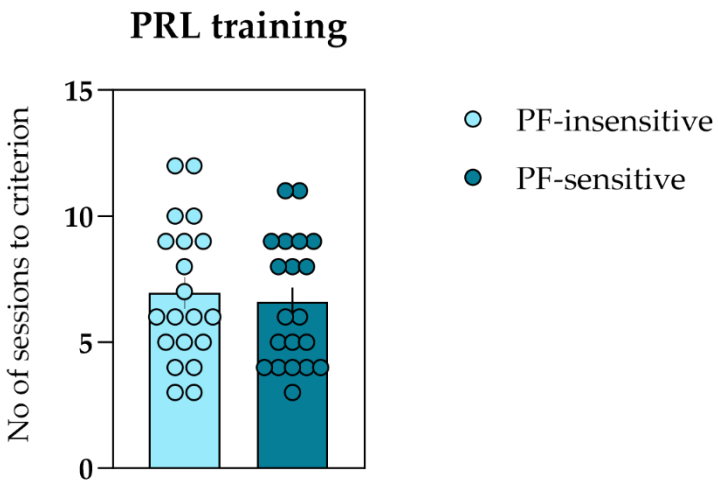


Figure S1. PRL training. The average number of tests needed to achieve PRL training criterion in PF-insensitive (N = 20) and PF-sensitive (N = 20) rats.

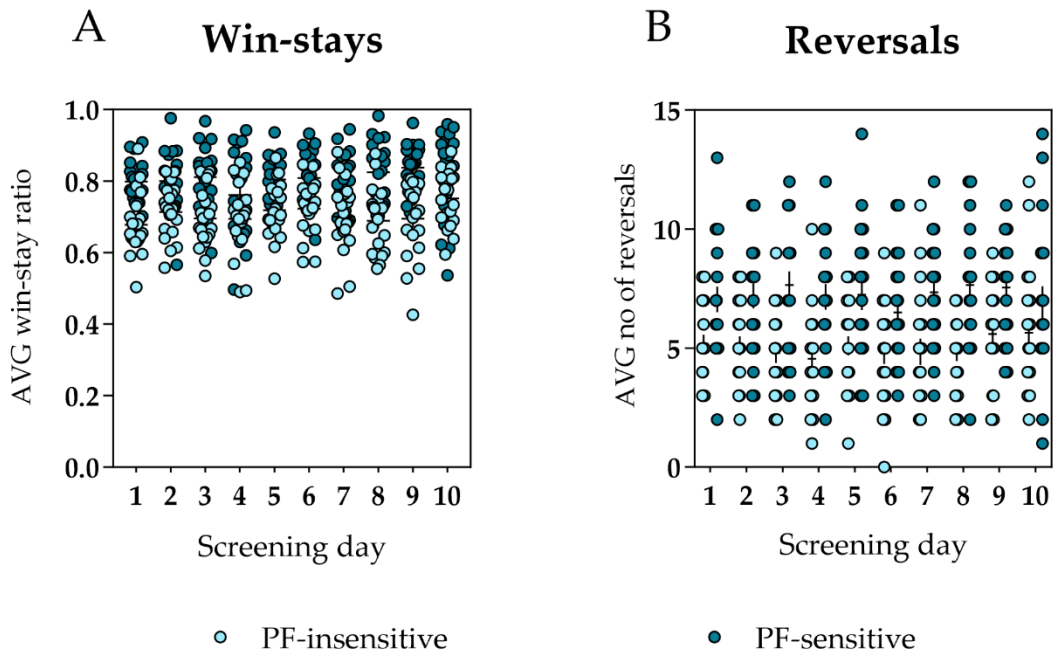


Figure S2. Positive feedback sensitivity screening. Individual data for (A) win-stay behaviours, and (B) reversal performance of all tested animals (20 PF-insensitive and 20 PF-sensitive) across 10 PRL screening tests.

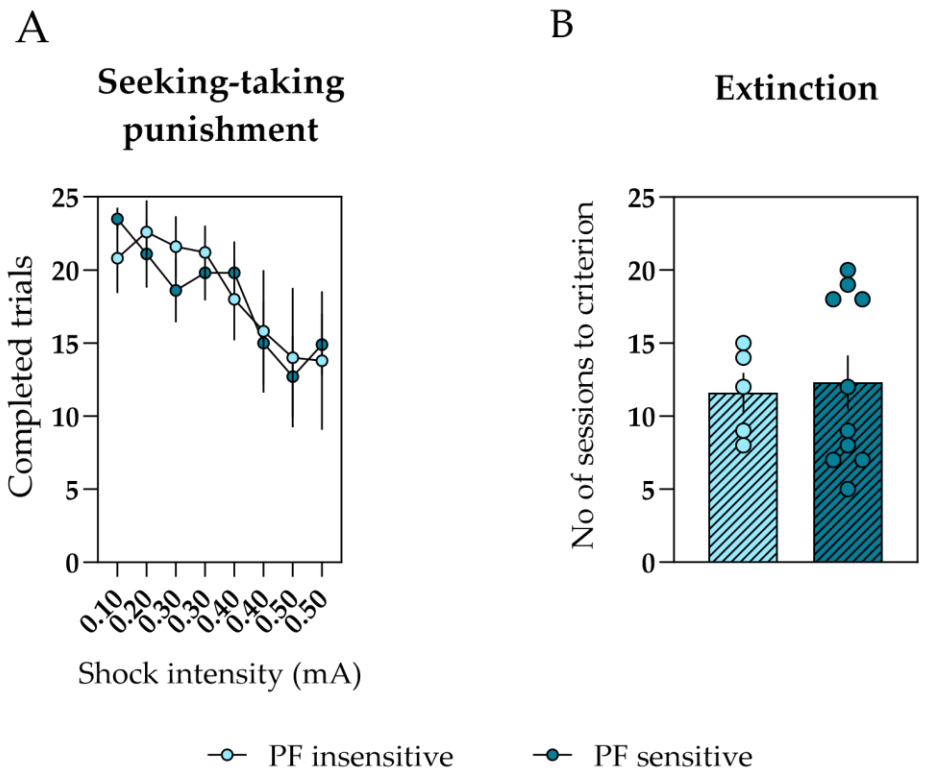


Figure S3. The effects of trait sensitivity to positive feedback (PF) on the alcohol-seeking in face of electric shock punishment (A), and on the length of alcohol-seeking extinction (B) in rats. A) As the shock intensity increased, the rats significantly decreased the number of completed trials. There were no significant differences in the number of trials completed between the PF-insensitive (N = 5) and PF-sensitive (N = 10) groups of animals. B) Following the seeking-taking punishment procedure, the rats underwent 5 baseline seeking-taking tests and were subsequently subjected to the extinction phase, during which seeking responses did not result in alcohol delivery. There was no statistical difference in length of extinction between PF-insensitive (N = 5) and PF-sensitive (N = 10) rats. Data are presented as the mean ± SEM

Table S1. The effects of trait sensitivity to PF and alcohol drinking on gene expression. Statistically significant effects and interactions are bolded.

Structure	Gene name	N	Interaction	Treatment	Sensitivity
ACC	*Adh1*	32	$F_{(1, 28)} = 0.988$ $p = 0.329$	$F_{(1, 28)} = 3.627$ $p = 0.067$	$F_{(1, 28)} = 0.019$ $p = 0.890$
	Cat	34	$F_{(1, 30)} = 0.019$ $p = 0.890$	$F_{(1, 30)} = 1.185$ $p = 0.285$	$F_{(1, 30)} = 0.035$ $p = 0.854$
	Comt	34	$F_{(1, 30)} = 0.387$ $p = 0.534$	**$F_{(1, 30)} = 5.223$** $p =$ **0.030 ***	$F_{(1, 30)} = 0.020$ $p = 0.888$
	Drd1	33	$F_{(1, 29)} = 3.969$ $p = 0.056$	$F_{(1, 29)} = 0.761$ $p = 0.390$	**$F_{(1, 29)} = 4.556$** $p =$ **0.041 ***
	Drd2	34	$F_{(1, 30)} = 0.282$ $p = 0.600$	$F_{(1, 30)} = 1.521$ $p = 0.227$	$F_{(1, 30)} = 0.349$ $p = 0.559$
	Gabbr1	34	$F_{(1, 30)} = 0.165$ $p = 0.688$	$F_{(1, 30)} = 2.581$ $p = 0.119$	$F_{(1, 30)} = 1.213$ $p = 0.280$
	Gabbr2	34	$F_{(1, 30)} = 0.001$ $p = 0.979$	$F_{(1, 30)} = 0.673$ $p = 0.416$	$F_{(1, 30)} = 0.052$ $p = 0.821$
	Gabra1	34	$F_{(1, 30)} = 0.899$ $p = 0.351$	$F_{(1, 30)} = 0.512$ $p = 0.480$	$F_{(1, 30)} = 0.611$ $p = 0.440$
	Gad1	34	$F_{(1, 30)} = 0.369$ $p = 0.548$	$F_{(1, 30)} = 0.752$ $p = 0.393$	$F_{(1, 30)} = 0.369$ $p = 0.548$
	Gad2	34	$F_{(1, 30)} = 0.467$ $p = 0.500$	$F_{(1, 30)} = 0.230$ $p = 0.635$	$F_{(1, 30)} = 0.343$ $p = 0.562$
	Gria1	34	$F_{(1, 30)} = 0.265$ $p = 0.611$	$F_{(1, 30)} = 0.143$ $p = 0.708$	**$F_{(1, 30)} = 4.809$** $p =$ **0.036 ***
	Grin2a	34	$F_{(1, 30)} = 0.899$ $p = 0.351$	$F_{(1, 30)} = 0.512$ $p = 0.480$	$F_{(1, 30)} = 0.611$ $p = 0.441$

	Grin2b	34	$F_{(1, 30)} = 0.143$ $p = 0.708$	$F_{(1, 30)} = 0.199$ $p = 0.660$	$F_{(1, 30)} = 0.033$ $p = 0.857$
	Grm2	34	$F_{(1, 30)} = 0.396$ $p = 0.534$	$F_{(1, 30)} = 0.031$ $p = 0.861$	$F_{(1, 30)} = 3.754$ $p = 0.062$
	Grm3	34	$F_{(1, 30)} = 0.026$ $p = 0.873$	$F_{(1, 30)} = 0.385$ $p = 0.539$	$F_{(1, 30)} < 0.001$ $p = 0.985$
	Grm5	34	$F_{(1, 30)} = 0.074$ $p = 0.788$	$F_{(1, 30)} = 1.217$ $p = 0.279$	$F_{(1, 30)} = 2.077$ $p = 0.160$
	Htr1a	34	$F_{(1, 30)} = 0.053$ $p = 0.820$	$F_{(1, 30)} = 2.300$ $p = 0.140$	$F_{(1, 30)} = 1.634$ $p = 0.211$
	Htr2a	34	$F_{(1, 30)} = 1.209$ $p = 0.280$	$F_{(1, 30)} = 0.012$ $p = 0.912$	$F_{(1, 30)} = 1.607$ $p = 0.215$
	Htr2b		-		
	Htr3a	34	$F_{(1, 30)} = 0.104$ $p = 0.749$	$F_{(1, 30)} = 0.126$ $p = 0.725$	**$F_{(1, 30)} = 5.855$** **$p = 0.022$ ***
	Npy	34	Kruskal–Wallis test: $p = 0.395$		
	Maoa	33	$F_{(1, 29)} = 0.458$ $p = 0.504$	**$F_{(1, 29)} = 4.732$** **$p = 0.038$ ***	$F_{(1, 29)} = 0.231$ $p = 0.635$
	Maob	34	$F_{(1, 30)} = 1.643$ $p = 0.210$	$F_{(1, 30)} = 1.510$ $p = 0.229$	$F_{(1, 30)} = 0.371$ $p = 0.547$
	Slc1a2	34	$F_{(1, 30)} = 1.064$ $p = 0.311$	$F_{(1, 30)} = 0.014$ $p = 0.907$	$F_{(1, 30)} = 0.019$ $p = 0.892$
	Slc6a3		-		
	Slc6a4	32	$F_{(1, 28)} = 2.125$ $p = 0.156$	$F_{(1, 28)} = 0.088$ $p = 0.769$	$F_{(1, 28)} = 0.539$ $p = 0.469$

	Th	34	$F_{(1, 30)} = 0.235$ $p = 0.631$	$F_{(1, 30)} = 1.553$ $p = 0.222$	$F_{(1, 30)} = 0.773$ $p = 0.386$
	Tph2	34	Kruskal–Wallis test: p = 0.081		
mPFC	***Adh1***	**33**	**$F_{(1, 29)} = 5.048$** **p = 0.032 ***	**$F_{(1, 29)} = 5.072$** **p = 0.032 ***	$F_{(1, 29)} = 3.423$ p = 0.075
	Cat	34	$F_{(1, 30)} = 0.037$ p = 0.849	**$F_{(1, 30)} = 7.312$** **p = 0.011 ***	**$F_{(1, 30)} = 9.431$** **p = 0.005 ****
	Comt	34	$F_{(1, 30)} = 0.447$ p = 0.509	**$F_{(1, 30)} = 18.320$** **p < 0.001 *****	$F_{(1, 30)} = 2.791$ p = 0.105
	Drd1	34	$F_{(1, 30)} = 0.033$ p = 0.856	$F_{(1, 30)} = 1.779$ p = 0.193	$F_{(1, 30)} = 0.958$ p = 0.336
	Drd2	34	$F_{(1, 29)} = 0.194$ p = 0.663	$F_{(1, 29)} = 0.517$ p = 0.478	$F_{(1, 29)} = 0.336$ p = 0.567
	Gabbr1	34	$F_{(1, 30)} = 0.002$ p = 0.965	$F_{(1, 30)} = 0.993$ p = 0.327	$F_{(1, 30)} = 2.292$ p = 0.141
	Gabbr2	34	Kruskal–Wallis test: p = 0.609		
	Gabra1	34	$F_{(1, 30)} = 1.029$ p = 0.318	$F_{(1, 30)} = 0.001$ p = 0.975	$F_{(1, 30)} = 2.779$ p = 0.106
	Gad1	34	$F_{(1, 30)} = 0.266$ p = 0.610	$F_{(1, 30)} = 1.918$ p = 0.176	$F_{(1, 30)} = 2.092$ p = 0.158
	Gad2	34	$F_{(1, 30)} = 0.108$ p = 0.745	$F_{(1, 30)} = 0.790$ p = 0.379	$F_{(1, 30)} = 2.224$ p = 0.146
	Gria1	34	$F_{(1, 30)} = 0.001$ p = 0.981	$F_{(1, 30)} = 0.702$ p = 0.409	$F_{(1, 30)} = 0.154$ p = 0.698
	Grin2a	34	Kruskal–Wallis test: p = 0.092		

	Grin2b	34	$F_{(1, 30)} = 0.195$ $p = 0.662$	$F_{(1, 30)} = 0.452$ $p = 0.506$	$F_{(1, 30)} = 0.456$ $p = 0.505$
	Grm2	34	$F_{(1, 30)} = 0.056$ $p = 0.815$	$F_{(1, 30)} = 0.386$ $p = 0.539$	$F_{(1, 30)} = 4.144$ $p = 0.051$
	Grm3	34	$F_{(1, 30)} = 0.084$ $p = 0.774$	$F_{(1, 30)} = 0.001$ $p = 0.974$	$F_{(1, 30)} = 0.405$ $p = 0.530$
	Grm5	34	Kruskal–Wallis test: $p = 0.989$		
	Htr1a	34	$F_{(1, 30)} = 0.066$ $p = 0.799$	$F_{(1, 30)} = 0.314$ $p = 0.580$	$F_{(1, 30)} = 0.315$ $p = 0.579$
	Htr2a	34	$F_{(1, 30)} = 0.010$ $p = 0.921$	$F_{(1, 30)} = 0.032$ $p = 0.859$	$F_{(1, 30)} = 2.933$ $p = 0.097$
	Htr2b	29	$F_{(1, 25)} = 0.885$ $p = 0.356$	$F_{(1, 25)} = 1.594$ $p = 0.218$	$F_{(1, 25)} = 1.171$ $p = 0.290$
	Htr3a	34	$F_{(1, 30)} = 0.676$ $p = 0.4174$	$F_{(1, 30)} = 0.716$ $p = 0.404$	$F_{(1, 30)} = 0.828$ $p = 0.370$
	Npy	34	$F_{(1, 30)} = 0.806$ $p = 0.376$	$F_{(1, 30)} = 3.297$ $p = 0.079$	$F_{(1, 30)} = 1.166$ $p = 0.289$
	Maoa	34	$F_{(1, 30)} = 0.002$ $p = 0.968$	$F_{(1, 30)} = 0.518$ $p = 0.477$	$F_{(1, 30)} = 1.151$ $p = 0.292$
	Maob	34	$F_{(1, 30)} = 0.010$ $p = 0.919$	$F_{(1, 30)} = 4.052$ $p = 0.053$	$F_{(1, 30)} = 3.084$ $p = 0.089$
	Slc1a2	34	$F_{(1, 30)} = 0.005$ $p = 0.946$	$F_{(1, 30)} = 1.438$ $p = 0.240$	$F_{(1, 30)} = 3.653$ $p = 0.066$
	Slc6a3	19	$F_{(1, 15)} = 0.103$ $p = 0.753$	$F_{(1, 15)} = 0.010$ $p = 0.921$	$F_{(1, 15)} = 0.530$ $p = 0.478$

	Slc6a4	34	$F_{(1, 30)} = 0.001$ $p = 0.972$	$F_{(1, 30)} = 0.012$ $p = 0.914$	$F_{(1, 30)} = 1.105$ $p = 0.302$
	Th	34	$F_{(1, 30)} = 0.018$ $p = 0.895$	$F_{(1, 30)} = 0.227$ $p = 0.637$	$F_{(1, 30)} = 0.373$ $p = 0.546$
	Tph2	33	$F_{(1, 29)} = 0.633$ $p = 0.433$	$F_{(1, 29)} = 1.428$ $p = 0.242$	$F_{(1, 29)} = 1.136$ $p = 0.295$
Amy	*Adh1*	32	Kruskal–Wallis test: p = 0.124		
	Cat	31	$F_{(1, 27)} = 0.019$ $p = 0.891$	$F_{(1, 27)} = 0.842$ $p = 0.367$	$F_{(1, 27)} = 0.650$ $p = 0.427$
	Comt	32	Kruskal–Wallis test: p = 0.274		
	Drd1	32	$F_{(1, 28)} = 0.680$ $p = 0.417$	$F_{(1, 28)} = 0.008$ $p = 0.932$	$F_{(1, 28)} = 1.007$ $p = 0.324$
	Drd2	**32**	**$F_{(1, 28)} = 6.200$** **$p = 0.019$ ***	**$F_{(1, 28)} = 5.092$** **$p = 0.032$ ***	$F_{(1, 28)} = 0.991$ $p = 0.328$
	Gabbr1	**31**	**$F_{(1, 27)} = 9.466$** $p =$ **0.005 ****	$F_{(1, 27)} = 3.727$ $p = 0.064$	$F_{(1, 27)} = 1.761$ $p = 0.196$
	Gabbr2	32	$F_{(1, 28)} = 0.043$ $p = 0.837$	$F_{(1, 28)} = 0.474$ $p = 0.497$	$F_{(1, 28)} = 0.886$ $p = 0.355$
	Gabra1	32	$F_{(1, 28)} = 0.187$ $p = 0.669$	$F_{(1, 28)} = 0.118$ $p = 0.734$	$F_{(1, 28)} = 1.525$ $p = 0.227$
	Gad1	32	$F_{(1, 28)} = 0.017$ $p = 0.897$	**$F_{(1, 28)} = 4.338$** **$p = 0.047$ ***	$F_{(1, 28)} = 1.216$ $p = 0.280$
	Gad2	32	$F_{(1, 28)} = 0.902$ $p = 0.350$	$F_{(1, 28)} = 3.643$ $p = 0.067$	$F_{(1, 28)} = 2.088$ $p = 0.160$
	Gria1	32	$F_{(1, 28)} = 1.170$	$F_{(1, 28)} = 0.274$	$F_{(1, 28)} = 0.707$

			p = 0.287	p = 0.605	p = 0.408
	Grin2a	32	$F_{(1, 28)}$ = 0.022 p = 0.884	$F_{(1, 28)}$ = 1.490 p = 0.232	$F_{(1, 28)}$ = 0.125 p = 0.727
	Grin2b	32	$F_{(1, 28)}$ = 0.144 p = 0.707	$F_{(1, 28)}$ = 0.197 p = 0.661	$F_{(1, 28)}$ = 0.217 p = 0.645
	Grm2	32	$F_{(1, 28)}$ = 1.206 p = 0.282	$F_{(1, 28)}$ = 0.423 p = 0.521	$F_{(1, 28)}$ = 2.257 p = 0.144
	Grm3	31	$F_{(1, 27)}$ = 0.055 p = 0.816	$F_{(1, 27)}$ = 0.003 p = 0.955	$F_{(1, 27)}$ = 2.095 p = 0.159
	Grm5	32	$F_{(1, 28)}$ = 0.664 p = 0.422	$F_{(1, 28)}$ = 2.104 p = 0.158	$F_{(1, 28)}$ = 0.201 p = 0.657
	Htr1a	32	$F_{(1, 28)}$ = 0.109 p = 0.743	$F_{(1, 28)}$ = 2.959 p = 0.096	$F_{(1, 28)}$ = 0.073 p = 0.790
	Htr2a	32	$F_{(1, 28)}$ = 1.209 p = 0.280	$F_{(1, 28)}$ = 0.012 p = 0.912	$F_{(1, 28)}$ = 1.607 p = 0.215
	Htr2b	26	$F_{(1, 22)}$ = 0.363 p = 0.553	$F_{(1, 22)}$ = 2.826 p = 0.107	$F_{(1, 22)}$ = 2.376 p = 0.138
	Htr3a	32	$F_{(1, 28)}$ = 0.274 p = 0.605	$F_{(1, 28)}$ = 3.046 p = 0.092	$F_{(1, 28)}$ = 2.541 p = 0.122
	Npy	32	$F_{(1, 28)}$ = 0.393 p = 0.536	$F_{(1, 28)}$ = 0.622 p = 0.437	$F_{(1, 28)}$ = 1.464 p = 0.236
	Maoa	32	Kruskal–Wallis test: p = 0.523		
	Maob	32	$F_{(1, 28)}$ = 0.347 p = 0.561	$F_{(1, 28)}$ = 0.583 p = 0.451	**$F_{(1, 28)}$ = 5.804 p = 0.023 ***
	Slc1a2	32	$F_{(1, 28)}$ = 0.238	$F_{(1, 28)}$ = 1.004	$F_{(1, 28)}$ = 2.490

			p = 0.630	p = 0.325	p = 0.126
	Slc6a3			-	
	Slc6a4	32	$F_{(1, 28)} = 0.424$ p = 0.520	$F_{(1, 28)} = 0.720$ p = 0.403	$F_{(1, 28)} = 3.774$ p = 0.062
	Th	32	Kruskal–Wallis test: p = 0.086		
	Tph2	**32**	**$F_{(1, 28)} = 4.732$** **p = 0.038 ***	$F_{(1, 28)} = 2.625$ p = 0.116	$F_{(1, 28)} = 0.989$ p = 0.329
OFC	***Adh1***	**34**	**Kruskal–Wallis test: p = 0.038 ***		
	Cat	34	$F_{(1, 30)} = 0.029$ p = 0.867	**$F_{(1, 30)} = 5.351$** p = **0.028 ***	$F_{(1, 30)} = 0.916$ p = 0.346
	Comt	34	$F_{(1, 30)} = 0.001$ p = 0.972	$F_{(1, 30)} = 1.396$ p = 0.247	$F_{(1, 30)} = 0.387$ p = 0.538
	Drd1	32	$F_{(1, 28)} = 0.077$ p = 0.784	$F_{(1, 28)} = 1.024$ p = 0.320	$F_{(1, 28)} = 0.236$ p = 0.631
	Drd2	33	$F_{(1, 29)} = 0.219$ p = 0.643	$F_{(1, 29)} = 0.787$ p = 0.382	$F_{(1, 29)} = 2.284$ p = 0.142
	Gabbr1	34	$F_{(1, 30)} = 0.230$ p = 0.635	$F_{(1, 30)} = 0.050$ p = 0.824	$F_{(1, 30)} = 0.846$ p = 0.365
	Gabbr2	34	$F_{(1, 30)} = 2.565$ p = 0.120	$F_{(1, 30)} = 1.091$ p = 0.305	$F_{(1, 30)} = 1.394$ p = 0.247
	Gabra1	34	$F_{(1, 30)} = 0.249$ p = 0.621	$F_{(1, 30)} = 0.054$ p = 0.818	$F_{(1, 30)} = 1.224$ p = 0.277
	Gad1	34	$F_{(1, 30)} = 1.199$ p = 0.282	$F_{(1, 30)} = 3.242$ p = 0.082	$F_{(1, 30)} = 2.275$ p = 0.142
	Gad2	34	$F_{(1, 30)} = 0.708$	**$F_{(1, 30)} = 6.329$**	$F_{(1, 30)} = 2.448$

			p = 0.407	**p = 0.018 ***	p = 0.128
	Gria1	34	$F_{(1, 30)} = 0.361$ p = 0.553	$F_{(1, 30)} = 2.001 \times 10^{-7}$ p = 1.00	$F_{(1, 30)} = 0.273$ p = 0.606
	Grin2a	34	$F_{(1, 30)} = 0.516$ p = 0.478	$F_{(1, 30)} = 0.163$ p = 0.690	$F_{(1, 30)} = 1.719$ p = 0.200
	Grin2b	34	$F_{(1, 30)} = 0.032$ p = 0.860	$F_{(1, 30)} = 0.377$ p = 0.544	$F_{(1, 30)} = 0.416$ p = 0.524
	Grm2	34	$F_{(1, 30)} = 0.707$ p = 0.407	$F_{(1, 30)} = 0.116$ p = 0.736	$F_{(1, 30)} = 2.909$ p = 0.098
	Grm3	34	$F_{(1, 30)} = 0.026$ p = 0.873	$F_{(1, 30)} = 0.385$ p = 0.540	$F_{(1, 30)} = 0.0003$ p = 0.985
	Grm5	34	$F_{(1, 30)} = 0.161$ p = 0.691	$F_{(1, 30)} = 3.220$ p = 0.083	$F_{(1, 30)} = 3.073$ p = 0.090
	Htr1a	34	$F_{(1, 30)} = 0.115$ p = 0.737	**$F_{(1, 30)} = 6.362$** **p = 0.017 ***	$F_{(1, 30)} = 2.551$ p = 0.121
	Htr2a	34	$F_{(1, 30)} = 0.694$ p = 0.411	$F_{(1, 30)} = 0.424$ p = 0.520	$F_{(1, 30)} = 3.298$ p = 0.079
	Htr2b	29	$F_{(1, 25)} = 0.542$ p = 0.468	$F_{(1, 25)} = 2.634$ p = 0.117	$F_{(1, 25)} = 0.265$ p = 0.611
	Htr3a	34	$F_{(1, 30)} = 0.004$ p = 0.953	$F_{(1, 30)} = 2.734$ p = 0.109	$F_{(1, 30)} = 0.763$ p = 0.390
	Npy	33	$F_{(1, 29)} = 0.244$ p = 0.625	$F_{(1, 29)} = 0.563$ p = 0.459	$F_{(1, 29)} = 0.739$ p = 0.397
	Maoa	34	$F_{(1, 30)} = 1.889$ p = 0.180	$F_{(1, 30)} = 1.091$ p = 0.305	$F_{(1, 30)} = 3.852$ p = 0.060

	Maob	34	$F_{(1, 30)} = 0.070$ p = 0.794	$F_{(1, 30)} = 0.320$ p = 0.576	$F_{(1, 30)} = 0.099$ p = 0.755
	Slc1a2	34	$F_{(1, 30)} = 0.080$ p = 0.780	$F_{(1, 30)} = 0.858$ p = 0.362	$F_{(1, 30)} = 1.532$ p = 0.225
	Slc6a3	33	$F_{(1, 29)} = 0.909$ p = 0.384	$F_{(1, 29)} = 0.123$ p = 0.728	$F_{(1, 29)} = 0.136$ p = 0.715
	Slc6a4	33	$F_{(1, 29)} = 0.223$ p = 0.641	$F_{(1, 29)} = 1.130$ p = 0.297	$F_{(1, 29)} = 0.036$ p = 0.851
	Th	34	$F_{(1, 30)} = 2.040$ p = 0.164	$F_{(1, 30)} = 0.146$ p = 0.705	$F_{(1, 30)} = 1.342$ p = 0.256
	Tph2	34	$F_{(1, 30)} = 0.170$ p = 0.683	$F_{(1, 30)} = 0.001$ p = 0.981	$F_{(1, 30)} = 0.652$ p = 0.426
Nacc	***Adh1***	31	$F_{(1, 27)} = 1.814$ p = 0.189	**$F_{(1, 27)} = 8.590$** **p = 0.007 ****	$F_{(1, 27)} = 4.137$ p = 0.052
	Cat	34	$F_{(1, 30)} = 0.409$ p = 0.527	$F_{(1, 30)} = 0.005$ p = 0.946	$F_{(1, 30)} = 0.819$ p = 0.373
	Comt	34	$F_{(1, 30)} = 0.314$ p = 0.580	$F_{(1, 30)} = 0.839$ p = 0.367	$F_{(1, 30)} = 0.458$ p = 0.504
	Drd1	34	$F_{(1, 30)} = 0.271$ p = 0.607	$F_{(1, 30)} = 0.030$ p = 0.864	$F_{(1, 30)} = 0.151$ p = 0.701
	Drd2	34	$F_{(1, 30)} = 0.046$ p = 0.832	$F_{(1, 30)} = 0.1174$ p = 0.734	$F_{(1, 30)} = 0.538$ p = 0.469
	Gabbr1	34	$F_{(1, 30)} = 0.126$ p = 0.725	$F_{(1, 30)} = 0.003$ p = 0.960	$F_{(1, 30)} = 0.966$ p = 0.333
	Gabbr2	33	$F_{(1, 29)} = 2.290$	$F_{(1, 29)} = 0.589$	**$F_{(1, 29)} = 6.557$**

			p = 0.141	p = 0.449	**p = 0.016 ***
	Gabra1	34	$F_{(1, 30)} = 3.502$ p = 0.071	$F_{(1, 30)} = 0.268$ p = 0.609	$F_{(1, 30)} = 1.199$ p = 0.282
	Gad1	34	$F_{(1, 30)} = 0.079$ p = 0.781	$F_{(1, 30)} = 0.402$ p = 0.531	$F_{(1, 30)} = 0.013$ p = 0.909
	Gad2	34	$F_{(1, 30)} = 0.460$ p = 0.503	$F_{(1, 30)} = 1.212$ p = 0.280	$F_{(1, 30)} = 0.786$ p = 0.382
	Gria1	34	$F_{(1, 30)} = 0.057$ p = 0.812	$F_{(1, 30)} = 0.048$ p = 0.829	$F_{(1, 30)} = 0.143$ p = 0.708
	Grin2a	34	$F_{(1, 30)} = 1.472$ p = 0.235	$F_{(1, 30)} = 0.045$ p = 0.833	$F_{(1, 30)} = 1.308$ p = 0.262
	Grin2b	34	$F_{(1, 30)} = 0.434$ p = 0.515	$F_{(1, 30)} = 0.467$ p = 0.500	$F_{(1, 30)} = 1.145$ p = 0.293
	Grm2	33	$F_{(1, 29)} = 1.724$ p = 0.200	$F_{(1, 29)} = 0.184$ p = 0.671	**$F_{(1, 29)} = 4.863$** **p = 0.036 ***
	Grm3	34	$F_{(1, 30)} = 0.219$ p = 0.643	$F_{(1, 30)} = 0.125$ p = 0.726	$F_{(1, 30)} = 0.458$ p = 0.504
	Grm5	34	$F_{(1, 30)} = 1.366$ p = 0.252	$F_{(1, 30)} = 0.263$ p = 0.612	$F_{(1, 30)} = 0.271$ p = 0.607
	Htr1a	34	$F_{(1, 30)} = 0.585$ p = 0.450	$F_{(1, 30)} = 0.031$ p = 0.863	**$F_{(1, 30)} = 6.452$** p = **0.017 ***
	Htr2a	**34**	**$F_{(1, 30)} = 4.606$** **p = 0.040 ***	$F_{(1, 30)} = 2.248$ p = 0.144	**$F_{(1, 30)} = 4.367$** **p = 0.045 ***
	Htr2b	23	$F_{(1, 19)} = 1.425$ p = 0.247	$F_{(1, 19)} = 0.643$ p = 0.433	$F_{(1, 19)} = 0.063$ p = 0.804

	Htr3a	34	$F_{(1, 30)} = 0.012$ $p = 0.913$	$F_{(1, 30)} = 0.082$ $p = 0.776$	$F_{(1, 30)} = 0.574$ $p = 0.455$
	Npy	34	$F_{(1, 30)} = 0.793$ $p = 0.380$	$F_{(1, 30)} = 0.063$ $p = 0.804$	**$F_{(1, 30)} = 10.020$** **$p = 0.004$ ****
	Maoa	34	Kruskal–Wallis test: $p = 0.972$		
	Maob	34	$F_{(1, 30)} = 0.182$ $p = 0.673$	$F_{(1, 30)} = 0.502$ $p = 0.484$	$F_{(1, 30)} = 0.0004$ $p = 0.983$
	Slc1a2	34	$F_{(1, 30)} = 0.009$ $p = 0.926$	$F_{(1, 30)} = 0.002$ $p = 0.965$	$F_{(1, 30)} = 0.061$ $p = 0.807$
	Slc6a3	34	$F_{(1, 30)} = 0.973$ $p = 0.332$	$F_{(1, 30)} = 2.639$ $p = 0.115$	**$F_{(1, 30)} = 5.166$** **$p = 0.030$ ***
	Slc6a4	**33**	**$F_{(1, 29)} = 4.977$** $p =$ **0.034 ***	**$F_{(1, 29)} = 6.895$** **$p = 0.014$ ***	$F_{(1, 29)} = 0.134$ $p = 0.717$
	Th	33	$F_{(1, 29)} = 0.027$ $p = 0.871$	$F_{(1, 29)} = 0.411$ $p = 0.527$	$F_{(1, 29)} = 1.548$ $p = 0.223$
	Tph2	34	$F_{(1, 30)} = 0.174$ $p = 0.680$	$F_{(1, 30)} = 0.020$ $p = 0.890$	$F_{(1, 30)} = 0.013$ $p = 0.908$

Abnormalities in the gene expression readings were detected in certain samples on the RT-PCR card, and these results were not included in the analysis.

Table S2. Western blot analysis. *Statistically significant effects and interactions are bolded*

Structure	Protein	N	Interaction	Treatment	Sensitivity
ACC	DRD1	27	$F_{(1, 23)} = 0.384$ p = 0.542	$F_{(1, 23)} = 2.454$ p = 0.131	$F_{(1, 23)} = 1.364$ p = 0.255
	5-HT3A	34	$F_{(1, 30)} = 0.009$ p = 0.925	$F_{(1, 30)} = 1.495$ p = 0.231	$F_{(1, 30)} = 0.08$ p = 0.778
mPFC	**ADH1**	33	$F_{(1, 29)} = 1.333$ p = 0.258	**$F_{(1, 29)} = 9.059$** **p = 0.005 ****	$F_{(1, 29)} = 0.569$ p = 0.457
	CAT	33	$F_{(1, 29)} = 0.051$ p = 0.822	$F_{(1, 29)} = 0.005$ p = 0.945	$F_{(1, 29)} = 0.113$ p = 0. 739
Amy	**MAO-B**	**34**	**$F_{(1, 30)} = 7.650$** **p = 0.010 ****	$F_{(1, 30)} = 0.104$ p = 0.750	$F_{(1, 30)} = 0.135$ p = 0. 716
OFC	**ADH1**	34	$F_{(1, 30)} = 0.063$ p = 0.803	**$F_{(1, 30)} = 4.753$** **p = 0.037 ***	$F_{(1, 30)} = 0.906$ p = 0.349
Nacc	**ADH1**	34	$F_{(1, 30)} = 3.572$ p = 0.069	**$F_{(1, 30)} = 7.287$** **p = 0.011 ***	$F_{(1, 30)} = 0.015$ p = 0.904
	GABABR2	32	$F_{(1, 28)} = 0.084$ p = 0.774	$F_{(1, 28)} = 3.136$ p = 0.088	**$F_{(1, 28)} = 5.422$** **p = 0.027 ***
	5-HT1A	33	$F_{(1, 29)} = 0.017$ p = 0.896	$F_{(1, 29)} = 2.306$ p = 0.140	$F_{(1, 29)} = 0.358$ p = 0. 554
	5-HT2A	34	$F_{(1, 30)} = 3.495$ p = 0.071	$F_{(1, 30)} = 2.384$ p = 0.133	**$F_{(1, 30)} = 6.689$** **p = 0.015 ***

Some protein bands were unsuitable for quantification due to a technical error.

Original Western blot images used for quantification of protein levels

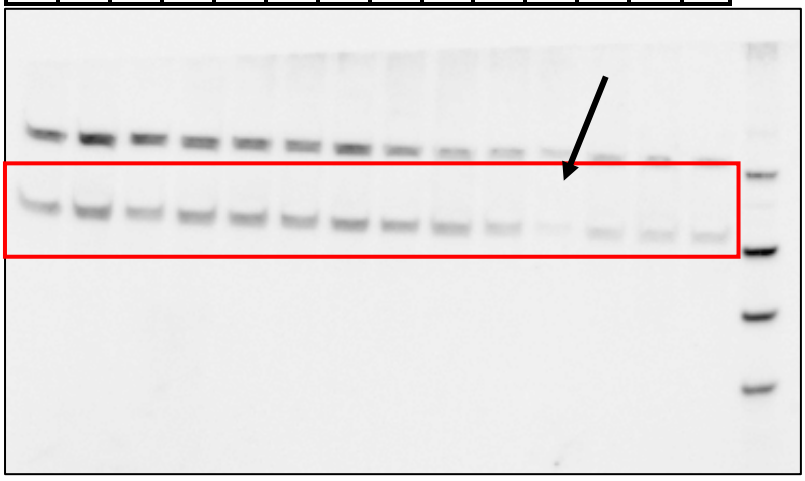
Legend:

I: PF-insensitive, S: PF-sensitive, bands that have not been quantified due to technical reasons are marked with an arrow.

ADH1_mPFC

ADH1_mPFC_membrane 1

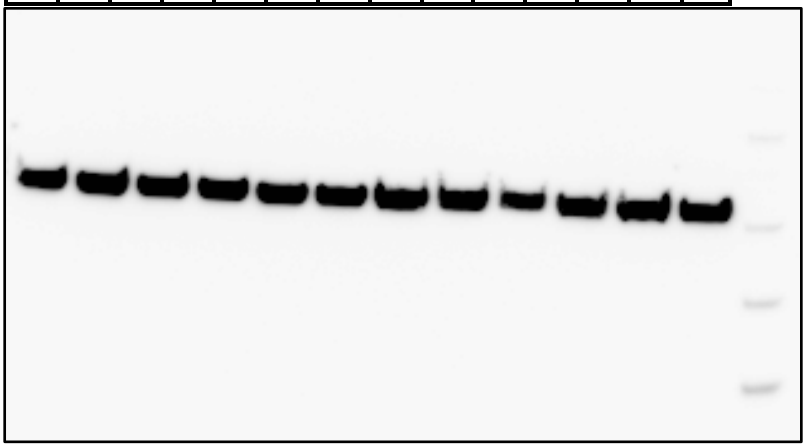
Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



40 kDa

βactin_mPFC_membrane 1 (ADH1)

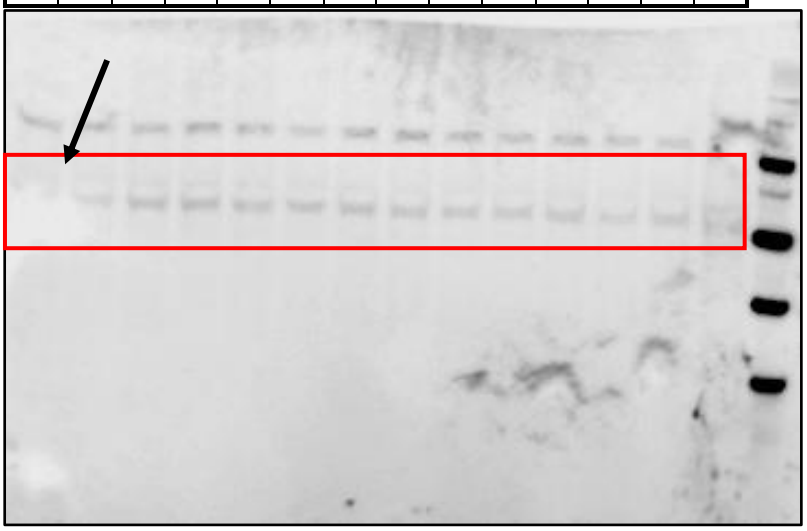
Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



42 kDa

ADH1_mPFC_membrane 2

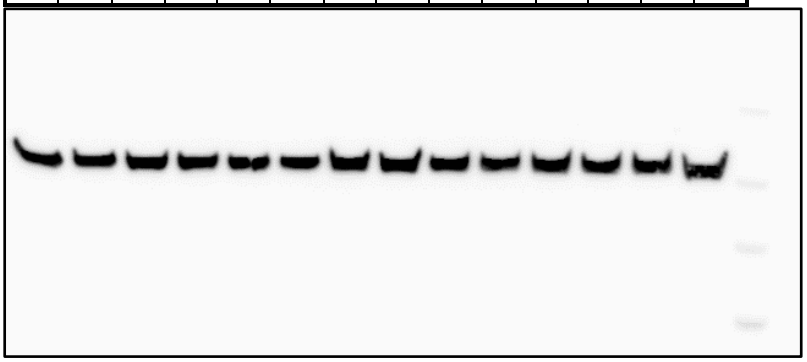
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



40 kDa

βactin_mPFC_membrane 2 (ADH1)

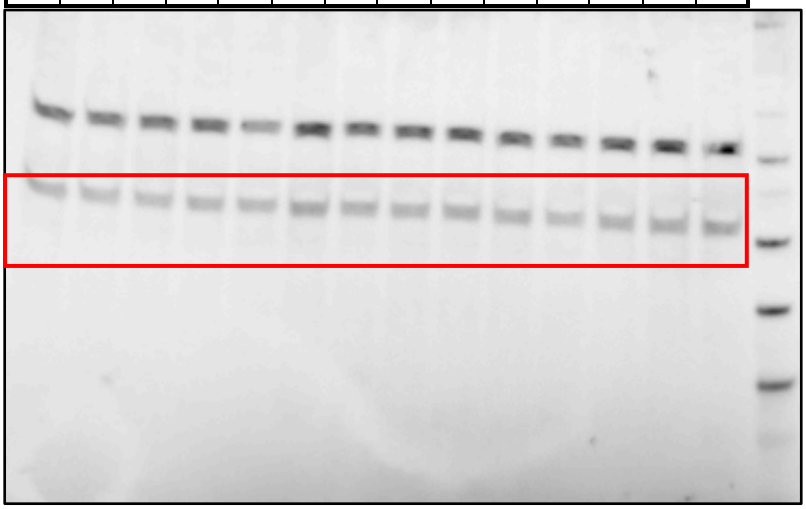
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



42 kDa

ADH1_mPFC_membrane 3

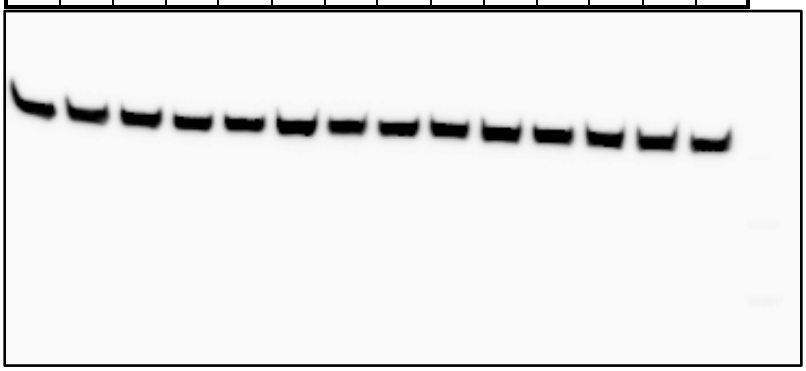
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10



40 kDa

βactin_mPFC_membrane 3 (ADH1)

Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10

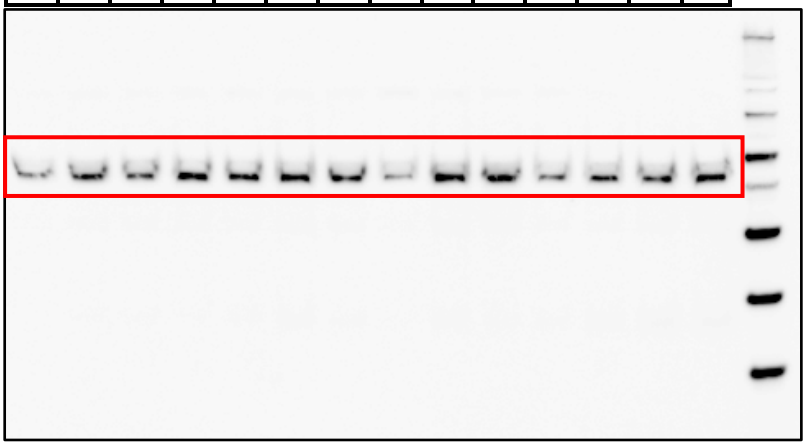


42 kDa

MAOB_AMY

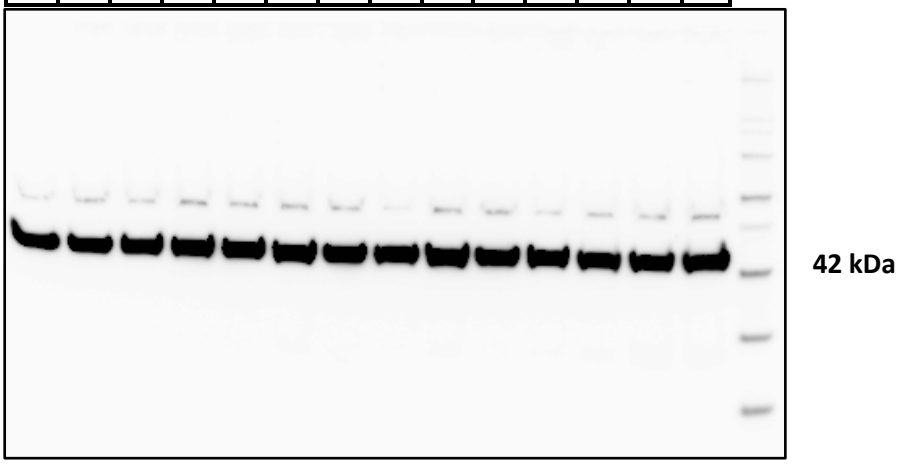
MAOB_AMY_membrane 1

Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



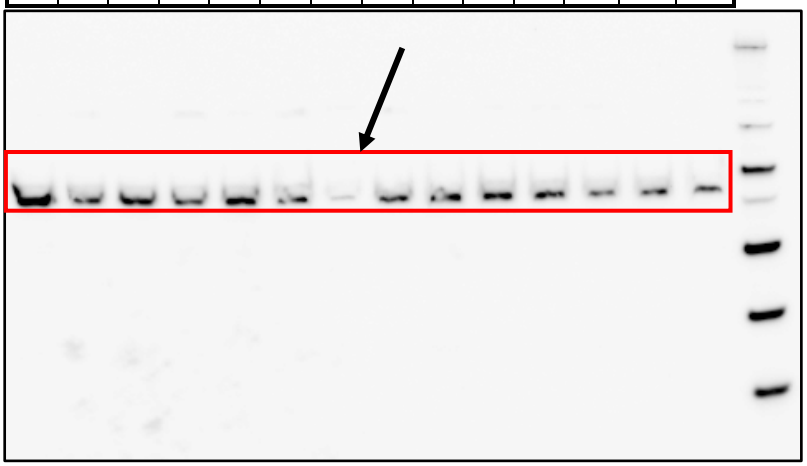
βactin_AMY_membrane 1 (MAOB)

Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



MAOB_AMY_membrane 2

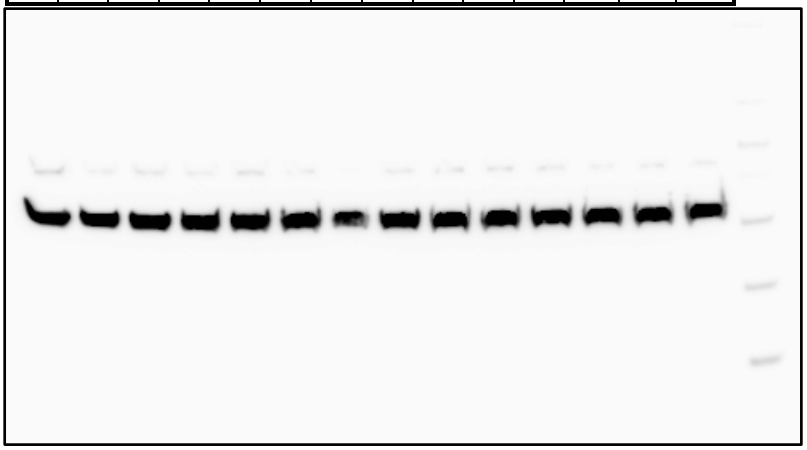
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



60 kDa

βactin_AMY_membrane 2 (MAOB)

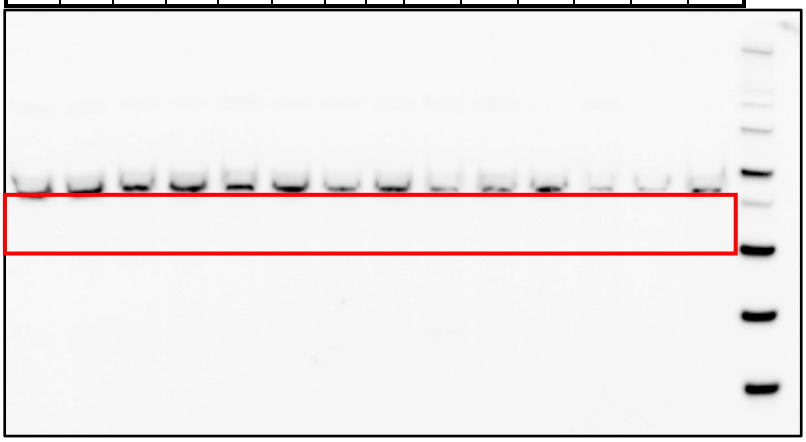
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



42 kDa

MAOB_AMY_membrane 3

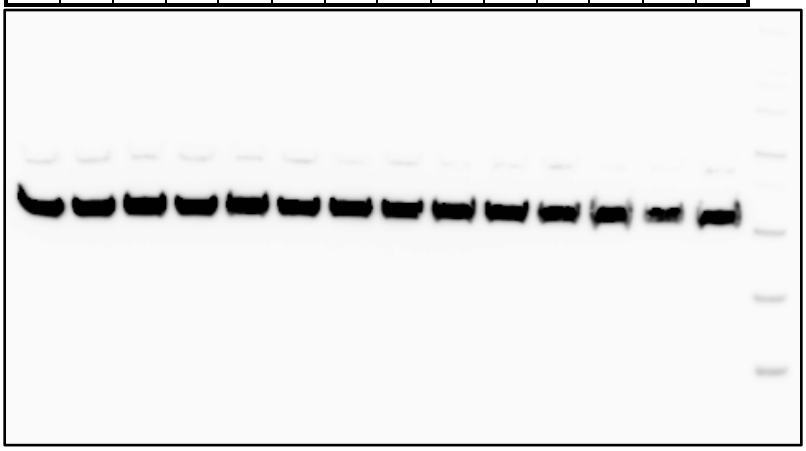
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10



60 kDa

βactin_AMY_membrane 3 (MAOB)

Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10

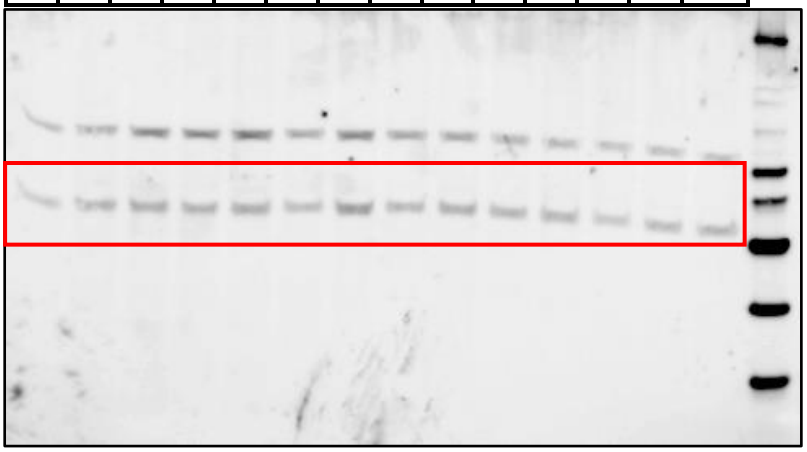


42 kDa

ADH1_OFC

ADH1_OFC_membrane 1

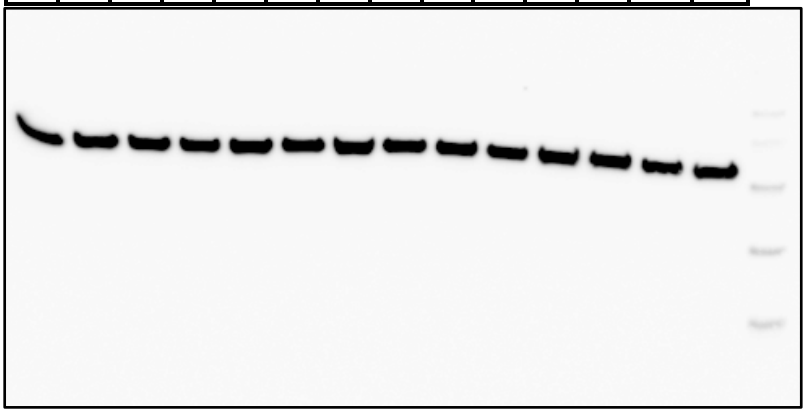
Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



40 kDa

βactin_OFC_membrane 1 (ADH1)

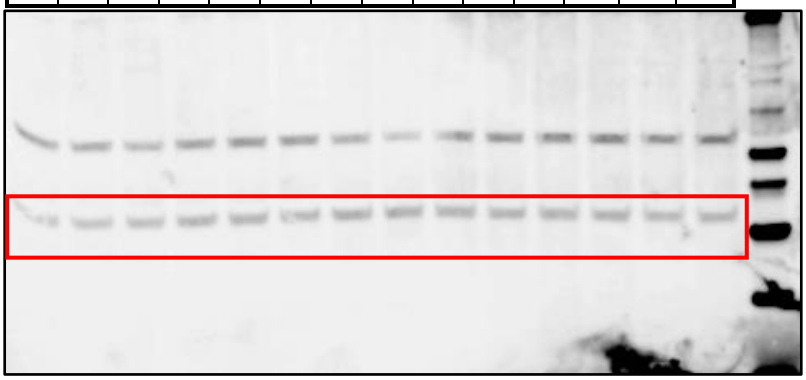
Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



42 kDa

ADH1_OFC_membrane 2

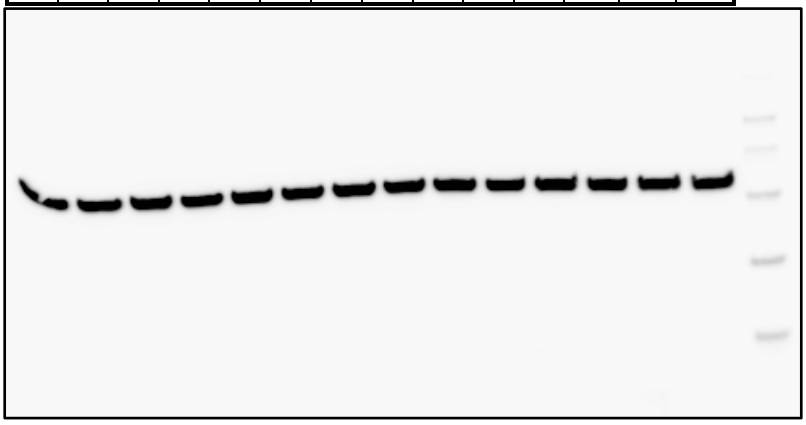
Treatment	EtOH				H2O									
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



40 kDa

βactin_OFC_membrane 2 (ADH1)

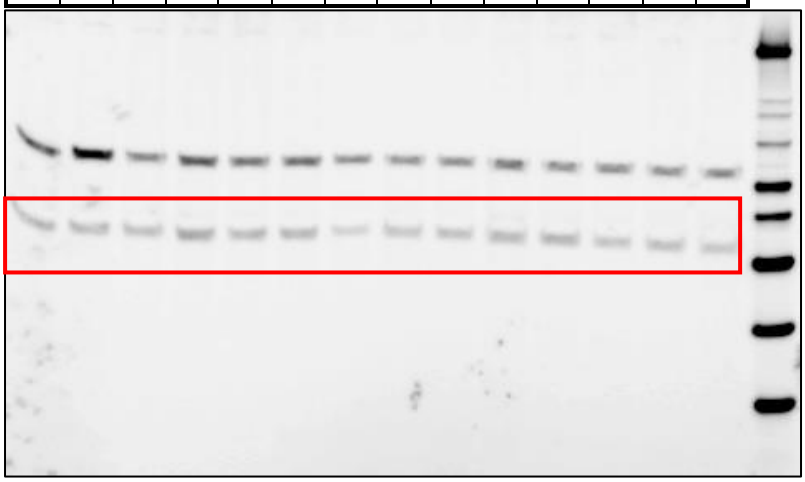
Treatment	EtOH				H2O									
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



42 kDa

ADH1_OFC_membrane 3

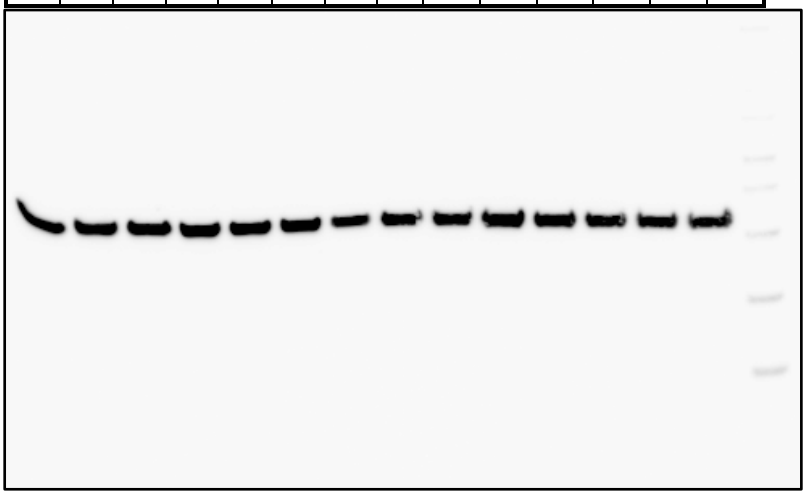
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10



40 kDa

βactin_OFC_membrane 3 (ADH1)

Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10

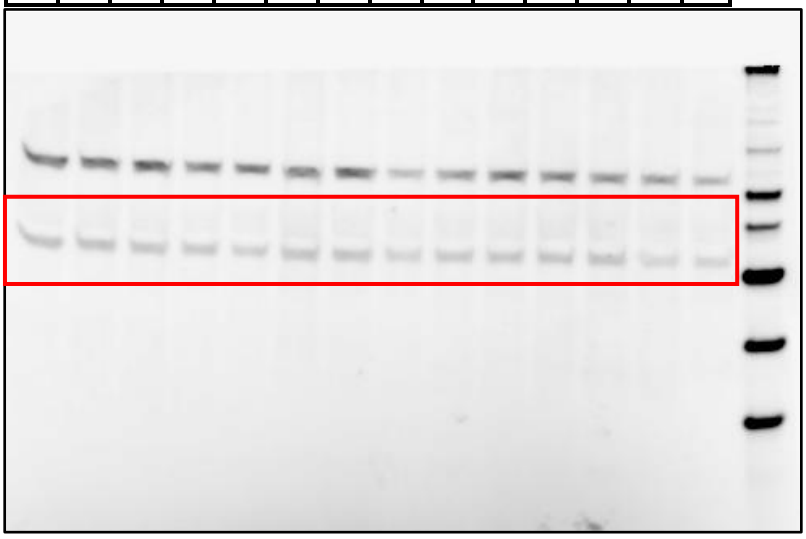


42 kDa

ADH1_Nacc

ADH1_Nacc_membrane 1

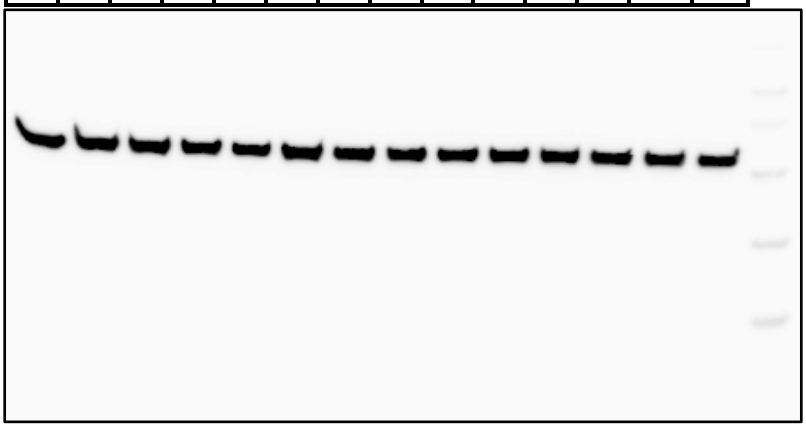
Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



40 kDa

βactin_Nacc_membrane 1 (ADH1)

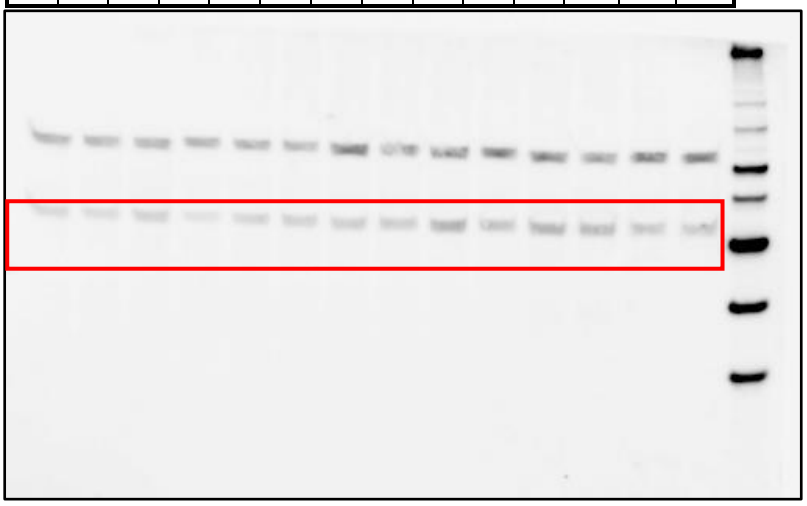
Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



42 kDa

ADH1_Nacc_membrane 2

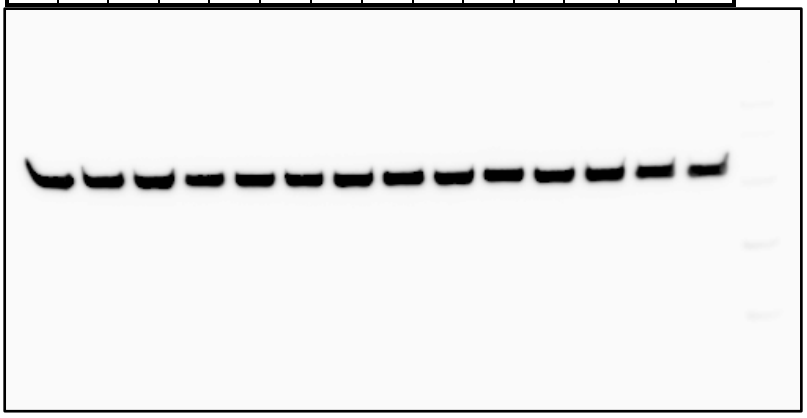
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



40 kDa

βactin_Nacc_membrane 2 (ADH1)

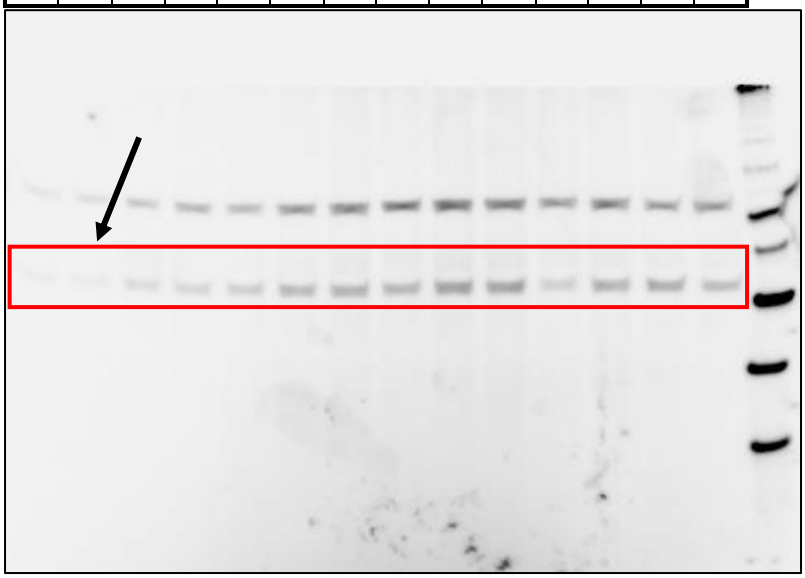
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



42 kDa

ADH1_Nacc_membrane 3

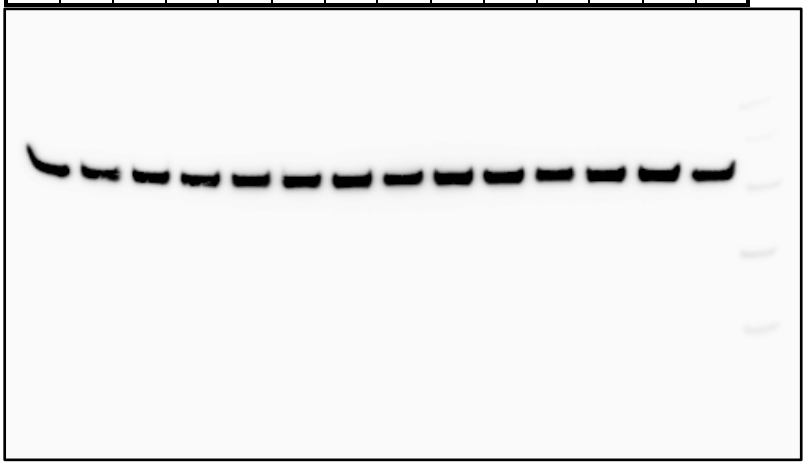
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10



40 kDa

βactin_Nacc_membrane 3 (ADH1)

Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10

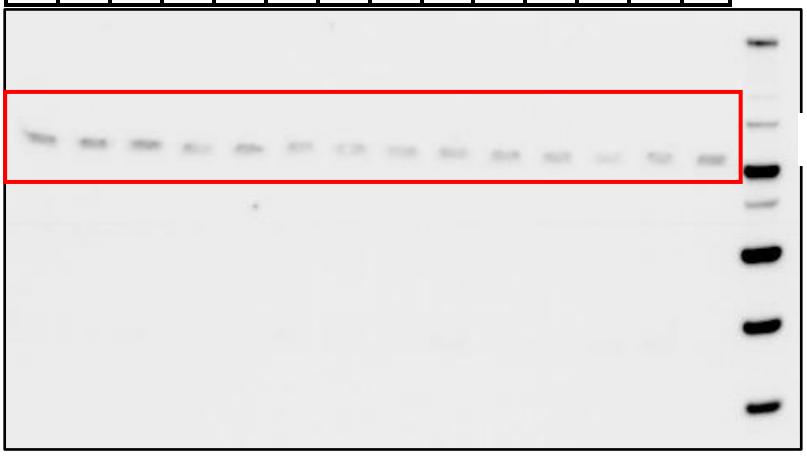


42 kDa

GABABR2_Nacc

GABABR2_Nacc_membrane 1

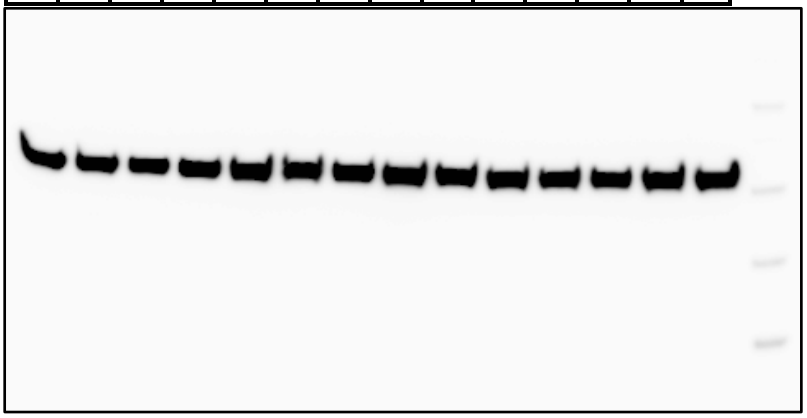
Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



90 kDa

βactin_Nacc_membrane 1 (GABABR2)

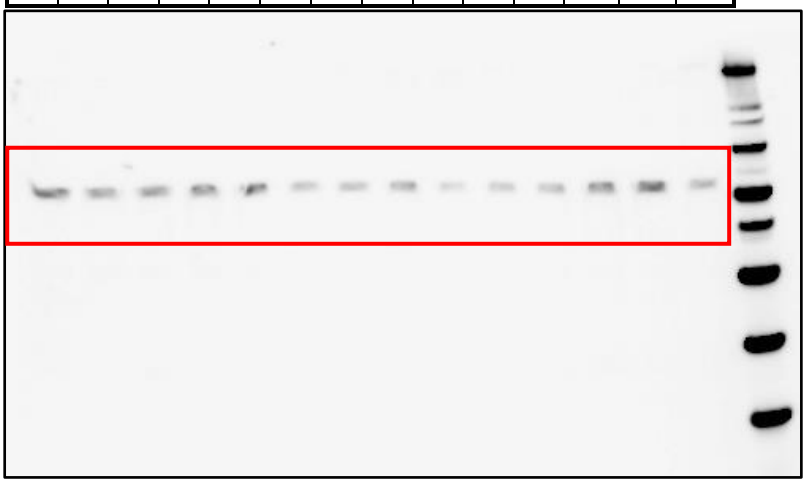
Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



42 kDa

GABABR2_Nacc_membrane 2

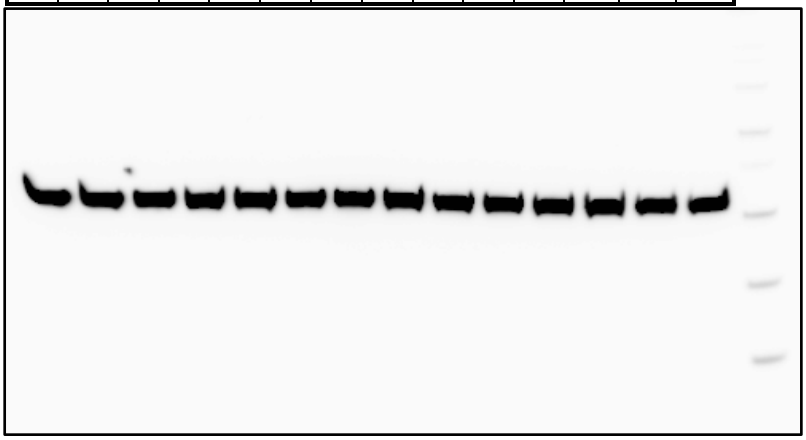
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



90 kDa

βactin_Nacc_membrane 2 (GABABR2)

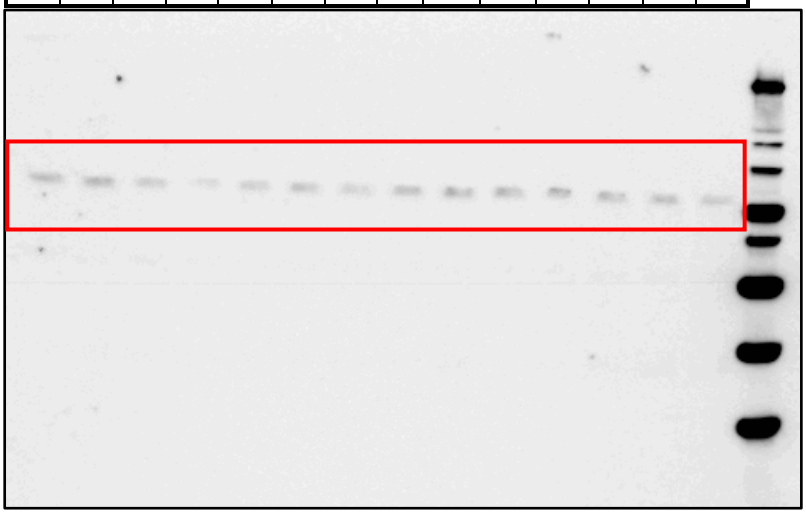
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



42 kDa

GABABR2_Nacc_membrane 3

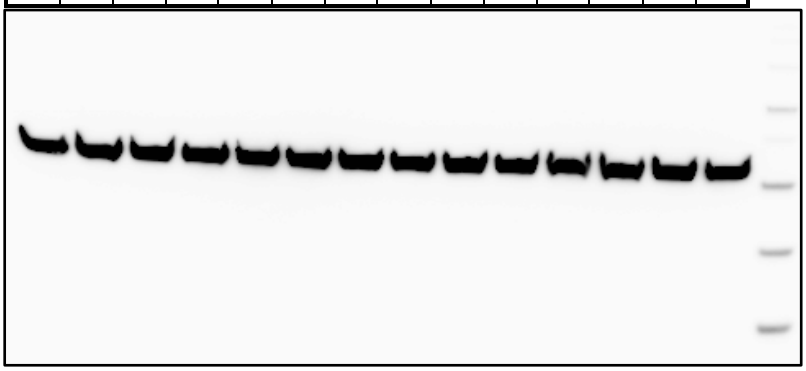
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10



90 kDa

βactin_Nacc_membrane 3 (GABABR2)

Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10

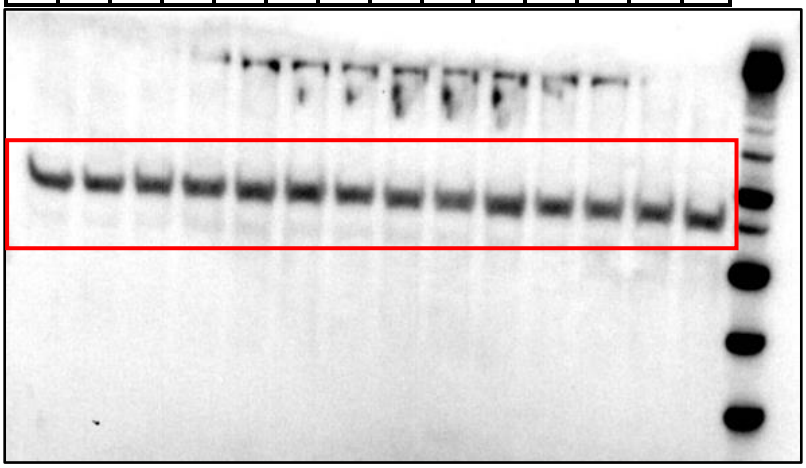


42 kDa

SERT_Nacc

SERT_Nacc_membrane 1

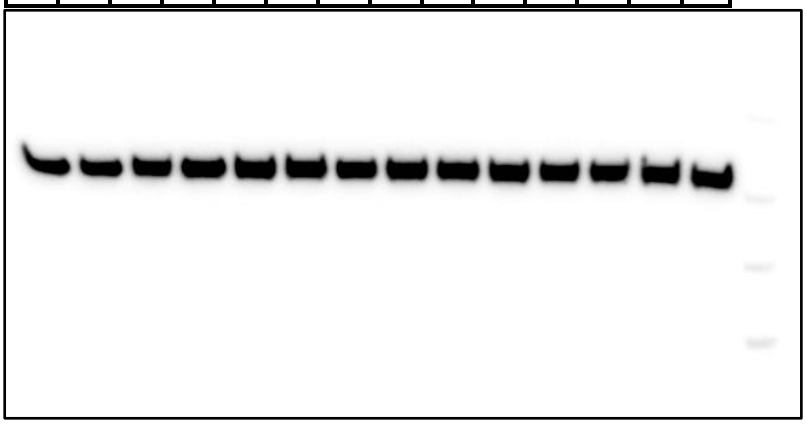
Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



80 kDa

βactin_Nacc_membrane 1 (SERT)

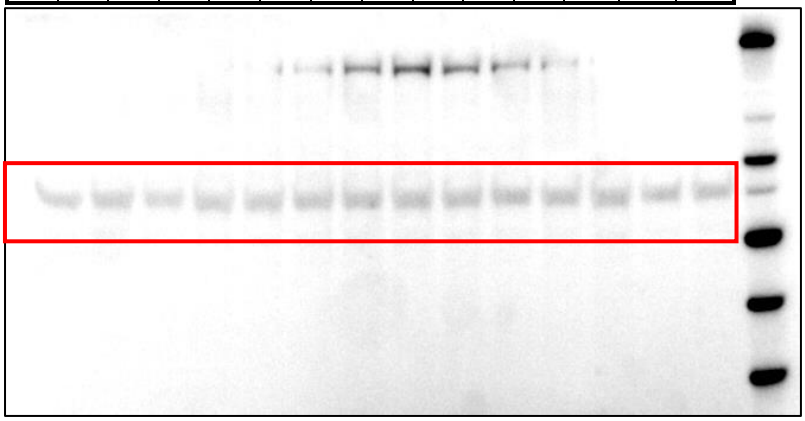
Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



42 kDa

SERT_Nacc_membrane 2

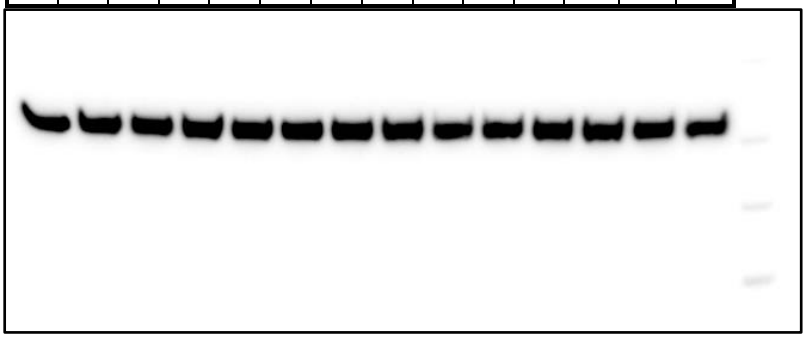
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



80 kDa

βactin_Nacc_membrane 1 (SERT)

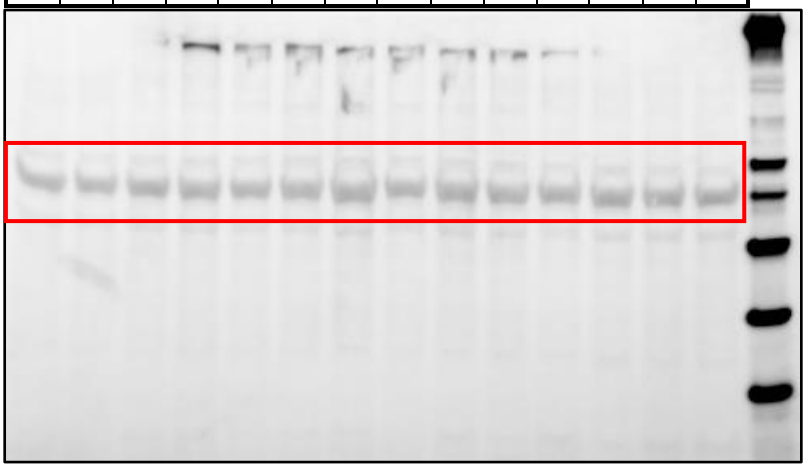
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



42 kDa

SERT_Nacc_membrane 3

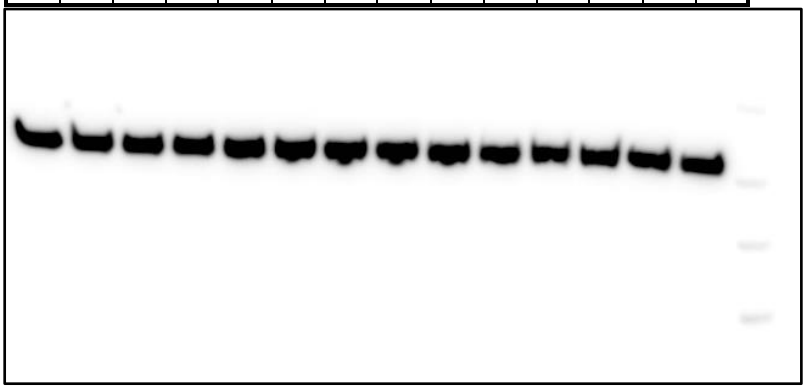
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10



80 kDa

βactin_Nacc_membrane 3 (SERT)

Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10

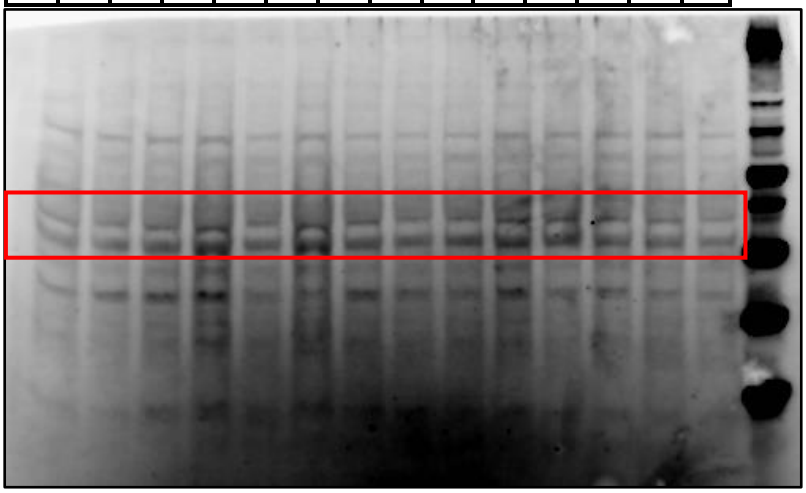


42 kDa

5-HT2A_Nacc

5-HT2A_Nacc_membrane 1

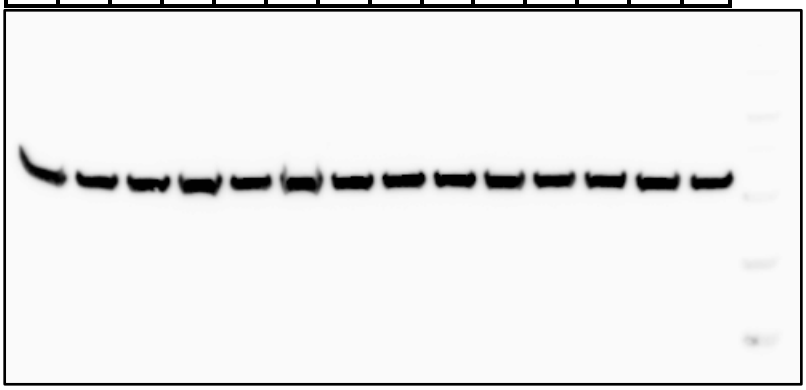
Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



53 kDa

βactin_Nacc_membrane 1 (5-HT2A)

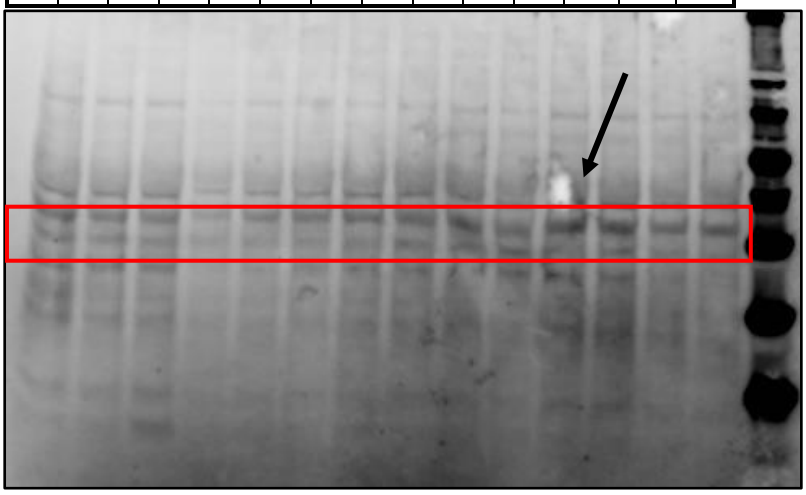
Treatment	EtOH						H2O							
PF sensitivity	I	I	S	S	I	S	S	S	S	S	S	I	I	I
Rat's nr	3	7	1	2	17	4	12	18	13	15	20	10	25	26



42 kDa

5-HT2A_Nacc_membrane 2

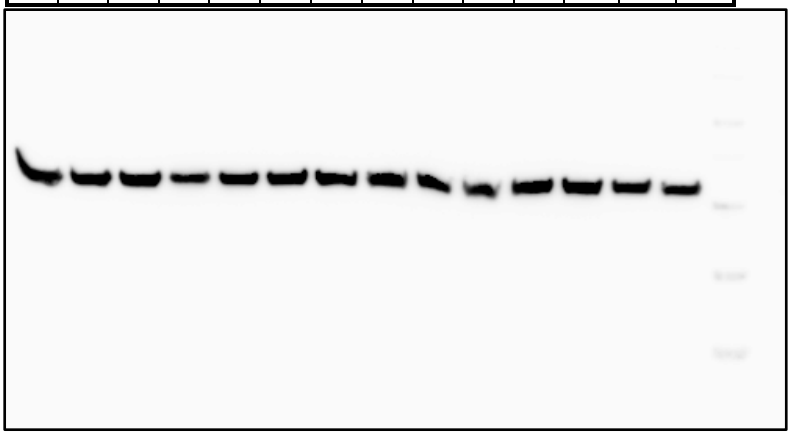
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



53 kDa

βactin_Nacc_membrane 2 (5-HT2A)

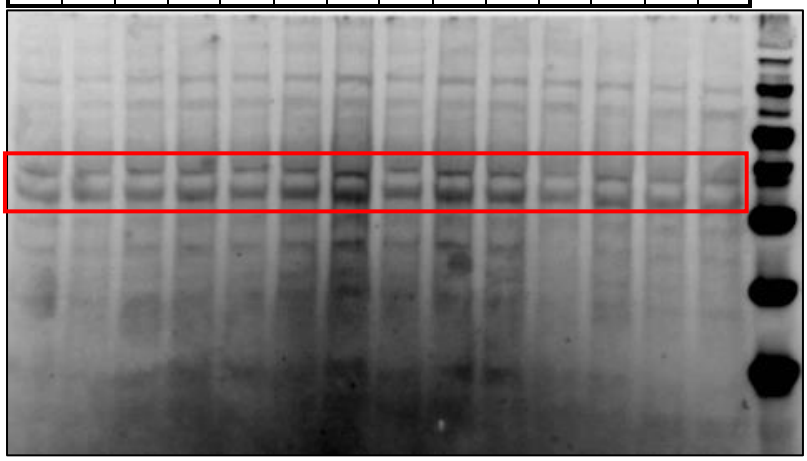
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	I	I	I	S	S	S	S
Rat's nr	2	5	9	29	17	19	16	22	28	36	30	24	38	39



42 kDa

5-HT2A_Nacc_membrane 3

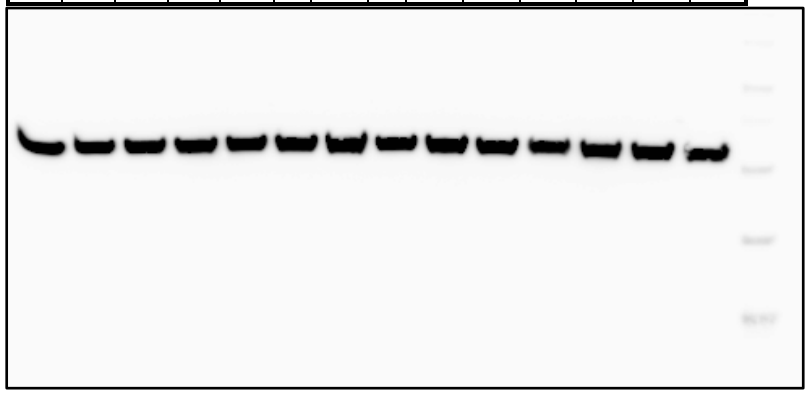
Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10



53 kDa

βactin_Nacc_membrane 3 (5-HT2A)

Treatment	EtOH							H2O						
PF sensitivity	S	S	S	S	I	I	I	S	S	S	I	I	S	I
Rat's nr	33	35	21	12	19	7	3	6	11	30	27	31	14	10



42 kDa

Research Paper

Trait sensitivity to negative feedback determines the intensity of compulsive alcohol seeking and taking in male rats

Agata Cieslik, MSc; Karolina Noworyta, PhD; Rafal Rygula, PhD

Background: Alcohol use disorder is one of the most common psychiatric disorders, and it is a leading cause of mortality worldwide. It has been demonstrated previously that people with alcohol use disorder are less sensitive to the negative outcomes of their actions and less able to use negative feedback to guide and adjust their ongoing behaviour. However, far less is known about the aberrant processing of negative feedback before the onset of alcohol use disorder. In this study, we investigated the theoretical claim that sensitivity to negative feedback — as a stable and enduring behavioural trait — can predict vulnerability to the development of compulsive alcohol consumption in rats. **Methods:** We trained and tested rats in a series of probabilistic reversal learning tests, and based on this "negative feedback sensitivity screening," we classified each rat as more or less sensitive to negative feedback. Then, in the intermittent-access 2-bottle choice paradigm, we measured alcohol consumption in the animals classified above. In the next step, using the instrumental second-order chained schedule of alcohol reinforcement task, we examined the influence of sensitivity to negative feedback on the development of compulsive alcohol seeking behaviour. Finally, we measured how trait sensitivity to negative feedback affected the extinction and reinstatement of alcohol seeking after a period of abstinence. **Results:** Trait sensitivity to negative feedback predicted the vulnerability of rats to the development of compulsive alcohol seeking and consumption. We also found significant differences between the more sensitive and less sensitive groups in their propensity to extinguish alcohol seeking behaviours when the alcohol was no longer available. **Limitations:** The findings from our study did not answer the question of whether individual differences in sensitivity to negative feedback have a genetic basis or develop in response to postnatal experiences. **Conclusion:** The results of our study suggest that negative feedback sensitivity screening could be used to evaluate individual vulnerability to the development and maintenance of alcohol use disorder.

Introduction

Alcohol use disorder is one of the most common psychiatric disorders, and it is a leading cause of mortality worldwide, contributing to 3 million deaths each year, and to disabilities and comorbidities.[1] It is a chronic, complex, relapsing disease, characterized by progressive escalation from moderate to excessive alcohol consumption and accompanied by cognitive, social and occupational impairments. According to the *Diagnostic and Statistical Manual for Mental Disorders*,[2] alcohol use disorder is a pattern of alcohol consumption with co-occurring symptoms such as high alcohol intake, uncontrollable seeking of alcohol and drinking despite adverse consequences. The latter symptom, a hallmark phenotypic characteristic of alcohol use disorder, is thought to be associated with deficient processing of negative feedback. Indeed, accumulating experimental evidence supports this idea. Several studies have demonstrated that people affected by chronic alcoholism recurrently make decisions that favour drinking, even in the face of mounting adverse consequences.[3–5]

It has been hypothesized that people with alcohol use disorder are less sensitive to the negative outcomes of their actions[6] and less able to use negative feedback to guide and adjust their ongoing behaviour,[6] suggesting a deficient feedback processing system.[7] However, despite an abundance of data linking alcohol use disorder to impaired decision-making, far less is known about the aberrant processing of negative feedback before the onset of alcohol use disorder. In fact, no study thus far has directly shown that biased sensitivity to feedback affects the transition from recreational to compulsive alcohol abuse, largely because it is difficult to obtain information about people's sensitivity to feedback before they develop an addiction.

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In humans, sensitivity to feedback can be investigated using a framework of neurophysiological[8–10] and neuropsychological measures.[11–15] One measure that offers an effective and fully translational way of assessing an individual's sensitivity to feedback is the probabilistic reversal learning test.[16] In this behavioural paradigm, participants are presented with 2 (and sometimes more) stimuli in each trial; using trial-and-error feedback after each response, they learn to select the stimulus that is usually correct (i.e., rewarded in more trials or unrewarded in fewer trials) and to avoid the stimulus that is usually incorrect (i.e., unrewarded in more trials or rewarded in fewer trials). This rule reverses intermittently, so that the stimulus that was usually rewarded becomes unrewarded, and vice versa, and responses must be adjusted to make favourable choices. Lose-shifts (i.e., unrewarded outcomes followed by a decision to change the choice) constitute a measure of sensitivity to negative feedback. Win-stay ratios (number of rewarded outcomes after which the subject repeated the choice divided by the total number of rewarded trials on a given stimulus) represent a measure of sensitivity to positive feedback. The use of probabilistic reinforcement increases the complexity of the task so that the information from any given choice is insufficient to guide future behaviour; participants must engage their cognitive function to track the outcome history for both types of stimuli to determine the stimulus that is more beneficial overall.[16]

The probabilistic reversal learning paradigm has been applied successfully in a number of studies that investigated the neuroanatomical and neurochemical correlates of reinforcement sensitivity in humans and animals.[11,16,17] It has also been used to demonstrate that sensitivity to feedback can be measured in animals as a stable and enduring cognitive trait.[18–21] These studies have opened a new and fascinating avenue of preclinical research that provides an opportunity to investigate the interplay between sensitivity to feedback and other cognitive processes and mental disorders. However, none of these studies has investigated sensitivity to feedback in the context of vulnerability to alcohol use disorder.

One of the main challenges in modern studies of alcohol addiction is the development of animal models that can be characterized by high ethanol intake and mimic the transition from controlled alcohol use to excessive alcohol abuse that occurs in human alcohol use disorder. A common method of achieving voluntary alcohol consumption in rats involves intermittent access to alcohol in the intermittent-access 2-bottle choice paradigm. Exposure to repeated cycles of free choice between 2 bottles (ethanol solution and water) and subsequent withdrawal causes a gradual increase in preference and voluntary alcohol consumption, reaching levels of 3–9 g/kg body weight per 24 hours, depending on the strain used (reviewed by Carnicella and colleagues[22]).

However, this procedure does not reflect all of the motivational and reinforcement processes responsible for alcohol seeking and consumption in humans. Therefore, animal models of high ethanol intake achieved through repeated cycles of alcohol intake and withdrawal must be complemented by procedures that involve instrumental training, such as the recently developed instrumental second-order chained schedule of alcohol reinforcement (ISOCSAR) task described by Giuliano and colleagues.[23] This model allows for measurement of the hallmark symptoms of alcohol use disorder, such as compulsive preparatory and consummatory behaviours, motivation for alcohol and persistence of alcohol intake in the face of aversive consequences.[23]

Because relapse is one of the important components of alcohol use disorder, it is also important to address an individual's predisposition to reinstating alcohol seeking behaviour after a period of forced abstinence. Although current animal models do not mimic self-imposed abstinence, the use of periods of forced abstinence followed by restoration of the alcohol-related environment has been shown to reflect the relapse observed in humans.

In the present study, we investigated the theoretical claim that sensitivity to negative feedback — as a stable and enduring behavioural trait — can predict subsequent vulnerability to the development of compulsive alcohol consumption in rats. For this purpose, we initially trained and tested the animals in a series of probabilistic reversal learning tests. Based on this "feedback sensitivity screening," we classified each rat as more or less sensitive to negative feedback. Then, using the 2-bottle choice paradigm, we measured alcohol consumption in the animals classified above. In the next step, using the ISOCSAR task,[23] we examined the influence of sensitivity to negative feedback on the development of compulsive alcohol seeking behaviour. Finally, we measured how trait sensitivity to negative feedback affects the extinction and reinstatement of alcohol seeking after a period of abstinence.

Methods

Animals and housing

We used 20 male Sprague Dawley rats (Charles River) weighing 176–200 g upon arrival at our facility. The rats were group-housed (4 animals per cage) in an enriched environment with controlled temperature (21 ± 1°C) and humidity (40%–50%) and using a 12-hour light–dark cycle (lights on at 7:00 am). Throughout the experiment, rats were mildly food-restricted to 85% of their free-feeding weight (according to the normal growth curve recommended by the laboratory rodent supplier) by providing 15 g of food pellets per rat per day (standard laboratory chow). Water was available ad libitum. All behavioural procedures and tests were performed during the light phase of the light–dark cycle.

Apparatus

The probabilistic reversal learning tests were conducted in operant conditioning chambers (Med Associates) enclosed in sound-attenuating boxes. Each chamber was equipped with a fan (which also served to eliminate extraneous noise), a house light, a speaker, a food dispenser set to deliver a sucrose pellet (Dustless Precision Pellets, 45 mg; Bio-Serv), a fluid receptacle and 2 retractable levers at the sides of the feeder.

Tests examining alcohol seeking behaviour in the seeking-taking task were conducted in the same operant chambers,

except that the levers were on the wall opposite to the liquid dispenser to create a new experimental setup that would not interfere with any habits the animals had acquired in the probabilistic reversal learning paradigm.

Measuring sensitivity to negative feedback with the probabilistic reversal learning test

After the initial instrumental training described in detail elsewhere,[19] and upon reaching the initial training criterion of fewer than 7.5% omissions on each lever (i.e., fewer than 15% total omissions but equally distributed between the 2 levers) for 3 consecutive training days, the rats were trained in the probabilistic reversal learning paradigm.

Briefly, each probabilistic reversal learning training session lasted until the completion of 200 trials, and each trial lasted for a maximum of 22 seconds. The start of a trial was signalled by the house light, which remained on until the end of the trial. Two seconds after the trial had started, both levers were presented; 1 lever was randomly assigned as the "correct" one, which delivered a reward (1 sucrose pellet) 80% of the times it was pressed. A press on the other lever (the "incorrect" lever) would result in a rewarding outcome only 20% of the times it was pressed. If the rat made no response in 10 seconds, the intertrial interval was triggered and the trial was counted as an omission. During the intertrial interval, both levers remained retracted and the house light was turned off. The same intertrial interval directly followed an unrewarded outcome (i.e., no reward on 20% of the "correct" and 80% of the "incorrect" lever presses). After every 8 consecutive "correct" lever presses (regardless of outcome), the criterion for the reversal of the outcome probabilities was reached; at that point, the previously "correct" lever became the "incorrect" lever, and vice versa. This pattern was followed until the end of the session. The probabilistic reversal learning training phase was repeated daily until the rats achieved sufficient performance levels (i.e., a minimum of 3 reversals in 3 consecutive training sessions, with fewer than 15% omissions per session).

Parameters measured in the probabilistic reversal learning test

To measure rats' sensitivity to negative feedback (as their ability to ignore an infrequent and misleading lack of reward), we monitored their decisions trial by trial. Unrewarded outcomes for the "correct" lever that were followed by the animal's decision to switch levers (probabilistic lose-shifts) were scored and expressed as a ratio of all unrewarded outcomes for that lever.

To measure rats' sensitivity to positive feedback, all rewarded outcomes (true and misleading) followed by a decision to stay with the lever that delivered them (win-stays) were counted jointly for the "correct" and "incorrect" levers and expressed as a ratio of all rewarded outcomes for that lever. This means of analyzing sensitivity to positive feedback follows the method described by Bari and colleagues[17] and was dictated by the fact that win-stay behaviours after misleading rewards on the incorrect lever were too uncommon to undergo robust analysis.

We used the number of reversals completed during the test as a measure of the animal's performance.

Feedback sensitivity screening

After the rats had achieved stable performance in the probabilistic reversal learning test (a minimum of 3 reversals in 3 consecutive sessions, with fewer than 15% omissions per session), they were then tested in 10 consecutive probabilistic reversal learning tests over 10 days. Based on this "sensitivity screening," the rats were classified (using the median as a cut-off) as more or less sensitive to negative feedback. We made the classification based on each animal's average ratio of lever changes after misleading unrewarded outcomes (probabilistic lose-shifts) across all 10 screening tests. The results of our previous studies clearly indicated that a dichotomous categorization based on median split was well suited for investigating negative feedback sensitivity as a stable and enduring cognitive trait in rats;[18–21] therefore, we extended this means of data analysis to the present study.

Intermittent-access 2-bottle choice paradigm

To induce drinking behaviour and determine the level of alcohol consumption in the rats, we conducted 18 sessions of the intermittent-access 2-bottle choice test every second day. During the 2-bottle choice test, rats were separated into individual cages for 24 hours, where they were presented with 1 bottle of 10% ethanol (wt/wt) and 1 bottle of water. We chose the percentage of the ethanol solution based on the findings of Giuliano and colleagues.[23] To avoid the potential effects of a side preference, we changed the position of the bottles after 12 hours. We weighed the bottles before and after each session to determine alcohol consumption (g ethanol per kg body weight). We calculated the volume of liquids consumed as the difference in bottle weights from the beginning and end of each session, subtracting volume lost as a result of dripping from bottles in empty cages.

Characterization of compulsive alcohol seeking behaviour

Taking task

Initially, the rats were trained to associate the pressing of the taking lever with alcohol delivery under a fixed-ratio-1 schedule of reinforcement. Each trial began with insertion of the randomly assigned taking lever and the house light on. Pressing on the lever resulted in presentation of the dipper on the opposite side of the box, delivery of 0.1 mL of 15% ethanol (wt/wt) and retraction of the taking lever. We chose the percentage of the ethanol solution based on the findings of Giuliano and colleagues.[23] Failure to respond to the lever within 10 seconds was considered an omission. Regardless of the result, each trial was followed by a 10-second intertrial interval, during which the taking lever was retracted and alcohol was not available. Rats were limited to a maximum of 60 rewards for a 30-minute training session. After they had achieved the performance criterion of at least

20 taking responses in 3 consecutive sessions, the animals were shifted to the seeking-taking phase of the training. The position of the taking lever was counterbalanced across animals.

Seeking-taking task
During this task, each trial began with insertion of the seeking lever next to the previously assigned taking lever (which remained retracted). Pressing on the seeking lever led to the extension of the taking lever after a random interval of 1–15 seconds. Pressing on the taking lever resulted in presentation of the dipper on the opposite side of the box, delivery of 0.1 mL of 15% ethanol (wt/wt) and retraction of both levers. Each trial was followed by a 10-second intertrial interval, during which both levers were retracted and alcohol was not available. Rats were limited to a maximum of 100 rewards for a 45-minute session. After they had achieved the performance criterion of at least 20 taking responses in 3 consecutive sessions, the animals were ready for the seeking-taking-punishment task.

Seeing-taking-punishment task
To measure the persistence of seeking behaviour in the face of aversive consequences, we used the seeking-taking-punishment task. In this paradigm, each trial began as described for the seeking-taking task — with the insertion of the seeking lever. The seeking lever response resulted in the extension of the taking lever after a random interval of 1–15 seconds, or in a 1 second electric shock (0.10–0.50 mA) administered through a grid floor. Each session consisted of 25 trials, of which 8 (30%) were punished with foot shock and 17 (70%) were reinforced by the delivery of 0.1 mL 15% ethanol after the taking lever response. When animals were punished after a seeking lever response, the taking lever and dipper were not presented, and no alcohol was available. The intensity of the shock increased gradually over consecutive test sessions according to the following pattern: 0.10, 0.20, 0.30, 0.30, 0.40, 0.40, 0.50 and 0.50 mA. Although punishment occurred randomly in each session, never more than 2 consecutive trials resulted in a foot shock, and the first trial of the session was always reinforced.

Extinction of alcohol seeking and taking behaviours

After the rats completed seeking-taking-punishment testing, they underwent 5 additional seeking-taking tests (baseline). They then underwent daily extinction sessions (lasting 15 minutes), during which the seeking lever response resulted in the extension of the taking lever (random interval of 1–15 seconds), but pressing the taking lever had no programmed consequences, and alcohol was not available. After 10 seconds of exposure, the lever was retracted and a 10-second intertrial interval began. After reaching the extinction criterion (fewer than 5 seeking responses in 3 consecutive sessions), the rats underwent 30 days of alcohol abstinence, during which they were not tested.

Reinstatement of alcohol seeking and taking

The reinstatement of alcohol seeking after extinction is one of the most common animal models for studying relapse and its underlying neural mechanisms.[24] The rate of operant responding (i.e., reinstatement) on the lever that was associated with alcohol delivery is taken as a measure of the animal's urge to obtain alcohol — a model of craving in patients.[25] After the extinction phase and 30 days of abstinence, the rats underwent a series of seeking-taking tests to measure how quickly they reinstated their alcohol seeking behaviour and brought their performance up to the pre-extinction baseline levels. The animals were not alcohol-primed, and apart from the context, no specific cue induced the seeking behaviour. The animals received response-contingent alcohol during the reinstatement sessions, and they were tested until they reached an average number of seeking responses from 5 tests that was equal to or higher than the average number of seeking responses from their 5 baseline seeking-taking tests.

Experimental schedule

The experimental schedule is summarized in Figure 1.

Statistical analysis

We analyzed the data using SPSS (version 25.0; SPSS Inc.). We verified the normality of the sensitivity to feedback data using the Kolmogorov–Smirnov test. We analyzed the data for the negative feedback sensitivity screening, 2-bottle choice, seeking-taking, seeking-taking-punishment and reinstatement tasks using 2-way repeated-measures analyses of variance; the within-subject factor was test day or session, and the between-subjects factor was feedback sensitivity.

To analyze the differences between the less sensitive and more sensitive groups in terms of average quantity of alcohol consumed and number of tests needed to achieve extinction and reinstatement criteria, we used *t* tests or, for nonparametric data, Mann–Whitney *U* tests. For pair-wise comparisons, we adjusted the values using Sidak correction for multiple comparisons.[26] We also computed a Pearson correlation coefficient to assess the relationship between negative feedback sensitivity and investigated measures of alcohol seeking and taking in rats.

All tests of significance were performed at $\alpha = 0.05$. We tested homogeneity of variance using a Levene test, and for repeated-measures analyses, we confirmed sphericity using a Mauchly test. Data are presented as mean ± standard error of the mean.

Results

Probabilistic reversal learning training and testing

All animals fulfilled the probabilistic reversal learning training criteria and qualified for the probabilistic reversal learning screening. On average, they reached the criteria after 6.8 ± 0.58 probabilistic reversal learning tests. The groups that were more or less sensitive to negative feedback did not differ significantly in terms of the number of probabilistic reversal learning tests needed to reach the criterion ($t_{18} = 0.338$, $p = 0.74$).

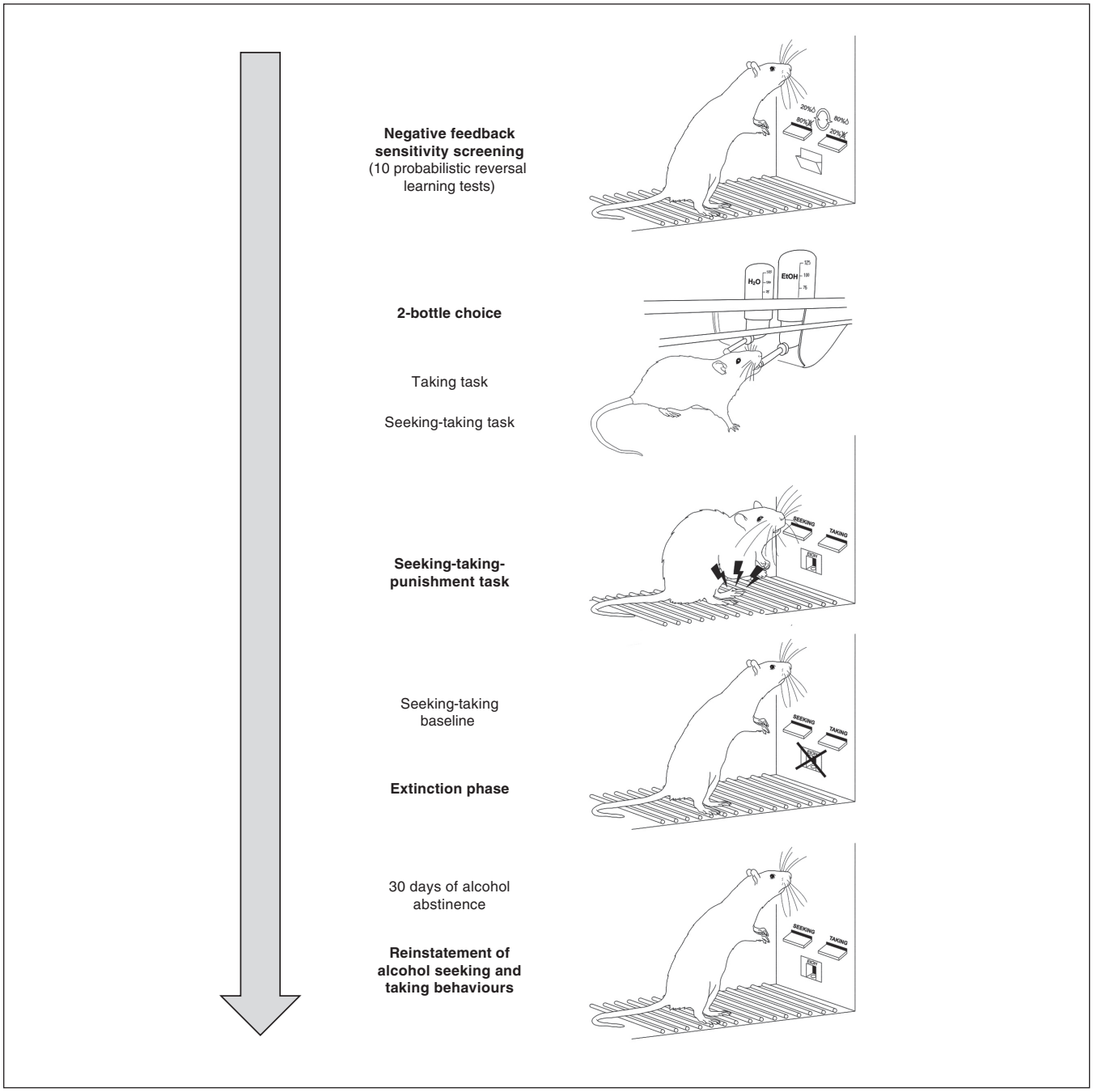

Figure 1: The experimental schedule. Initially, we trained and tested the animals in a series of probabilistic reversal learning tests. Based on this "negative feedback sensitivity screening," we classified each rat as more or less sensitive to negative feedback. Then, in the 2-bottle choice paradigm, we measured the consumption of alcohol in the animals classified above. In the next step, using the alcohol seeking-taking-punishment task, we examined the influence of sensitivity to negative feedback on the development of compulsive alcohol seeking behaviour. We then measured how trait sensitivity to negative feedback affected the extinction of alcohol seeking and the reinstatement of this behaviour after a period of abstinence.

Negative feedback sensitivity screening

For the animals classified as less sensitive to negative feedback, the average proportion of probabilistic lose-shift behaviours after misleading negative feedback ranged from 0.358 to 0.532, with an average of 0.453 ± 0.018. For the animals classified as more sensitive to negative feedback, the average proportion of probabilistic lose-shift behaviours ranged from 0.537 to 0.698, with an average of 0.583 ± 0.015.

The between-group difference in sensitivity to negative feedback was stable across the screening period (i.e., no significant interaction between screening day and sensitivity to negative feedback; $F_{9,162} = 0.566$, $p = 0.82$) — a significant sensitivity effect ($F_{1,18} = 31.19$, $p < 0.001$; Figure 2A). The more and less sensitive groups did not differ significantly in terms of average sensitivity to positive feedback ($F_{1,18} = 1.149$, $p = 0.30$; Figure 2B) or average number of reversals made during the screening tests ($F_{1,18} = 1.984$, $p = 0.18$; Figure 2C).

Induction and assessment of drinking behaviour

During the 18 intermittent-access 2-bottle choice sessions, the rats significantly ($p < 0.05$) increased their alcohol intake (Figure 3). Average intake in the first session was 3.49 ± 0.58 g/kg per 24 hours, increasing to an average of 4.95 ± 0.41 g/kg per 24 hours in the last session (significant main effect of session; $F_{17,221} = 2.774$, $p < 0.001$). We observed no significant differences in alcohol consumption between the less and more sensitive groups (nonsignificant effect of sensitivity; $F_{1,13} = 0.1661$, $p = 0.69$) and found a nonsignificant session × sensitivity interaction ($F_{17,221} = 1.016$, $p = 0.44$).

Because only 15 of the 20 rats achieved the criteria for the taking and seeking-taking tasks, we analyzed alcohol consumption during the 2-bottle choice sessions in only these animals.

Characterization of compulsive alcohol seeking behaviour

In the next step, the animals were trained to associate the pressing of the taking lever with alcohol delivery under a fixed-ratio-1 schedule of reinforcement.

The number of sessions needed to achieve the taking task criterion ranged from 4 to 39, with an average of 16.3 ± 3.9. The animals from the less sensitive group reached the taking task criterion after 11.9 ± 4.6 sessions; animals from the more sensitive group needed 22.8 ± 6.3 sessions.

The number of sessions needed to achieve the seeking-taking task criterion ranged from 4 to 31, with an average of 17.5 ± 1.7. To achieve the seeking-taking task criterion, animals from the less sensitive group needed 17.6 ± 2.9 sessions, and animals from the more sensitive group needed 17.3 ± 0.8 sessions.

We observed no significant differences between the 2 groups in terms of number of sessions needed to achieve the taking test criterion ($U = 17.50$, $p = 0.24$) or the seeking-taking test criteria ($t_{13} = 0.061$, $p = 0.95$).

After the taking and seeking-taking training, the rats were tested in the seeking-taking-punishment task. Completion of trials during the seeking-taking-punishment task was an indicator of the animals' persistence in seeking alcohol in the face of aversive consequences. Two-way repeated-measures analysis of variance revealed a significant sensitivity × shock intensity interaction ($F_{7,77} = 3.427$, $p = 0.003$,

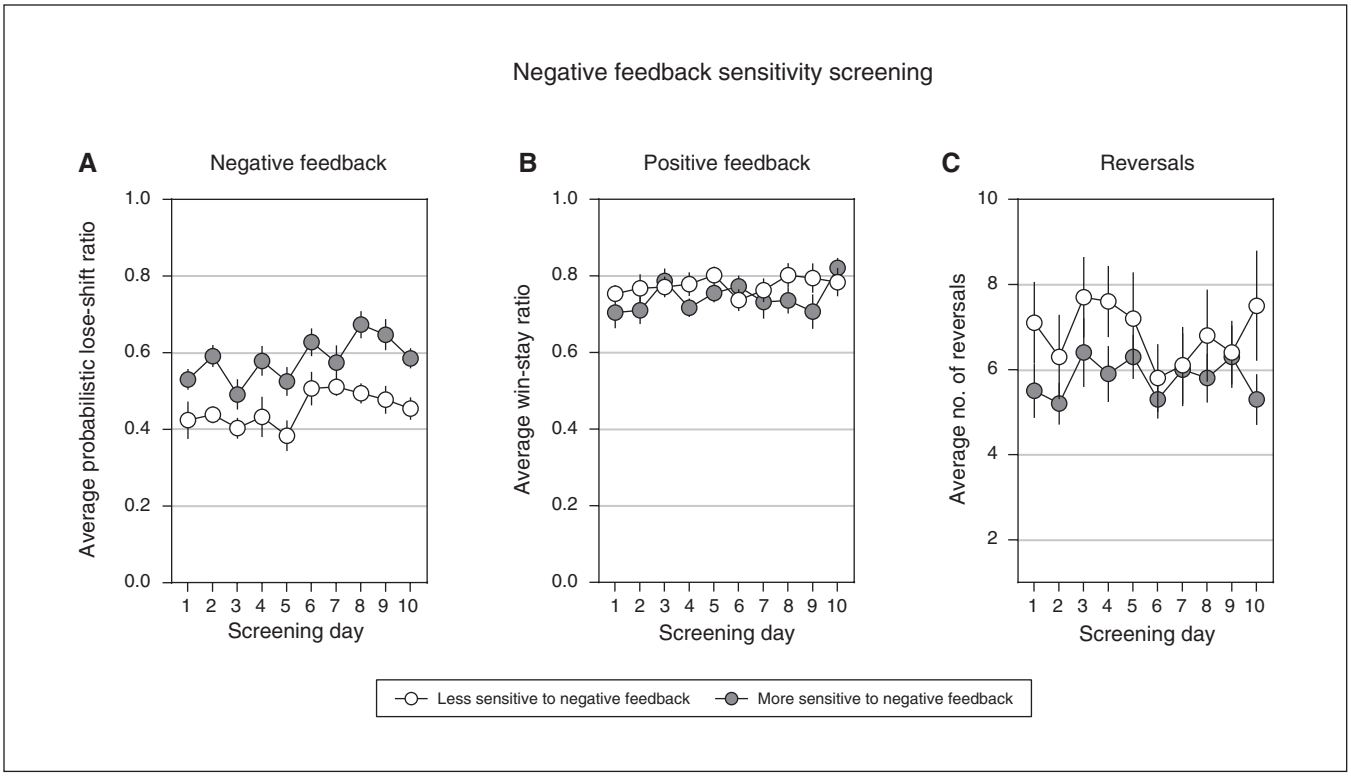

Figure 2: Results of negative feedback sensitivity screening. (A) Average proportion of lose-shift behaviours after misleading unrewarded outcomes; (B) average proportion of win-stay behaviours after a reward; and (C) average number of reversals in animals classified as less sensitive (open circles, $n = 10$) and more sensitive (filled circles, $n = 10$) to negative feedback during the 10 screening probabilistic reversal learning tests. Data are presented as the mean ± standard error of the mean.

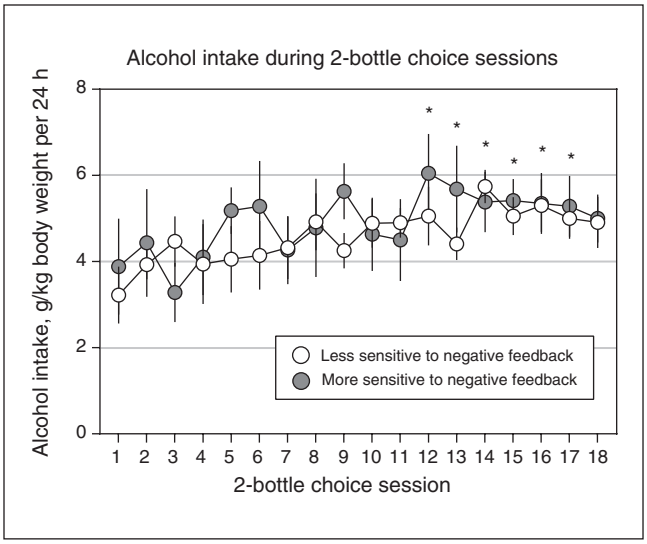

Figure 3: Alcohol intake during the intermittent-access 2-bottle choice sessions. Average daily alcohol intake (g/kg of body weight) in groups of rats classified as less sensitive (open circles, $n = 9$) and more sensitive (filled circles, $n = 6$) to negative feedback. Data are presented as mean ± standard error of the mean. *Significant ($p < 0.05$) difference in average alcohol consumption (for the entire cohort) between a given 2-bottle choice session and the first 2-bottle choice session.

and $F_{7,77} = 2.494$, $p = 0.023$ for seeking responses and completed trials, respectively).

Two rats (1 less sensitive to negative feedback and 1 more sensitive) that showed a significantly different pattern of behaviour on the seeking-taking-punishment task were excluded from the analysis based on the Grubbs test for outliers. Because the behaviour of these 2 rats differed only during the seeking-taking-punishment tests, their data were excluded for those tests only, and included in the analyses for other parts of the study.

As the shock intensity increased from 0.10 to 0.50 mA during consecutive sessions, the rats classified as more sensitive to negative feedback significantly decreased their number of seeking responses (Figure 4A) compared to their initial performance ($p = 0.012$ at 0.4 mA and $p < 0.001$ at 0.50 mA) and compared to their less sensitive counterparts ($p = 0.045$ and $p = 0.002$ at 0.5 mA). We observed similar differences between the less and more sensitive groups in the number of completed trials (Figure 4B). As the shock intensity increased from 0.10 to 0.50 mA over consecutive sessions, the rats classified as more sensitive to negative feedback significantly lowered their number of completed trials compared to their initial performance ($p < 0.001$ at 0.5 mA) and compared to their less sensitive counterparts ($p = 0.035$ at 0.5 mA).

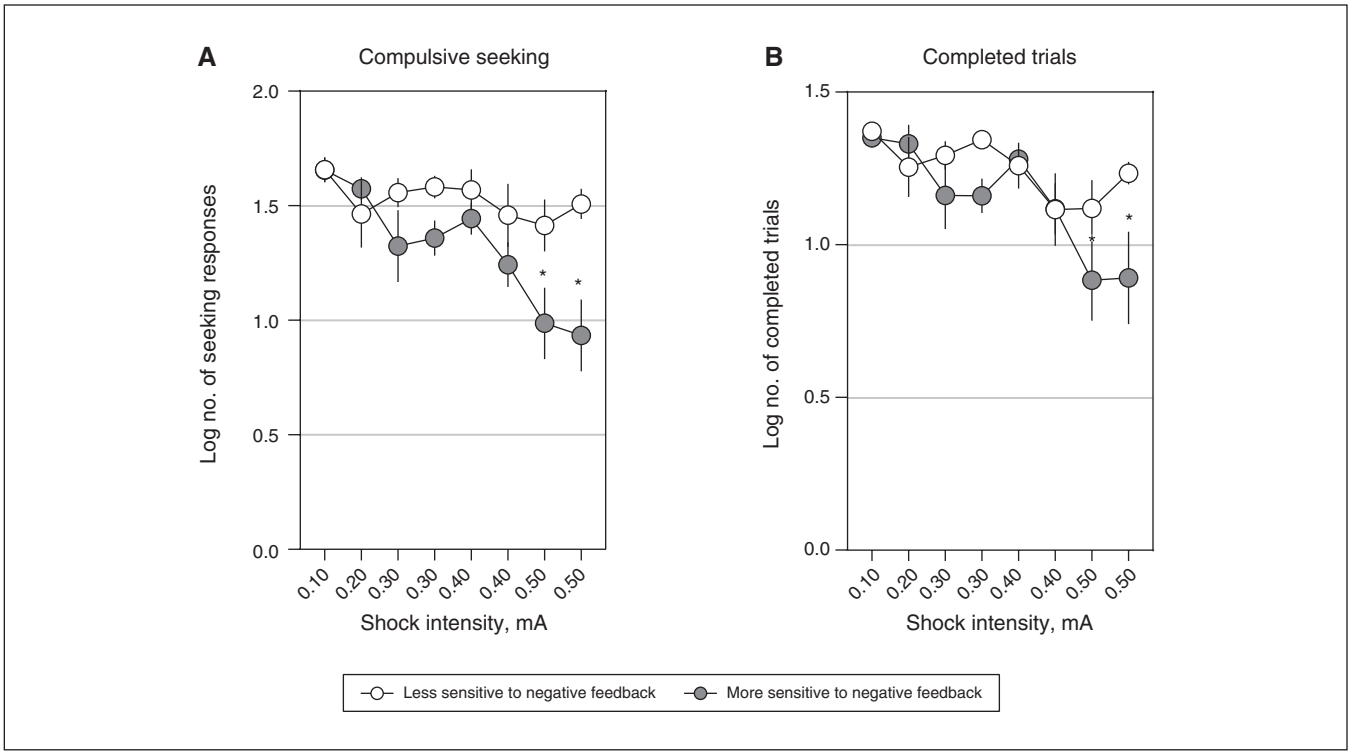

Figure 4: Trait sensitivity to negative feedback determines compulsive alcohol seeking and taking in rats. Rats were trained on an instrumental second-order chained schedule of alcohol reinforcement task to work for alcohol, and then their seeking responses were punished by mild electric foot shocks of increasing intensity (from 0.1 through 0.2, 0.3 and 0.4 up to 0.5 mA). As the shock intensity increased, the rats classified as more sensitive to negative feedback (filled circles, $n = 5$) significantly decreased (A) their number of seeking responses and (B) their number of completed trials compared to their baseline performance and to the less sensitive cohort (open circles, $n = 8$). Data are presented as mean ± standard error of the mean. *Significant ($p < 0.05$) difference between the less sensitive and more sensitive groups.

After seeking-taking-punishment testing, all animals underwent 5 baseline seeking-taking tests before the start of the extinction phase. We found no significant differences between groups in the average number of seeking and taking responses during the baseline seeking-taking tests ($t_{13} = 0.695$, $p = 0.49$, and $U = 25$, $p = 0.84$, respectively).

Extinction and reinstatement of alcohol seeking behaviour

The number of sessions needed to achieve the extinction criterion ranged from 4 to 20, with an average of 11 ± 1.32. All rats extinguished their seeking lever responses, but those more sensitive to negative feedback needed significantly fewer sessions than their less sensitive counterparts to cease their seeking behaviour (7.67 ± 1.17 sessions v. 13.22 ± 1.71 sessions; $t_{13} = 2.39$, $p = 0.033$; Figure 5A and inset).

We assessed the effect of sensitivity to negative feedback on the reinstatement of alcohol seeking after a 30-day abstinence interval. Over the course of 10 seeking-taking tests, most of the animals (apart from 2 less sensitive rats and 1 more sensitive rat) reinstated their pre-extinction baseline level of seeking responses. We observed no significant differences in the number of seeking responses across the reinstatement phase between those less and more sensitive to negative feedback (nonsignificant effect of sensitivity; $F_{1,13} = 0.1928$, $p = 0.67$), and we found a nonsignificant session × sensitivity interaction ($F_{9,117} = 0.6824$, $p = 0.72$; Figure 5B). The average number of sessions needed to achieve the reinstatement criterion was 6.1670 ± 0.5752. The rats from the less and more sensitive groups did not differ significantly in the number of sessions needed to reinstate the baseline levels of seeking responses ($U = 13$, $p = 0.39$).

Correlation between negative feedback sensitivity and measures of alcohol seeking and taking

We computed a Pearson correlation coefficient to assess the relationship between negative feedback sensitivity and measures of alcohol seeking and taking in rats. We found a negative correlation between negative feedback sensitivity and the log number of seeking responses during the seeking-taking-punishment task at 0.30 mA ($r_{13} = -0.6576$, $p = 0.015$) and in both 0.50 mA trials ($r_{13} = -0.6701$, $p = 0.012$; $r_{13} = -0.7043$, $p = 0.007$). We also found a trend toward statistical significance for the negative correlation between negative feedback sensitivity and number of seeking responses during the trial of the extinction criterion ($r_{13} = -0.4878$, $p = 0.06$). The analysis revealed no significant correlations between sensitivity to negative feedback and other investigated measures of alcohol seeking and taking. Findings are presented in Appendix 1, Table S1, available at www.jpn.ca/lookup/doi/10.1503/jpn.210220/tab-related-content.

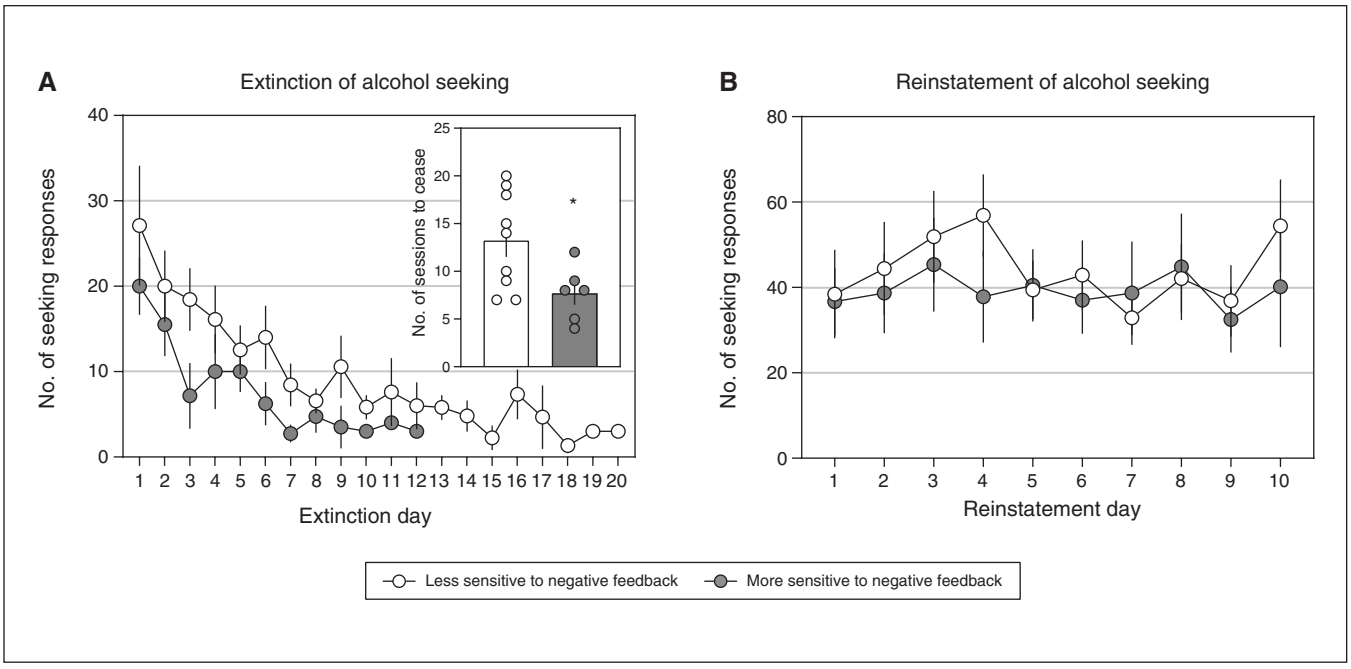

Figure 5: The effects of trait sensitivity to negative feedback on the length of extinction and reinstatement of compulsive alcohol seeking in rats. (A) After the punishment tests, the rats underwent 5 baseline seeking-taking tests and then underwent the extinction phase, during which seeking responses did not result in alcohol delivery. All rats extinguished their seeking lever responses, but animals more sensitive to negative feedback (filled circles, $n = 6$) needed significantly fewer sessions to cease their seeking behaviour than their less sensitive counterparts (open circles, $n = 9$; inset). *Significant ($p < 0.05$) difference between the more sensitive and less sensitive groups. (B) After a 30-day abstinence interval, rats from the less sensitive (open circles, $n = 9$) and more sensitive (filled circles, $n = 6$) groups underwent 10 seeking-taking tests to measure how quickly they reinstated their baseline level of alcohol seeking responses. Data are presented as mean ± standard error of the mean.

Discussion

The results of the present study demonstrated that trait sensitivity to negative feedback predicts the vulnerability of rats to the development of compulsive alcohol seeking and consumption in a situation when these behaviours are punished. Our findings also showed significant differences between animals classified as less and more sensitive to negative feedback in their propensity to extinguish alcohol seeking behaviours after the termination of alcohol availability. Finally, our findings complement the existing literature, proving that the development of compulsive alcohol seeking and taking behaviours in Sprague Dawley rats can be achieved with intermittent free access and instrumental alcohol drinking paradigms.

Apart from excessive drinking to the point of intoxication, people addicted to alcohol also devote much time and effort to compulsively seeking alcohol, in spite of the consequences. Although several preclinical studies have reported on procedures mimicking the persistence of alcohol consumption in the face of aversive consequences,[27–29] none of them directly addressed the compulsive nature of alcohol seeking, which occurs before drinking and is mechanistically dissociable from the acute intoxicating effects of the drug.

A breakthrough occurred in 2015, together with the development of a behavioural procedure allowing for the temporal separation of seeking and taking instrumental responses for alcohol. In their study, Giuliano and colleagues[30] introduced a new behavioural paradigm allowing for the above-mentioned separation and demonstrated for the first time that in rats, a propensity to consume and spontaneously prefer alcohol is dissociable from the propensity to compulsively seek it. This observation suggested that in rats, individual vulnerability to compulsive seeking of alcohol may depend on cognitive mechanisms other than a simple preference.

The experiments in the present study have confirmed the above assumption. Although all rats displayed similar initial alcohol consumption, those with lower sensitivity to negative feedback were more vulnerable to compulsive alcohol seeking than their more sensitive counterparts. This increased vulnerability was demonstrated by their weaker reaction to the unpredictable punishment of seeking responses (i.e., foot shock intensity increasing from 0.1 to 0.5 mA over repeated sessions) and their prolonged extinction of instrumental alcohol seeking responses when alcohol was no longer available.

In contrast, rats classified as being more sensitive to negative feedback progressively decreased their alcohol seeking, significantly reducing it at shock intensities of 0.4 to 0.5 mA, and they needed significantly fewer alcohol-free instrumental sessions to extinguish their alcohol seeking behaviours. These intuitive results were in line with studies in humans showing that individuals with a high sensitivity to adverse outcomes tend to use less alcohol than those who are less sensitive.[31] Our results were also consistent with those of studies in students; among those who drank heavily and received an infraction for their alcohol use, those with a higher sensitivity to punishment were more likely to reduce their drinking.[32] To the best of our knowledge, this is the first direct evidence that in an animal model of alcohol dependence, sensitivity to negative feedback interacts with the development of compulsive intake of alcohol.

Although further studies are needed to directly pinpoint the neurobiological correlates of the interaction we observed, our results may be at least partially explained using the framework of psychobiological models of motivation, reinforcement sensitivity theory being one of the most influential.[33,34] According to these models, input from the basal ganglia, mesolimbic dopamine projections from the ventral tegmental area to the ventral striatum, the nucleus accumbens, and mesocortical dopamine projections to the prefrontal cortex (constituting the neural circuit of the behavioural activation system) mediate the rewarding effects of alcohol and the reactions associated with seeking it. In turn, differences in sensitivity to negative feedback, which interacts with compulsive alcohol seeking, could account for differences in the activity of the behavioural inhibition and fight-flight-freeze systems, which are neuroanatomically bound to the septohippocampal system, periaqueductal grey matter, medial hypothalamus, amygdala, cingulate cortex, and dorsal and ventral prefrontal cortices.[35]

Limitations

Based on the data from the present study, we could not unequivocally infer whether the differences in the length of extinction of alcohol seeking were parallel or secondary to the differences in persistent drinking despite negative consequences, but this second-level validation confirms the role of trait sensitivity to negative feedback in the development of compulsive alcohol consumption. Because the extinction was based on a lack of reward, the results from this phase also exclude the unlikely possibility that the differences we observed in the seeking-taking-punishment task could have resulted from the various sensitivities to electric foot shocks.

In contrast to previous studies,[23,30] the present study used an outbred Sprague Dawley strain rather than inbred, alcohol-preferring rats. This strain has been reported to demonstrate moderate alcohol consumption in the intermittent-access 2-bottle choice paradigm (reviewed by Carnicella and colleagues[22]), and to our knowledge, it has never been tested with the ISOCSAR task. The use of a strain without a genetic predisposition to alcohol preference demonstrated naturally occurring differences in alcohol consumption, but also accounted for the fact that almost a quarter of the tested animals failed to meet task criteria. The fact that most of the excluded rats came from the more sensitive group lent strength to the results of our experiments, suggesting that a high sensitivity to negative feedback could be associated with a generally weaker vulnerability to the effects of alcohol. However, this concept calls for further investigation.

Several other issues should be investigated further. The findings from our study did not answer the question of whether individual differences in sensitivity to negative feedback have a genetic basis, develop in response to postnatal experiences, or both. We also do not know whether a similar difference in sensitivity to negative feedback could be observed in females,

or the relationship between sensitivity to negative feedback and social hierarchy. Finally, considering the caloric value of alcohol, we do not know if food restriction affected alcohol consumption in rats. Although alcohol has a fairly high caloric value, these are so-called "empty calories" with no hunger-quenching potential, and the literature suggests mixed effects: a 2001 study found that alcohol decreases the level of leptin, a hormone involved in the regulation of energy balance by inhibiting hunger,[36] but in 2005, Calissendorff and colleagues[37] found that alcohol inhibits appetite-stimulating ghrelin secretion.

Conclusion

Using multiple, consecutive probabilistic reversal learning tests, we confirmed our previous observation that sensitivity to negative feedback in rats is a stable and enduring behavioural trait. We also showed that this trait may determine the rats' vulnerability to the development of compulsive alcohol seeking, maintained despite the risk of punishment. Trait sensitivity to negative feedback was also associated with a better ability to cease alcohol seeking behaviour when it was no longer available. Our results call for further investigation of the neurobiological mechanisms involved. Future studies should also determine whether trait sensitivity to negative feedback interacts with molecular and physiologic correlates of compulsive alcohol intake. Finally, it is possible that negative feedback sensitivity screening could be used to evaluate individual differences in response to the therapeutic effects of drugs used in alcohol use disorder.

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Competing interests: None declared.

Contributors: R. Rygula designed the study. A. Cieslik and K. Noworyta acquired the data. A. Cieslik wrote the article, which K. Noworyta and R. Rygula reviewed. All authors approved the final version to be published, agree to be accountable for all aspects of the work and can certify that no other individuals not listed as authors have made substantial contributions to the paper.

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References

1. Peacock A, Leung J, Larney S, et al. Global statistics on alcohol, tobacco and illicit drug use: 2017 status report. *Addiction* 2018;113:1905-26.
2. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders. Fifth edition.* Arlington (VA): American Psychiatric Association Publishing; 2013.
3. Cantrell H, Finn PR, Rickert ME, et al. Decision making in alcohol dependence: insensitivity to future consequences and comorbid disinhibitory psychopathology. *Alcohol Clin Exp Res* 2008;32:1398-407.
4. Mazas CA, Finn PR, Steinmetz JE. Decision-making biases, antisocial personality, and early-onset alcoholism. *Alcohol Clin Exp Res* 2000;24:1036-40.
5. Miranda R Jr, MacKillop J, Meyerson LA, et al. Influence of antisocial and psychopathic traits on decision-making biases in alcoholics. *Alcohol Clin Exp Res* 2009;33:817-25.
6. Bechara A, Dolan S, Hindes A. Decision-making and addiction (part II): myopia for the future or hypersensitivity to reward? *Neuropsychologia* 2002;40:1690-705.
7. Kamarajan C, Rangaswamy M, Tang Y, et al. Dysfunctional reward processing in male alcoholics: an ERP study during a gambling task. *J Psychiatr Res* 2010;44:576-90.
8. Beauchaine T. Vagal tone, development, and Gray's motivational theory: toward an integrated model of autonomic nervous system functioning in psychopathology. *Dev Psychopathol* 2001;13:183-214.
9. Fowles DC. Psychophysiology and psychopathology: a motivational approach. *Psychophysiology* 1988;25:373-91.
10. Iaboni F, Douglas VI, Ditto B. Psychophysiological response of ADHD children to reward and extinction. *Psychophysiology* 1997;34:116-23.
11. Cools R, Clark L, Owen AM, et al. Defining the neural mechanisms of probabilistic reversal learning using event-related functional magnetic resonance imaging. *J Neurosci* 2002;22:4563-7.
12. Evers EA, Cools R, Clark L, et al. Serotonergic modulation of prefrontal cortex during negative feedback in probabilistic reversal learning. *Neuropsychopharmacology* 2005;30:1138-47.
13. Paulus MP, Hozack N, Frank L, et al. Error rate and outcome predictability affect neural activation in prefrontal cortex and anterior cingulate during decision-making. *Neuroimage* 2002;15:836-46.
14. Paulus MP, Hozack N, Frank L, et al. Decision making by methamphetamine dependent subjects is associated with error-rate-independent decrease in prefrontal and parietal activation. *Biol Psychiatry* 2003;53:65-74.
15. Slaney C, Hinchcliffe JK, Robinson ESJ. Translational shifts in preclinical models of depression: implications for biomarkers for improved treatments. *Curr Top Behav Neurosci* 2018;40:169-93.
16. Rygula R, Noworyta-Sokolowska K, Drozd R, et al. Using rodents to model abnormal sensitivity to feedback in depression. *Neurosci Biobehav Rev* 2018;95:336-46.
17. Bari A, Theobald DE, Caprioli D, et al. Serotonin modulates sensitivity to reward and negative feedback in a probabilistic reversal learning task in rats. *Neuropsychopharmacology* 2010;35:1290-301.
18. Noworyta K, Rygula R. Phenotypes of reinforcement sensitivity as predictors of the response to acute antidepressant treatment in rats. *Eur Neuropsychopharmacol* 2021;43:102-15.
19. Noworyta-Sokolowska K, Kozub A, Jablonska J, et al. Sensitivity to negative and positive feedback as a stable and enduring behavioural trait in rats. *Psychopharmacology (Berl)* 2019;236:2389-403.
20. Rygula R, Popik P. Trait "pessimism" is associated with increased sensitivity to negative feedback in rats. *Cogn Affect Behav Neurosci* 2016;16:516-26.
21. Surowka P, Noworyta K, Rygula R. Trait sensitivity to negative and positive feedback does not interact with the effects of acute antidepressant treatment on hedonic status in rats. *Front Behav Neurosci* 2020;14:147.
22. Carnicella S, Ron D, Barak S. Intermittent ethanol access schedule in rats as a preclinical model of alcohol abuse. *Alcohol* 2014;48:243-52.
23. Giuliano C, Pena-Oliver Y, Goodlett CR, et al. Evidence for a long-lasting compulsive alcohol seeking phenotype in rats. *Neuropsychopharmacology* 2018;43:728-38.
24. Epstein DH, Preston KL, Stewart J, et al. Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology (Berl)* 2006;189:1-16.
25. Domi E, Domi A, Adermark L, et al. Neurobiology of alcohol seeking behavior. *J Neurochem* 2021;157:1585-614.
26. Howell DC. *Statistical methods for psychology*. 4th ed. Belmont (CA): Wadsworth; 1997.
27. Marchant NJ, Khuc TN, Pickens CL, et al. Context-induced relapse to alcohol seeking after punishment in a rat model. *Biol Psychiatry* 2013;73:256-62.
28. Seif T, Chang SJ, Simms JA, et al. Cortical activation of accumbens hyperpolarization-active NMDARs mediates aversion-resistant alcohol intake. *Nat Neurosci* 2013;16:1094-100.

29. Vengeliene V, Celerier E, Chaskiel L, et al. Compulsive alcohol drinking in rodents. *Addict Biol* 2009;14:384-96.
30. Giuliano C, Goodlett CR, Economidou D, et al. The novel mu-opioid receptor antagonist GSK1521498 decreases both alcohol seeking and drinking: evidence from a new preclinical model of alcohol seeking. *Neuropsychopharmacology* 2015;40:2981-92.
31. Bijttebier P, Beck I, Claes L, et al. Gray's reinforcement sensitivity theory as a framework for research on personality-psychopathology associations. *Clin Psychol Rev* 2009;29:421-30.
32. Wray TB, Simons JS, Dvorak RD. Alcohol-related infractions among college students: associations with subsequent drinking as a function of sensitivity to punishment. *Psychol Addict Behav* 2011;25:352-7.
33. Corr PJ. Reinforcement sensitivity theory and personality. *Neurosci Biobehav Rev* 2004;28:317-32.
34. Corr PJ. The reinforcement sensitivity theory of personality and psychopathology. *Int J Psychophysiol* 2008;69:151-2.
35. McNaughton N, Gray JA. Anxiolytic action on the behavioural inhibition system implies multiple types of arousal contribute to anxiety. *J Affect Disord* 2000;61:161-76.
36. Röjdmark S, Calissendorff J, Brismar K. Alcohol ingestion decreases both diurnal and nocturnal secretion of leptin in healthy individuals. *Clin Endocrinol (Oxf)* 2001;55:639-47.
37. Calissendorff J, Danielsson O, Brismar K, et al. Inhibitory effect of alcohol on ghrelin secretion in normal man. *Eur J Endocrinol* 2005; 152:743-7.

Experimental phase			Number of pairs	Variable	Correlation (r)	p-value
Two-bottle choice		T1	15	g EtOH/ kg b.w./ 24h	-0.002434	0.9931
		T2			-0.03751	0.8944
		T3			-0.4645	0.0811
		T4			-0.004159	0.9883
		T5			0.2216	0.4274
		T6			0.009118	0.9743
		T7			-0.253	0.363
		T8			-0.2304	0.4088
		T9			0.09377	0.7396
		T10			-0.02986	0.9159
		T11			-0.2892	0.2958
		T12			-0.09365	0.7399
		T13			0.1937	0.4891
		T14			-0.05449	0.8471
		T15			0.2087	0.4553
		T16			0.1111	0.6933
		T17			0.09762	0.7293
		T18			-0.1548	0.5818
Taking criterion			15	Number of tests	0.3277	0.2331
Seeking-taking criterion			15	Number of tests	0.1565	0.5775
Seeking-taking punishment	Seeking responses	0.10 mA	13	LOG Number of seeking responses	-0.3358	0.2619
		0.20 mA			0.3802	0.2
		0.30 mA			**-0.5502**	**# 0.0514**
		0.30 mA			**-0.6576**	*** 0.0146**
		0.40 mA			-0.2439	0.422
		0.40 mA			-0.3869	0.1915
		0.50 mA			**-0.6701**	*** 0.0122**
		0.50 mA			**-0.7043**	*** 0.0072**

	Trials	0.10 mA		LOG Number of completed trials	-0.1111	0.7179
		0.20 mA			0.4516	0.1213
		0.30 mA			-0.3547	0.2343
		0.30 mA			-0.5175	0.0701
		0.40 mA			0.1892	0.5358
		0.40 mA			-0.03833	0.9011
		0.50 mA			-0.4711	0.1041
		0.50 mA			-0.4794	0.0974
Extinction criterion			15	Number of tests	**-0.4878**	**# 0.0651**
Reinstatement criterion			12	Number of tests	-0.4811	0.1133

Table S1.) Correlation between the NF sensitivity and measured variables of alcohol seeking and taking. *NF sensitivity. expressed as the average probabilistic lose-shift ratio was correlated with investigated measured of alcohol-seeking ang taking in rats. * indicates a significant ($p < 0.05$) correlation between NF sensitivity and the given variable of interest. # indicates a trend toward a statistically significant correlation between NF sensitivity and the given variable of interest.*

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SHORT COMMUNICATION

Identification of genes regulated by trait sensitivity to negative feedback and prolonged alcohol consumption in rats

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Abstract

Background The results of our previous studies demonstrated that low sensitivity to negative feedback (NF) is associated with increased vulnerability to the development of compulsive alcohol-seeking in rats. In the present study, we investigated the molecular underpinnings of this relationship.

Methods Using TaqMan Gene Expression Array Cards, we analyzed the expression of the genes related to NF sensitivity and alcohol metabolism in three cortical regions (medial prefrontal cortex [mPFC], anterior cingulate cortex [ACC], orbitofrontal cortex [OFC]) and two subcortical regions (nucleus accumbens [Nacc], amygdala [Amy]). Gene expression differences were confirmed at the protein level with Western blot.

Results Sensitivity to NF was characterized by differences in *Gad2*, *Drd2*, and *Slc6a4* expression in the ACC, *Maoa* in the mPFC, and *Gria1*, *Htr3a*, and *Maoa* in the OFC. Chronic alcohol consumption was associated with differences in the expression of *Comt* and *Maoa* in the ACC, *Comt*, *Adh1*, and *Htr2b* in the mPFC, *Adh1,* and *Slc6a4* in the Nacc, *Gad2,* and *Htr1a* in the OFC, and *Drd2* in the Amy. Interactions between the sensitivity to NF and alcohol consumption were observed in the expression of *Gabra1*, *Gabbr2*, *Grin2a*, *Grin2b*, and *Grm3* in the ACC, and *Grin2a* in the OFC. The observed differences were confirmed at the protein level for MAO-A in the mPFC, and ADH1 in the mPFC and Nacc.

Conclusions Our findings contribute to a better understanding of the molecular mechanisms underlying the relationship between trait sensitivity to NF and compulsive alcohol consumption.

Keywords Feedback sensitivity · Animal model · Alcohol · Rat · Genes · MAO-A

Introduction

Alcohol use disorder (AUD) is a chronic psychiatric condition characterized by the progression from occasional, moderate drinking to compulsive alcohol abuse. AUD is a significant global health issue, predominantly affecting men, leading to a high number of deaths each year. The economic burden of alcohol abuse on a global scale is enormous [1, 2]. The intricate nature of this disorder and the inter-individual differences between people suffering from alcohol dependence implies that individual traits may play a role in determining susceptibility to the development of compulsive drinking and subsequent addiction. Previous studies have indicated that people with symptoms of alcohol dependence often exhibit reduced responsiveness to the adverse consequences of their actions, as well as a decreased capacity to utilize negative feedback (NF) for regulating and adapting current behavior [3].This hints at a potential deficit in their feedback processing [3, 4]. Increased sensitivity to NF manifests itself in inadequate responses to negative outcomes of one's actions and deficits in adjusting behavior following failures or errors [5, 6]. However, until recently, it remained unclear whether this biased processing preceded the onset of alcohol dependence or was a consequence of it.

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In a recent publication from our laboratory [7], we presented findings highlighting the significant influence of trait sensitivity to NF on the development and maintenance of an alcohol-dependent-like state in rats. Our research demonstrated that trait sensitivity to NF can modulate alcohol-seeking behavior in response to punishment or the absence of expected rewards. Specifically, we found that rats with lower sensitivity to NF exhibited a higher propensity for compulsive alcohol-seeking compared to their more sensitive conspecifics. While these results shed light on the role of NF sensitivity in the development of an alcohol-dependent-like state, they did not elucidate the molecular mechanisms that could account for the observed effects.

Ethanol (EtOH) is a small, water-soluble molecule that is easily distributed throughout the body, allowing it to affect tissues and organs. The molecular effects of EtOH on the brain are intricate and encompass a multitude of mechanisms and signaling pathways. To gain further insight into the previously reported relationship between trait sensitivity to NF and prolonged alcohol consumption [7], the present study aimed to analyze differences in the expression of various genes in five brain regions: three cortical (medial prefrontal cortex [mPFC], anterior cingulate [ACC], and orbitofrontal cortex [OFC]) and two subcortical areas (nucleus accumbens [Nacc] and amygdala [Amy]). All the above-mentioned brain regions have been previously demonstrated to be involved in mediating sensitivity to feedback [8–11]. The selected genes were potentially linked to the modulation of NF sensitivity and the effects of alcohol. By extensively reviewing existing literature and analyzing the consequences of various genetic and pharmacological interventions on feedback sensitivity, four groups of genes were identified for scrutiny.

1. The first group encompassed genes responsible for the functioning and regulation of the serotonin (5-HT) system, such as serotonin receptors (5-HT_{1A}, 5-HT_{2A}), serotonin transporter (SERT), and tryptophan hydroxylase [12–15].
2. The second group of genes was selected based on their involvement in dopaminergic neurotransmission, as dopamine (DA) is the secondary neurotransmitter crucially implicated in feedback-based learning [10, 16]. This group included genes like dopamine receptors (D_1, D_2, D_4), dopamine transporter (DAT), tyrosine hydroxylase, monoamine oxidase (MAO) A and B, and catechol-*O*-methyltransferase (COMT).
3. Because changes in brain DA neurotransmission often result from secondary neuroadaptations in other neurotransmitter systems, such as glutamate [17] and γ-aminobutyric acid (GABA) [18], genes associated with these 2 neurotransmitter systems, e.g., the ionotropic glutamate receptors NMDA and AMPA, the metabotropic glutamate receptors $mGLU_2$, $mGLU_3$, and $mGLU_5$, glutamate decarboxylase (GAD), and $GABA_A$ and $GABA_B$ receptors, constituted the third analyzed group.
4. Genes implicated in EtOH metabolism, including catalase and alcohol dehydrogenase [19], constituted the fourth group.
5. Additionally, ribosomal protein L32 (*Rpl32*) and peptidylprolyl isomerase A (*Ppia*) were employed as reference genes, as described previously [20].

Materials and methods

In a previously published behavioral study, we analyzed differences in susceptibility to various aspects of compulsive alcohol consumption between 20 male Sprague–Dawley rats classified as less/more sensitive to NF [7]. This study had a non-drinking control group ($N = 20$) that could not be used for comparison in behavioral tests that used alcohol as a reward and was therefore not reported. In the current study, the brain tissue from these 20 additional rats along with the brain tissue of the 20 rats described in the previous study, was used to analyze the differences in the expression of a variety of genes related to feedback sensitivity and alcohol metabolism in rats with a lower and higher level of sensitivity to NF. This analysis was conducted within the groups of animals subjected to long-term exposure to alcohol and their non-drinking counterparts. The experimental schedule is summarized in Fig. 1.

Ethical statement

All experiments were conducted following the European Union guidelines for the care and use of laboratory animals (2010/63/EU). Experimental protocols were reviewed and approved by the 2nd Local Institutional Animal Care and Use Committee, Institute of Pharmacology Polish Academy of Sciences in Krakow (Agreement: No. 230/2019, dated 10.10.2019). The authors declare that every effort has been made to minimize the animals' suffering and the number of animals used.

Subjects and behavioral procedures

We used 40 male Sprague–Dawley rats. Rats from the control group ($N = 20$) underwent probabilistic reversal learning (PRL) paradigm training together with EtOH rats ($N = 20$), for which the procedure was previously described in detail [7]. Briefly, the tests were conducted in the operant conditioning boxes, and each PRL session had 200 trials. During each trial, both levers were presented. One lever was randomly set as the "correct" lever, yielding an 80% reward rate, while the other, the "incorrect" lever, had

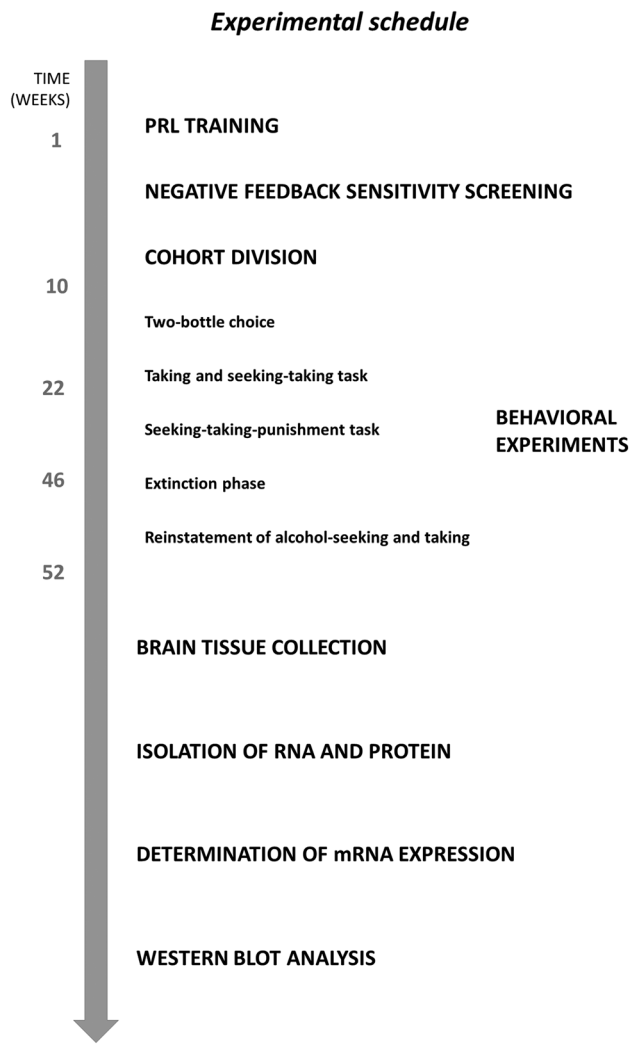

Fig. 1 Experimental schedule. To determine the effects of lower and higher sensitivity to negative feedback (NF) and prolonged alcohol consumption on gene expression and protein levels, a cohort of rats was trained and tested in a series of Probabilistic Reversal Learning (PRL) tests. Based on this "Negative feedback sensitivity screening", rats were classified as less sensitive and more sensitive to NF. The cohort was further divided into alcohol (EtOH) and water (H_2O) drinking groups. Rats from the EtOH group were then subjected to a series of behavioral tests measuring hallmark symptoms of alcohol use disorder (behavioral data previously published [7]). H_2O rats were handled daily throughout the entire experiment. At the end of these behavioral procedures, the rats were sacrificed, and the effects of prolonged alcohol consumption on gene expression and protein levels were compared between animals less sensitive and more sensitive to NF

a 20% reward rate. A 5 s intertrial interval (ITI) followed reward delivery. No response within 10 s was counted as an omission and also triggered the ITI. The same ITI followed unrewarded outcomes. After eight consecutive "correct" lever presses, the outcome probabilities were reversed. To evaluate rats' sensitivity to NF, indicating their ability to disregard occasional lack of reward, trial-by-trial decisions were monitored. Probabilistic lose-shifts, where rats switched levers after unrewarded "correct" lever press, were tallied as a ratio of all such outcomes on that lever. Using the results of 10 PRL tests from 10 consecutive days as a "sensitivity screening," the rats were divided into two groups based on their sensitivity to NF, using the median to split them into less sensitive and more sensitive groups. This split was based on the average ratio of probabilistic in all 10 screening tests.

Rats from the EtOH group were then tested in a series of experiments measuring the hallmark features of alcohol addiction: alcohol intake, alcohol-seeking in the face of aversive consequences, and extinguishing and reinstating alcohol-seeking behavior. The results obtained during these behavioral procedures have been previously published along with a detailed description of the applied tests [7]. A detailed description of subjects, housing conditions, and behavioral procedures is presented in Supplementary materials S1. Control rats were handled daily for the entire duration of the experiment.

Tissue collection

The day after the last alcohol intake and the last behavioral test, between 9:00 AM and 12:00 PM, the rats from both groups were decapitated in a counterbalanced manner (EtOH and control animal from the same NF sensitivity group at the same time), and five brain structures were collected for analyses: ACC, mPFC, Amy, Nacc, and OFC. Tissue was collected based on the "Rat Brain Atlas" of Paxinos and Watson [21] and according to Achterberg and colleagues [22]. The total number of samples for mRNA and protein analyses came from 34 animals: 18 less sensitive to NF (9 control and 9 EtOH) and 16 more sensitive to NF (10 control and 6 EtOH). The structures were frozen on dry ice and stored at −70 °C for further analysis.

Isolation of RNA and protein from the brain structures

The purification procedure for total RNA isolated from collected tissues was performed according to the instructions provided with the commercially available RNeasy Plus Mini Kit (Qiagen, Germantown, MD, US). In addition, the protein was obtained during RNA isolation by cold acetone precipitation and then dissolved in urea buffer. The quality and quantity of the isolated total RNA were evaluated by a NanoDrop ND-1000 (Thermo Fisher Scientific) and an Experion microcapillary electrophoresis system (Bio-Rad, Hercules, California, US). Samples that passed the quality threshold (RIN > 8.0) were used for further experiments.

Determination of mRNA expression by TaqMan Gene Expression Array Cards

The isolated RNAs were used to synthesize cDNA transcripts according to the manufacturer's protocol of the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). The amount of RNA was equalized for all samples depending on the structure. The obtained cDNA was mixed with TaqMan Universal PCR Master Mix, No AmpErase UNG (Thermo Fisher Scientific) for RT-qPCRs using Custom TaqMan Gene Expression Array Cards (Thermo Fisher Scientific). One Array Card was used to examine the mRNA expression of four samples in triplicate. The RT-qPCRs were run on a QuantStudio 12K Flex System (Applied Biosystems, Waltham, Massachusetts, US). Data were further analyzed with QuantStudio 12K Flex Software (Applied Biosystems). A Ct value above 34 was considered undetectable. The same threshold equal to 0.20 was set for all samples for comparison. Then, the data were analysed with qBasePLUS 3.1 software (Biogazelle, Zwijnaarde, Belgium) [23]. *Rpl32* and *Ppia* were selected for normalization.

Western blot analysis

The concentration of proteins was determined using the Bradford Reagent (Sigma-Aldrich, Saint Louis, MO, USA) following the manufacturer's protocol. Equal concentrations of proteins were mixed with 4 × Bolt® LDS Sample Buffer (Invitrogen, Waltham, MA, USA) and 10× Bolt® Sample Reducing Agent (Invitrogen) and then denatured at 70 °C for 10 min. Samples were separated on Bolt™ 4–12% Bis–Tris Plus Gels (Invitrogen) under reducing conditions in 20× Bolt® MES SDS Running Buffer (Invitrogen), incubated in 20% ethanol for 10 min, and transferred to immunoblot nitrocellulose membranes (iBlot® 2 Transfer Stacks, nitrocellulose, Invitrogen, Waltham, MA, USA) following the manufacturer's protocol. Primary and secondary antibodies were suspended in an iBind™ Solution Kit followed by membrane incubation on iBind™ Cards using the iBind™ Western Device (SLF1000, Invitrogen, Waltham, MA, USA) for 2.5 h or overnight. Due to the lack of high-quality primary antibodies, we were unable to verify differences in the expression of several genes at the protein level. Western blot analysis was performed for the following proteins: MAO-A, ADH1, 5-$HT_{3A,}$ and SERT. The following concentrations of primary antibodies were used to determine protein levels: 1:2000 for MAO-A (rabbit, cat. number: PA579623, Invitrogen)), 1:2000 for ADH1 (rabbit, cat. number PA5-78,730, Invitrogen), 1:1000 for 5-HT_{3A} (rabbit, cat. number: bs-2126R Bioss antibodies), and 1:2000 for SERT (rabbit, cat. number: PA5-80032, Invitrogen). The secondary anti-rabbit (cat. number: ab6721, Abcam) antibodies were used at concentrations of 1:20 000. As a loading control, β-actin (monoclonal anti-β-actin antibody produced in mouse, A5441, Sigma-Aldrich, Saint Louis, MO, USA) was applied at a concentration of 1:20 000, and its corresponding secondary antibody (anti-mouse IgG, A9044, Sigma−Aldrich, Saint Louis, MO, USA) was applied at a concentration of 1:20 000. The electrophoretic bands were detected using the Clarity™ Western ECL Substrate (Bio-Rad, Hercules, CA, USA) and FUJIFILM LAS-4000 (Fujifilm Life Science, USA) device. Blot analysis was performed using ImageJ 1.53e software (Wayne Rusband and NIH, USA). Due to limited gel spots, a minimum of three samples from different groups were included in each blot.

Statistics

The data were analyzed using SPSS (version 25.0, SPSS Inc., Chicago, IL, USA). The normality of the data was assessed using the Shapiro–Wilk test. For gene expression and protein level data, two-way ANOVAs were conducted. For pairwise comparisons, the values were compared using Sidak's post-hoc tests. Nonparametric data were normalized by applying the square root transformation and, if necessary, outliers were removed. In cases where data could not be normalized, the Kruskal–Wallis test was employed followed by Dunn's post hoc test. Feedback sensitivity screening data were analyzed using two-way repeated-measures ANOVA, with the within-subject factor being the test day/session and the between-subject factor being the sensitivity to NF. All significance tests were conducted with $\alpha = 0.05$. The homogeneity of variance was examined using Levene's test, and for repeated-measures analyses, sphericity was confirmed using Mauchly's test. The data are presented as the mean ± SEM (standard error of the mean) for parametric data, or as the median and interquartile range for nonparametric data.

Results

NF sensitivity screening

All animals fulfilled the PRL training criteria and qualified for the PRL screening. Screening data for the EtOH group have been previously published [7]. Screening data for the whole cohort are presented in Fig. 2. For the animals classified as less sensitive to NF, the average proportion of lose-shift behaviors following misleading NF ranged from 0.36 to 0.54, with an average of 0.46 ± 0.01. For those classified as more sensitive to NF, the average proportion of probabilistic lose-shift behaviors ranged from 0.54 to 0.71, with an average of 0.59 ± 0.01. The difference in sensitivity to NF between both groups was stable across the screening period (non-significant interaction between screening day and NF

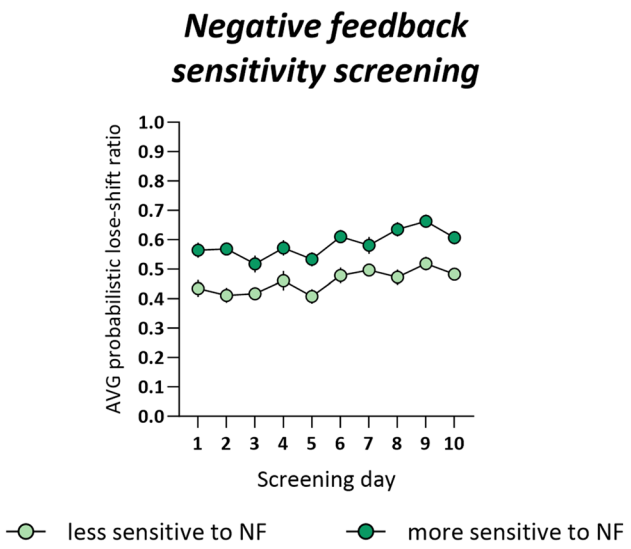

Fig. 2 Negative feedback (NF) sensitivity screening. The average proportion of lose-shift behaviors following misleading unrewarded outcomes in rats classified as less sensitive ($n=20$) and more sensitive to NF ($n=20$) across all 10-screening probabilistic reversal learning tests. Data are presented as the mean ± SEM

sensitivity ($F_{9,342}=0.331$, $p=0.542$), a significant sensitivity effect ($F_{1,38}=62.36$, $p<0.001$), Fig. 2).

Gene expression

Analysis of the gene expression revealed statistically significant lower levels of mRNA in rats more sensitive to NF compared to the less sensitive group, for *Gad2* ($F_{1, 29}=7.533$, $p=0.010$) in ACC (Fig. 3A), for *Maoa* ($F_{1, 30}=5.229$, $p=0.029$) in mPFC (Fig. 3B) and *Gria1* ($F_{1, 30}=6.268$, $p=0.018$), *Htr3a* ($F_{1, 30}=6.514$, $p=0.016$), and *Maoa* ($F_{1, 29}=4.734$, $p=0.038$) in OFC (Fig. 3E).

In the ACC, the level of mRNA for *Drd2* ($F_{1, 30}=4.920$, $p=0.034$) and *Slc6a4* ($F_{1, 28}=5.254$, $p=0.030$) was significantly higher in the more sensitive to NF group (Fig. 3A). There were no significant effects of NF sensitivity on the expression of genes of interest in Amy and Nacc.

The mRNA levels were higher in the EtOH group compared to control, for *Comt* ($F_{1, 29}=10.220$, $p=0.003$) and *Maoa* ($F_{1, 29}=4.368$, $p=0.046$) in ACC, for *Comt* ($F_{1, 30}=13.270$, $p=0.001$), and *Htr2b* ($F_{1, 23}=6.437$, $p=0.018$) in mPFC (Fig. 3B), for *Adh1* ($F_{1, 27}=9.895$, $p=0.004$) in Nacc (Fig. 3D), and *Gad2* ($F_{1, 30}=4.390$, $p=0.045$) in OFC (Fig. 3E).

In mPFC, the mRNA level of *Adh1* was higher in the EtOH group than in the control group only for rats more sensitive to NF (Kruskal–Wallis test: $p=0.033$, Fig. 3B). The expression of *Drd2* ($F_{1, 28}=4.436$, $p=0.044$) in Amy (Fig. 3C), *Slc6a4* ($F_{1, 29}=5.258$, $p=0.029$) in Nacc (Fig. 3D), and *Htr1a* ($F_{1, 30}=8.506$, $p=0.007$) in OFC (Fig. 3E) was lower in the EtOH group compared to the controls.

The analysis also revealed significant interactions between the effects of sensitivity to NF and the effects of prolonged alcohol exposure on the expression of *Gabra1* ($F_{1, 30}=4.629$ $p=0.040$), *Gabbr2* ($F_{1, 30}=5.772$ $p=0.023$), *Grin2a* ($F_{1, 30}=4.629$, $p=0.040$), *Grin2b* ($F_{1, 30}=9.156$, $p=0.005$), and *Grm3* ($F_{1, 30}=9.867$, $p=0.004$) in ACC (Fig. 3A), and on the expression of *Grin2a* ($F_{1, 30}=4.629$, $p=0.040$) in OFC (Fig. 3E). In the group of rats more sensitive to NF, the mRNA level of *Grm3* in ACC was lower in the EtOH group than in their control conspecifics. Additionally, within the control group, rats more sensitive to NF exhibited lower levels of *Grm3* expression compared to their less sensitive to NF counterparts. For *Grin2b* in ACC within the control group, rats more sensitive to NF showed higher mRNA expression compared to their less sensitive counterparts. For the EtOH group in ACC, the mRNA level of *Gabbr2* was lower in rats more sensitive to NF compared to their less sensitive conspecifics. The post-hoc tests did not reveal significant inter-group differences in the expression of *Garba1*, *Grin2a* in ACC, and *Grin2a* in OFC.

The results of statistical analyses of the expression of all genes are listed in Table S1 (Supplementary Materials S2). Two samples from Amy failed to pass the RNA quality threshold. Abnormalities in the gene expression readings were detected in certain samples on the RT-PCR card, specifically: for *Htr2b* in Amy, mPFC, ACC, NaCC, and OFC; for *Slc6a3* in Amy, mPFC, ACC, and OFC; and *Slc6a4* in ACC and Nacc. These results were not included in the analysis.

Protein expression

The observed differences in the mRNA levels were further explored at the protein level using the Western blot technique. Statistically significant effect of alcohol treatment on ADH1 protein (Alcohol dehydrogenase 1, gene: *Adh1*) level was detected in the mPFC ($F_{1,31}=7.650$, $p=0.010$; Fig. 4A) and in the Nacc ($F_{1,31}=7.650$, $p=0.010$; Fig. 4B).

Statistical analysis revealed also a significant interaction between the effects of sensitivity to NF and prolonged alcohol exposure on MAO-A protein level (Monoamine oxidase A, gene: *Maoa*) in mPFC ($F_{1,31}=7.650$, $p=0.010$; Fig. 4A), with a higher level of MAO-A in rats more sensitive to NF within the control group and significantly lower level of this protein in alcohol drinking group within the group of animals more sensitive to NF.

There were no statistically significant differences in the expression of other analyzed proteins. The results of statistical analyses of differences in the expression of all proteins of interest are listed in the Supplementary Table S2 (Supplementary materials S2). Original Western blot images

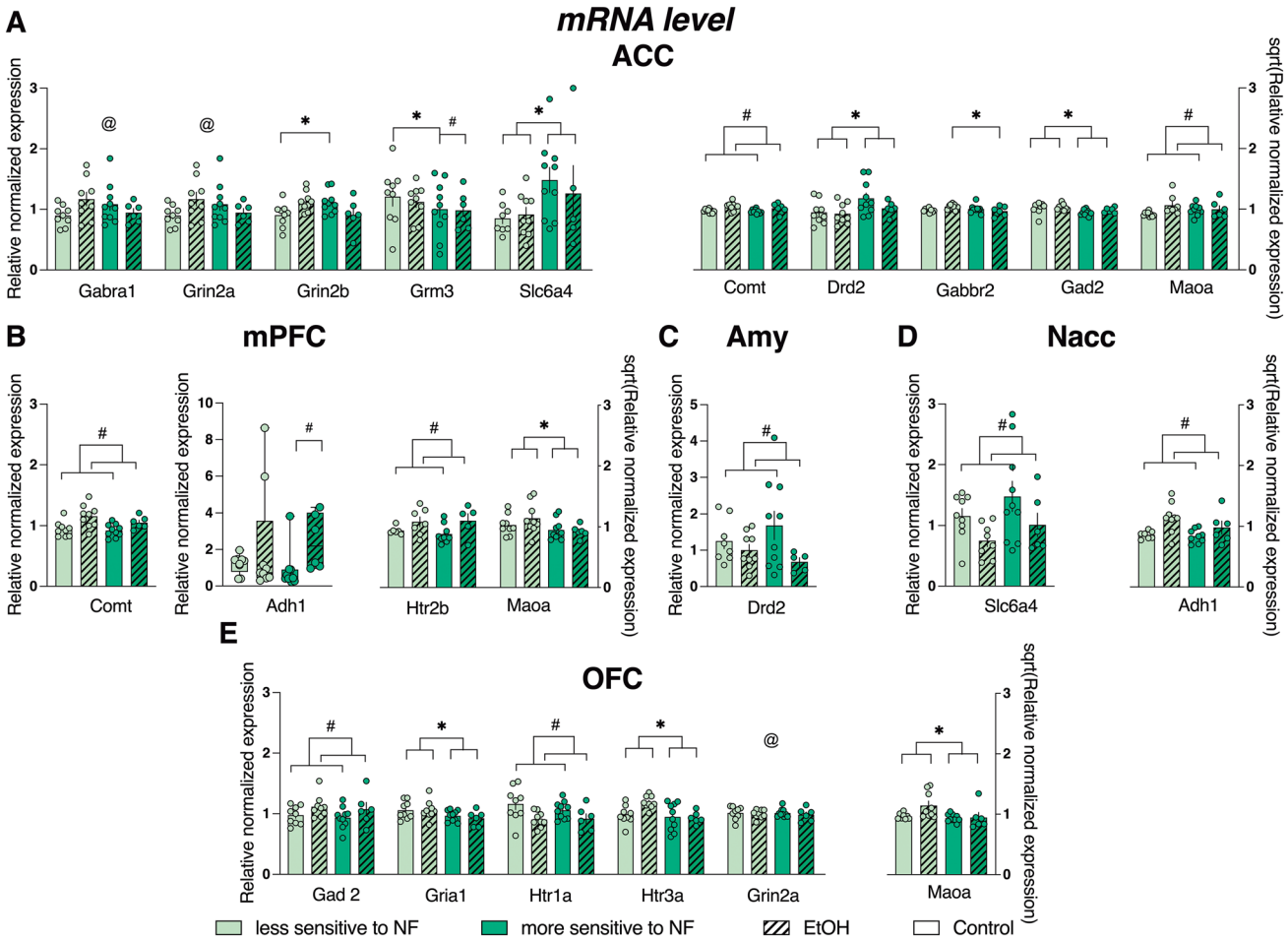

Fig. 3 Genes expression following chronic alcohol exposure in the brains of male Sprague Dawley rats with higher or lower sensitivity to negative feedback (NF). Bar graphs represent a relative normalized expression of the genes assessed with TaqMan Gene Expression Array Cards in animals less sensitive to NF (light green bars) and more sensitive to NF (dark green bars) belonging to control (open bars) and EtOH (dashed bars) groups in **A** anterior cingulate cortex (ACC), **B** medial prefrontal cortex (mPFC), **C** amygdala (Amy), **D** nucleus accumbens (Nacc) and **E** orbitofrontal cortex (OFC); Total number of samples: $n = 34$ (less sensitive to NF: 9 control (8 in Amy), 9 EtOH; more sensitive to NF: 10 control, 6 EtOH [5 in Amy]). For some genes, single samples were excluded due to abnormalities in the gene expression readings or the removal of outliers during data normalization. The scale for normally distributed data is shown on the left *Y*-axis. The scale of the right *Y*-axis corresponds to the data normalized by square root transformation. Data are presented as the mean ± SEM (**A**–**E**) or as a median and interquartile range (B: *Adh1*) * indicates a significant ($p < 0.05$) difference between animals less and more sensitive to NF. # indicates a significant ($p < 0.05$) difference between the EtOH and control group. @ indicates significant NF sensitivity × alcohol exposure interaction with non-significant intergroup differences in post hoc tests (2-way ANOVA, Sidak's post hoc test; for *Adh1* in mPFC Kruskal–Wallis test, Dunn's post hoc test)

are included in Supplementary materials S3. Some protein bands were unsuitable for quantification due to technical errors and are indicated by black frames (Supplementary materials S3).

Discussion

The results of the study described above are complementary to and need to be discussed in the light of the results published in our earlier paper [7] in which we tested the hypothesis that in rats, individual vulnerability to compulsive seeking of alcohol may be linked to cognitive mechanisms based on sensitivity to NF. The experiments described in the mentioned study have confirmed the above assumption. Although initially the rats classified as NF less and more sensitive did not differ in voluntary alcohol consumption, the NF less sensitive animals turned out to be more vulnerable to compulsive alcohol seeking than their more NF-sensitive counterparts. This increased vulnerability was demonstrated by their weaker reaction to the unpredictable punishment of seeking responses (i.e., foot shock intensity increasing from 0.1 to 0.5 mA over repeated sessions) and their prolonged extinction of

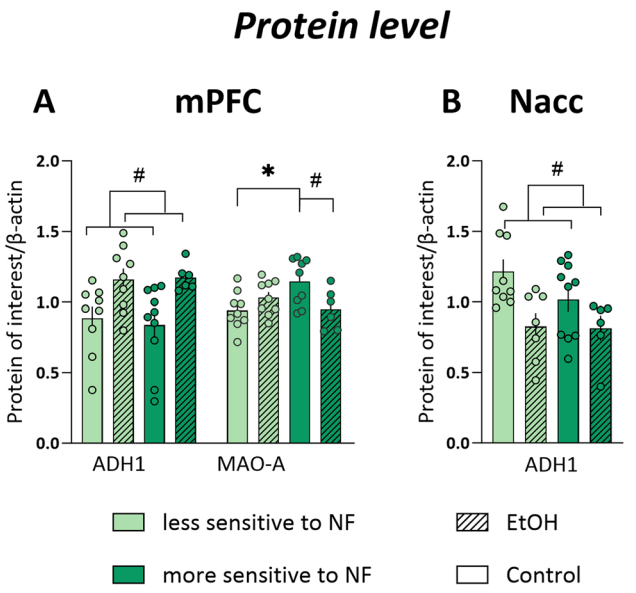

Fig. 4 Protein levels following chronic alcohol exposure in the brains of male Sprague Dawley rats with higher or lower sensitivity to negative feedback (NF). **A** Alcohol dehydrogenase 1 (ADH1) and Monoamine oxidase A (MAO-A) to β-actin ratio in animals less sensitive to NF (light green bars) and more sensitive to NF (dark green bars) belonging to control (open bars) and EtOH (dashed bars) groups in mPFC **B**) ADH1 to β-actin ratio in animals less sensitive to NF (light green bars) and more sensitive to NF (dark green bars) belonging to control (open bars) and EtOH (dashed bars) groups in Nacc. Total number of samples included in Western blot analysis was n = 34 (less sensitive to NF: 9 control, 9 EtOH; more sensitive to NF: 10 control, 6 EtOH). For MAOA-A in mPFC, one sample was excluded (control, more sensitive to NF) and for ADH1 in Nacc, two samples (EtOH, less sensitive to NF) were excluded due to bands not being quantifiable. *Data are presented as the mean ± SEM.* * indicates a significant ($p < 0.05$) difference between animals less and more sensitive to NF # indicates a significant ($p < 0.05$) difference between the EtOH and control group (2-way ANOVA, Sidak's post hoc test)

instrumental alcohol-seeking responses when alcohol was no longer available (for details see [7]).

The findings of our present study suggest that differences in the expression of certain genes and proteins, within several brain regions, can be linked to individual differences in sensitivity to NF, and the mechanisms determining the NF-linked vulnerability to compulsive alcohol-seeking and taking in rats. Specifically, we found that in the ACC, the rats that were more sensitive to NF had a lower level of mRNA expression of the *Gad2, a* gene, that is involved in the production of GABA [24], than their less NF-sensitive conspecifics. Conversely, rats that were more sensitive to NF had a higher mRNA expression level of *Drd2*, which encodes for a dopamine D_2 receptor, and *Slc6a4*, which encodes for a serotonin transporter, than the rats from the NF less sensitive group. Similarly, in the mPFC and the OFC, the rats that were more sensitive to NF had a lower *Maoa* mRNA expression level than those, that were less sensitive to NF. Moreover, in the OFC, the level of mRNA expression of *Gria1*, which encodes for a subunit of a glutamate NMDA receptor, and the level of *Htr3a*, which encodes for a serotonin 5-HT_{3A} receptor, was lower in rats more sensitive to NF than in their NF less sensitive counterparts.

Our study also confirmed that chronic alcohol exposure leads to significant changes in gene expression in different brain regions of rats, which may contribute to the behavioral and physiological effects of alcohol. In the ACC, rats exposed to prolonged alcohol consumption had significantly higher mRNA levels of *Comt* and *Maoa* as compared to their non-drinking controls. *Comt* encodes for catechol *O*-methyltransferase (COMT), an enzyme involved in the breakdown of DA and other catecholamines [25], while *Maoa* encodes for monoamine oxidase A (MAO-A), an enzyme involved in the breakdown of neurotransmitters such as 5-HT and DA [26]. Higher levels of these enzymes may reflect the increased activity of the neurotransmitter systems they break down, possibly as a compensatory response to chronic alcohol exposure. In the mPFC, the mRNA levels of *Comt* and *Htr2b*, which encodes for a serotonin 5-HT_{2B} receptor, were higher in the rats from the EtOH group as compared to their non-drinking controls. In the Nacc, alcohol-exposed rats had significantly higher levels of *Adh1*, which encodes for an alcohol dehydrogenase enzyme, and lower levels of the mentioned already above *Slc6a4*. In the OFC, alcohol-exposed rats had higher mRNA levels for *Gad2*, compared to control rats. However, they also had lower levels of *Htr1a*, which encodes for the serotonin 5-HT_{1A} receptor.

Our findings also showed that there are significant interactions between the sensitivity to NF and the effects of prolonged alcohol exposure on the expression of specific genes in different cortical regions, namely the ACC and OFC. The expression levels of *Gabra1, Gabbr2, Grin2a, Grin2b*, and *Grm3* were found to be significantly affected by both sensitivity to NF and prolonged alcohol exposure in the ACC. *Gabra1* and *Gabbr2* are involved in the regulation of GABA, while *Grin2a*, *Grin2b*, and *Grm3* are involved in glutamatergic neurotransmission. In the OFC, only the expression level of *Grin2a* was found to be affected by both sensitivity to NF and prolonged alcohol exposure. Alcohol has the potential to disrupt the delicate balance between GABA, the major inhibitory neurotransmitter, and glutamate, the principal excitatory neurotransmitter within the central nervous system [27]. The differences in gene expression related to GABAergic neurotransmission observed in ACC align with prior research findings that have demonstrated how alterations in GABA signaling can influence reward processes and the reinforcing effects of alcohol. Given that the mRNA expression analysis was conducted after a period of forced abstinence and reinstatement in the EtOH group, it should come as no surprise that there are differences in the expression of

genes related to the glutamatergic system. In fact, numerous studies have indicated that alcohol withdrawal is associated with disturbances in excitatory amino acid transmission, and modulating it can alleviate withdrawal symptoms [28, 29].

In our investigation, most inter-trait differences in gene expression failed to manifest at the protein level. The lack of alignment between differences in gene expression and protein levels is not entirely surprising and could have several reasons, including post-transcriptional modifications, alternative splicing, translational regulation, and post-translational modifications. These processes introduce complexities that can obscure the direct relationship between gene activity and protein abundance and require further investigation. Despite this, we confirmed that animals more sensitive to NF within the control group had higher levels of MAO-A in mPFC than their NF less sensitive conspecifics. Though the precise function of MAO-A in influencing sensitivity to feedback remains uncertain, its heightened activity could potentially accelerate the breakdown of biogenic amines. This, in turn, may reduce their accessibility to receptors and hinder the processing of adverse information. It is worth mentioning that reversible monoamine oxidase inhibitors are commonly used in the treatment of depression and may potentially reduce sensitivity to NF, a trait commonly observed in individuals with depression [30–32]. Additionally, our study found that in animals more sensitive to NF, chronic alcohol consumption led to lower levels of MAO-A in the mPFC. This suggests that alcohol consumption downregulates MAO-A expression only in animals with higher sensitivity to NF, not in those with lower NF sensitivity. These findings are in line with previously published behavioral data where rats more sensitive to NF were less likely to seek alcohol when it was associated with punishment and after the termination of alcohol availability, compared to their less sensitive conspecifics [7]. Previous studies have shown that genetic variants of *Maoa* and epigenetic mechanisms are strongly associated with the occurrence of AUD in both humans and animals [33–35]. Although the exact mechanism by which alcohol regulates MAO-A expression is unknown, this finding presents a promising avenue for further research in identifying individual differences between animals less and more sensitive to NF and their susceptibility to the development of alcohol dependence. Future studies should aim to investigate the cellular mechanisms underlying MAO-A-driven susceptibility to alcohol dependence and explore epigenetic and regulatory mechanisms that may mediate the effects of chronic alcohol exposure on *Maoa* expression.

The second gene, the differences in expression of which were confirmed at the protein level, was the gene encoding alcohol dehydrogenase. Although alcohol-induced differences in the expression of this gene and protein levels were not unexpected, their presence confirms the effectiveness of the model used and positively verifies the effects of alcohol.

In conclusion, this study provides further evidence for the relationship between trait sensitivity to NF and compulsive alcohol consumption in rats. Our findings demonstrate significant differences in the expression of genes and (some) proteins related to NF sensitivity and alcohol metabolism in various cortical and subcortical regions of the brain between rats less and more sensitive to NF that consumed alcohol and their non-drinking counterparts. Because of the wide range of neurotransmitter and neuromodulator systems affected by alcohol, the effectiveness of current pharmacotherapies aimed at treating alcohol dependence is constrained. The imperative for reducing the harmful use of alcohol in a public health context requires the development of successful therapeutic strategies. Our research aimed to address this need by identifying potential molecular targets for new drugs to treat AUD. Our findings contribute to a better understanding of the molecular mechanisms underlying compulsive alcohol consumption in rats and therefore may have implications for the development of treatments for alcohol use disorders.

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Data availability The data that support the findings of this study are available from the corresponding author, [RR], upon reasonable request.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Rehm J, Imtiaz S. A narrative review of alcohol consumption as a risk factor for global burden of disease. Subst Abuse Treat Prev Policy. 2016;11:37.
2. Molina PE, Gardner JD, Souza-Smith FM, Whitaker AM. Alcohol abuse: critical pathophysiological processes and contribution to disease burden. Physiology (Bethesda). 2014;29:203–15.
3. Bechara A, Damasio H. Decision-making and addiction (part I): impaired activation of somatic states in substance dependent individuals when pondering decisions with negative future consequences. Neuropsychologia. 2002;40:1675–89.
4. Kamarajan C, Rangaswamy M, Tang Y, Chorlian DB, Pandey AK, Roopesh BN, et al. Dysfunctional reward processing in male alcoholics: an ERP study during a gambling task. J Psychiatry Res. 2010;44:576–90.
5. Santesso DL, Steele KT, Bogdan R, Holmes AJ, Deveney CM, Meites TM, et al. Enhanced negative feedback responses in remitted depression. NeuroReport. 2008;19:1045–8.
6. Ruchsow M, Herrnberger B, Beschoner P, Grön G, Spitzer M, Kiefer M. Error processing in major depressive disorder: evidence from event-related potentials. J Psychiatry Res. 2006;40:37–46.
7. Cieslik A, Noworyta K, Rygula R. Trait sensitivity to negative feedback determines the intensity of compulsive alcohol seeking and taking in male rats. J Psychiatry Neurosci. 2022;47:E252–62.
8. Clarke HF, Cardinal RN, Rygula R, Hong YT, Fryer TD, Sawiak SJ, et al. Orbitofrontal dopamine depletion upregulates caudate dopamine and alters behavior via changes in reinforcement sensitivity. J Neurosci. 2014;34:7663–76.
9. Cools R, Clark L, Owen AM, Robbins TW. Defining the neural mechanisms of probabilistic reversal learning using event-related functional magnetic resonance imaging. J Neurosci. 2002;22:4563–7.
10. Cools R, Frank MJ, Gibbs SE, Miyakawa A, Jagust W, D'Esposito M. Striatal dopamine predicts outcome-specific reversal learning and its sensitivity to dopaminergic drug administration. J Neurosci. 2009;29:1538–43.
11. Dalton GL, Phillips AG, Floresco SB. Preferential involvement by nucleus accumbens shell in mediating probabilistic learning and reversal shifts. J Neurosci. 2014;34:4618–26.
12. Chamberlain SR, Müller U, Blackwell AD, Clark L, Robbins TW, Sahakian BJ. Neurochemical modulation of response inhibition and probabilistic learning in humans. Science. 2006;311:861–3.
13. Cools R, Roberts AC, Robbins TW. Serotoninergic regulation of emotional and behavioural control processes. Trends Cogn Sci. 2008;12:31–40.
14. Rygula R, Clarke HF, Cardinal RN, Cockcroft GJ, Xia J, Dalley JW, et al. Role of central serotonin in anticipation of rewarding and punishing outcomes: effects of selective amygdala or orbitofrontal 5-HT depletion. Cereb Cortex. 2015;25:3064–76.
15. Golebiowska J, Rygula R. Effects of acute dopaminergic and serotonergic manipulations in the ACI paradigm depend on the basal valence of cognitive judgement bias in rats. Behav Brain Res. 2017;327:133–43.
16. Pessiglione M, Seymour B, Flandin G, Dolan RJ, Frith CD. Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. Nature. 2006;442:1042–5.
17. Kauer JA, Malenka RC. Synaptic plasticity and addiction. Nat Rev Neurosci. 2007;8:844–58.
18. Volkow ND, Wang GJ, Fowler JS, Tomasi D, Telang F, Baler R. Addiction: decreased reward sensitivity and increased expectation sensitivity conspire to overwhelm the brain's control circuit. BioEssays. 2010;32:748–55.
19. Hipólito L, Sánchez MJ, Polache A, Granero L. Brain metabolism of ethanol and alcoholism: an update. Curr Drug Metab. 2007;8:716–27.
20. Gąska M, Kuśmider M, Solich J, Faron-Górecka A, Krawczyk MJ, Kułakowski K, et al. Analysis of region-specific changes in gene expression upon treatment with citalopram and desipramine reveals temporal dynamics in response to antidepressant drugs at the transcriptome level. Psychopharmacology. 2012;223:281–97.
21. Paxinos G, Watson C. The rat brain in stereotaxic coordinates: hard. cover. Amsterdam: Elsevier; 2006.
22. Achterberg EJ, van Kerkhof LW, Damsteegt R, Trezza V, Vanderschuren LJ. Methylphenidate and atomoxetine inhibit social play behavior through prefrontal and subcortical limbic mechanisms in rats. J Neurosci. 2015;35:161–9.
23. Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 2007;8:R19.
24. Erlander MG, Tillakaratne NJ, Feldblum S, Patel N, Tobin AJ. Two genes encode distinct glutamate decarboxylases. Neuron. 1991;7:91–100.
25. Männistö PT, Ulmanen I, Lundström K, Taskinen J, Tenhunen J, Tilgmann C, et al. Characteristics of catechol O-methyl-transferase (COMT) and properties of selective COMT inhibitors. Prog Drug Res. 1992;39:291–350.
26. Abell CW, Kwan SW. Molecular characterization of monoamine oxidases A and B. Prog Nucleic Acid Res Mol Biol. 2001;65:129–56.
27. Valenzuela CF. Alcohol and neurotransmitter interactions. Alcohol Health Res World. 1997;21:144–8.
28. Narita M, Soma M, Mizoguchi H, Tseng LF, Suzuki T. Implications of the NR2B subunit-containing NMDA receptor localized in mouse limbic forebrain in ethanol dependence. Eur J Pharmacol. 2000;401:191–5.
29. Bienkowski P, Krzascik P, Koros E, Kostowski W, Scinska A, Danysz W. Effects of a novel uncompetitive NMDA receptor antagonist, MRZ 2/579 on ethanol self-administration and ethanol withdrawal seizures in the rat. Eur J Pharmacol. 2001;413:81–9.
30. Beats BC, Sahakian BJ, Levy R. Cognitive performance in tests sensitive to frontal lobe dysfunction in the elderly depressed. Psychol Med. 1996;26:591–603.
31. Taylor Tavares JV, Drevets WC, Sahakian BJ. Cognition in mania and depression. Psychol Med. 2003;33:959–67.
32. Taylor Tavares JV, Clark L, Furey ML, Williams GB, Sahakian BJ, Drevets WC. Neural basis of abnormal response to negative feedback in unmedicated mood disorders. Neuroimage. 2008;42:1118–26.
33. Philibert RA, Sandhu H, Hollenbeck N, Gunter T, Adams W, Madan A. The relationship of 5HTT (SLC6A4) methylation and genotype on mRNA expression and liability to major depression and alcohol dependence in subjects from the Iowa Adoption Studies. Am J Med Genet B Neuropsychiatr Genet. 2008;147B:543–9.
34. Cervera-Juanes R, Wilhem LJ, Park B, Lee R, Locke J, Helms C, et al. MAOA expression predicts vulnerability for alcohol use. Mol Psychiatry. 2016;21:472–9.
35. Bendre M, Granholm L, Drennan R, Meyer A, Yan L, Nilsson KW, et al. Early life stress and voluntary alcohol consumption in relation to Maoa methylation in male rats. Alcohol. 2019;79:7–16.

Supplementary materials S1

Methods

Subjects and housing

We used male Sprague Dawley rats housed in groups of 4 rats per cage, in an enriched environment (wooden blocks and plastic pipes 25 cm long), with controlled temperature (21 ± 1 °C) and humidity (40 - 50%) and water always available *ad libitum*. Both groups of rats (EtOH and control) were delivered on the same day with an initial body weight of 176–200 g (5 weeks old according to the Growth Chart provided by the laboratory rodent supplier Charles River). They were kept on a 12-hour light/dark cycle, with lights turned on at 7:00 AM. All procedures were conducted during the light phase. The rats were mildly food-restricted throughout the experiment to facilitate instrumental training [1-3], receiving 15 g of standard laboratory chow per day, which was equivalent to 85% of their free-feeding weight based on the normal growth curve recommended by the laboratory rodent supplier, Charles River Research Models and Services Catalogue.

Experimental Apparatus

PRL tests were conducted in operant conditioning chambers (Med Associates; St Albans, Vermont, USA) enclosed within a sound-attenuating box. Each chamber was equipped with a fan, house light, speaker, a food dispenser set to deliver a sucrose pellet (Dustless Precision Pellets, 45 mg; Bio-Serv, New Jersey, USA), fluid receptacle, and two retractable levers located at the sides of the feeder.

Measuring sensitivity to feedback using the PRL test

After the initial instrumental training described in detail elsewhere [4] and upon reaching the initial training criterion of less than 7.5% omissions on each lever (i.e., less than 15% total omissions but equally distributed between the 2 levers) for 3 consecutive training days, the rats from both groups were trained in the PRL paradigm. In brief, each PRL training session consisted of 200 trials, and each trial lasted for a maximum of 22 s. The start of a trial was signaled by the house light, which remained on until the end of the trial. Two seconds after the trial had started, both levers were presented, and one of them was randomly assigned as the "correct" lever, which delivered a reward (one sucrose pellet) 80% of the time it was pressed. A press on the other lever - the "incorrect" lever - would result in a rewarding outcome only 20% of the time it was pressed. A 5 s intertrial interval (ITI) followed reward delivery. During the ITI, both levers remained retracted, and the house light was turned off. No response in 10 s triggered the ITI and was counted as an omission. The same ITI directly followed an unrewarded outcome, i.e., no reward on 20% of the "correct" and 80% of the "incorrect" lever presses. After every 8 consecutive "correct" lever presses (regardless of the outcome), the criterion for the reversal of the outcome probabilities was reached. The previously "correct" lever now became "incorrect" and vice versa. This pattern was followed until the end of the session. The PRL training phase was repeated daily until the individual animals achieved sufficient performance levels. The criteria to be met were a minimum of 3 reversals completed during 3 consecutive training sessions, with less than 15% omissions per session.

Parameters measured in the PRL test

To assess rats' sensitivity to negative feedback (NF), which reflects their ability to disregard occasional and misleading lack of reward, their decisions were tracked trial by trial. The number of unrewarded outcomes for the "correct" lever that were followed by the animal switching to the other lever (probabilistic lose-shifts) were recorded, and expressed as a ratio of all unrewarded outcomes for that lever. To measure rats' sensitivity to positive feedback, all rewarded outcomes (both true and misleading) that were followed by a decision to stick with the lever that produced them (win-stays) were counted for both the "correct" and "incorrect" levers, and expressed as a ratio of all rewarded outcomes for that lever. This method of analyzing sensitivity to positive feedback was inspired by Bari et al.'s approach and was based on the infrequency of win-stay behavior after misleading rewards on the incorrect lever[2](2)(2)(2)(2)(3). The number of reversals completed during the test was used as an indicator of the animals' performance.

Feedback sensitivity screening

Once the rats achieved a stable performance in the PRL test, with a minimum of 3 reversals and less than 15% omissions in three consecutive sessions, they underwent 10 consecutive PRL tests over 10 days. Using the results of these tests as a "sensitivity screening," the rats were divided into two groups based on their sensitivity to NF, using the median to split them into less sensitive and more sensitive groups. This division was determined by calculating the average ratio of lever changes following misleading unrewarded outcomes (probabilistic lose-shifts) made by the animals across all 10 screening tests.

Procedures measuring alcohol-related behaviors

Intermittent access 2BC paradigm

To induce drinking behavior and to determine the level of alcohol consumption in the rats, 18 sessions of the intermittent access 2BC procedure were conducted every second day. During the 2BC test, animals were separated into individual cages for 24 hours, where they were presented with one bottle of 10% ethanol (EtOH) (w/w) and one bottle of water. The bottles were weighed before and after each session to determine alcohol consumption (g EtOH/kg).

TAKING TASK

Initially, the rats were trained to associate the pressing of the taking lever with alcohol delivery under a fixed-ratio 1 (FR1) schedule of reinforcement. Each trial started with the insertion of the randomly assigned taking lever and the house light on. Pressing on the lever resulted in the dipper presentation on the opposite side of the box, delivery of 0.1 ml of 15% EtOH (w/w), and simultaneous retraction of the taking lever. Rats were limited to a maximum of 60 rewards for a 30 min training session. After achieving the performance criterion of a minimum of 20 taking responses in 3 consecutive sessions, the animals were shifted to the ST phase of the training.

ST TASK

During this task, each trial started with the insertion of the seeking lever, next to the previously assigned taking lever, which remained retracted. Pressing on the seeking lever led to the extension of the taking lever following a random interval of 1 to 15 s (RI 1-15 s). Pressing on the taking lever resulted in the presentation of the dipper on the opposite side of the box, delivery of 0.1 ml of 15% EtOH (w/w), and simultaneous retraction of both levers. Rats were limited to a maximum of 100 rewards for a 45 min session. After achieving the performance

criterion of a minimum of 20 taking responses in 3 consecutive sessions, the animals were ready to be tested on the seeking taking punishment (STP) task.

STP TASK

In this paradigm, each trial started as described for the ST task, with the insertion of the seeking lever. The seeking lever response resulted either in a 1 s electric shock (0.10-0.50 mA), administered through a grid floor, or the extension of the taking lever after a random interval (RI 1-15 s). Each session consisted of 25 trials, of which 8 (30%) were punished with foot shock and 17 (70%) were reinforced by the delivery of 0.1 ml 15% EtOH following the taking lever response. The intensity of the shock increased gradually in the consecutive test sessions according to the following pattern: 0.10, 0.20, 0.30, 0.30, 0.40, 0.40, 0.50, and 0.50 mA. Although punishment occurred randomly in each session, never more than two consecutive trials resulted in a foot shock, and the first trial of the session was always reinforced.

Extinction of alcohol-seeking and taking behaviors

After the completion of STP testing, the animals underwent 5 additional ST tests (baseline) and were then subjected to daily extinction sessions (lasting 15 min), during which the seeking lever response resulted in the extension of the taking lever (under RI 1-15 s); however, the pressing of that lever had no programmed consequences, and alcohol was not available. After reaching less than 5 seeking responses in 3 consecutive sessions, the rats were not tested for the following 30 days of alcohol abstinence.

Reinstatement of alcohol-seeking and taking behaviors

After the 30 days of abstinence, the rats underwent a series of ST tests to measure how quickly they reinstated their alcohol-seeking behavior. The animals were tested until they reached the criterion of an average number of seeking responses from 5 tests that was equal to or higher than the average number of seeking responses from the 5 baseline ST tests.

1. Boulougouris, V., A. Castañé, and T.W. Robbins, *Dopamine D2/D3 receptor agonist quinpirole impairs spatial reversal learning in rats: investigation of D3 receptor involvement in persistent behavior.* Psychopharmacology (Berl), 2009. **202**(4): p. 611-20.
2. Bari, A., et al., *Serotonin modulates sensitivity to reward and negative feedback in a probabilistic reversal learning task in rats.* Neuropsychopharmacology, 2010. **35**(6): p. 1290-301.
3. Izquierdo, A., et al., *Genetic and dopaminergic modulation of reversal learning in a touchscreen-based operant procedure for mice.* Behav Brain Res, 2006. **171**(2): p. 181-8.
4. Noworyta-Sokolowska, K., et al., *Sensitivity to negative and positive feedback as a stable and enduring behavioural trait in rats.* Psychopharmacology, 2019. **236**(8): p. 2389-2403.

Table S1. The effects of trait sensitivity to NF and alcohol drinking on gene expression.

Statistically significant effects and interactions are bolded (2-way ANOVA).

Structure	Gene name	*n*	*Interaction*	*Treatment*	Sensitivity
ACC	*Adh1*	31	$F_{1, 27} = 2.658$ $p = 0.115$	$F_{1, 27} = 1.267$ $p = 0.253$	$F_{1, 27} = 3.765$ $p = 0.063$
	Cat	34	$F_{1, 30} = 2.230$ $p = 0.137$	$F_{1, 30} = 0.670$ $p = 0.419$	$F_{1, 30} = 3.746$ $p = 0.062$
	Comt	33	$F_{1, 29} = 0.166$ $p = 0.686$	**$F_{1, 29} = 10.22$** **$p = 0.003$ ****	$F_{1, 29} = 0.728$ $p = 0.401$
	Drd1	34	$F_{1, 30} = 1.759$ $p = 0.195$	$F_{1, 30} = 0.235$ $p = 0.631$	$F_{1, 30} = 1.128$ $p = 0.297$
	Drd2	34	$F_{1, 30} = 0.988$ $p = 0.328$	$F_{1, 30} = 1.665$ $p = 0.207$	**$F_{1, 30} = 4.920$** **$p = 0.034$ ***
	Gabbr1	34	$F_{1, 30} = 1.164$ $p = 0.289$	$F_{1, 30} = 1.660$ $p = 0.207$	$F_{1, 30} = 0.060$ $p = 0.808$
	Gabbr2	34	**$F_{1, 30} = 5.772$** **$p = 0.023$ ***	$F_{1, 30} = 0.507$ $p = 0.482$	$F_{1, 30} = 1.358$ $p = 0.253$
	Gabra1	34	**$F_{1, 30} = 4.629$** **$p = 0.040$ ***	$F_{1, 30} = 0.492$ $p = 0.488$	$F_{1, 30} = 0.036$ $p = 0.851$
	Gad1	34	$F_{1, 30} = 2.303$ $p = 0.140$	$F_{1, 30} = 0.272$ $p = 0.606$	$F_{1, 30} = 1.177$ $p = 0.287$
	Gad2	33	$F_{1, 29} = 0.317$ $p = 0.578$	$F_{1, 29} = 1.259$ $p = 0.271$	**$F_{1, 29} = 7.533$** **$p = 0.01$ ***
	Gria1	34	$F_{1, 30} = 0.883$ $p = 0.355$	$F_{1, 30} = 1.352$ $p = 0.254$	$F_{1, 30} = 2.289$ $p = 0.141$
	Grin2a	34	**$F_{1, 30} = 4.629$** **$p = 0.04$ ***	$F_{1, 30} = 0.492$ $p = 0.488$	$F_{1, 30} = 0.036$ $p = 0.851$
	Grin2b	34	**$F_{1, 30} = 9.156$** **$p = 0.005$ ****	$F_{1, 30} = 0.038$ $p = 0.847$	$F_{1, 30} = 0.011$ $p = 0.915$
	Grm2	34	$F_{1, 30} = 0.059$ $p = 0.810$	$F_{1, 30} = 0.144$ $p = 0.707$	$F_{1, 30} = 1.548$ $p = 0.223$
	Grm3	34	**$F_{1, 30} = 9.867$** **$p = 0.004$ ****	$F_{1, 30} = 0.283$ $p = 0.599$	$F_{1, 30} = 0.305$ $p = 0.585$
	Grm5	34	$F_{1, 30} = 0.013$ $p = 0.910$	$F_{1, 30} = 0.480$ $p = 0.494$	$F_{1, 30} = 0.564$ $p = 0.459$
	Htr1a	34	$F_{1, 30} = 1.159$ $p = 0.290$	$F_{1, 30} = 3.805$ $p = 0.061$	$F_{1, 30} = 0.020$ $p = 0.887$
	Htr2a	34	$F_{1, 30} = 0.268$ $p = 0.609$	$F_{1, 30} = 0.046$ $p = 0.831$	$F_{1, 30} = 0.360$ $p = 0.553$
	Htr2b	20	$F_{1, 16} = 0.574$ $p = 0.460$	$F_{1, 16} = 0.045$ $p = 0.835$	$F_{1, 16} = 0.187$ $p = 0.671$
	Htr3a	34	$F_{1, 30} = 1.068$ $p = 0.310$	$F_{1, 30} = 0.042$ $p = 0.840$	$F_{1, 30} = 0.335$ $p = 0.567$
	Npy	34	$F_{1, 30} = 2.918$ $p = 0.098$	$F_{1, 30} = 1.631$ $p = 0.211$	$F_{1, 30} = 0.482$ $p = 0.493$
	Maoa	33	$F_{1, 29} = 2.956$ $p = 0.096$	**$F_{1, 29} = 4.368$** **$p = 0.046$ ***	$F_{1, 29} = 0.007$ $p = 0.934$
	Maob	34	$F_{1, 30} = 0.829$	$F_{1, 30} = 1.103$	$F_{1, 30} = 0.916$

			p = 0.370	p = 0.302	p = 0.346
	Slc1a2	34	$F_{1, 30} = 0.522$ p = 0.476	$F_{1, 30} = 0.001$ p = 0.974	$F_{1, 30} = 0.111$ p = 0.742
	Slc6a3		-		
	Slc6a4	32	$F_{1, 28} = 0.443$ p = 0.511	$F_{1, 28} = 0.134$ p = 0.717	**$F_{1, 28} = 5.254$** **p = 0.030 ***
	Th	34	$F_{1, 30} = 1.870$ p = 0.182	$F_{1, 30} = 1.025$ p = 0.319	$F_{1, 30} = 0.302$ p = 0.587
	Tph2	34	$F_{1, 30} = 0.428$ p = 0.518	$F_{1, 30} = 1.932$ p = 0.175	$F_{1, 30} = 0.083$ p = 0.776
mPFC	***Adh1***	34	**Kruskal-Wallis test: p = 0.033 ***		
	Cat	34	$F_{1, 30} = 0.010$ p = 0.922	$F_{1, 30} = 2.654$ p = 0.114	$F_{1, 30} = 0.884$ p = 0.355
	Comt	34	$F_{1, 30} = 0.854$ p = 0.363	**$F_{1, 30} = 13.27$** **p = 0.001 ****	$F_{1, 30} = 1.816$ p = 0.188
	Drd1	34	$F_{1, 30} = 1.053$ p = 0.313	$F_{1, 30} = 2.524$ p = 0.123	$F_{1, 30} = 0.193$ p = 0.664
	Drd2	34	$F_{1, 30} = 3.976$ p = 0.055	$F_{1, 30} = 0.003$ p = 0.958	$F_{1, 30} = 0.008$ p = 0.929
	Gabbr1	34	$F_{1, 30} = 0.016$ p = 0.900	$F_{1, 30} = 0.399$ p = 0.533	$F_{1, 30} = 0.004$ p = 0.950
	Gabbr2	33	$F_{1, 29} = 0.090$ p = 0.766	$F_{1, 29} = 1.590$ p = 0.693	$F_{1, 29} = 0.002$ p = 0.963
	Gabra1	34	Kruskal-Wallis test: p = 0.817		
	Gad1	34	$F_{1, 30} = 1.421$ p = 0.243	$F_{1, 30} = 1.249$ p = 0.273	$F_{1, 30} = 0.058$ p = 0.812
	Gad2	34	$F_{1, 30} = 0.367$ p = 0.549	$F_{1, 30} = 1.475$ p = 0.234	$F_{1, 30} = 0.461$ p = 0.502
	Gria1	34	$F_{1, 30} = 0.744$ p = 0.395	$F_{1, 30} = 0.522$ p = 0.476	$F_{1, 30} = 0.178$ p = 0.676
	Grin2a	34	$F_{1, 30} = 3.586$ p = 0.068	$F_{1, 30} = 0.064$ p = 0.802	$F_{1, 30} = 0.264$ p = 0.611
	Grin2b	33	$F_{1, 29} = 1.125$ p = 0.298	$F_{1, 29} = 0.965$ p = 0.334	$F_{1, 29} = 0.033$ p = 0.857
	Grm2	34	$F_{1, 30} = 0.104$ p = 0.749	$F_{1, 30} = 0.047$ p = 0.831	$F_{1, 30} = 0.065$ p = 0.801
	Grm3	34	$F_{1, 30} = 0.913$ p = 0.347	$F_{1, 30} = 0.074$ p = 0.787	$F_{1, 30} = 0.151$ p = 0.701
	Grm5	32	$F_{1, 28} = 0.646$ p = 0.429	$F_{1, 28} = 0.687$ p = 0.414	$F_{1, 28} = 1.256$ p = 0.272
	Htr1a	34	$F_{1, 30} = 0.676$ p = 0.417	$F_{1, 30} = 0.145$ p = 0.706	$F_{1, 30} = 0.018$ p = 0.895
	Htr2a	34	$F_{1, 30} = 0.098$ p = 0.756	$F_{1, 30} = 0.476$ p = 0.496	$F_{1, 30} = 0.258$ p = 0.615
	Htr2b	27	$F_{1, 23} = 0.282$ p = 0.601	**$F_{1, 23} = 6.437$** **p = 0.018 ***	$F_{1, 23} = 0.062$ p = 0.806
	Htr3a	34	$F_{1, 30} = 3.179$ p = 0.085	$F_{1, 30} = 0.382$ p = 0.541	$F_{1, 30} = 0.022$ p = 0.883
	Npy	34	$F_{1, 30} = 0.280$ p = 0.601	$F_{1, 30} = 1.708$ p = 0.201	$F_{1, 30} = 1.889$ p = 0.180
	Maoa	34	$F_{1, 30} = 1.061$	$F_{1, 30} = 0.427$	**$F_{1, 30} = 5.229$**

			p = 0.311	p = 0.518	**p = 0.029 ***
	Maob	34	$F_{1, 30} = 0.303$ p = 0.587	$F_{1, 30} = 2.103$ p = 0.157	$F_{1, 30} = 0.373$ p = 0.546
	Slc1a2	34	$F_{1, 30} = 0.088$ p = 0.769	$F_{1, 30} = 0.391$ p = 0.537	$F_{1, 30} = 0.296$ p = 0.590
	Slc6a3	19	$F_{1, 15} = 0.227$ p = 0.640	$F_{1, 15} = 0.064$ p = 0.804	$F_{1, 15} = 0.656$ p = 0.431
	Slc6a4	34	$F_{1, 30} = 0.076$ p = 0.785	$F_{1, 30} = 0.026$ p = 0.873	$F_{1, 30} = 2.704$ p = 0.111
	Th	34	$F_{1, 30} = 0.001$ p = 0.977	$F_{1, 30} = 0.375$ p = 0.545	$F_{1, 30} = 0.197$ p = 0.661
	Tph2	33	$F_{1, 29} = 0.0003$ p = 0.985	$F_{1, 29} = 1.301$ p = 0.263	$F_{1, 29} = 2.364$ p = 0.135
Amy	*Adh1*	31	$F_{1, 27} = 1.335$ p = 0.258	$F_{1, 27} = 2.556$ p = 0.122	$F_{1, 27} = 4.158$ p = 0.051
	Cat	31	$F_{1, 27} = 0.535$ p = 0.471	$F_{1, 27} = 0.564$ p = 0.459	$F_{1, 27} = 0.095$ p = 0.761
	Comt	32	$F_{1, 28} = 0.069$ p = 0.795	$F_{1, 28} = 0.366$ p = 0.550	$F_{1, 28} = 1.749$ p = 0.197
	Drd1	32	$F_{1, 28} = 3.270$ p = 0.081	$F_{1, 28} = 0.054$ p = 0.818	$F_{1, 28} = 2.768$ p = 0.107
	Drd2	32	$F_{1, 28} = 1.579$ p = 0.219	**$F_{1, 28} = 4.436$** **p = 0.044 ***	$F_{1, 28} = 0.034$ p = 0.856
	Gabbr1	31	$F_{1, 27} = 0.054$ p = 0.818	$F_{1, 27} = 0.483$ p = 0.493	$F_{1, 27} = 3.117$ p = 0.089
	Gabbr2	32	$F_{1, 28} = 0.248$ p = 0.622	$F_{1, 28} = 0.862$ p = 0.361	$F_{1, 28} = 0.386$ p = 0.539
	Gabra1	32	$F_{1, 28} = 0.001$ p = 0.973	$F_{1, 28} = 4.136 \times 10^{-6}$ p = 0.998	$F_{1, 28} = 0.260$ p = 0.614
	Gad1	32	$F_{1, 28} = 1.069$ p = 0.310	$F_{1, 28} = 2.542$ p = 0.122	$F_{1, 28} = 0.562$ p = 0.460
	Gad2	32	Kruskal-Wallis test: p = 0.190		
	Gria1	32	$F_{1, 28} = 0.010$ p = 0.921	$F_{1, 28} = 0.039$ p = 0.845	$F_{1, 28} = 0.172$ p = 0.682
	Grin2a	32	$F_{1, 28} = 0.200$ p = 0.658	$F_{1, 28} = 1.348$ p = 0.255	$F_{1, 28} = 0.077$ p = 0.783
	Grin2b	32	$F_{1, 28} = 1.567$ p = 0.221	$F_{1, 28} = 0.001$ p = 0.973	$F_{1, 28} = 0.434$ p = 0.516
	Grm2	32	$F_{1, 28} = 0.439$ p = 0.513	$F_{1, 28} = 0.928$ p = 0.343	$F_{1, 28} = 0.062$ p = 0.805
	Grm3	32	$F_{1, 28} = 0.012$ p = 0.911	$F_{1, 28} = 0.406$ p = 0.529	$F_{1, 28} = 0.786$ p = 0.383
	Grm5	32	$F_{1, 28} = 3.764$ p = 0.063	$F_{1, 28} = 2.298$ p = 0.141	$F_{1, 28} = 1.017$ p = 0.322
	Htr1a	32	$F_{1, 28} = 1.339$ p = 0.257	$F_{1, 28} = 3.505$ p = 0.072	$F_{1, 28} = 0.177$ p = 0.678
	Htr2a	32	$F_{1, 28} = 0.005$ p = 0.942	$F_{1, 28} = 0.040$ p = 0.844	$F_{1, 28} = 1.719$ p = 0.201
	Htr2b	26	$F_{1, 22} = 2.124$ p = 0.159	$F_{1, 22} = 1.577$ p = 0.222	$F_{1, 22} = 1.334$ p = 0.261

	Htr3a	32	$F_{1, 28} = 0.484$ $p = 0.492$	$F_{1, 28} = 3.476$ $p = 0.073$	$F_{1, 28} = 0.637$ $p = 0.432$
	Npy	32	$F_{1, 28} = 0.001$ $p = 0.970$	$F_{1, 28} = 0.037$ $p = 0.849$	$F_{1, 28} = 0.778$ $p = 0.385$
	Maoa	31	$F_{1, 27} = 0.263$ $p = 0.612$	$F_{1, 27} = 0.483$ $p = 0.493$	$F_{1, 27} = 3.117$ $p = 0.089$
	Maob	32	$F_{1, 28} = 3.812 \times 10^{-5}$ $p = 0.995$	$F_{1, 28} = 0.148$ $p = 0.703$	$F_{1, 28} = 0.924$ $p = 0.345$
	Slc1a2	32	$F_{1, 28} = 0.021$ $p = 0.885$	$F_{1, 28} = 1.374$ $p = 0.350$	$F_{1, 28} = 0.902$ $p = 0.350$
	Slc6a3	24	$F_{1, 20} = 0.383$ $p = 0.543$	$F_{1, 20} = 3.661$ $p = 0.070$	$F_{1, 20} = 3.568 \times 10^{-8}$ $p = 0.999$
	Slc6a4	32	$F_{1, 28} = 0.330$ $p = 0.570$	$F_{1, 28} = 0.022$ $p = 0.884$	$F_{1, 28} = 0.367$ $p = 0.550$
	Th	32	$F_{1, 28} = 2.016$ $p = 0.167$	$F_{1, 28} = 1.813$ $p = 0.189$	$F_{1, 28} = 0.805$ $p = 0.377$
	Tph2	32	$F_{1, 28} = 2.02 \times 10^{-5}$ $p = 0.996$	$F_{1, 28} = 2.390$ $p = 0.133$	$F_{1, 28} = 0.301$ $p = 0.588$
OFC	*Adh1*	34	Kruskal-Wallis test: $p = 0.967$		
	Cat	34	$F_{1, 30} = 0.187$ $p = 0.669$	$F_{1, 30} = 3.842$ $p = 0.059$	$F_{1, 30} = 1.268$ $p = 0.269$
	Comt	34	$F_{1, 30} = 0.0004$ $p = 0.984$	$F_{1, 30} = 0.771$ $p = 0.387$	$F_{1, 30} = 3.034$ $p = 0.092$
	Drd1	34	$F_{1, 30} = 0.023$ $p = 0.880$	$F_{1, 30} = 3.916$ $p = 0.057$	$F_{1, 30} = 0.081$ $p = 0.777$
	Drd2	32	$F_{1, 28} = 0.249$ $p = 0.622$	$F_{1, 28} = 0.352$ $p = 0.558$	$F_{1, 28} = 0.169$ $p = 0.684$
	Gabbr1	34	$F_{1, 30} = 0.180$ $p = 0.674$	$F_{1, 30} = 0.284$ $p = 0.598$	$F_{1, 30} = 1.273$ $p = 0.268$
	Gabbr2	34	$F_{1, 30} = 0.003$ $p = 0.954$	$F_{1, 30} = 2.157$ $p = 0.152$	$F_{1, 30} = 0.079$ $p = 0.780$
	Gabra1	34	$F_{1, 30} = 0.523$ $p = 0.475$	$F_{1, 30} = 0.006$ $p = 0.938$	$F_{1, 30} = 1.166$ $p = 0.289$
	Gad1	34	$F_{1, 30} = 0.275$ $p = 0.604$	$F_{1, 30} = 2.158$ $p = 0.152$	$F_{1, 30} = 0.171$ $p = 0.683$
	Gad2	34	$F_{1, 30} = 6.23 \times 10^{-6}$ $p = 0.998$	**$F_{1, 30} = 4.390$** **$p = 0.045$ ***	$F_{1, 30} = 0.337$ $p = 0.566$
	Gria1	34	$F_{1, 30} = 0.236$ $p = 0.631$	$F_{1, 30} = 0.191$ $p = 0.665$	**$F_{1, 30} = 6.268$** **$p = 0.018$ ***
	Grin2a	34	**$F_{1, 30} = 4.629$** **$p = 0.040$ ***	$F_{1, 30} = 0.492$ $p = 0.488$	$F_{1, 30} = 0.036$ $p = 0.851$
	Grin2b	34	$F_{1, 30} = 0.032$ $p = 0.860$	$F_{1, 30} = 0.898$ $p = 0.351$	$F_{1, 30} = 0.881$ $p = 0.356$
	Grm2	34	$F_{1, 30} = 0.427$ $p = 0.518$	$F_{1, 30} = 0.006$ $p = 0.937$	$F_{1, 30} = 0.948$ $p = 0.338$
	Grm3	34	$F_{1, 30} = 0.236$ $p = 0.631$	$F_{1, 30} = 0.014$ $p = 0.907$	$F_{1, 30} = 2.046$ $p = 0.163$
	Grm5	34	$F_{1, 30} = 1.221$ $p = 0.278$	$F_{1, 30} = 1.933$ $p = 0.175$	$F_{1, 30} = 0.023$ $p = 0.882$

	Htr1a	34	$F_{1, 30} = 0.689$ $p = 0.413$	**$F_{1, 30} = 8.506$** **$p = 0.007$ ****	$F_{1, 30} = 0.467$ $p = 0.450$
	Htr2a	34	$F_{1, 30} = 0.080$ $p = 0.780$	$F_{1, 30} = 1.440$ $p = 0.240$	$F_{1, 30} = 0.825$ $p = 0.371$
	Htr2b	29	$F_{1, 25} = 0.102$ $p = 0.753$	$F_{1, 25} = 4.067$ $p = 0.055$	$F_{1, 25} = 0.804$ $p = 0.378$
	Htr3a	34	$F_{1, 30} = 3.393$ $p = 0.057$	$F_{1, 30} = 2.905$ $p = 0.099$	**$F_{1, 30} = 6.514$** **$p = 0.016$ ***
	Npy	34	Kruskal-Wallis test: p = 0.898		
	Maoa	33	$F_{1, 29} = 2.643$ $p = 0.115$	$F_{1, 29} = 3.321$ $p = 0.079$	**$F_{1, 29} = 4.734$** **$p = 0.038$ ***
	Maob	34	$F_{1, 30} = 0.756$ $p = 0.392$	$F_{1, 30} = 0.157$ $p = 0.695$	$F_{1, 30} = 1.749$ $p = 0.196$
	Slc1a2	34	$F_{1, 30} = 0.682$ $p = 0.415$	$F_{1, 30} = 0.568$ $p = 0.457$	$F_{1, 30} = 0.009$ $p = 0.926$
	Slc6a3	33	$F_{1, 29} = 0.747$ $p = 0.395$	$F_{1, 29} = 0.099$ $p = 0.755$	$F_{1, 29} = 1.854$ $p = 0.184$
	Slc6a4	34	$F_{1, 30} = 0.968$ $p = 0.333$	$F_{1, 30} = 2.197$ $p = 0.149$	$F_{1, 30} = 0.841$ $p = 0.367$
	Th	34	$F_{1, 30} = 0.741$ $p = 0.396$	$F_{1, 30} = 0.823$ $p = 0.372$	$F_{1, 30} = 3.450$ $p = 0.073$
	Tph2	34	$F_{1, 30} = 0.244$ $p = 0.625$	$F_{1, 30} = 0.014$ $p = 0.906$	$F_{1, 30} = 0.071$ $p = 0.792$
Nacc	***Adh1***	31	$F_{1, 27} = 1.126$ $p = 0.298$	**$F_{1, 27} = 9.895$** **$p = 0.004$ ****	$F_{1, 27} = 1.793$ $p = 0.192$
	Cat	34	$F_{1, 30} = 0.409$ $p = 0.528$	$F_{1, 30} = 0.044$ $p = 0.835$	$F_{1, 30} = 0.257$ $p = 0.616$
	Comt	34	$F_{1, 30} = 1.301$ $p = 0.263$	$F_{1, 30} = 1.301$ $p = 0.263$	$F_{1, 30} = 2.589$ $p = 0.118$
	Drd1	34	$F_{1, 30} = 0.017$ $p = 0.898$	$F_{1, 30} = 0.011$ $p = 0.916$	$F_{1, 30} = 1.127$ $p = 0.297$
	Drd2	34	$F_{1, 30} = 0.839$ $p = 0.832$	$F_{1, 30} = 0.297$ $p = 0.590$	$F_{1, 30} = 0.478$ $p = 0.495$
	Gabbr1	34	$F_{1, 30} = 0.080$ $p = 0.780$	$F_{1, 30} = 0.002$ $p = 0.968$	$F_{1, 30} = 0.474$ $p = 0.497$
	Gabbr2	34	Kruskal-Wallis test: p = 0.800		
	Gabra1	34	$F_{1, 30} = 0.051$ $p = 0.822$	$F_{1, 30} = 0.152$ $p = 0.699$	$F_{1, 30} = 0.018$ $p = 0.895$
	Gad1	34	$F_{1, 30} = 0.088$ $p = 0.769$	$F_{1, 30} = 0.303$ $p = 0.586$	$F_{1, 30} = 0.475$ $p = 0.496$
	Gad2	34	$F_{1, 30} = 0.277$ $p = 0.603$	$F_{1, 30} = 1.223$ $p = 0.278$	$F_{1, 30} = 1.425$ $p = 0.242$
	Gria1	34	$F_{1, 30} = 0.245$ $p = 0.624$	$F_{1, 30} = 0.064$ $p = 0.802$	$F_{1, 30} = 0.004$ $p = 0.948$
	Grin2a	34	$F_{1, 30} = 0.012$ $p = 0.913$	$F_{1, 30} = 0.042$ $p = 0.840$	$F_{1, 30} = 3.122$ $p = 0.087$
	Grin2b	34	$F_{1, 30} = 0.130$ $p = 0.721$	$F_{1, 30} = 0.612$ $p = 0.440$	$F_{1, 30} = 0.450$ $p = 0.508$
	Grm2	32	$F_{1, 28} = 1.289$ $p = 0.266$	$F_{1, 28} = 0.167$ $p = 0.686$	$F_{1, 28} = 0.012$ $p = 0.915$
	Grm3	34	$F_{1, 30} = 0.481$	$F_{1, 30} = 0.001$	$F_{1, 30} = 0.344$

			p = 0.494	p = 0.977	p = 0.562
	Grm5	34	$F_{1, 30}$ = 0.018 p = 0.893	$F_{1, 30}$ = 0.447 p = 0.509	$F_{1, 30}$ = 0.098 p = 0.756
	Htr1a	34	$F_{1, 30}$ = 0.040 p = 0.843	$F_{1, 30}$ = 0.315 p = 0.579	$F_{1, 30}$ = 2.250 p = 0.144
	Htr2a	34	$F_{1, 30}$ = 0.981 p = 0.330	$F_{1, 30}$ = 1.415 p = 0.244	$F_{1, 30}$ = 0.009 p = 0.923
	Htr2b	23	$F_{1, 19}$ = 0.011 p = 0.918	$F_{1, 19}$ = 0.693 p = 0.416	$F_{1, 19}$ = 3.959 p = 0.061
	Htr3a	34	$F_{1, 30}$ = 2.149 p = 0.153	$F_{1, 30}$ = 0.051 p = 0.823	$F_{1, 30}$ = 0.029 p = 0.866
	Npy	34	$F_{1, 30}$ = 0.460 p = 0.503	$F_{1, 30}$ = 0.051 p = 0.823	$F_{1, 30}$ = 0.097 p = 0.758
	Maoa	34	$F_{1, 30}$ = 0.243 p = 0.626	$F_{1, 30}$ = 0.594 p = 0.447	$F_{1, 30}$ = 1.694 p = 0.203
	Maob	34	$F_{1, 30}$ = 1.089 p = 0.305	$F_{1, 30}$ = 0.756 p = 0.392	$F_{1, 30}$ = 0.554 p = 0.462
	Slc1a2	34	$F_{1, 30}$ = 0.707 p = 0.407	$F_{1, 30}$ = 0.046 p = 0.832	$F_{1, 30}$ = 1.002 p = 0.325
	Slc6a3	34	$F_{1, 30}$ = 0.143 p = 0.708	$F_{1, 30}$ = 3.698 p = 0.064	$F_{1, 30}$ = 1.647 p = 0.209
	Slc6a4	33	$F_{1, 29}$ = 0.027 p = 0.872	**$F_{1, 29}$ = 5.258 p = 0.029 ***	$F_{1, 29}$ = 2.349 p = 0.136
	Th	33	$F_{1, 29}$ = 0.895 p = 0.352	$F_{1, 29}$ = 0.699 p = 0.410	$F_{1, 29}$ = 3.685 p = 0.065
	Tph2	34	$F_{1, 30}$ = 3.384 p = 0.076	$F_{1, 30}$ = 0.004 p = 0.952	$F_{1, 30}$ = 0.002 p = 0.963

Table S2. The effects of trait sensitivity to NF and alcohol drinking on protein level.

Statistically significant effects and interactions are bolded (2-way ANOVA).

Structure	Protein	*n*	*Interaction*	*Treatment*	*Sensitivity*
ACC	SERT	34	$F_{1, 30}$ = 0.992 p = 0.327	$F_{1, 30}$ = 0.009 p = 0.925	$F_{1, 30}$ = 0.424 p = 0.520
mPFC	**ADH1**	34	$F_{1, 30}$ = 0.136 p = 0.715	**$F_{1, 30}$ = 13.28 p = 0.001 ****	$F_{1, 30}$ = 0.043 p = 0.836
	MAO-A	33	**$F_{1, 29}$ = 8.168 p = 0.008 ****	$F_{1, 29}$ = 1.135 p = 0.296	$F_{1, 29}$ = 1.532 p = 0.226
OFC	MAO-A	34	$F_{1, 30}$ = 0.344 p = 0.562	$F_{1, 30}$ = 0.787 p = 0.382	$F_{1, 30}$ = 0.251 p = 0.620
	5-HT3A	33	$F_{1, 29}$ = 0.343 p = 0.563	$F_{1, 29}$ = 2.915 p = 0.099	$F_{1, 29}$ = 0.055 p = 0.816
Nacc	**ADH1**	32	$F_{1, 28}$ = 1.022 p = 0.321	**$F_{1, 28}$ = 10.38 p = 0.003 ****	$F_{1, 28}$ = 1.304 p = 0.263
	SERT	28	$F_{1, 24}$ = 0. 086 p = 0.772	$F_{1, 24}$ = 3.485 p = 0.074	$F_{1, 24}$ = 0.004 p = 0.951

Original Western blot images used for quantification of protein levels.

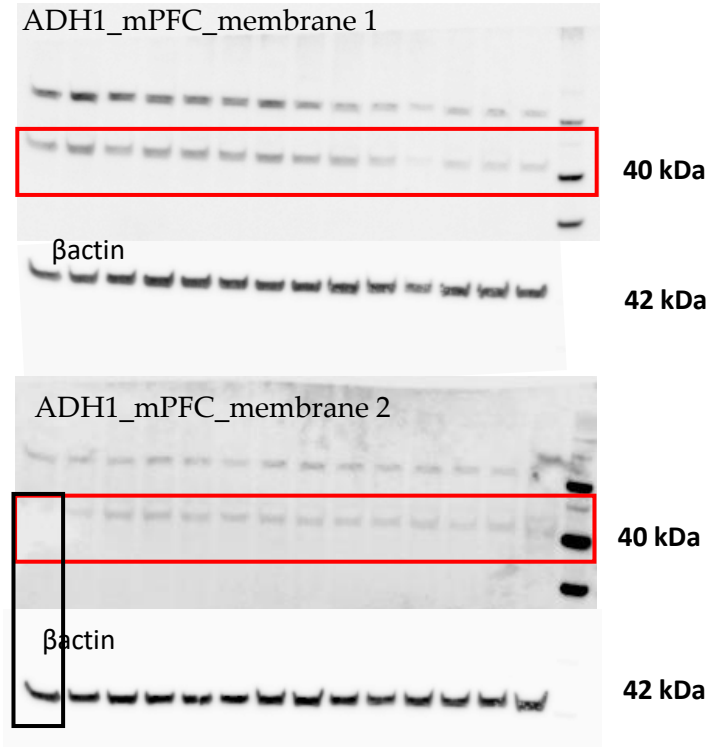
Some protein bands were unsuitable for quantification due to technical errors and these are indicated by black frames

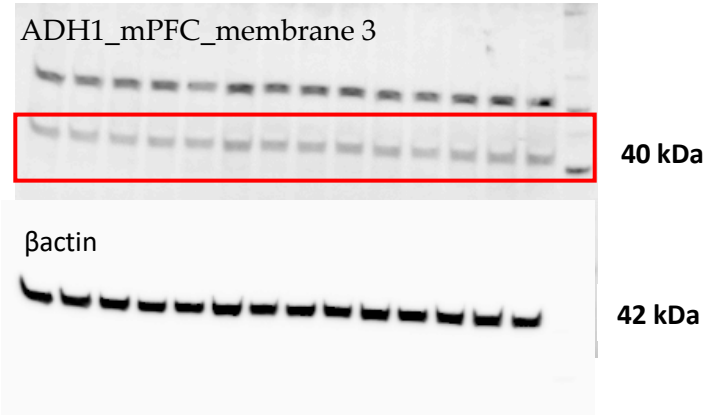
	EtOH							**H_2O**						
Membrane 1	NFL	NFL	NFL	NFL	NFM	NFM	NFM	NFM	NFL	NFL	NFL	NFM	NFM	NFM
Rat's number	3	7	1	2	17	4	12	18	13	15	20	10	25	26
Membrane 2	NFL	NFL	NFL	NFL	NFM	NFM	NFM	NFM	NFM	NFM	NFM	NFL	NFL	NFL
Rat's number	2	5	9	29	17	19	16	22	28	36	30	24	38	39
Membrane 3	NFL	NFL	NFM	NFM	NFM	NFL	NFL	NFL	NFL	NFL	NFM	NFM	NFM	NFM
Rat's number	33	35	21	12	19	7	3	6	11	30	27	31	14	10

NFL – less sensitive to negative feedback

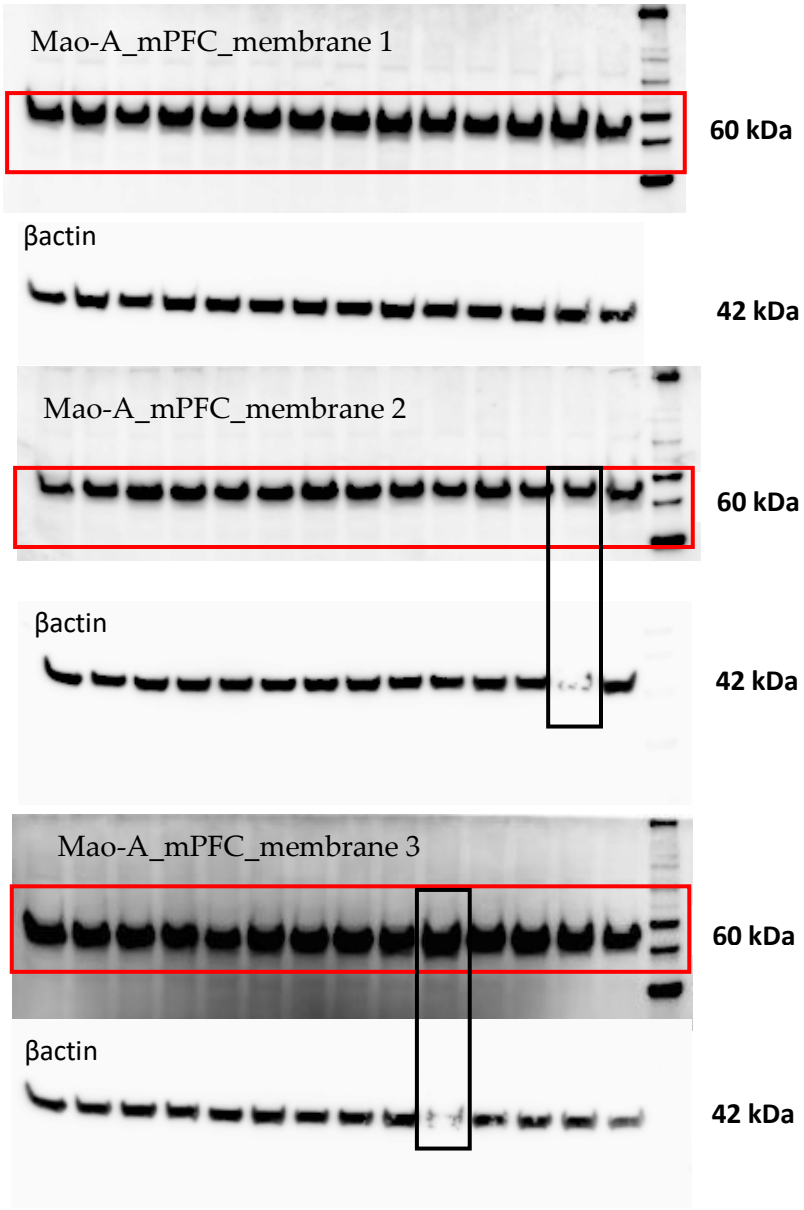
NFM – more sensitive to negative feedback

ADH1_mPFC


ADH1_mPFC_membrane 1
40 kDa
βactin
42 kDa
ADH1_mPFC_membrane 2
40 kDa
βactin
42 kDa

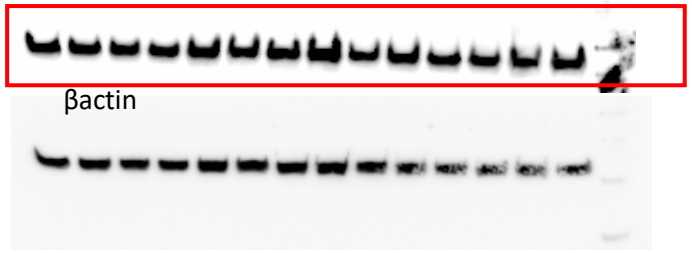

ADH1_mPFC_membrane 3
40 kDa
βactin
42 kDa

Mao-A_mPFC


Mao-A_mPFC_membrane 1
60 kDa
βactin
42 kDa
Mao-A_mPFC_membrane 2
60 kDa
βactin
42 kDa
Mao-A_mPFC_membrane 3
60 kDa
βactin
42 kDa

Mao-A_OFC

Mao-A_OFC_membrane 1



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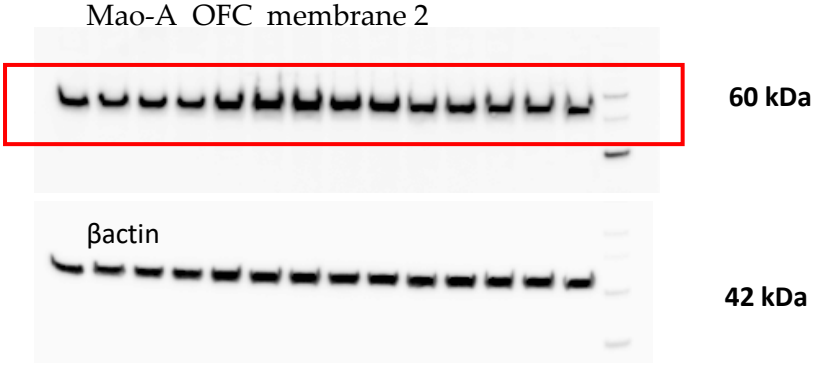

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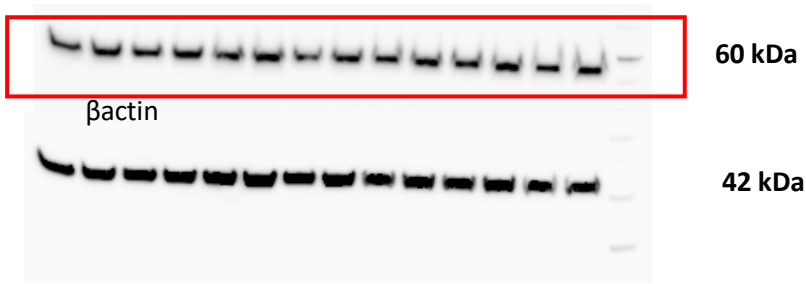

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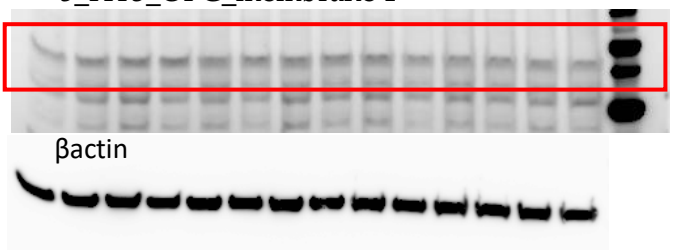

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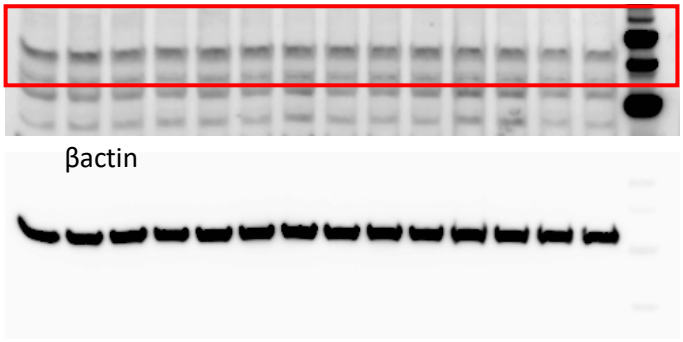

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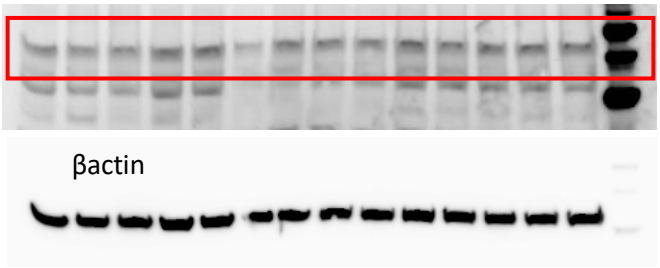
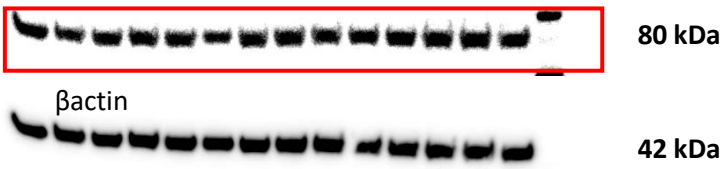


SERT_ACC

SERT_ACC_membrane 1



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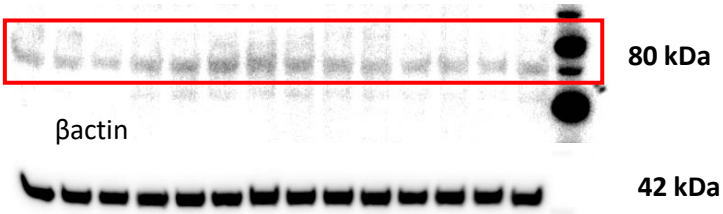

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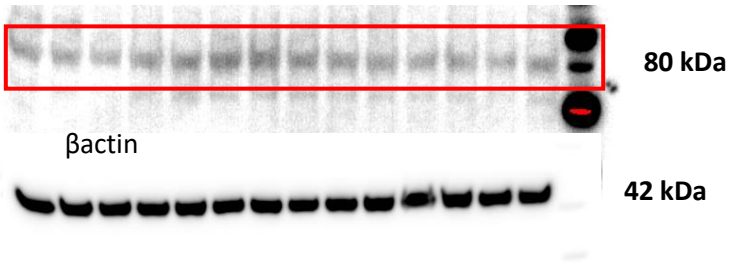

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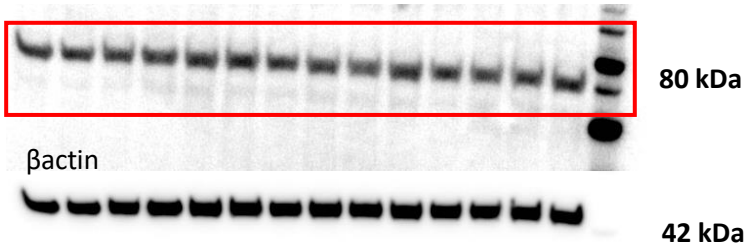

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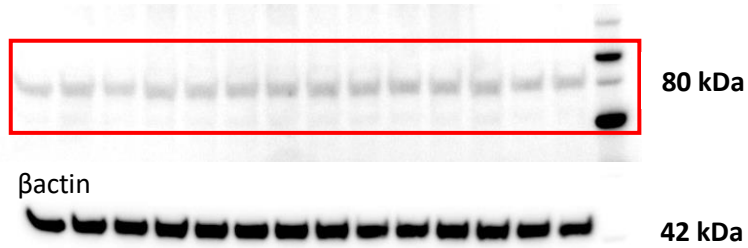

ADH1_Nacc

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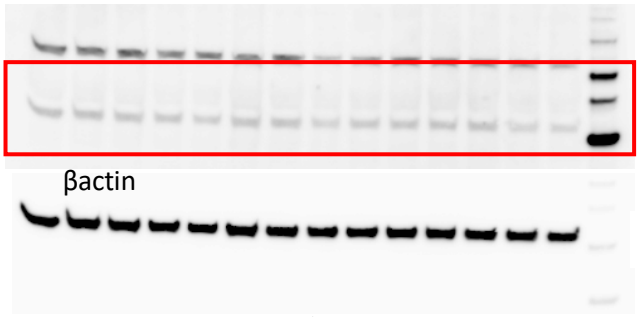

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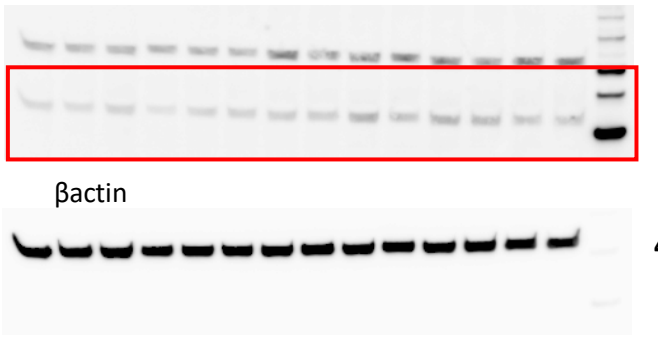

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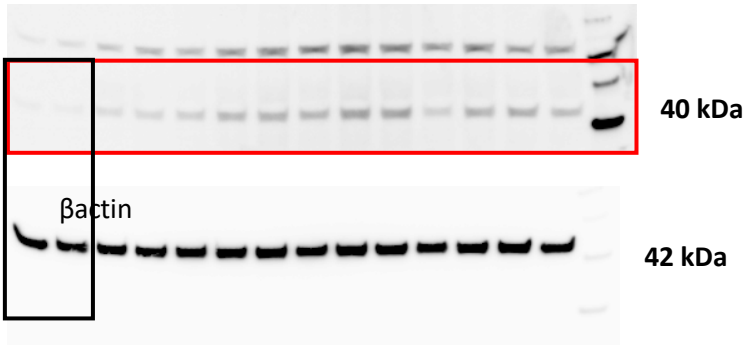

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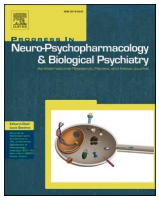

Unveiling the power of optimism: Exploring behavioral and neuromolecular correlates of alcohol seeking and drinking in rats with biased judgement

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A B S T R A C T

Alcohol use disorder (AUD) is a common psychiatric condition with substantial global mortality. Despite extensive research into its pathophysiology, the cognitive predispositions driving alcohol dependence are less understood. This study explores whether biased cognition, specifically traits of optimism and pessimism, predicts susceptibility to alcohol-seeking behaviors using an animal model.

Rats were initially tested for judgement bias through Ambiguous Cue Interpretation tests. Those identified as 'optimistic' or 'pessimistic' were further examined for their tendency to escalate alcohol intake using the intermittent access 2-bottle choice (2BC) paradigm. Additionally, we assessed how judgement bias influenced the development of compulsive alcohol-seeking behavior in a Seeking-Taking (ST) and Seeking-Taking Punishment tasks, alcohol-seeking motivation in the Progressive Ratio Schedule of Reinforcement paradigm, the speed of extinction, and reinstatement after abstinence. Neurochemical analyses were conducted to investigate trait-specific differences in neurotransmitter-related gene expression and receptor densities in the brain. We used TaqMan Gene Expression Array Cards to analyze expression levels of genes linked to serotonergic, dopaminergic, glutamatergic, and GABAergic pathways, and alcohol metabolism in various brain regions. Receptor densities for 5-HT_{1A}, 5-HT_{2A}, and D_2 were measured using autoradiography analysis.

Behaviorally, 'optimistic' rats showed significantly lower alcohol consumption in the 2BC paradigm compared to 'pessimistic' rats. This lowered intake correlated with decreased monoamine oxidase-A (*Maoa*) expression in the medial prefrontal cortex (mPFC) and increased metabotropic glutamate receptor 2 (*Grm2*) expression in the amygdala (Amy). Additionally, we observed significant interactions between judgement bias and alcohol intake in the expression of several genes in the mPFC, nucleus accumbens (Nacc), orbitofrontal cortex (OFC), and Amy, as well as in 5-HT_{2A} receptor binding in the Nacc.

Overall, these results suggest that optimism is linked to lower alcohol consumption and related neurochemical changes, indicating a potential cognitive mechanism in AUD risk.

1. Introduction

Oscar Wilde once observed, 'We are all in the gutter, but some of us are looking at the stars'. This insightful metaphor not only captures the essence of human resilience and our varied responses to life's challenges but also beautifully illustrates the concept of optimism. The construct of optimism has been defined as a general expectation that good things will happen in the future, coupled with a positively biased judgement that interprets events and outcomes in a favorable light (Carver et al., 2010). It involves a tendency to focus on the most hopeful aspects of a situation, maintaining a belief that one can influence positive outcomes even in challenging circumstances (Carver et al., 2010). This positively biased perspective can significantly enhance emotional well-being, promote effective coping strategies, and foster resilience, enabling individuals to navigate life's obstacles with a constructive and hopeful outlook (Carver and Scheier, 2014; Scheier and Carver, 2018). The current paper explores the intriguing possibility that trait optimism—defined as a positive judgement bias—may play an important role in resilience to the development of compulsive alcohol-seeking and consumption behaviors, core symptoms of the alcohol use disorder (AUD, (Everitt and Robbins, 2016; Koob and Volkow, 2010)). By investigating the psychological and neurobiological mechanisms through which cognitive judgement bias could potentially act as a buffer against the development AUD we aim to contribute to a broader understanding of preventive strategies in the realm of substance abuse.

The important role of optimistically/pessimistically biased

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judgement in the etiology and maintenance of various psychiatric disorders (Alloy and Ahrens, 1987; Hart et al., 2008; Hirsch et al., 2007; Strunk et al., 2006), including AUD has long been hypothesized (Noworyta et al., 2022). Moreover, antidepressant treatment has been shown to effectively modulate negative cognitive bias (Chamberlain et al., 2006), suggesting it may also be beneficial for patients with AUD. Judgement bias influences how individuals perceive and respond to risks and rewards, potentially skewing their decision-making processes towards the short-term gratification provided by alcohol, despite long-term negative consequences. Indeed, individuals exhibiting an optimism bias may underestimate the risks associated with excessive drinking while overestimating their ability to control their drinking habits (Blume et al., 2003; Fromme and D'Amico, 2000). On the other hand, negative judgement biases, such as a heightened focus on stressors or negative emotions, can drive the maintenance of alcohol use as a coping mechanism (Veilleux et al., 2014). Therefore, understanding these cognitive biases may provide important insights into why some individuals develop and sustain AUD, offering potential targets for cognitive-behavioral interventions aimed at correcting maladaptive thought patterns and promoting recovery.

Animal studies provide further evidence about a role of negatively/positively biased judgement in psychiatric disorders. Recent studies from our laboratory revealed that, in rats, the vector of bias in judgement predicts the vulnerability of animals to stress-induced anhedonia (Rygula et al., 2013) and stress-induced motivational deficits (Drozd et al., 2017). Other studies using rats have shown that a bias in judgement is associated with differences in motivation to gain a reward in progressive ratio schedule of reinforcement task (Rygula et al., 2015), sensitivity to negative feedback (Rygula and Popik, 2016), risk-taking behavior (Drozd et al., 2016), and immunological profile (Curzytek et al., 2018) – all of which could be associated with various aspects of AUD.

Preclinical measurement of cognitive judgement bias in rats can be achieved through the Ambiguous cue interpretation (ACI) test. In this paradigm, rats are trained to press one lever in an operant chamber to receive a food reward when a specific tone is played and to press another lever in response to a different tone to avoid punishment by a mild electric foot shock. The tones acquire positive and negative valences, and the training continues until the rats achieve a stable, correct discrimination ratio. Once stable discrimination performance is attained, the animals are ready for testing. Ambiguous cue interpretation includes a discrimination task, as previously described, with the addition of tone(s) that have a frequency intermediate between the positive and negative tones. The pattern of lever press responses to this ambiguous cue serves as an indicator of the rats' expectation of a positive or negative event, representing 'optimism' or 'pessimism', respectively.

In the current study, we employed the ambiguous cue interpretation paradigm to assess the cognitive judgement bias in an animal model. Through a series of ambiguous cue interpretation tests, we identified two groups of rats that exhibited significant differences in their cognitive judgement bias index, classifying them as exhibiting stable 'optimistic' or 'pessimistic' traits. To investigate the role of judgement bias in predisposing individuals to develop an alcohol-dependent-like state, we utilized a mixed regimen of intermittent free access and instrumental alcohol exposure. An initial increase in ethanol consumption was induced using intermittent access two-bottle choice (2BC) sessions (Cieslik et al., 2022). Subsequent phases were dedicated to evaluating hallmark symptoms of human AUD, including compulsive alcohol seeking despite potential punishment, the motivation to seek alcohol, the extinction of alcohol seeking, and the reinstatement of this behavior after a period of enforced abstinence (Giuliano et al., 2018).

In our study, we chose specific biochemical markers associated with serotonergic, dopaminergic, glutamatergic and GABAergic pathways as these neurotransmitters play key roles in the regulation of mood, reward, impulsivity and inhibitory control that are relevant in the context of alcohol dependence (Uhl et al., 2019). Alterations in the transmission of these neurotransmitters have been linked to addiction and relapse behavior, making them critical to understanding the mechanisms underlying AUD (Koob and Volkow, 2010). The research presented in this paper aims to illuminate the pathways through which optimism and pessimism may influence alcohol consumption, enhancing our comprehension of AUD and providing novel insights to inform more effective prevention and treatment strategies.

2. Experimental procedures

2.1. Ethics statement

All experiments were conducted following the European Union guidelines for the care and use of laboratory animals (2010/63/EU). Experimental protocols were reviewed and approved by the 2nd Local Institutional Animal Care and Use Committee, Institute of Pharmacology Polish Academy of Sciences in Krakow (Agreement: No. 230/2019, dated 10.10.2019). The authors declare that every effort has been made to minimize the animals' suffering and the number of animals used.

2.2. Subjects and housing

This study involved 96 male Sprague Dawley rats (175–200 g upon arrival, Charles River, Germany) housed in groups of 4 in an enriched environment (wooden blocks 9.5 × 4 × 4 cm and 25 cm long plastic pipes). They were kept in a temperature and humidity-controlled room (21 ± 1 °C, 30–50% humidity) with a 12/12-h light/dark cycle (lights on at 07:00 AM). Throughout the experiment, rats were mildly food-restricted to 85% of their free-feeding weight, receiving 15 g of food pellet per rat per day (according to the normal growth curve recommended by the laboratory rodent supplier). Food restriction was introduced 7 days before the first ACI training. Water was available ad libitum, and all behavioral procedures were carried out during the light phase.

Of the 96 rats, only 87 finished the ambiguous cue interpretation testing described below. They were then screened for judgement bias and categorized as 'optimistic' ($N = 43$) or 'pessimistic' ($N = 44$). Animals were also divided according to treatment into EtOH ($N = 50$) and control ($N = 37$) groups, resulting in four experimental groups: optimistic control ($N = 18$), optimistic EtOH ($N = 25$), pessimistic control ($N = 19$), and pessimistic EtOH (N = 25). For analysis, we selected the upper and lower quartiles of the judgement bias index from 69 rats that completed the experiment (32 EtOH and 37 control), resulting in four groups: optimistic$_{control}$ ($N = 8$), optimistic$_{EtOH}$ ($N = 9$), pessimistic$_{control}$ ($N = 13$), and pessimistic$_{EtOH}$ (N = 5).

2.3. Experimental apparatus

The behavioral tests were conducted in computer-controlled operant conditioning chambers (Med Associates, St Albans, Vermont, USA), enclosed within sound-attenuating boxes. Each chamber was equipped with a fan, house light, speaker, and a fluid dispenser (set to deliver 0.01 ml of a 5% sucrose solution (during ACI testing and screening) or 0.1 ml of 10% ethanol (EtOH, during instrumental testing with alcohol reinforcement)), a grid floor through which scrambled electric shocks could be administered, and 2 retractable levers positioned at the sides of the fluid dispenser. Tests measuring alcohol-seeking and taking behaviors were executed in the same operant chambers, except that the levers were relocated to the wall opposite the liquid dispenser. This adjustment aimed to create a novel experimental environment to avoid interference with the habits acquired by the animals during ambiguous cue interpretation training and screening.

2.4. Experimental schedule

The full series of experiments is schematically summarized in Fig. 1.

2.5. Ambiguous cue interpretation test

This experimental stage consisted of the following phases: positive tone training, negative tone training, discrimination training and ambiguous cue interpretation testing.

During positive tone training, sessions lasted 30 min, with a 10-s intertrial interval (ITI). Rats initially associated the tone (50 s, 2000 Hz at 75 dB or 9000 Hz at 75 dB, counterbalanced) with a 5% sucrose reward (0.01 ml). In the subsequent step, each trial started with the extension of the left lever. Pressing the lever triggered the tone signal and the delivery of the sucrose solution. Training continued until stable performance (≥80 responses over 3 sessions) was achieved. In the final step, each trial began with the tone presentation, followed by a lever extension. Pressing the lever during this phase triggered reward delivery and the termination of the tone. This 'positive tone' acquired positive valence due to its association with palatable reward, and the associated lever was termed the 'positive lever'. Animals progressed to negative tone training after achieving ≥70% responses to 45 tone presentations over 3 sessions.

During negative tone training, sessions lasted 30 min with 30 tone presentations and 10-s ITIs. Each trial began with a tone (9000 Hz at 75 dB or 2000 Hz at 75 dB, counterbalances) and right lever extension. The negative tone signaled electric shocks (0.20, 0.30. 0.40, 0.50 mA), terminated by lever press. Training continued until stable performance (≥70% responses at each shock intensity). In the next stage, rats were trained to press the lever to avoid an electric shock (0.50 mA, 10 s). Pressing before shock onset ended the tone. Pressing after shock onset terminated both tone and shock. No response was counted as an omission. Each trial was followed by a 10-s ITI. Due to its association with a concomitant punishment, this tone acquired a negative valence and was referred to as the 'negative tone' and the associated lever was termed the 'negative lever'. After achieving ≥70% prevention responses over 3 sessions, animals proceeded to discrimination training.

During the discrimination training stage, rats were trained to distinguish between positive and negative tones by responding to the appropriate levers. 20 positive and 20 negative tones were presented in pseudo-randomized order with 10-s ITIs. Pressing the positive lever during a positive tone led to immediate reward delivery while pressing the negative lever during a negative tone terminated the tone. Incorrect presses or omissions were considered failed trials. Animals progressed to the ambiguous cue interpretation test after achieving ≥70% correct responses with each lever in 3 consecutive sessions.

In each testing session, 20 positive, 20 negative, and 10 ambiguous tones (5000 Hz at 75 dB) were presented in a pseudo-randomized order with 10 s ITI. If any lever was pressed during the ambiguous tone, the tone terminated without consequences. No response within 50 s was considered an omission. Responses to ambiguous tones were analyzed for the proportion of overall responses to that tone. The judgement bias index was computed by subtracting the proportion of negative responses from the proportion of positive responses, resulting in values between −1 and 1. Values above 0 indicated an overall positive judgement or 'optimistic' interpretation, while values below 0 indicated a negative judgement or 'pessimistic' interpretation of the ambiguous cue.

2.6. Judgement bias screening and cohort division

To assess cognitive judgement bias as a stable trait, rats underwent 10 consecutive ambiguous cue interpretation tests, conducted at 3-day intervals and alternated with discrimination tests. Rats were categorized as 'optimistic' or 'pessimistic' based on their average judgement bias index score across the 10 tests, with 'optimistic' rats having an average score above 0, and 'pessimistic' rats having an average score below 0. Each group was further divided into ethanol- (EtOH) and water-drinking (control) groups.

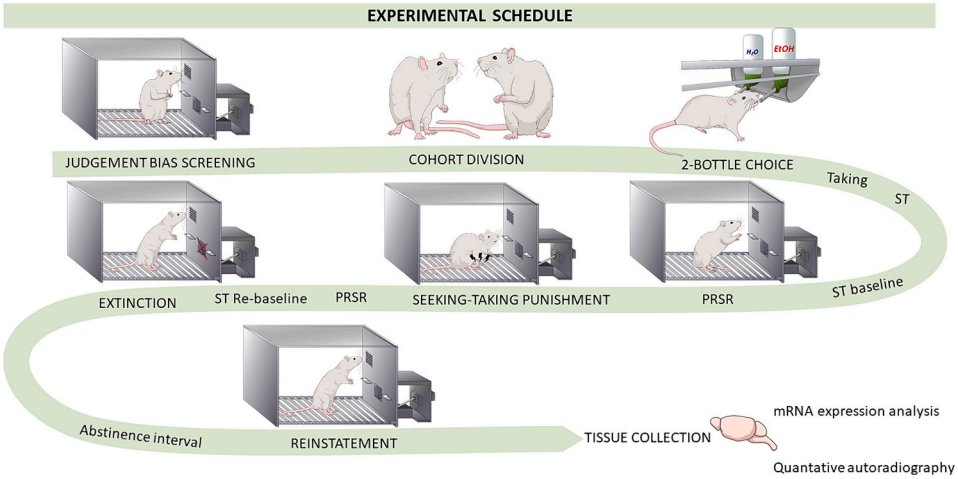

Fig. 1. The schedule of performed experiments. To determine the effects of optimism and pessimism as stable behavioral traits on the susceptibility to transition from controlled use to alcohol abuse, a cohort of rats was subjected to ACI training. This involved associating positive and negative tones with reward and punishment, respectively. Subsequently, the rats learned to differentiate between positive and negative tones by responding to corresponding levers. During judgement bias screening, an ambiguous tone was introduced to identify which rats displayed optimistic and pessimistic interpretations of the ambiguous cue. Based on this screening, each rat was categorized as either optimistic or pessimistic. The cohort was further divided into alcohol (EtOH) and water (control) drinking groups. To induce alcohol consumption and track its progression, the rats underwent testing in the 2-bottle choice (2BC) intermittent access paradigm. Following this, the rats' motivation to drink alcohol was measured using the progressive ratio schedule of reinforcement (PRSR) paradigm after initial training in the Seeking-Taking (ST) task. In the next steps, the rats were subjected to 3 Seeking-Taking baseline tests and the influence of the judgement bias on alcohol-seeking behavior was measured in the instrumental Seeking-Taking Punishment task, following which the animals' motivation to drink alcohol was evaluated again using PRSR. Then, alcohol-seeking behavior was again assessed with 3 Seeking-Taking tests. Following this re-baseline, the effects of trait optimism/pessimism on alcohol-seeking behavior were evaluated following the termination of alcohol availability (extinction phase) and following 1 month of abstinence (reinstatement). Following the re-baseline, the impact of trait optimism/pessimism on alcohol-seeking behavior was assessed during the extinction phase, following the termination of alcohol availability, and after 1 month of abstinence (reinstatement). At the end of the experiment, the animals were sacrificed, and the effects of prolonged alcohol consumption on gene expression and receptor density were examined in rats exhibiting optimistic and pessimistic judgement bias.

2.7. Induction and assessment of alcohol drinking behavior

The first stage of measuring alcohol-related behaviors aimed to induce and assess the differences in spontaneous alcohol consumption between 'optimistic' and 'pessimistic' rats. For this, all animals underwent 12 sessions of the 2BC procedure, conducted for 24 h every other day, with alternating 24-h periods without alcohol. During the 24 h test, animals were housed individually, with access to one bottle of 10% ethanol (*w*/w) and one bottle of water for the EtOH group, and 2 bottles of water for the control group. To prevent side preferences, the position of the bottles was switched every 12 h. The bottles were weighed before and after each session to calculate the total fluid intake (g/24 h) and specifically alcohol consumption for the EtOH group (g EtOH/kg of body weight (b.w.)/24 h). The volume of liquid consumed was determined by the weight difference of the bottles at the start and end of each session, with adjustments made for any dripping in an empty cage.

2.8. Development of alcohol-seeking behavior

Rats were trained to associate pressing a lever with receiving 0.1 ml of 15% EtOH (EtOH group) or water (control group) under a fixed-ratio 1 (FR1) schedule of reinforcement. Each trial began with the extension of a randomly assigned taking lever (left/right counterbalanced), accompanied by a house light. No response within 10 s was considered an omission. Following each trial, there was a 10-s ITI with the lever retracted. Rats were limited to a maximum of 60 rewards during a 30-min session. After achieving the performance criterion of at least 20 taking responses in 3 consecutive sessions, the rats transitioned to the Seeking-Taking task.

During the Seeking-Taking task, trials began with the insertion of the seeking lever opposite the taking lever. A seeking lever response triggered the taking lever extension after a random interval of 1 to 15 s (RI 1–15 s). Pressing the taking lever under FR1 resulted in the delivery of 0.1 ml of 15% EtOH (*w*/w), followed by the retraction of both levers. Each trial was followed by a 10-s ITI with levers retracted. Rats were limited to a maximum of 100 rewards in a 45-min session. After achieving the performance criterion of a minimum of 20 taking responses in 3 consecutive sessions, the animals progressed to the progressive ratio schedule of reinforcement task to assess their motivation for alcohol.

In this task, each trial started with the seeking lever extension. Pressing it led to the taking lever extension after a RI 1–15 s. The number of seeking lever presses required to produce this effect increased exponentially with each successive taking lever response and EtOH delivery, as determined by the following equation: response ratio $= \left(5 \times e^{(0.2 \times \text{taking lever response number})}\right) - 5$, rounded to the nearest integer (Roberts and Bennett, 1993; Rygula et al., 2015). Thus, the values of the steps were 1, 2, 4, 6, 9, 12, 15, 20, 25, 32 etc. Each trial was followed by a 10-s ITI with both levers retracted. Sessions lasted 30 min. The breakpoint, indicating motivation, was the maximum number of seeking lever presses a rat was willing to exert.

Subsequently, rats underwent three baseline Seeking-Taking tests and the persistence of seeking behavior in the face of aversive consequences was measured using the Seeking-Taking Punishment (STP) task. Each trial in this paradigm began with the insertion of the seeking lever, pressing on which lead to either a 1-s electric shock through a grid floor or the extension of the taking lever after RI 1–15 s. Sessions were capped at 25 trials, with 17 reinforced by EtOH delivery and 8 punished with foot shock. Shock intensity increased daily (0.10, 0.20, 0.30, 0.40, 0.50, 0.50, 0.50 mA). Although punishment occurred randomly in each session, never >2 consecutive trials resulted in a foot shock, and the first trial was always reinforced. Upon completion of the Seeking-Taking Punishment task, rats were re-challenged in the progressive ratio schedule of reinforcement test and re-baselined in 5 Seeking-Taking test sessions.

After the re-baseline procedure, all animals underwent daily 15-min extinction sessions, during which the seeking lever response under RI 1–15 s resulted in taking lever extension, however, the taking lever presses had no programmed consequences and alcohol was not available. After reaching the extinction criterion (<5 seeking responses in 3 consecutive sessions), the rats were alcohol deprived and not tested for the following 30 days.

After the thirty days of abstinence, the rats underwent a series of Seeking-Taking tests to measure how quickly they reinstated their alcohol-seeking behavior and brought their performance up to the basal level. Testing continued until reaching a criterion of an average number of seeking responses from five tests equal to or higher than the average number of seeking responses from the 5 re-baseline Seeking-Taking tests.

2.9. Tissue collection

The day after the final reinstatement test, rats were euthanized by decapitation in a counterbalanced manner (i.e. a rat from the EtOH group together with a control rat from the same judgement bias group). The procedure was conducted from 09:00 to 12:00.

To avoid potential disruptions in the analysis of receptor binding related to lateralization, randomly right brain hemispheres were collected from some animals, while left brain hemispheres were collected from others (counterbalanced between groups). The collected brain hemispheres were snap-frozen in heptane with dry ice and stored at −70 °C until autoradiographic analysis was conducted. Next the brain hemispheres were sectioned into appropriate slices according to the rat brain atlas (Paxinos and Watson, 2006) using a Leica cryostat. Five brain structures designated for gene expression analysis by RT-PCR were isolated from the remaining hemispheres: 3 cortical (mPFC, anterior cingulate (Acc), and OFC) and 2 subcortical areas (Nacc and amygdala). Tissue was taken based on the 'Rat brain atlas' of Paxinos and Watson (Paxinos and Watson, 2006), and according to Achterberg (Achterberg et al., 2015). The structures were frozen on dry ice and stored at −70 °C for further analysis.

2.10. Autoradiography

Autoradiography of dopamine D_2 receptors was performed using [^{3}H]Methylspiperone with a specific activity of 80 Ci/mmol (Perkin Elmer, USA). Tissue sections were preincubated in a 50 mM Tris-HCl buffer (pH 7.4) for 15 min. The radioligand binding at a dissociation constant (Kd) of 0.3 nM, was conducted in incubation buffer (IB1) containing 50 mM Tris-HCl, 5 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 μM ketanserin (Sigma, Inc.) to block 5-HT_{2A} receptors, pH 7.4, for 60 min at room temperature. Non-specific binding was determined simultaneously using IB1 supplemented with 10 μM (+) butaclamol (Sigma, Inc.). The incubation was terminated by two washes in 50 mM Tris-HCl (pH 7.4) at 4 °C for 5 min, followed by a brief rinse with ice-cold distilled water.

For the analysis of serotonin 5-HT_{2A} receptors, [^{3}H]ketanserin was used, with a specific activity of 47.3 Ci/mmol (Perkin Elmer, USA) and a Kd of 2 nM. Tissue sections were preincubated for 15 min in a buffer (IB2) containing 50 mM Tris-HCl, 120 mM NaCl, and 4 mM $CaCl_2$. Receptor binding analysis was then performed in IB2 with the appropriate concentration of radioligand for 60 min at room temperature. Non-specific binding was assessed using IB2 supplemented with an additional 10 μM mianserin (Tocris, Inc.). The process was completed with 2 washes in 50 mM Tris–HCl (pH 7.4) at 4 °C for 10 min, followed by a rinse with ice-cold distilled water.

For the serotonin 5-HT_{1A} receptors, tissue sections were preincubated in a 50 mM Tris-HCl buffer for 30 min at room temperature. They were then incubated in IB3 buffer, which contains 50 mM Tris-HCl, 4 mM $CaCl_2$, 0.1% ascorbic acid, and [^{3}H]8-OH-DPAT (specific activity: 250 Ci/mmol; Perkin Elmer, USA; Kd: 2 nM), for 60 min at the

same temperature. Non-specific binding was determined using IB3 supplemented with 10 μM serotonin (Sigma, Inc.). The incubation was completed with three washes in ice-cold 50 mM Tris-HCl for 5 min each, followed by immersion in cold water. The labelled tissue slices were placed against an imaging plate (Fujifilm, Japan) with autoradiographic microscales (GE Healthcare) for seven days. The autoradiograms were analyzed and quantified using ImageGauge software (Fujifilm, Japan). The specific binding of the radioligand to dopamine D_2, 5-HT_{1A}, and 5-HT_{2A} receptors was quantified by subtracting the images representing non-specific binding in adjacent brain slices from the total binding signal. The results are expressed as femtomoles of bound radioligand per milligram of tissue (fmol/mg tissue) in each examined brain structure.

2.11. Gene expression analysis

This analysis assessed the interaction between the effects of the traits 'optimism' and 'pessimism' and the effects of alcohol consumption on gene expression in specific brain regions using TaqMan Low-Density Arrays. The arrays screened 30 genes—28 candidates and 2 references. An extensive literature review facilitated the selection of 5 groups of genes possibly associated with the modulation of the relationship between 'optimism'/'pessimism' and alcohol effects. The first group included genes involved in the functioning and regulation of the serotonin system. The second group consisted of genes involved in dopaminergic neurotransmission. The third group comprised genes related to the glutamatergic system. The fourth group consisted of genes associated with γ-aminobutyric acid (GABA) neurotransmission. The fifth group included genes implicated in ethanol (EtOH) metabolism. Additionally, ribosomal protein L32 (*Rpl32*) and peptidylprolyl isomerase A (*Ppia*) were used as reference genes.

The total RNA was isolated from collected tissues using the RNeasy Plus Mini Kit (Qiagen, Germantown, MD, US) according to the manual's instructions. The samples (8–11 per group) were homogenized with 600 μl of the buffer RTL Plus with β-mercaptoethanol for 4 min at 50 Hz with TissueLyser LT (Qiagen, Germantown, MD, US). Then the gDNA Eliminator spin columns were used. The 600 μl of 70% ethanol was added to each sample and transferred to the RNeasy spin column. After washing the column, 30 μl of RNase-free water was added to the column for the RNA elution. The quality and quantity of the isolated total RNA were evaluated by a NanoDrop ND-1000 (Thermo Fisher Scientific) and Experion microcapillary electrophoresis system (Bio-Rad, Hercules, California, US). Samples that passed the quality threshold (RIN > 8.0) were used for further experiments.

Isolated RNAs were reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific), with RNA quantities normalized across samples based on the specific brain structure. The resulting cDNA was then mixed with TaqMan Universal PCR Master Mix, No AmpErase UNG (Thermo Fisher Scientific), to perform the RT-qPCR reactions on custom TaqMan Low-Density Arrays which included the 28 selected genes. One Array Card was used to examine the mRNA expression of 4 samples in triplicate. The RT-qPCR was conducted on a QuantStudio 12 K Flex System (Applied Biosystems, Waltham, Massachusetts, US), with data analysis performed using the QuantStudio 12 K Flex Software (Applied Biosystems). Ct values exceeding 34 were classified as representing undetectable mRNA levels. For comparison purposes, a consistent threshold of 0.2 was applied to all samples. The analysis was executed with qBasePLUS 3.1 software (Biogazelle, Zwijnaarde, Belgium) (Hellemans et al., 2007), with normalization against Ribosomal protein L32 (Rpl32) and peptidylprolyl isomerase A (Ppia).

2.12. Statistics

The statistical analysis was conducted using GraphPad Prism (version 10.1.0, GraphPad Software, Inc., San Diego, CA). The Shapiro-Wilk test confirmed the normality of data distribution. For nonparametric datasets, normalization was achieved through square root transformation and exclusion of outliers. Behavioral and molecular data were examined using two-way ANOVA. For the judgement bias screening, 2 BCE, and STP, the within-subject factor was 'test day/session' and the between-subject factor was 'judgement bias'. For the gene expression and autoradiography data, the between-subject factors were 'judgement bias' and 'treatment'. *t*-tests and Mann-Whitney *U* tests (for nonparametric data) compared the 'optimistic' and 'pessimistic' groups regarding average consumption in 2 BCE and the number of sessions required for extinction and reinstatement criteria. Pairwise comparisons were adjusted for multiple testing using Sidak correction. Significance tests were performed at an alpha level of 0.05. Variance homogeneity was verified using Levene's test, and sphericity for repeated-measures analyses was confirmed with Mauchly's test. Data are presented as mean ± SEM or, for nonparametric data, as median with interquartile range.

3. Results

3.1. Cohort division

During the discrimination training phase, nine rats failed to show progress and were subsequently excluded from further analysis. The remaining 87 animals met the criteria and qualified for the judgement bias screening. Based on the average judgement bias index from 10 screening tests, the animals were separated into two groups: 'optimistic' ($N = 43$) and 'pessimistic' ($N = 44$). The cohort was also unequally divided based on the applied treatment into EtOH ($N = 50$) and control ($N = 37$) groups. These divisions resulted in 4 experimental groups: $optimistic_{control}$ ($N = 18$), $optimistic_{EtOH}$ ($N = 25$), $pessimistic_{control}$ ($N = 19$) and $pessimistic_{EtOH}$ ($N = 25$). This asymmetry was dictated by our previous experiments in which approximately 25% of rats receiving alcohol showed no increase in consumption throughout the experiments (Cieslik et al., 2022). Indeed, in the present study, only 32 of 50 rats from the EtOH group completed all of the experimental stages measuring alcohol-seeking behavior.

To highlight changes resulting from differences in the judgement bias index, from among the 69 rats that completed the experiment (32 EtOH and 37 control animals), we distinguished 2 groups of animals whose scores were in the upper and lower quartiles of judgement bias index scores which resulted in four experimental groups: $optimistic_{control}$ ($N = 8$), $optimistic_{EtOH}$ ($N = 9$), $pessimistic_{control}$ ($N = 13$) and $pessimistic_{EtOH}$ ($N = 5$). All analyses described below concern these 4 groups of animals.

3.2. Ambiguous cue interpretation training

The number of tests performed to achieve criterion in each phase of the training is presented in the supplementary table S1. There were no differences between 'optimistic' and 'pessimistic' rats in number of tests to achieve the positive tone ($U = 122.5$, $p = 0.24$), negative tone ($U = 148.5$, $p = 0.89$) and discrimination training ($t(33) = 0.31$, $p = 0.76$) criteria.

3.3. Judgement bias screening

Following the cognitive judgement bias screening, the animals with an average judgement bias index above 0 were classified as 'optimistic', while those with an average below 0 were classified as 'pessimistic'. Screening results for the entire cohort are presented in the supplementary Fig. S1. To emphasize the often subtle changes resulting from differences in the level of 'optimism', in this study, we selected 2 groups of animals which were distinct in the interpretation of ambiguous cues over time and were classified as permanently 'optimistic' (top quartile of the judgement bias index scores) and permanently 'pessimistic' (lower quartile of the judgement bias index scores).

Although the cognitive bias index fluctuated in both groups of animals (significant main effect of screening day ($F(6.818, 225.0) = 2.14$, $p = 0.04$)), the differences between 'optimistic' and 'pessimistic' groups did not significantly change across the screening period (no significant screening day × judgement bias interaction ($F(9, 297) = 0.88$, $p = 0.54$)) indicating stability of the traits (Fig. 2A). For animals classified as 'optimistic' ($N = 17$) an average judgement bias index equaled 0.36 ± 0.03, whereas the judgement bias index in the 'pessimistic' group ($N = 18$) was −0.41 ± 0.03 (Fig. 2B).

Compared with their 'pessimistic' counterparts, 'optimistic' animals responded significantly more often to the positive lever (significant judgement bias × tone interaction ($F(2,99) = 24.98$, $p < 0.05$; Fig. 2C))

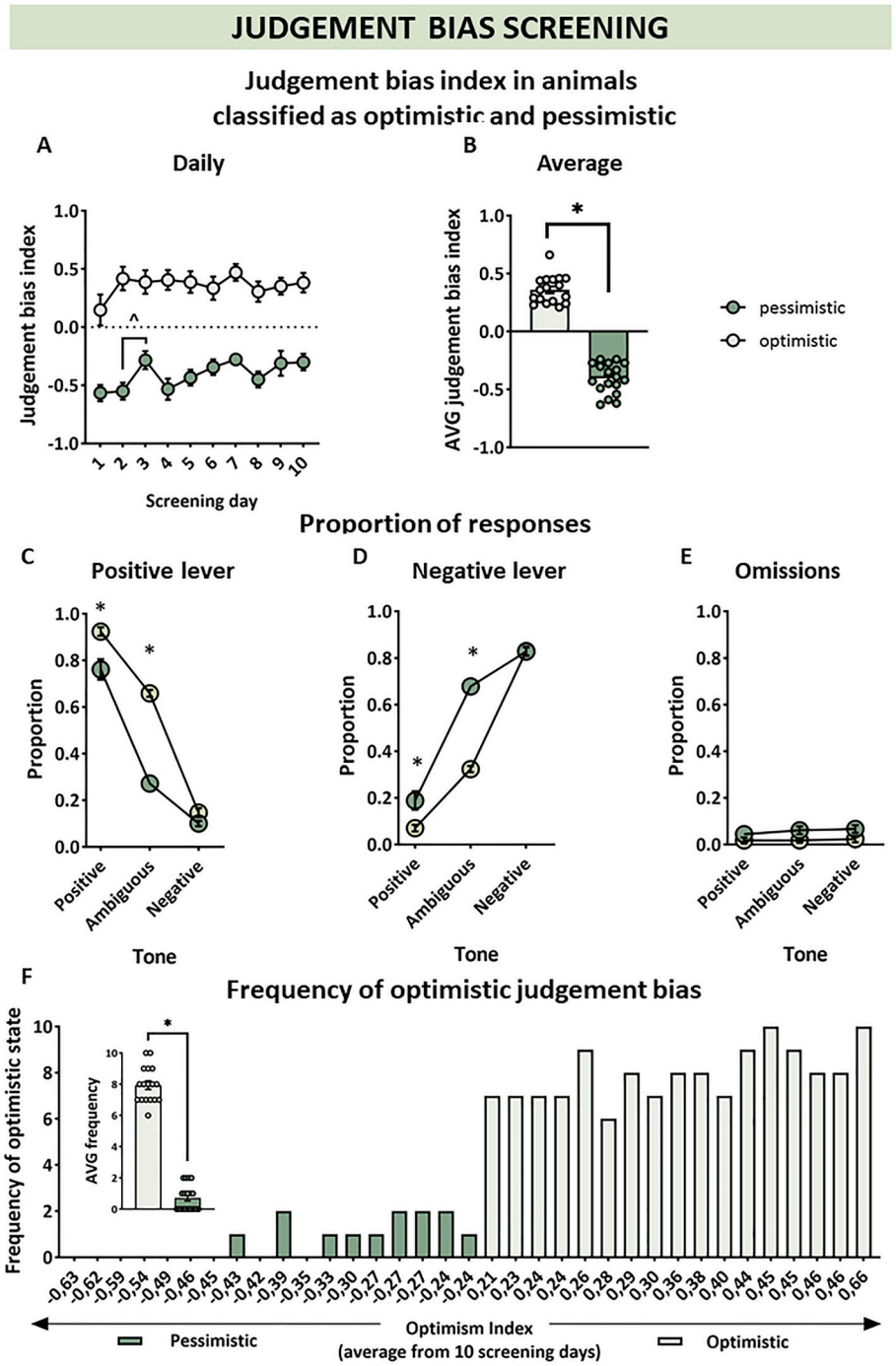

Fig. 2. Cognitive bias as a stable and long-lasting behavioral trait. Daily (A) and average (B) judgement bias index of the animals classified as 'pessimistic' (dark green bars and circles) and 'optimistic' (light green bars and circles), based on 10 ACI screening tests. A judgement bias index below 0 indicates an overall negative judgement and a pessimistic interpretation of the ambiguous cue. The mean ± SEM proportions of positive (C), negative (D) and omitted (E) responses to the trained and ambiguous tones in the 'pessimistic' (dark green circles) and 'optimistic' groups. Average frequency of pessimistic judgement bias (F), expressed as the number of ACI tests (out of the 10 comprising screening) in which an animal displayed a value of judgement bias index below 0. Average frequency of optimistic judgement bias (F,Inset) in animals classified as 'optimistic' and 'pessimistic'. Data are presented as a mean ± SEM. * indicates significant ($p < 0.05$) differences between the 'optimistic' and 'pessimistic' groups.^ indicates significant ($p < 0.05$) difference between screening days. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and less often to the negative lever in response to the ambiguous tone (significant judgement bias × tone interaction ($F(2,99) = 32.76$, $p < 0.05$; Fig. 2D)). The 'optimistic' animals also responded less often to the negative lever in response to the positive tone, compared to the 'pessimistic" group ($p < 0.05$, Fig. 2D). 'Optimistic' and 'pessimistic' rats did not differ in the number of omissions made (non-significant judgement bias × tone interaction ($F(2,99) = 1.38$, $p = 0.26$, Fig. 2E)).

The average frequency of 'optimistic' judgement bias in the group of animals classified as 'optimistic' was significantly higher ($p < 0.05$) than that in the 'pessimistic' group (Mann-Whitney $U = 0$; Fig. 2F and inset).

3.4. Induction and assessment of alcohol drinking behavior

During 12 two-bottle choice sessions, rats from the 'optimistic' group consumed significantly less alcohol than their 'pessimistic' conspecifics (significant judgement bias effect ($F(1,12) = 5.93$, $p = 0.03$), Fig. 3A). The raw consumption data are included in the supplementary Fig. S2.

Moreover, EtOH rats consumed significantly more fluids than the control group ($U = 32$, $p < 0.0001$, Fig. 3B). There was no significant difference in water consumption between 'optimistic' and 'pessimistic' rats from the control group ($U = 49$, $p = 0.86$, Fig. 3C).

There were no differences in the initial weight of the animals before the first two-bottle choice session, with an average of 405.4 ± 7.9. The initial weights in four experimental groups were as follows: $optimistic_{control} = 402.5 \pm 8.8$, $optimistic_{EtOH} = 409.6 \pm 21.8$, $pessimistic_{control} = 414.9 \pm 13.3$ and $pessimistic_{EtOH} = 378.0 \pm 14$.

3.5. Development of alcohol-seeking behavior

In the next step, the animals from the EtOH and control groups were trained to associate the pressing of the taking lever with the alcohol or water delivery under FR1. None of the rats from the control group met the criteria. After reaching the Seeking-Taking criterion, the rats from EtOH group were tested in the Seeking-Taking Punishment task. As the shock intensity increased from 0.10 to 0.50 mA during consecutive sessions, all rats gradually decreased the number of trials completed compared to the initial session (main shock intensity effect ($F(2.597, 31.17) = 18.12$, $p < 0.0001$)). However, there were no significant differences in the number of completed trials between 'optimistic' and 'pessimistic' rats (non-significant judgement bias effect ($F(1,12) = 0.01$, $p = 0.92$) and non-significant shock intensity x judgement bias interaction ($F(9, 108) = 0.56$, $p = 0.83$), Fig. 3E).

Additionally, to measure the impact of punishment in the Seeking-Taking Punishment task on rats' motivation for alcohol-seeking, we conducted 2 progressive ratio schedule of reinforcement tests. In the first one, executed before Seeking-Taking Punishment tests, the rats' average break point was 11.23 ± 1.03, while in the second one, performed the day after last Seeking-Taking Punishment test, the average break point was 4.46 ± 1.22. All rats' motivation significantly decreased following the experience of punishment in the Seeking-Taking Punishment task (significant test effect ($F(1,12) = 14.96$, $p = 0.002$)), however, there were no differences in the breaking point between 'optimistic' and 'pessimistic' rats (non-significant judgement bias effect ($F(1,12) = 0.003$, $p = 0.96$) and non-significant test x judgement bias interaction ($F(1,12) = 1.08$, $p = 0.32$), Fig. 3D).

After the second progressive ratio schedule of reinforcement test, all animals were re-baselined in 3 ST tests. Following the re-baseline, rats were tested under Seeking-Taking extinction conditions. The number of sessions needed to achieve the extinction criterion ranged from 5 to 16, with an average of 9.14 ± 0.94. Judgement bias had no significant impact on the length of extinction ($t(12) = 0.66$, $p = 0.52$, Fig. 3F).

The effects of judgement bias on the reinstatement of alcohol-seeking were assessed following 30 days of forced abstinence. Animals needed between 3 and 9 Seeking-Taking tests, with an average of 4.21 ± 0.56, to bring their alcohol-seeking responses to the basal, pre-extinction levels. However, 'optimistic' and 'pessimistic' rats did not significantly differ in

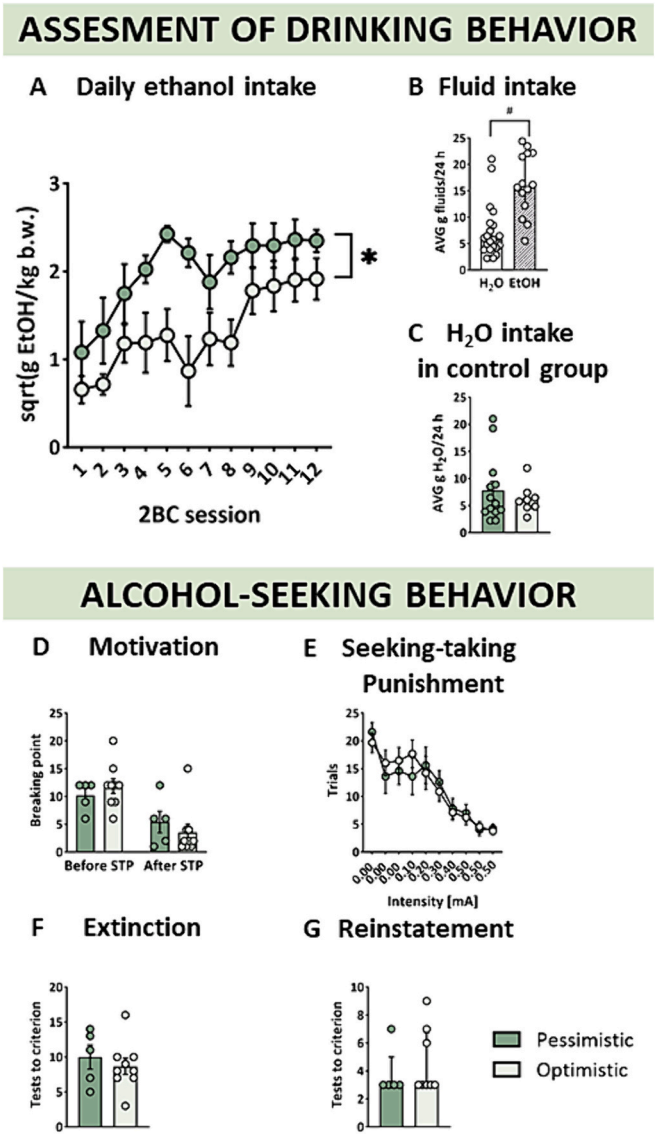

Fig. 3. Alcohol-related behaviors. Daily ethanol intake (A) during all 12 2BC sessions in 'pessimistic' (dark green circles) and 'optimistic' (light green circles) rats from the EtOH group; Average fluid intake (B) from all 12 2BC sessions in control (H2O; white bars) and alcohol-drinking group (EtOH; dashed bars) groups; Average H2O intake (C) from all 12 2BC sessions in 'optimistic' and 'pessimistic' rats from the control group; The effects of judgement bias on motivation (D) to seek alcohol. The breaking point in the Progressive Ratio Schedule of Reinforcement tests conducted before and after the Seeking-Taking Punishment sessions in rats classified as 'pessimistic' (dark green bars) and 'optimistic' (light green bars) from EtOH group; Daily number of trials (E) completed during Seeking-Taking Punishment sessions in 'optimistic' and 'pessimistic' rats from EtOH group; Average number of tests needed to achieve the extinction (F) criterion (<5 taking responses in three consecutive sessions) in optimistic and pessimistic rats from EtOH group; Average number of tests needed to reinstate (G) the alcohol-seeking behavior (average from 3 reinstatement test ≥ average from re-baseline) sessions in rats classified as 'optimistic' and 'pessimistic' from EtOH group. Data with normal distribution are presented as a mean ± SEM. Non-parametric data are presented as a median and interquartile range (G). * indicates a significant ($p < 0.05$) difference between 'optimistic' and 'pessimistic' groups. # indicates a significant ($p < 0.05$) difference between EtOH and H2O groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the number of tests needed to achieve criterion (U = 19.50, $p = 0.78$, Fig. 3G).

Operant, alcohol-related behavior expressed as the amount of alcohol consumed in g per kg of body weight in 'pessimistic' and 'optimistic' rats from the EtOH group is presented in supplementary Fig. S3.

3.6. The effects of 'optimism' and alcohol consumption on gene expression

3.6.1. Serotonergic system

Statistical analysis of the effects of trait 'optimism' on the expression of genes related to serotonergic neurotransmitter system revealed statistically significant interactions between the effects of judgement bias and alcohol drinking on the expression of *Slc6a4* in mPFC (F(1,24) = 11.82, $p = 0.002$; Fig. 4A) and Nacc (F(1,16) = 8.18, $p = 0.01$; Fig. 4A) and *Maoa* in OFC (F(1,31) = 4.45, $p = 0.04$; Fig. 4A). In mPFC, the level of *Slc6a4* expression, within the 'optimistic' rats, was lower in the EtOH group compared to the control. Moreover, in the EtOH group, 'optimistic' rats showed lower expression of *Slc6a4* compared to 'pessimistic' ones. Finally, in the control group, the expression of Slc6a4 was higher in the 'optimistic' rats. In Nacc, within the control group, 'optimistic' rats showed lower expression of *Slc6a4*. What is more, the level of expression was also higher in the 'pessimistic' control group, compared to 'pessimistic' EtOH rats.

The statistically significant effects of the judgement bias effect were revealed in the expression of *Maoa* in mPFC (F(1,30) = 4.45, $p = 0.04$; Fig. 4A) and *Htr2b* in OFC (F(1,17) = 7.45, $p = 0.01$; Fig. 4A), with the level of expression of *Htr2b* higher in 'optimistic' animals, compared to their 'pessimistic' conspecifics. Compared to 'pessimistic' rats, the expression of *Maoa* in mPFC was lower in 'optimistic' group.

3.6.2. Glutamatergic system

Statistical analysis revealed statistically significant judgement bias x treatment interactions in the expression of *Grm3* in amygdala (F(1,31) = 9.68, $p = 0.004$; Fig. 4B) and *Slc1a2* in Nacc (F(1,31) = 5.47, $p = 0.03$; Fig. 4B). In amygdala, in the EtOH group, the level of *Grm3* was higher in 'optimistic' rats than in the 'pessimistic' ones. In Nacc, the level of *Slc1a2* was significantly higher in the EtOH group than in the control one, within 'optimistic' rats.

The significant effects of prolonged alcohol drinking (main treatment effect) were revealed in the level of expression of *Grm2* in Acc (F(1,31) = 11.73, p = 0.002; Fig. 4B) and amygdala (F(1,31) = 7.16, $p = 0.01$; Fig. 4B), and the level of *Grin2b* in Nacc (F(1,29) = 4.50, $p = 0.04$; Fig. 4B), with the expression lower in EtOH group compared to control. Conversely, the EtOH group showed higher expression levels than the controls of *Grin2a* (F(1,31) = 7.70, $p = 0.009$; Fig. 4B) and *Slc1a2* (F(1,31) = 7.26, p = 0.01; Fig. 4B) in the mPFC.

The analysis also revealed statistically significant intergroup differences (main judgement bias effect) in the expression of *Grm2* (F(1,31) = 4.65, p = 0.04; Fig. 4B) and *Grm3* (F(1,31) = 7.04, p = 0.01; Fig. 4B) in amygdala, with the level of expression higher in 'optimistic' animals, compared to their 'pessimistic' conspecifics.

3.6.3. Dopaminergic system

Statistically significant differences between EtOH and control groups were observed in the expression of the *Drd2* gene in OFC, with the level of expression higher in EtOH rats (main effect of treatment: F(1,28) = 8.20, $p = 0.008$; Fig. 4C).

3.6.4. GABAergic system

Statistical analysis of the effects of trait 'optimism' on the expression of genes related to GABAergic neurotransmitter system revealed statistically significant interactions between the effects of judgement bias and alcohol drinking on the expression of *Gabbr2* in mPFC (F(1,31) = 4.72, p = 0.04), with the expression lower, within EtOH group, in the 'optimistic' animals, compared to the 'pessimistic' ones.

3.6.5. Alcohol metabolism

Finally, there was a significant judgement bias x treatment interaction in the expression of *Adh1* in mPFC (F(1,30) = 7.67, p = 0.01; Fig. 4E), with the expression lower in 'optimistic' rats, compared to the 'pessimistic' ones, in the control group. Moreover, within the 'pessimistic' animals, the level of expression was higher in controls, compared to the EtOH group.

3.7. Autoradiography

The autoradiographic analysis of serotonin 5-HT_{2A} receptors showed statistically significant interactions of judgement bias and prolonged alcohol consumption (treatment) in [^{3}H]ketanserin binding in Nacc core (F(1,29) = 6.43, $p = 0.02$; Fig. 4G) and shell (F(1,29) = 5.90, p = 0.02; Fig. 4H). In both structures, there was stronger 5-HT_{2A} receptor binding within the EtOH group in 'optimistic' rats, compared to the 'pessimistic' ones. The analysis also revealed an increase in 5-HT_{2A} binding in 'optimistic' EtOH animals – when compared to their 'pessimistic' conspecifics from the control group.

The autoradiographic analysis of dopamine D_2 receptors using [^{3}H] methylspiperone radioligand and of the 5-HT_{1A} receptor using [^{3}H]8-OH-DPAT did not reveal significant intergroup differences in any of the examined structures.

4. Discussion

Recent research has emphasized the importance of investigating human cognitive traits in animal models as a valuable method for identifying cognitive markers associated with various psychiatric disorders (Anderson et al., 2013; Hales et al., 2017; Rygula et al., 2014; Stracke et al., 2017). One such trait is the pessimistic or optimistic judgement bias. In humans, this trait may impair an individual's ability to accurately assess risks and benefits, leading to an overemphasis on the rewarding effects of alcohol and an underestimation of its negative consequences (Blume et al., 2003; Leeman et al., 2009). However, it remains unknown whether biased judgement measured as a stable and enduring cognitive trait, can determine the manner, in which different individuals consume alcohol, and thus their susceptibility to the transition from recreational to compulsive drinking. This uncertainty arises from the challenges of gathering data on past cognitive biases in patients diagnosed with AUD, and resulting practical impossibility of conducting longitudinal studies in humans. The present research utilizing an animal model, as presented in this paper, permits us to infer causative links between the cognitive judgement bias, measured as a stable cognitive trait, and factors related to the development of alcohol addiction. By employing sophisticated behavioral tests, we were able to ascertain the vector and magnitude of this trait in rats. Subsequently, using a comprehensive suite of behavioral assays, we evaluated how this trait influenced the rats' alcohol consumption patterns. Obtained results indicate that rats exhibiting trait 'optimism' drink significantly less alcohol than their 'pessimistic' counterparts. This lower alcohol consumption is evident solely in the 2BC paradigm and does not translate into significant differences in the development of compulsive alcohol-seeking behavior, increased motivation for drinking alcohol, speed of extinction of alcohol seeking behaviors, or difference in the reinstatement of alcohol-seeking after a period of abstinence. On the neuromolecular level, the decreased alcohol consumption observed in 'optimistic' animals is associated with a decreased level of *Maoa* expression in the mPFC and an increased level of *Grm2* expression in the amygdala. Significant interactions between judgement bias and alcohol intake are also observed in the expression of *Slc6a4*, *Gabbr2*, and *Adh1* in the mPFC; *Slc1a2* and *Slc6a4* in the Nacc; *Maoa* in the *OFC*; and *Grm3* in the amygdala. There are also notable interactions between the effects of judgement bias and alcohol consumption on 5-HT_{2A} receptor binding in the Nacc. Alcohol consumption is generally associated with changes in the expression of *Grm2* in the Acc and amygdala, Grin2a in the mPFC,

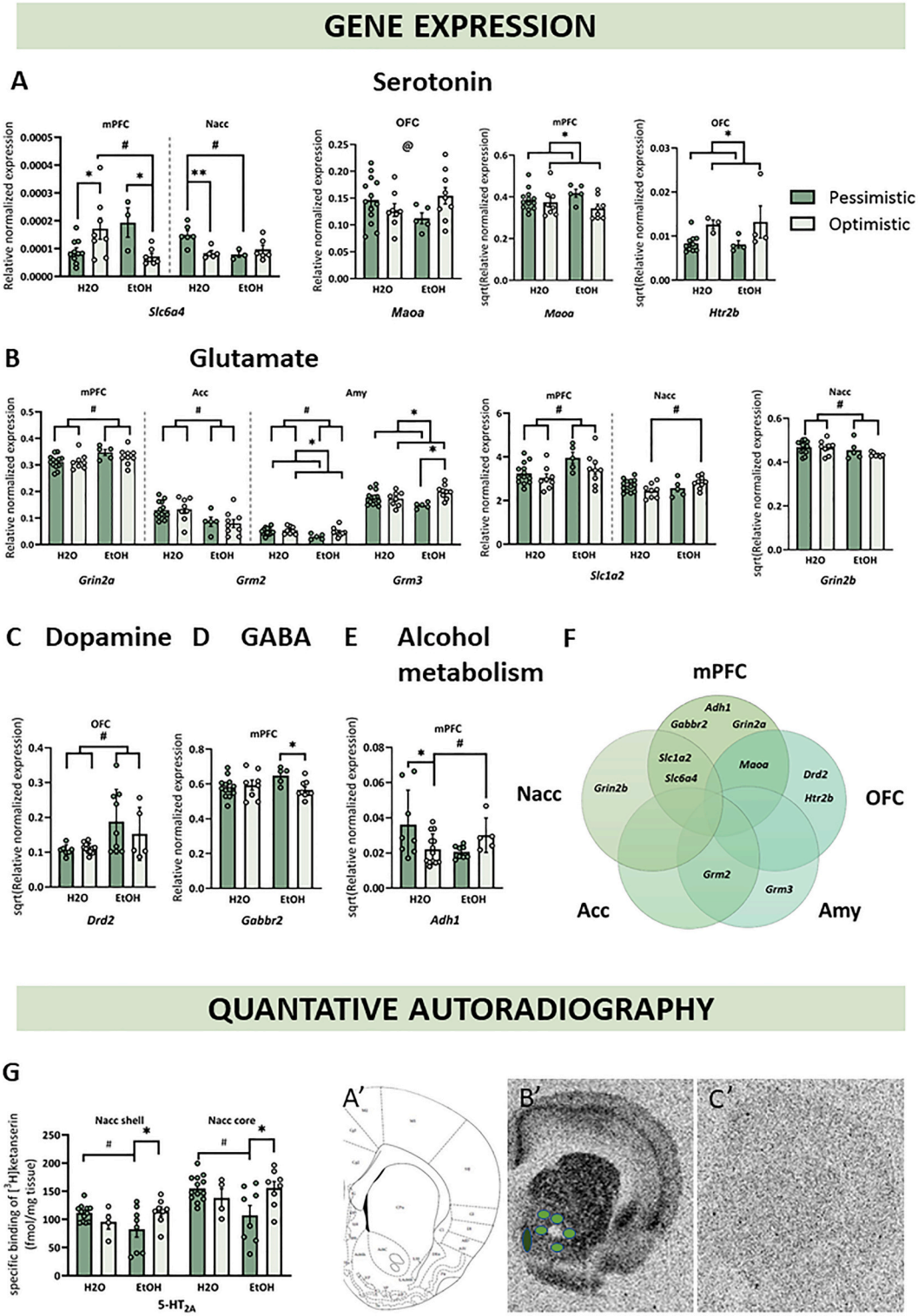

Fig. 4. Molecular differences associated with pessimistic and optimistic judgement bias and alcohol drinking in rats. Relative normalized expression in selected brain regions of genes (A-E) related to serotonergic system (A), glutamatergic system (B), dopaminergic system (C), GABAergic system (D) and alcohol metabolism (E) in 'pessimistic' (dark green bars) and 'optimistic' rats (light green bars) belonging to control (H2O) and EtOH groups. Venn diagram (F) illustrating genes altered by judgement bias and/or alcohol consumption (H2O, EtOH) in the selected brain structures (mPFC, OFC, Amy, Acc, Nacc). Differences in [3H]ketanserin binding in Nacc shell and core, and representative autoradiogram of [3H]ketanserin binding (G) to rat brain. Analyzed section (A') based on the Atlas of the Rat Brain (Paxinos and Watson, 2006) Example image (B') of total [3H]ketanserin binding to the rat brain, colors (bright green –Nacc core; dark green Nacc shell) indicate areas taken for analysis, on-specific binding of [3H]ketanserin (C'). * indicates a significant ($p < 0.05$) difference between optimistic and pessimistic groups. # indicates a significant ($p < 0.05$) difference between EtOH and H_2O groups. @ indicates a significant ($p < 0.05$) judgement bias x treatment interaction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Drd2 in the OFC, and *Grin2b* in the Nacc.

These results should be interpreted within a broader context, starting with the outcomes of our previous research, which demonstrated how reinforcement sensitivity predicts rats' susceptibility to various aspects of AUD that were also measured in the current experiments (Cieslik et al., 2022; Cieslik-Starkiewicz et al., 2024a; Cieslik-Starkiewicz et al., 2024b). In these studies, we have shown that trait sensitivity to negative feedback, which has been linked to a pessimistic judgement bias (Rygula and Popik, 2016), predicts rats' vulnerability to developing compulsive alcohol-seeking and consumption behaviors, particularly when these behaviors are punished (Cieslik et al., 2022). These studies also revealed significant differences in the propensity to extinguish alcohol-seeking behaviors after the cessation of alcohol availability between animals classified as less or more sensitive to negative feedback. Further studies from our laboratory have indicated that sensitivity to positive feedback, in contrast, determines the degree of motivation to seek alcohol following the experience of its adverse effects and the tendency to resume alcohol-seeking behaviors after a period of enforced abstinence (Cieslik-Starkiewicz et al., 2024b). The aforementioned effects were accompanied by differences in blood stress hormone levels, as well as differences in the cortical and subcortical expression of genes and proteins associated with dopaminergic, serotonergic, and GABAergic neurotransmitter systems (Cieslik-Starkiewicz et al., 2024a; Cieslik-Starkiewicz et al., 2024b). Taken collectively and in light of the current and previously mentioned results, it seems that various forms of biased cognition, previously defined as reinforcement-based cognitive biases (Noworyta et al., 2022), exert a comprehensive and complementary influence on, and modulate, animals' susceptibility to various facets of alcoholism.

Given that cognitive judgement bias reflects an individual's affective state, with those in a negative affective state displaying pessimism, and those in positive affective state tending towards optimism, the heightened alcohol consumption in 'pessimistic' rats, as observed in this study, could mirror one of the hypothesized human drinking patterns, specifically, 'drinking for relief' (Grodin et al., 2024). This pattern of consumption, is displayed by individuals who drink to mitigate negative emotions or to alleviate stress and could be parallel to the behavior of the 'pessimistic' rats who may also show an increased consumption of alcohol in an attempt to escape a negative affective state. Building on this premise, the lack of noticeable effects of judgement bias on the more effortful aspects of alcohol seeking and drinking can be attributed to the overriding impact of the mentioned negative affective state that likely resulted in motivational deficits among the rats. This would particularly affect their engagement in tasks that require higher levels of effort and active decision-making. Unfortunately, our experiment did not include direct measurements of motivational levels, which limits our ability to definitively pinpoint this as the underlying cause. However, it appears highly plausible that the effects of pessimism were predominantly observable during spontaneous drinking sessions because these sessions required less cognitive effort and decision-making from the rats. This scenario suggests that when the cognitive load is reduced, the rats' inherent pessimistic biases have a more pronounced influence on their drinking behavior.

Alcohol interacts with numerous neurotransmitter systems, disrupting brain function and creating an imbalance in the regulation of inhibitory and excitatory neurotransmitters (Koob, 1992). This altered neurotransmission is linked to the reinforcing effects of alcohol (Koob, 2004) and results in adaptive changes in neural circuits, ultimately leading to addiction (Koob, 1992; Koob and Volkow, 2010). Indeed, in the present study, multiple differences in gene expression associated with chronic alcohol exposure were confirmed in various brain regions of interest. Prolonged alcohol intake resulted in higher expression of genes related to the glutamatergic system, namely *Grin2a*, which encodes the 2 A subunit of glutamate ionotropic NMDA receptor and *Slc1a2*, which encodes solute carrier family 1 member 2 (EAAT2) in mPFC in EtOH group compared to control. The same pattern was observed for *Drd2*, which encodes dopamine receptor D_2, in OFC. The involvement of the above-mentioned genes in alcohol-related behaviors has been extensively discussed in the literature so far (Bice et al., 2008; Daut et al., 2015; Kalluri et al., 1998; McColl and Piquette-Miller, 2020; Sari, 2014). In contrast, expression was lower in the EtOH group, compared to the water-drinking control, for other genes involved in glutamatergic neurotransmission, such as *Grm2* which encodes glutamate metabotropic receptor 2, in Acc and amygdala, as well as *Grin2b*, which encodes subunit 2B of glutamate ionotropic NMDA receptor, in Nacc. The pivotal role of these components in the prolonged impacts of ethanol has been well-documented in previous research (Healey et al., 2023; Nagy, 2008).

Efforts to identify genes associated with complex behavioral traits like pessimism and optimism have faced challenges, mainly due to the presence of multiple causative variants and the heterogeneity of groups exhibiting a given trait. Our findings provide novel insights into the molecular mechanisms associated with these traits in rats. The level of *Maoa* expression, responsible for encoding Monoamine Oxidase A, was observed to be higher in the mPFC of 'pessimistic' rats compared to their 'optimistic' counterparts. While the role of MAO-A in modulating the level of optimism is not straightforward, heightened MAO-A activity could accelerate the degradation of biogenic amines, leading to reduced availability for receptors and impacting the affective state. Lower expression of *Maoa* was also recently shown for rats characterized by increased negative feedback sensitivity (Cieslik et al., 2022), a trait that co-occurs with pessimism (Rygula and Popik, 2016). Furthermore, as previously noted, pessimism often correlates with depressive symptoms. It is noteworthy that reversible monoamine oxidase inhibitors are commonly used in the treatment of depression and may potentially reshape pessimistic bias towards a more positive orientation. The serotonergic system stands out as a prominent neurotransmitter system in cognitive research with 5-HT playing a pivotal role in cognitive functions and the regulation of affective states. Our research has shown that the *Htr2b* expression level in OFC was lower in 'pessimistic' rats compared to their 'optimistic' counterparts. *Htr2b* encodes the 5-HT_{2B} receptor, which directly contributes to the control of 5-HT levels and regulates serotonin transporter activity (Launay et al., 2006). Moreover, these receptors are essential for the therapeutic effects of selective serotonin reuptake inhibitors, underscoring their involvement in affective state regulation. Therefore, the reduced expression of *Htr2b* in 'pessimistic' rats may be associated with lower 5-HT availability, contributing to a negative affective state. Another significant difference between 'pessimistic' and 'optimistic' animals lies in the expression of *Grm2* in the amygdala, with lower expression observed in 'pessimistic' animals. Research indicates that *Grm2* is implicated in the regulation of susceptibility to stress and anhedonia, crucial for maintaining positive affective states (Nasca et al., 2015). The amygdala is known for its involvement in mood disorders and AUD, which is reflected by the substantial influence of both judgement bias and prolonged alcohol consumption on *Grm2* expression in this structure (Price and Drevets, 2010).

To unravel the complicated relationship between levels of 'pessimism'/'optimism' and susceptibility to alcohol abuse, success hinges on understanding the interaction of these two factors in regulating the expression levels of various genes. In the EtOH group, we observed that the expression of *Grm3* in the amygdala was lower in 'pessimistic' rats compared to their 'optimistic' counterparts. The physiological studies of group II mGlu receptor subtypes suggest that modulating the mGluR2/3 function could attenuate alcohol consumption (Bäckström and Hyytiä, 2005; Griffin et al., 2014), while activation of mGluR3 effectively reduces ethanol-motivated behavior (Rodd et al., 2006). Additional behavioral findings indicate that the activation of mGlu2/3 may play a role in mitigating the behavioral effects of ethanol, pointing towards the involvement of amygdala in this process (Cannady et al., 2011). Perhaps in more 'optimistic' rats, *Grm3* expression is modulated in such a way that increases levels of mGluR3 which serves as a protective agent against increased alcohol consumption. Another crucial component of

the glutamatergic system that may prevent an increase in alcohol intake is EAAT2. It was proven that inducing the expression of EAAT2 in the Nacc of rats decreases their voluntary alcohol intake in the 2BC paradigm (Sari et al., 2011). Indeed, we observed higher expression of this gene in Nacc in the EtOH group compared to the controls, within 'optimistic' rats.

The unexpected difference observed in the expression of the $GABA_B$ receptor 2 subunit in the mPFC is intriguing. Research has shown that activation of the $GABA_B$ receptor attenuates the rewarding effects of ethanol and reduces alcohol intake (Colombo et al., 2004; Loi et al., 2013). This study showed that in the mPFC the expression level of this receptor was higher in 'pessimists' than in 'optimists' of the EtOH group. Although these results seem counterintuitive, we cannot rule out the existence of additional compensatory mechanisms or regulations that occur at the translation stage. Since *Gabbr2* encodes only one of the $GABA_B$ receptor subunits, it is conceivable that it is upregulated due to positive feedback in response to insufficient expression of the other receptor subunits. Another interesting interaction occurred also in mPFC, in the expression level of *Adh1*, which encodes alcohol dehydrogenase, an enzyme involved in ethanol metabolism (Edenberg, 2007). ADH1 catalyzes the reversible oxidation of ethanol to acetaldehyde. Different levels of Adh1 expression between 'optimists' and 'pessimists' in the control group and between EtOH and controls in 'pessimistic' rats suggest that differences in the individual susceptibility of rats to consuming large amounts of alcohol may result from different rates of alcohol metabolism. Last, but not least, there were also significant differences in *Slc6a4* expression, which encodes 5-HT transporter (SERT), in mPFC and Nacc. The impact of alcohol on 5-HTT mRNA levels has been demonstrated in various models of alcohol consumption and dependency. In animal studies, alcohol exposure resulted in elevated concentrations of 5-HTT mRNA in serotonergic brain areas, as well as in regions associated with reward circuitry, such as Nacc, and information processing, like mPFC (Chen et al., 2023; Diehl and Redish, 2023). Several studies have shown that up-regulation of the SERT leads to an increased sensitivity to aversive outcomes, a trait correlated with pessimistic judgement bias (den Ouden et al., 2013; Ineichen et al., 2012).

The serotonin 5-HT_{2A} receptor emerges as another pivotal component in the complicated interplay between pessimistic judgement bias and alcohol abuse. Indeed, the activation of various serotonin receptor subtypes, including 5-HT_{2A}, has been demonstrated to decrease ethanol consumption (Maurel et al., 1999). Other findings indicate that alcohol-preferring rats exhibit lower levels of serotonin 5-HT_{2A} receptor binding sites in several brain regions compared to non-preferring controls (Ciccocioppo et al., 1999). In the current study, quantitative autoradiography uncovered a reduced serotonin 5-HT_{2A} receptor density in 'pessimistic' rats exposed to ethanol, compared to their 'optimistic' conspecifics and counterparts in the control group. It is therefore plausible that alcohol-induced downregulation of this receptor expression occurs specifically in 'pessimistic' rats, potentially constituting an important genetic factor contributing to the susceptibility of this group to heightened alcohol intake.

5. Limitations

Over the past decade, a growing body of research has pointed to the potential use of assessing cognitive correlates of human personality traits in animals to identify biomarkers of various psychiatric disorders (Noworyta et al., 2021). Although optimism and pessimism have been shown to be excellent candidates for this role, their assessment in animal models has certain limitations. This classification is anthropomorphic, attributing human-like emotional states to animals, which may not reflect their true cognitive processes. Although animals can have pessimistic and optimistic expectations, unlike humans, these are not based on considerations about the future but rather serve as strategies for coping with uncertainty in current actions. When an animal must decide on its behavior, the optimal decision maximizes expected gain and minimizes the risk of loss. In guiding behavior, animals must assess the extent to which environmental cues indicate specific situations, such as the presence of a predator or the availability of food (Houston et al., 2012). Therefore, from an evolutionary perspective, 'optimistic' and 'pessimistic' interpretations of environmental cues serve survival and future reproductive success (McNamara et al., 2011). Although for the purposes of clarity in this paper animals have been classified as 'optimistic' and 'pessimistic', it is important that in both humans and animals this is not a completely binary trait, but rather a spectrum. The classification used was intended to simplify the concept in order to highlight the differences observed between groups.

Another potential limitation of our study is the generalization of the negative response bias, which in the case of rats classified as pessimistic, included not only the ambiguous tone but also the positive referent tones. This generalization can be interpreted as a general decrease in sensitivity to stimuli predicting the arrival of a reward. Indeed, similar effects have been observed in earlier studies and even in the landmark work published by Enkel et al. in 2010. This effect does not diminish the value of the obtained results but rather indicates that the bias in evaluation is more general in nature.

There is also the question of potential differences between 'optimistic' and 'pessimistic' rats in their response to the shock. This, however, is unlikely because neither the duration of the negative tone training (data included in supplementary table S1), nor the final response rate to the shock-predicting negative tone (Fig. 1C and D) significantly differed between the 'optimistic' and 'pessimistic' rats.

When it comes to the design of the experiment and the method of data analysis, it is worth discussing our decision to analyze only animals whose screening results were in the upper quartiles of values for optimism and pessimism. This approach was dictated by the relatively flat distribution of these traits across the population, which can obscure subtle but important behavioral differences when including the entire range. Animals with pronounced traits provide clearer, and more distinct behavioral patterns. Additionally, this selection strategy helps in increasing the statistical power of our analyses by magnifying effect sizes, which are more discernible in animals with extreme scores. This method also reduces intra-group variability, leading to more consistent and reliable results. Thus, selecting animals from the upper quartiles is not merely a methodological convenience but a strategic choice to ensure meaningful and actionable findings. On the other hand, the chosen method of analysis significantly reduces the number of animals in each group, particularly among the alcohol-drinking rats, thereby limiting the generalizability of our findings. Additionally, the relatively small differences observed in only one of the alcohol consumption paradigms require caution in interpreting the results, making it challenging to draw robust conclusions applicable to the general population.

In sum, our investigation presents pioneering evidence that traits of optimism and pessimism significantly influence alcohol intake in rodent models. Our findings reveal that rats with a 'pessimistic' disposition, which could reflect the human tendency to consume alcohol as a coping mechanism (relief drinking), exhibit higher levels of alcohol consumption compared to their more 'optimistic' counterparts. This suggests that akin to humans, rats may engage in increased alcohol consumption driven by negative emotional states, implying a form of self-administration. Additionally, our research elucidates the relationship between these cognitive tendencies and specific neurochemical alterations within the brain. It is particularly noteworthy that rats characterized by 'pessimism' display altered gene expression profiles in neurotransmitter pathways, which likely underpin their elevated alcohol use.

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CRediT authorship contribution statement

Agata Cieslik-Starkiewicz: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Michal Piksa:** Writing – review & editing, Investigation. **Karolina Noworyta:** Writing – review & editing, Methodology, Investigation. **Joanna Solich:** Writing – review & editing, Methodology, Investigation. **Paulina Pabian:** Writing – review & editing, Investigation. **Katarzyna Latocha:** Writing – review & editing, Investigation. **Agata Faron-Górecka:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rafal Rygula:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2024.111124.

References

Achterberg, E.J., van Kerkhof, L.W., Damsteegt, R., Trezza, V., Vanderschuren, L.J., 2015. Methylphenidate and atomoxetine inhibit social play behavior through prefrontal and subcortical limbic mechanisms in rats. J. Neurosci. 35, 161–169.

Alloy, L.B., Ahrens, A.H., 1987. Depression and pessimism for the future: biased use of statistically relevant information in predictions for self versus others. J. Pers. Soc. Psychol. 52, 366–378.

Anderson, M.H., Munafo, M.R., Robinson, E.S., 2013. Investigating the psychopharmacology of cognitive affective bias in rats using an affective tone discrimination task. Psychopharmacology 226, 601–613.

Bäckström, P., Hyytiä, P., 2005. Suppression of alcohol self-administration and cue-induced reinstatement of alcohol seeking by the mGlu2/3 receptor agonist LY379268 and the mGlu8 receptor agonist (S)-3,4-DCPG. Eur. J. Pharmacol. 528, 110–118.

Bice, P.J., Liang, T., Zhang, L., Strother, W.N., Carr, L.G., 2008. Drd2 expression in the high alcohol-preferring and low alcohol-preferring mice. Mamm. Genome 19, 69–76.

Blume, A.W., Lostutter, T.W., Schmaling, K.B., Marlatt, G.A., 2003. Beliefs about drinking behavior predict drinking consequences. J. Psychoactive Drugs 35, 395–399.

Cannady, R., Grondin, J.J., Fisher, K.R., Hodge, C.W., Besheer, J., 2011. Activation of group II metabotropic glutamate receptors inhibits the discriminative stimulus effects of alcohol via selective activity within the amygdala. Neuropsychopharmacology 36, 2328–2338.

Carver, C.S., Scheier, M.F., 2014. Dispositional optimism. Trends Cogn. Sci. 18, 293–299.

Carver, C.S., Scheier, M.F., Segerstrom, S.C., 2010. Optimism. Clin. Psychol. Rev. 30, 879–889.

Chamberlain, S.R., Müller, U., Blackwell, A.D., Clark, L., Robbins, T.W., Sahakian, B.J., 2006. Neurochemical modulation of response inhibition and probabilistic learning in humans. Science 311, 861–863.

Chen, G., Lai, S., Bao, G., Ke, J., Meng, X., Lu, S., Wu, X., Xu, H., Wu, F., Xu, Y., Xu, F., Bi, G.Q., Peng, G., Zhou, K., Zhu, Y., 2023. Distinct reward processing by subregions of the nucleus accumbens. Cell Rep. 42, 112069.

Ciccocioppo, R., Angeletti, S., Colombo, G., Gessa, G., Massi, M., 1999. Autoradiographic analysis of 5-HT2A binding sites in the brain of Sardinian alcohol-preferring and nonpreferring rats. Eur. J. Pharmacol. 373, 13–19.

Cieslik, A., Noworyta, K., Rygula, R., 2022. Trait sensitivity to negative feedback determines the intensity of compulsive alcohol seeking and taking in male rats. J. Psychiatry Neurosci. 47, E252–e262.

Cieslik-Starkiewicz, A., Noworyta, K., Solich, J., Korlatowicz, A., Faron-Gorecka, A., Rygula, R., 2024a. Identification of genes regulated by trait sensitivity to negative feedback and prolonged alcohol consumption in rats. Pharmacol. Rep. 76, 207–215.

Cieslik-Starkiewicz, A., Noworyta, K., Solich, J., Korlatowicz, A., Faron-Górecka, A., Rygula, R., 2024b. Trait sensitivity to positive feedback is a predisposing factor for several aspects of compulsive alcohol drinking in male rats: behavioural, physiological, and molecular correlates. Psychopharmacology 241, 33–47.

Colombo, G., Addolorato, G., Agabio, R., Carai, M.A., Pibiri, F., Serra, S., Vacca, G., Gessa, G.L., 2004. Role of GABA(B) receptor in alcohol dependence: reducing effect of baclofen on alcohol intake and alcohol motivational properties in rats and amelioration of alcohol withdrawal syndrome and alcohol craving in human alcoholics. Neurotox. Res. 6, 403–414.

Curzytek, K., Kubera, M., Trojan, E., Wójcik, K., Basta-Kaim, A., Detka, J., Maes, M., Rygula, R., 2018. The effects of pessimism on cell-mediated immunity in rats. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 80, 295–303.

Daut, R.A., Busch, E.F., Ihne, J., Fisher, D., Mishina, M., Grant, S.G., Camp, M., Holmes, A., 2015. Tolerance to ethanol intoxication after chronic ethanol: role of GluN2A and PSD-95. Addict. Biol. 20, 259–262.

den Ouden, H.E., Daw, N.D., Fernandez, G., Elshout, J.A., Rijpkema, M., Hoogman, M., Franke, B., Cools, R., 2013. Dissociable effects of dopamine and serotonin on reversal learning. Neuron 80, 1090–1100.

Diehl, G.W., Redish, A.D., 2023. Differential processing of decision information in subregions of rodent medial prefrontal cortex. Elife 12.

Drozd, R., Cieslak, P.E., Rychlik, M., Rodriguez Parkitna, J., Rygula, R., 2016. Cognitive judgment Bias interacts with risk based decision making and sensitivity to dopaminergic challenge in male rats. Front. Behav. Neurosci. 10, 163.

Drozd, R., Rojek-Sito, K., Rygula, R., 2017. The trait 'pessimism' does not interact with cognitive flexibility but makes rats more vulnerable to stress-induced motivational deficits: results from the attentional set-shifting task. Behav. Brain Res. 335, 199–207.

Edenberg, H.J., 2007. The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res. Health 30, 5–13.

Everitt, B.J., Robbins, T.W., 2016. Drug addiction: updating actions to habits to compulsions ten years on. Annu. Rev. Psychol. 67, 23–50.

Fromme, K., D'Amico, E.J., 2000. Measuring adolescent alcohol outcome expectancies. Psychol. Addict. Behav. 14, 206–212.

Giuliano, C., Pena-Oliver, Y., Goodlett, C.R., Cardinal, R.N., Robbins, T.W., Bullmore, E. T., Belin, D., Everitt, B.J., 2018. Evidence for a long-lasting compulsive alcohol seeking phenotype in rats. Neuropsychopharmacology 43, 728–738.

Griffin, W.C., Haun, H.L., Hazelbaker, C.L., Ramachandra, V.S., Becker, H.C., 2014. Increased extracellular glutamate in the nucleus accumbens promotes excessive ethanol drinking in ethanol dependent mice. Neuropsychopharmacology 39, 707–717.

Grodin, E.N., Baskerville, W.A., Meredith, L.R., Nieto, S., Ray, L.A., 2024. Reward, relief, and habit drinking profiles in treatment seeking individuals with an AUD. Alcohol Alcohol. 59.

Hales, C.A., Houghton, C.J., Robinson, E.S.J., 2017. Behavioural and computational methods reveal differential effects for how delayed and rapid onset antidepressants effect decision making in rats. Eur. Neuropsychopharmacol. 27, 1268–1280.

Hart, S.L., Vella, L., Mohr, D.C., 2008. Relationships among depressive symptoms, benefit-finding, optimism, and positive affect in multiple sclerosis patients after psychotherapy for depression. Health Psychol. 27, 230–238.

Healey, K., Waters, R.C., Knight, S.G., Wandling, G.M., Hall, N.I., Jones, B.N., Shobande, M.J., Melton, J.G., Pandey, S.C., Scott Swartzwelder, H., Maldonado-Devincci, A.M., 2023. Adolescent intermittent ethanol exposure alters adult exploratory and affective behaviors, and cerebellar Grin2b expression in C57BL/6J mice. Drug Alcohol Depend. 253, 111026.

Hellemans, J, Mortier, G, De Paepe, A, Speleman, F, Vandesompele, J, 2007. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol 8, R19.

Hirsch, J.K., Conner, K.R., Duberstein, P.R., 2007. Optimism and suicide ideation among young adult college students. Arch. Suicide Res. 11, 177–185.

Houston, A.I., Trimmer, P.C., Fawcett, T.W., Higginson, A.D., Marshall, J.A., McNamara, J.M., 2012. Is optimism optimal? Functional causes of apparent behavioural biases. Behav. Process. 89, 172–178.

Ineichen, C., Sigrist, H., Spinelli, S., Lesch, K.P., Sautter, E., Seifritz, E., Pryce, C.R., 2012. Establishing a probabilistic reversal learning test in mice: evidence for the processes mediating reward-stay and punishment-shift behaviour and for their modulation by serotonin. Neuropharmacology 63, 1012–1021.

Kalluri, H.S., Mehta, A.K., Ticku, M.K., 1998. Up-regulation of NMDA receptor subunits in rat brain following chronic ethanol treatment. Brain Res. Mol. Brain Res. 58, 221–224.

Koob, G.F., 1992. Neural mechanisms of drug reinforcement. Ann. N. Y. Acad. Sci. 654, 171–191.

Koob, G.F., 2004. A role for GABA mechanisms in the motivational effects of alcohol. Biochem. Pharmacol. 68, 1515–1525.

Koob, G.F., Volkow, N.D., 2010. Neurocircuitry of addiction. Neuropsychopharmacology 35, 217–238.

Launay, J.M., Schneider, B., Loric, S., Da Prada, M., Kellermann, O., 2006. Serotonin transport and serotonin transporter-mediated antidepressant recognition are controlled by 5-HT2B receptor signaling in serotonergic neuronal cells. FASEB J. 20, 1843–1854.

Leeman, R.F., Toll, B.A., Taylor, L.A., Volpicelli, J.R., 2009. Alcohol-induced disinhibition expectancies and impaired control as prospective predictors of problem drinking in undergraduates. Psychol. Addict. Behav. 23, 553–563.

Loi, B., Maccioni, P., Lobina, C., Carai, M.A., Gessa, G.L., Thomas, A.W., Malherbe, P., Colombo, G., 2013. Reduction of alcohol intake by the positive allosteric modulator of the GABA(B) receptor, rac-BHFF, in alcohol-preferring rats. Alcohol 47, 69–73.

Maurel, S., De Vry, J., De Beun, R., Schreiber, R., 1999. 5-HT2A and 5-HT2C/5-HT1B receptors are differentially involved in alcohol preference and consummatory behavior in cAA rats. Pharmacol. Biochem. Behav. 62, 89–96.

McColl, E.R., Piquette-Miller, M., 2020. SLC neurotransmitter transporters as therapeutic targets for alcohol use disorder: a narrative review. Alcohol. Clin. Exp. Res. 44, 1965–1976.

McNamara, J.M., Trimmer, P.C., Eriksson, A., Marshall, J.A., Houston, A.I., 2011. Environmental variability can select for optimism or pessimism. Ecol. Lett. 14, 58–62.
Nagy, J., 2008. Alcohol related changes in regulation of NMDA receptor functions. Curr. Neuropharmacol. 6, 39–54.
Nasca, C., Bigio, B., Zelli, D., Nicoletti, F., McEwen, B.S., 2015. Mind the gap: glucocorticoids modulate hippocampal glutamate tone underlying individual differences in stress susceptibility. Mol. Psychiatry 20, 755–763.
Noworyta, K., Cieslik, A., Rygula, R., 2021. Neuromolecular underpinnings of negative cognitive Bias in depression. Cells 10.
Noworyta, K., Cieslik, A., Rygula, R., 2022. Reinforcement-based cognitive biases as vulnerability factors in alcohol addiction: from humans to animal models. Br. J. Pharmacol. 179, 4265–4280.
Paxinos, G, Watson, C, 2006. The rat brain in stereotaxic coordinates: hard cover edition. Elsevier.
Price, J.L., Drevets, W.C., 2010. Neurocircuitry of mood disorders. Neuropsychopharmacology 35, 192–216.
Roberts, D.C., Bennett, S.A., 1993. Heroin self-administration in rats under a progressive ratio schedule of reinforcement. Psychopharmacology 111, 215–218.
Rodd, Z.A., McKinzie, D.L., Bell, R.L., McQueen, V.K., Murphy, J.M., Schoepp, D.D., McBride, W.J., 2006. The metabotropic glutamate 2/3 receptor agonist LY404039 reduces alcohol-seeking but not alcohol self-administration in alcohol-preferring (P) rats. Behav. Brain Res. 171, 207–215.
Rygula, R., Popik, P., 2016. Trait “pessimism” is associated with increased sensitivity to negative feedback in rats. Cogn. Affect. Behav. Neurosci. 16, 516–526.
Rygula, R., Papciak, J., Popik, P., 2013. Trait pessimism predicts vulnerability to stress-induced anhedonia in rats. Neuropsychopharmacology 38, 2188–2196.
Rygula, R., Papciak, J., Popik, P., 2014. The effects of acute pharmacological stimulation of the 5-HT, NA and DA systems on the cognitive judgement bias of rats in the ambiguous-cue interpretation paradigm. Eur. Neuropsychopharmacol. 24, 1103–1111.
Rygula, R., Golebiowska, J., Kregiel, J., Kubik, J., Popik, P., 2015. Effects of optimism on motivation in rats. Front. Behav. Neurosci. 9, 32.
Sari, Y., 2014. Role of glutamate transporter 1 in the attenuation of alcohol intake. Front. Neurosci. 8, 200.
Sari, Y., Sakai, M., Weedman, J.M., Rebec, G.V., Bell, R.L., 2011. Ceftriaxone, a beta-lactam antibiotic, reduces ethanol consumption in alcohol-preferring rats. Alcohol Alcohol. 46, 239–246.
Scheier, M.F., Carver, C.S., 2018. Dispositional optimism and physical health: a long look back, a quick look forward. Am. Psychol. 73, 1082–1094.
Stracke, J., Otten, W., Tuchscherer, A., Puppe, B., Düpjan, S., 2017. Serotonin depletion induces pessimistic-like behavior in a cognitive bias paradigm in pigs. Physiol. Behav. 174, 18–26.
Strunk, D.R., Lopez, H., DeRubeis, R.J., 2006. Depressive symptoms are associated with unrealistic negative predictions of future life events. Behav. Res. Ther. 44, 861–882.
Uhl, G.R., Koob, G.F., Cable, J., 2019. The neurobiology of addiction. Ann. N. Y. Acad. Sci. 1451, 5–28.
Veilleux, J.C., Skinner, K.D., Reese, E.D., Shaver, J.A., 2014. Negative affect intensity influences drinking to cope through facets of emotion dysregulation. Personal. Individ. Differ. 59, 96–101.

Supplementary Materials

	Positive tone training	Negative tone training	Discrimination training
Min	7	14	38
Max	14	67	93
Mean ± SEM	8 ± 0.21	27 ± 1.9	64 ± 2.5

Table S1 *Minimum, maximum and average number of tests needed to achieve criteria of positive tone training, negative tone training and discrimination phase.*

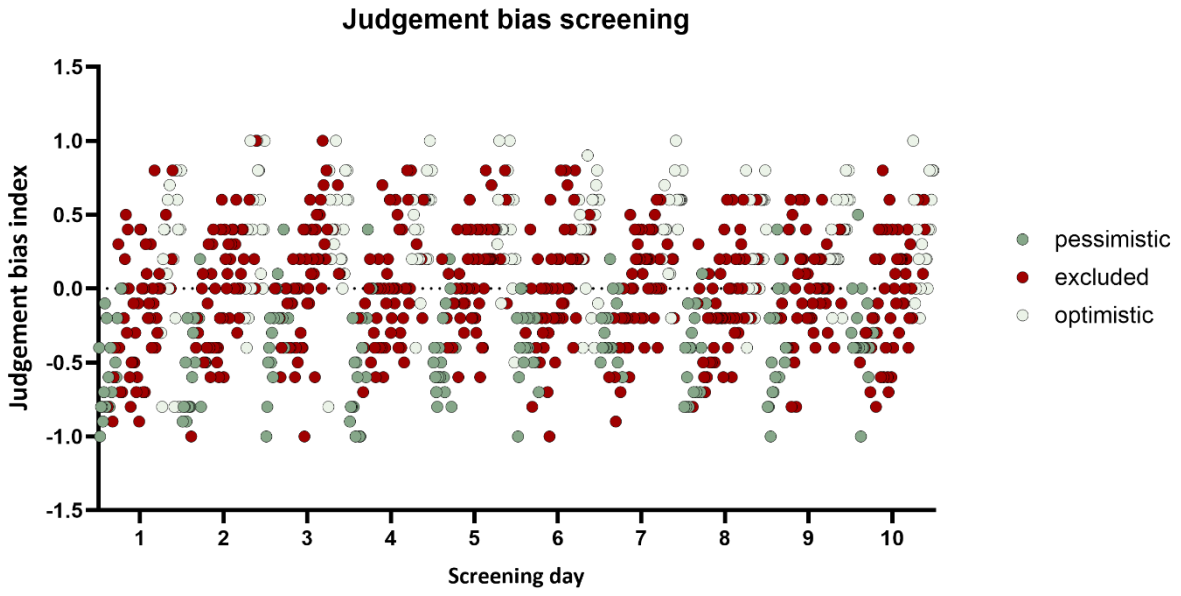

Figure S1 *Daily judgment bias index of all animals during the judgment bias screening. The rats classified as 'pessimistic' are depicted as dark green circles, while 'optimistic' rats are shown as light green circles. The animals marked as red circles were excluded from the analysis*

based either on insufficient performance during the behavioral tests or because their scores were within the two middle quartiles of the judgment bias index.

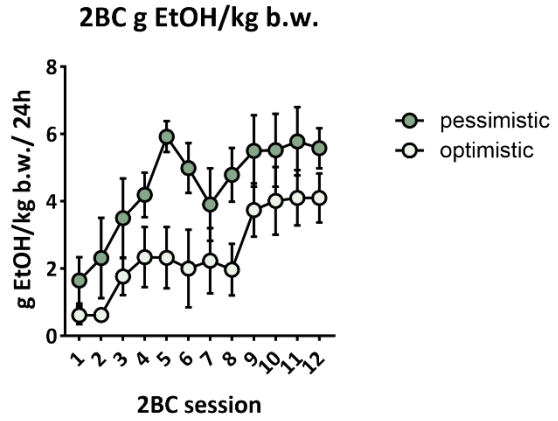

Figure S2 *Raw (untransformed) data for daily ethanol intake during all 12 2BC sessions in 'pessimistic' (dark green circles) and 'optimistic' (light green circles) rats from the EtOH group.*

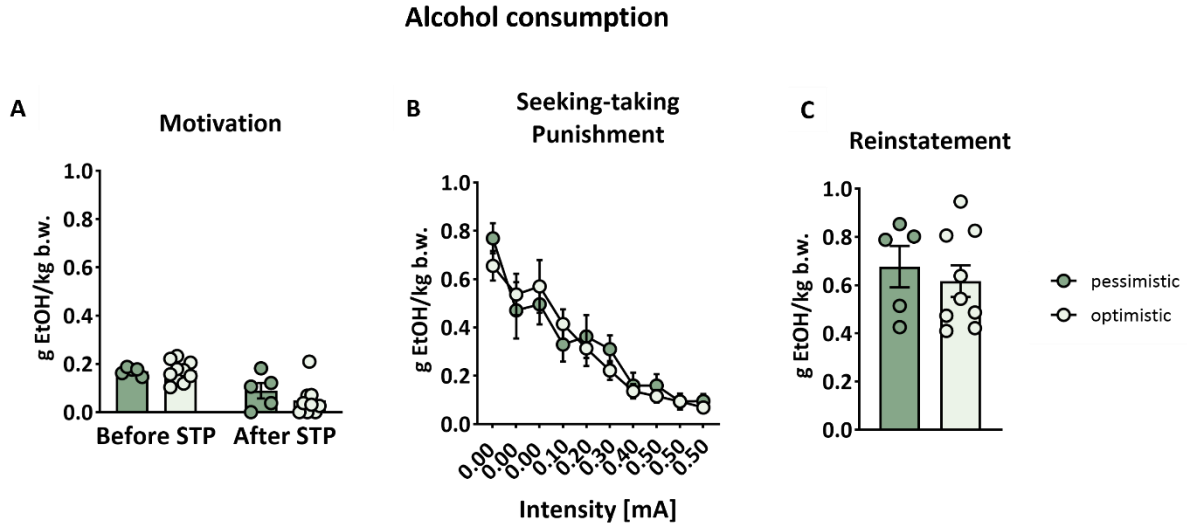

Figure S3 *Operant, alcohol-related behavior expressed as the amount of alcohol consumed in g per kg of body weight in 'pessimistic' (dark green circles and bars) and 'optimistic' (light green*

circles and bars) rats from the EtOH group. Average alcohol consumption in the Progressive Ratio Schedule of Reinforcement tests conducted before and after the Seeking-Taking Punishment sessions (A); during Seeking-Taking Punishment sessions (B); and in the reinstatement tests (C).