Genetic inactivation of midkine, not pleiotrophin, facilitates extinction of alcohol-induced conditioned place preference

Marta Vicente-Rodríguez, Carmen Pérez-García, Esther Gramage, Gonzalo Herradón

Pleiotrophin (PTN) and midkine (MK) are growth factors that modulate alcohol consumption and reward. Since both PTN and MK limit the rewarding effects of alcohol, pharmacological potentiation of the PTN and MK signaling pathways has been proposed for the treatment of alcohol use disorders (AUD). Although the use of this therapy in the prevention of alcohol relapse is important, the potential role of these cytokines in extinguishing alcohol-induced seeking behavior is a key question that remains unanswered. To fill this gap, we have now studied the extinction of the conditioned place preference (CPP) induced by different doses of alcohol in Ptn knockout ($Ptn^{-/-}$) and Mk knockout ($Mk^{-/-}$) mice. The data confirm a higher sensitivity of $Ptn^{-/-}$ mice to the conditioning effects of a low dose (1 g/kg) and a rewarding dose (2 g/kg) of alcohol, while $Mk^{-/-}$ mice are only more susceptible to the conditioning effects of the low dose of this drug. More importantly, the percentage of $Mk^{-/-}$ mice, not $Ptn^{-/-}$ mice, that efficiently extinguished alcohol-induced CPP was significantly higher than that of Wt mice. Taken together, the data presented here confirm that Ptn and Mk are genetic factors that determine the conditioning effects of alcohol in mice and that Mk is a novel factor that plays an important role in the extinction of alcohol-induced CPP.

Keywords: Abstinence Alcohol CPP Extinction MK PTN Relapse

1. Introduction

According to the world health organization (WHO), the harmful use of alcohol causes approximately 3 million deaths every year. In young adults (20–39 years), around 13.5% of the total deaths are attributed to alcohol. These staggering and unacceptable numbers have led the WHO to develop a global strategy to reduce the harmful use of alcohol as a public health priority during the next decade. This includes the devel-opment of new therapeutics and biomarkers to treat more effectively alcohol use disorder (AUD).

As with other substance use disorders, the risk of developing AUD is strongly influenced by environmental and genetic factors. Among the latter, neurotrophic factors have attracted increasing attention over the past two decades [1]. Several neurotrophic factors, such as brain- derived neurotrophic factor (BDNF), have been found to be upregu-lated in the brain following alcohol administration [2,3], and, it was initially hypothesized that they may play a role in the homeostatic, neuroprotective response to the harmful effects of alcohol in the brain.

In addition, they have been shown to also be key factors in the regulation of alcohol consumption and the rewarding effects of this drug [1], opening unexpected avenues for the development of new biomarkers and therapeutics in AUD. Recent neurotrophic factors discovered to play an important role in alcohol consumption and reward include pleio-trophin (PTN) and midkine (MK).

Pleiotrophin and MK are cytokines that are upregulated in different brain areas after administration of different drugs such as cannabis, opioids, nicotine and amphetamine [4]. Midkine is also found upregu-lated in the frontal cortex of human alcoholics [5,6] and PTN is upre-gulated in rodents after a single administration of alcohol [7]. Midkine knockout mice ($Mk^{-/-}$) are more susceptible to the conditioning effects of a low dose of alcohol (1 g/kg) [8] and they consume higher amounts of this drug in the “drinking in the dark” paradigm (DID) [9]. Accord-ingly, specific genetic inactivation of Mk in the mouse ventral tegmental area (VTA) resulted in increased alcohol consumption [9]. On the other hand, the conditioning and sedative effects of alcohol are enhanced in pleiotrophin knockout ($Ptn^{-/-}$) mice [7,10]. Taken together, these

results suggest the pharmacological potentiation of PTN and MK signaling pathways for the treatment of AUD.

Midkine and PTN signal through different receptors, including Receptor Protein Tyrosine Phosphatase (RPTP) β/ζ [11,12], syndecan-3 [13], and anaplastic lymphoma kinase (ALK) [14]. RPTPβ/ζ (a.k.a. RPTPβ, PTPRZ and PTPζ) is highly expressed in the Central Nervous System (CNS) [15,16], where it is thought to be one of the main mediators of PTN and MK actions [17]. Pleiotrophin and MK inactivate the phosphatase activity of RPTPβ/ζ, increasing the phosphorylation levels of its substrates [12,18]. Some of these RPTPβ/ζ substrates such as ALK [19], also a proposed receptor for PTN and MK [14], and Fyn kinase [20] are known to be important regulators of alcohol behavioral effects [1,21]. Importantly, small-molecule blood–brain barrier (BBB)-permeable inhibitors of RPTPβ/ζ [22] have recently been shown to reduce alcohol-induced conditioned place preference (CPP) and alcohol consumption in mice [10,23], and to reduce alcohol consumption in an operant self-administration paradigm in rats [24]. These findings therefore support the hypothesis of potentiating PTN and MK signaling pathways for the treatment of AUD.

Despite all the existing evidence pointing to significant roles of PTN and MK in modulating alcohol-induced behavioral effects, the possible role of these cytokines in extinguishing alcohol-induced seeking behavior has not yet been investigated. This is crucial because memories of the learned association between cues and the rewarding properties of drugs are difficult to extinguish and contribute substantially to drug relapse [25]. To address this gap in our knowledge, we have now examined the extinction of conditioned place preference induced by alcohol in $Ptn^{-/-}$ and $Mk^{-/-}$ mice.

2. Methods

2.1. Animals

$Mk^{-/-}$ and $Ptn^{-/-}$ mice on a C57BL/6J background were generated using previously described methods [26,27]. Briefly, the Ptn gene has five exons encoding an 18-kDa protein with a 32 amino acid signal peptide. The replacement targeting vector generated a PTN null allele (PTN 2-4neo) by deleting exons 2 to 4. $Ptn^{-/-}$ mouse genotypes were confirmed by polymerase chain reaction (PCR) using 5′-GAT TGA ACA AGA TGG ATT GC-3′ forward and 5′-CAT TTA GGC AAA CAG GAA GGA CG-3′ reverse as primers to generate a cDNA of ∼ 0.7 kb, which was detected in agarose gels of genomic DNA extracted from tails of $Ptn^{-/-}$ and Wild type (Wt) mice. $Mk^{-/-}$ mice were generated by using a basic vector to target a part of exon 1, intron 1, and part of exon 2 of the Mk gene. Genotypes of $Mk^{-/-}$ mice were confirmed by PCR using 5′-ATC GGT TCC AAG TCC TCC CTC CGT C-3′ forward and 5′-CAC CTT CCT CAG TTG ACA AAG ACA AGC-3′ reverse as primers to generate cDNA of ∼ 0.7 kb from genomic DNA extracted from tails of $Mk^{-/-}$ and Wt mice.

We used male $Ptn^{-/-}$, $Mk^{-/-}$ and Wt animals of 9–10 weeks of age (20–25 g). Mice were housed under controlled environmental conditions (22 ± 1 C and a 12-h light/12-h dark cycle) with free access to food and water.

All the animals used in this study were maintained in accordance with the European Union Laboratory Animal Care Rules (2010/63/EU directive) and protocols were approved by the Animal Research Committee of Universidad San Pablo-CEU.

2.2. Conditioned place preference (CPP)

2.2.1. Apparatus

A biased apparatus was used, consisting of two equally sized square Plexiglas compartments (20 cm long × 14 cm high × 27 cm wide). One compartment had a black Plexiglas floor and walls and the other had a black Plexiglas floor and white walls.

2.2.2. Subjects and alcohol doses

We performed different sets of experiments. In the first set, mice were only tested for induction of CPP with 1 g/kg alcohol i.p. (Wt, n = 24; $Ptn^{-/-}$, n = 12; $Mk^{-/-}$, n = 13) or with 2 g/kg alcohol (Wt, n = 6; $Ptn^{-/-}$, n = 9; $Mk^{-/-}$, n = 5). The higher dose of alcohol (2 g/kg) was chosen because it is proved to be effective in the induction of CPP in similar studies [28,29]. Despite the capacity of the lower dose of alcohol (1 g/kg) to induce CPP in mice is very limited [29], we used it in case genotypic differences could be masked by the robust rewarding effects of the higher dose of alcohol (2 g/kg) used. The results obtained led us to design a second set of experiments to test for genotypic differences in alcohol CPP extinction. Given the limited capacity of 1 g/kg alcohol to induce CPP in mice, in this second set of experiments, mice were tested for induction and extinction of CPP with 2 g/kg alcohol (Wt, n = 11; $Ptn^{-/-}$, n = 9; $Mk^{-/-}$, n = 13).

2.2.3. Induction of CPP

The procedure consisted of a 5-day schedule with three phases: preconditioning (Pre-C, day 1), conditioning (days 2–4) and testing (CPP, day 5) (Fig. 1). During preconditioning, mice were free to explore the two compartments for 15 min; their behaviour was monitored using a video tracking system (San Diego, California, USA) to calculate the time spent in each compartment. We have previously used this "biased" apparatus and subject assignment, i.e., mice were paired with alcohol in the non-preferred compartment, to study genotype differences in CPP studies with different drugs such as amphetamine, cocaine and alcohol [7,8,30]. As in these previous studies, the compartment with white walls was the non-preferred compartment, where mice of the three genotypes spent a similar amount of time on day 1 (∼30 % stay of total time in the preconditioning phase).

We performed a 3-day conditioning phase routinely used by our group [31], consisting of two conditioning sessions. The first included a morning session, starting at 8 am, in which the animals received a single injection of saline i.p. (10 ml/kg) and were immediately confined to the initially preferred compartment for 5 min. In the evening session, which began at 3 pm, animals were injected (i.p.) with 1 g/kg or 2 g/kg alcohol (20% v/v in isotonic saline) and confined to the initially non-preferred compartment for 5 min. The same procedure was used on the following two days, but the order of treatments (morning/evening) was changed to avoid the influence of circadian variability.

In the testing phase on day 5, mice received a drug-free, 15-min preference test. The percentage of time-spent (stay) in the non-preferred (alcohol-paired) compartment was calculated in all cases. The increase in the percentage of time-spent in this compartment during this phase (day 5, CPP) compared with the time spent in the same compartment during the preconditioning (day 1, Pre-C) was considered indicative of the degree of conditioning induced by alcohol.

2.2.4. Extinction of CPP

The extinction procedure of this study consisted of several sessions as outlined in Fig. 1. Following the acquisition of CPP (day 5) extinction sessions began. Mice that showed less than 15% increase in the percentage of stay in the alcohol-paired compartment, in day 5 compared to day 1 were considered unable to acquire CPP and were excluded from the extinction sessions. After the testing phase (day 5), mice that had acquired CPP according to this criterion were returned to their cages and were neither injected nor re-exposed to the CPP apparatus until the extinction trials, which began on day 7 (Fig. 1). As in the preconditioning (day 1) and testing phases (day 5), the mice performed a drug-free, 15-min preference test in each of the 9 extinction sessions. Mice remained in their cages and were not exposed to the CPP apparatus during the days between the first 8 extinction sessions and during the 3 days between extinction sessions 8 and 9. Mice that returned to basal (Pre-C) levels in each extinction session (+10%) were considered to have extinguished the initial CPP response and left the study. The criteria for assuming that a mouse has acquired or extinguished CPP and

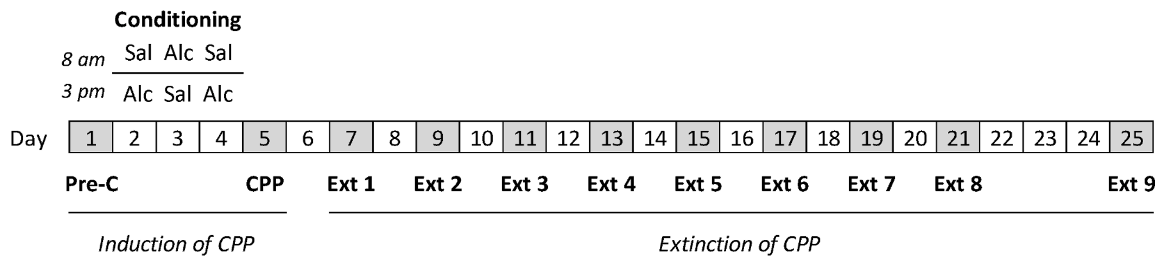


Fig. 1. Timeline of the study.

the extinction paradigm used here follow successful protocols previously used with other drugs, with some modifications [32].

2.3. Statistics

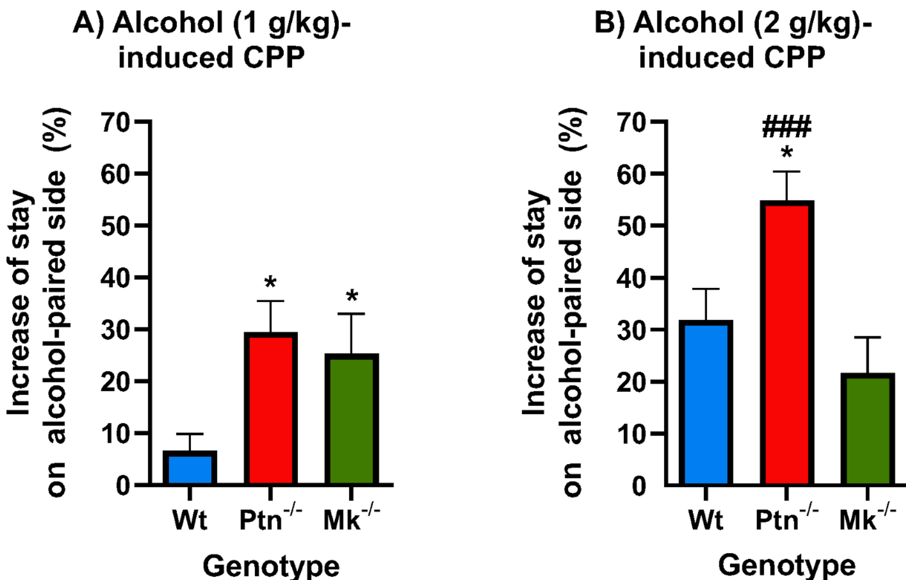
Data, presented as mean ± SEM of the increase in the percentage of stay in the alcohol-paired compartment before and after the conditioning sessions were analysed by 1-way ANOVA, followed by Tukey's post-hoc tests, considering genotype as a variable. Data, presented as mean ± SEM of the percentage of stay in the alcohol-paired compartment in the testing phase (CPP, day 5) and in each extinction session were analysed by 2-way ANOVA followed by Tukey's post-hoc tests.

Contingency tables were built for the analysis of data where the outcome of the experiment was categorical variables (animals from each genotype that were efficiently conditioned or not, and that did or did not extinguish CPP). In these cases, data were analysed using chi-square test.

A p value of less than 0.05 was considered a statistically significant difference. All statistical analyses were performed using Graphpad prism 8 program (La Jolla, CA, USA).

3. Results

To test for genotypic differences in the acquisition of alcohol CPP, we jointly analyzed data from the different sets of experiments described in the Methods. As mentioned before, mice from the three genotypes spent a similar amount of time (~30 % stay of total time in the pre-conditioning phase) in the less-preferred (drug-paired) compartment on day 1. A significant effect of genotype was observed in the acquisition of CPP after conditioning with 1 g/kg alcohol (Fig. 2A) ($F (2, 65) = 5.076$; $p = 0.009$). Conditioning with the lowest dose of alcohol (1 g/kg) resulted in significantly greater increases in the percentage of time-spent in the alcohol-paired compartment in $Ptn^{-/-}$ and $Mk^{-/-}$ mice compared to Wt mice during the testing phase (day 5, CPP) (Fig. 2A). The percentage of mice that were efficiently conditioned by 1 g/kg alcohol (mice that showed a 10% or greater increase in the percentage of stay in the drug-paired compartment at day 5 compared with day 1) differed between genotypes (Fig. 2A, Table). Induction of CPP by 1 g/kg alcohol was observed in more $Ptn^{-/-}$ mice (65 % of the animals that started in the Pre-C phase) and more $Mk^{-/-}$ mice (57,1%) than in Wt mice (18,9%) (Fig. 2A). As expected, the use of the lower dose of alcohol in the



Genotype	Conditioned mice (CPP, day 5)
Wt	7/27 (18,9%)
$Ptn^{-/-}$	13/20 (65%) **
$Mk^{-/-}$	12/21 (57,1%) *

Genotype	Conditioned mice (CPP, day 5)
Wt	13/17 (76,4%)
$Ptn^{-/-}$	16/18 (88,9%) #
$Mk^{-/-}$	10/18 (55,5%)

Fig. 2. Alcohol-induced place preference. Results are presented as the mean ± SEM of the increases in the percentages of the time spent by Wt, $Ptn^{-/-}$ and $Mk^{-/-}$ mice in the 1 g/kg alcohol-paired compartment (A) or 2 g/kg alcohol-paired compartment (B) in the testing phase (CPP, day 5) compared to the time spent during pre-conditioning (Pre-C, day 1). Tables represent the number and the percentage of mice from all genotypes that were efficiently conditioned after a short conditioning phase (days 2 to 4) with 1 g/kg alcohol (A) and 2 g/kg alcohol (B). * $P < 0.05$ vs. Wt; ** $P < 0.01$ vs. Wt; # $P < 0.05$ vs. $Mk^{-/-}$; ### $P < 0.001$ vs. $Mk^{-/-}$.

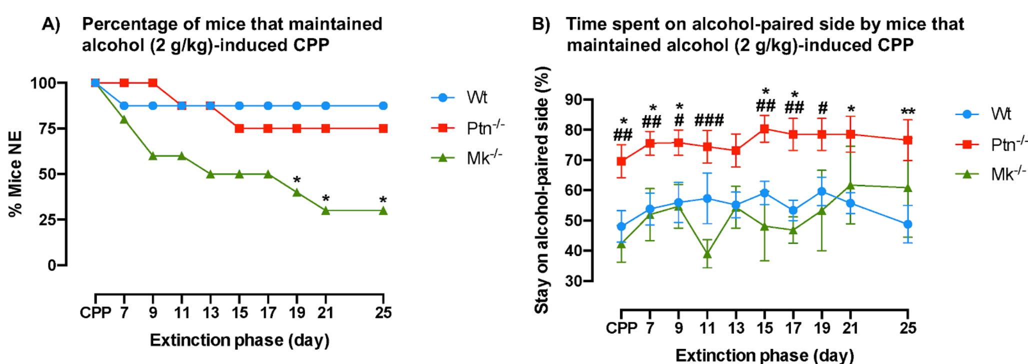
conditioning phase facilitated the observation of genotypic differences. The data confirmed the higher sensitivity of $Ptn^{-/-}$ mice and, to a lesser extent, $Mk^{-/-}$ mice to the conditioning effects of a low dose of alcohol [7,8]. However, at the higher dose of alcohol tested (2 g/kg), we observed genotypic differences in a different manner (Fig. 2B) ($F (2, 50) = 7.75$; $p = 0.001$). Conditioning with the rewarding dose of alcohol (2 mg/kg) resulted in a significantly higher increase in the percentage of time-spent in the alcohol-paired compartment in $Ptn^{-/-}$ mice compared to $Mk^{-/-}$ and Wt mice during the testing phase (day 5, CPP) (Fig. 2B). Interestingly, the percentage of mice that acquired alcohol (2 g/kg)-induced CPP was significantly higher in $Ptn^{-/-}$ mice than in $Mk^{-/-}$ mice, whereas Wt mice were intermediate (Fig. 2B, Table). Overall, the data demonstrate a higher vulnerability of $Ptn^{-/-}$ mice to the conditioning effects of alcohol at low and rewarding doses, whereas $Mk^{-/-}$ mice are more susceptible to the conditioning effects of alcohol only at lower doses.

In the second set of experiments, we tested the possible effect of genotype in extinguishing alcohol CPP with the rewarding dose of alcohol (2 mg/kg). In this study, 8 of the 11 wt mice, 8 of the 9 $Ptn^{-/-}$ mice, and 10 of the 13 $Mk^{-/-}$ mice acquired CPP. Very interestingly, the percentage of $Mk^{-/-}$ mice that did not extinguish alcohol (2 g/kg) CPP at the end of the experiment, 3 of 10 (30%), was significantly lower than the percentage of Wt mice (87.5%) and the percentage of $Ptn^{-/-}$ mice (75%) that did not extinguish alcohol (2 g/kg) CPP (Fig. 3A, C). Mice of all genotypes that extinguished alcohol CPP in each extinction session abandoned the experiment, but we were also interested in the evolution of the time that mice that maintained alcohol CPP across extinction sessions spent in the alcohol (2 g/kg)-paired compartment (Fig. 3B). ANOVA showed a significant effect of genotype ($F (2, 164) = 44.44$; $p < 0.0001$). $Ptn^{-/-}$ mice, that began the extinction session on day 5 (CPP) with a significantly higher percentage of time-spent in the alcohol-paired compartment (Fig. 3B) maintained this strong preference (70–80% of the time in the drug-paired compartment) across all extinction sessions (Fig. 3B). $Mk^{-/-}$ mice and Wt mice that did not extinguish alcohol CPP also maintained the alcohol preference with which they began the extinction sessions (40–60% of the time in the drug-paired compartment). Overall, the data suggest that genetic deletion of Mk facilitates extinction of the conditioning effects of alcohol.

4. Discussion

Compulsive drug-seeking behavior is related to an individual's association of environmental stimuli with the effects of the drug [33,34]. The motivational properties of the drug-associated stimuli play an important role in this process and are related to the risk of relapse and the ability of the substance to generate addiction. Therefore, discovering the factors that determine an individuals ability to extinguish these appetitive memories is important for uncovering biomarkers for withdrawal treatments and to develop new treatments for addictive disorders. Previously, the growth factor PTN was shown to facilitate the extinction of amphetamine seeking behavior [30] while MK facilitated the extinction of the conditioning effects of cocaine [32]. Importantly, PTN and MK are known to modulate the conditioning effects of alcohol [7,8]. Whether these genetic factors also determine the extinction of alcohol-seeking behaviors remained to be studied.

In this work, we first used a low dose of alcohol (1 g/kg) which has been reported to be unable to induce CPP in mice or to produce moderate conditioning effects [29]. In our hands, this was confirmed as the increase in time spent in the alcohol-paired compartment by Wt mice was very small and only 18.9% of the tested subjects acquired CPP. In contrast, the increase in time spent in the alcohol-paired compartment was significantly higher in $Ptn^{-/-}$ and $Mk^{-/-}$ mice than in Wt mice. Furthermore, we found that 65% of $Ptn^{-/-}$ mice and 57.1% of $Mk^{-/-}$ mice tested were efficiently conditioned with alcohol (1 g/kg), which tripled the percentage of Wt mice that acquired CPP. When a higher, rewarding, dose of alcohol was used (2 g/kg), we found that the increase in time spent by $Ptn^{-/-}$ mice in the alcohol-paired compartment was significantly higher than that of Wt and $Mk^{-/-}$ mice. The time spent by Wt mice in the alcohol (2 g/kg)-paired compartment was higher than that caused by conditioning with 1 g/kg alcohol in Wt mice. Surprisingly, this was not observed in $Mk^{-/-}$ mice that remained similarly long after conditioning with both doses of alcohol, suggesting a ceiling effect of alcohol only in mice lacking Mk. Accordingly, the percentage of $Mk^{-/-}$ mice that acquired CPP induced by alcohol (2 g/kg) was the lowest of the three genotypes, whereas nearly 90% of $Ptn^{-/-}$ mice acquired CPP. It is important to note that $Ptn^{-/-}$ and $Mk^{-/-}$ mice do not show gross changes in locomotor activity [7,8]. In addition, $Ptn^{-/-}$ mice did not show differences in working memory compared to Wt mice in the Y-maze, which is relevant to the extinction procedures discussed below



C) Number of mice that maintained alcohol (2 g/kg)-induced CPP

Genotype	n (CPP, day 5)	Extinction phase (day)								
		7	9	11	13	15	17	19	21	25
Wt	8	7/8	7/8	7/8	7/8	7/8	7/8	7/8	7/8	7/8
$Ptn^{-/-}$	8	8/8	8/8	7/8	7/8	6/8	6/8	6/8	6/8	6/8
$Mk^{-/-}$	10	8/10	6/10	6/10	5/10	5/10	5/10	4/10	3/10	3/10

Fig. 3. Extinction of alcohol (2 g/kg)-induced place preference. The evolution of the percentages of mice from all genotypes that continue without extinguishing (No extinguish, NE) alcohol-induced CPP after each of the 9 extinction phases (days 7, 9, 11, 13, 15, 17, 19, 21 and 25) are represented (A). The evolution of the percentages of time spent in the alcohol (2 g/kg)-paired compartment of mice from all genotypes is represented in the testing phase (CPP, day 5) and each of the 9 extinction phases (B). Table represents the numbers of mice from the total conditioned mice (n (CPP)) of all genotypes that did not extinguish alcohol-induced CPP in each extinction phase (C). * $P < 0.05$ vs. Wt; ** $P < 0.01$ vs. Wt; # $P < 0.05$ vs. $Mk^{-/-}$; ## $P < 0.01$ vs. $Mk^{-/-}$; ### $P < 0.01$ vs. $Mk^{-/-}$.

[32]. Also, it is important to note that 4-week-old $Mk^{-/-}$ mice showed a transient deficit in working memory in the Y-maze [27]. However, 8-week-old $Mk^{-/-}$ mice, similar to the ones used in our studies, do not show significant changes in working memory compared to Wt mice [27]. The data support that PTN limits the rewarding effects of alcohol at low and rewarding doses and that MK can limit the rewarding effects of alcohol only at low doses of the drug [7,8].

To study the extinction of the conditioning effects of alcohol in individuals who had efficiently acquired CPP, preference for the alcohol-paired compartment was assessed after repeated exposure to cues previously paired with the drug in the absence of alcohol [28,34,35]. Extinction was pursued through repeated exposures to the CPP apparatus. This is a low demanding protocol by which we pretended to dissect genotypic differences in extinction that could be masked in more demanding protocols by repeated pairing of animals with saline injection into the previously drug-paired compartment [32]. Accordingly, we observed that only one Wt mouse extinguished alcohol (2 g/kg)-induced CPP after all extinction sessions of this protocol. The most important finding of the present study is that the percentage of $Mk^{-/-}$ mice (70%) that efficiently extinguished alcohol (2 g/kg)-induced CPP was significantly higher than that of Wt mice. It should be noted that Wt and $Mk^{-/-}$ mice that began the extinction sessions had a similar amount of time spent in the alcohol (2 g/kg)-paired compartment at day 5, suggesting that genotypic differences in extinction sessions are unlikely to be influenced by differences in the acquisition of alcohol-induced CPP. As mentioned previously, adult Wt and $Mk^{-/-}$ mice show similar performance on working memory tasks [27], suggesting that genotypic differences in extinction of alcohol-induced CPP are not related to an enhanced working memory of $Mk^{-/-}$ mice. The data demonstrate that endogenous MK, not PTN, plays a role in the extinction of alcohol-induced CPP. Although PTN and MK are cytokines that highly overlap in structure and functions [36], the data presented here on the induction and extinction of alcohol CPP demonstrate that the modulation of the conditioning effects of alcohol by PTN and MK are distinct and suggest that these cytokines may use different receptors and signaling cascades to exert their modulatory actions on alcohol behavioral effects [4]. Moreover, in contrast to the data presented here, genetic inactivation of Mk has been shown to prevent the extinction of cocaine-induced CPP [32]. Alcohol and cocaine differ in their molecular actions, suggesting different mechanisms triggered by MK to modulate the extinction of the rewarding effects depending on the drug. Importantly, Fyn kinase and TrkA are two substrates of the MK receptor RPTPβ/ζ which are known to regulate alcohol and cocaine seeking behaviors [23,37–39]. As mentioned before, MK is an endogenous inhibitor of the tyrosine phosphatase activity of RPTPβ/ζ. Therefore, it is possible that MK regulates the kinase activity of Fyn and TrkA by increasing the phosphorylation of key tyrosine residues in these proteins, which could be different from those regulated by PTN. In addition, phosphorylation of $GABA_A$ receptors plays an essential role in the assembly, trafficking and cell surface stability of these receptors [40], which may influence the pharmacological effects of $GABA_A$-active drugs such as alcohol. Interestingly, Fyn is a kinase that contributes to phosphorylation of tyrosine residues in the $GABA_A$ receptor [41], suggesting the possibility that an impaired MK-RPTPβ/ζ-Fyn axis in $Mk^{-/-}$ mice may underlie the differential behavioral responses of $Mk^{-/-}$ mice to alcohol activation of $GABA_A$ receptors by affecting the phosphorylation of these receptors. Additional studies are needed to dissect the possible contribution of TrkA and/or Fyn kinase to the mechanisms triggered by MK in the modulation of the behavioral responses to alcohol and to explain the different roles of MK in alcohol and cocaine-seeking behaviors.

Pharmacological strategies that reduce the behavioral impact of drug-paired cues by enhancing and maintaining extinction of these cues could potentially reduce the possibility of relapse [42,43]. Thus, the data presented here suggest that inactivation of MK may facilitate extinction of alcohol seeking behaviors. However, a limitation of the present study is that we did not perform extinction/reinstatement procedures and/or models more associated to the motivational properties of alcohol, such as self-administration. Therefore, based on the data presented here, we cannot conclude whether inactivation of MK influences the risk of relapse. Pharmacological modulation of the effects of MK for the treatment of AUD appears to be more complex, as genetic inactivation of MK leads to enhanced conditioning effects of low doses of alcohol. This dual role of MK may require different approaches for potential clinical treatments of AUD. As we have already mentioned, MK is an endogenous inhibitor of RPTPβ/ζ. Accordingly, small-molecule inhibitors of RPTPβ/ζ mimic the actions of MK and reduce alcohol (2 g/kg) CPP in mice [7,23] and reduce alcohol intake in mice and rats [9,23,24]. Thus, pharmacological potentiation of MK actions could be a novel therapy for excessive alcohol consumption. However, the opposite, i.e., inhibition of MK actions, might also be required to maintain abstinence and prevent relapse in alcoholics. It is important to note that small inhibitors of MK and blocking monoclonal antibodies against MK are being developed for other indications, mainly related to cardiovascular and autoimmune diseases and malignancies [4].

5. Conclusion

The data confirm that Ptn and Mk are genetic factors that determine the conditioning effects of alcohol in mice. The data demonstrate that Mk is a novel genetic factor that plays a role in the extinction of alcohol-induced CPP.

Author contributions

GH and CP-G, conceptualization. MVR and EG, investigation. GH, MVR and CP-G, data curation and formal analysis. GH, writing - original draft. MVR, CP-G and EG, writing - review & editing. GH, funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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