Sexually dimorphic influence of the circadian clock gene *Bmal1* in the striatum on alcohol intake

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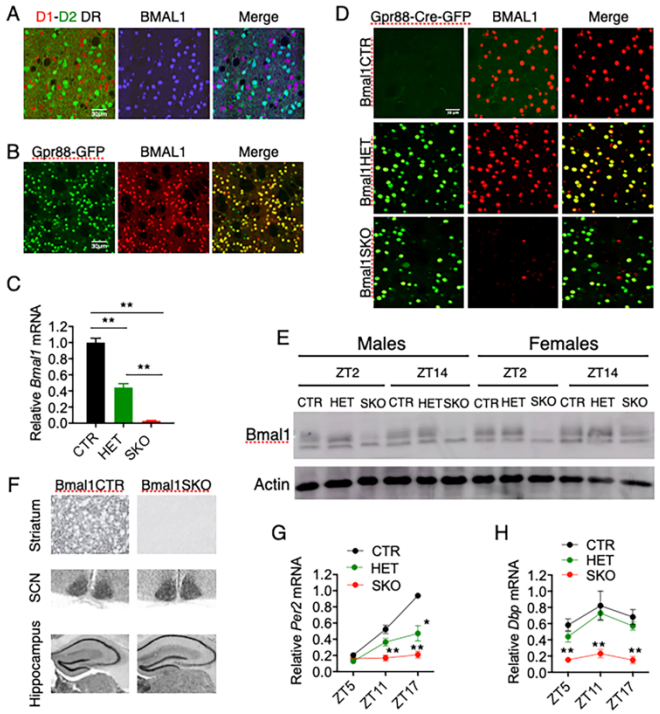
Summary
The clock gene *Bmal1* plays an obligatory role in the generation of circadian rhythms in gene expression, physiology, and behavior in mammals [1-4]. In mice, perturbations in *Bmal1* expression in the brain are associated with loss of circadian rhythmicity and various physiological and behavioral disturbances, including disrupted sleep architecture and deficits in cognitive and affective behaviors [5-14]. Gene association studies in both humans and animals suggest that *Bmal1* may also play a role in the control of appetitive behaviors such as alcohol preference and consumption [15-19]. Although there is evidence that genes that interact with *Bmal1* in the molecular circadian clock, such as *Per2* and *Clock* influence alcohol intake and preference [15, 20-23], experimental evidence of a causal role of *Bmal1* is lacking. In addition, the specific brain regions where *Bmal1* might affect alcohol consumption are not known. We investigated voluntary alcohol consumption in conditional knockout mice that lack BMAL1 protein exclusively in the striatum, which is an important structure in the control of alcohol intake and preference [24-29]. Particular emphasis was attributed to the investigation of male and female mice because of known sex differences in alcohol intake and preference [30-32], and the impact of a sexually dimorphic constitution of circadian clocks on behavior [33, 34]. We found that deletion of BMAL1 from the principal medium spiny neurons (MSNs) of the striatum significantly altered voluntary alcohol intake and preference, without affecting total fluid intake, sucrose preference, body weight, or circadian rhythms in behavior. Strikingly, there were major sex differences in the effect of striatal BMAL1 deletion on alcohol consumption. While striatal BMAL1 deletion augmented alcohol intake and preference in males, the same deletion suppressed intake and preference in females. Interestingly, striatal deletion of PER2, a clock gene that interacts with Bmal1 in the circadian clock, and which has been shown to limit alcohol consumption in mice [22], mimicked the effect of striatal BMAL1 deletion, albeit only in males. Together, our results reveal that BMAL1 in MSNs of the striatum plays a sexually dimorphic role in the control of alcohol intake in mice, restraining consumption in males, possibly by interacting with PER2, and promoting intake in females, independently of PER2. We therefore hypothesize that a sex-specific mechanism in the function of BMAL1 in MSNs of the striatum regulates differences between male and female mice in the propensity to consume alcohol.

Expression of BMAL1 in Striatal MSNs and Generation of Striatal *Bmal1* Knockout Mice

MSNs constitute approximately 95% of the entire striatal neuronal population [35, 36] and play a critical role in alcohol neuroadaptation and alcohol intake and preference [25, 37-39]. They receive excitatory glutamatergic inputs from the cortex and thalamus and dopaminergic input from midbrain dopamine (DA) nuclei, and project either directly (striatonigral) or indirectly (striatopallidal) to the output nuclei of the basal ganglia [40]. Striatonigral MSNs are distinguished by the expression of D1 DA receptor and striatopallidal MSNs by the expression of D2 DA receptors [41]. Moreover, both MSNs subtypes express Gpr88, a striatum-specific G-protein coupled receptor [42-44]. Using fluorescence immunohistochemistry in tdTomato-D1/GFP-D2 double transgenic reporter mice [45] we confirmed that D1 and D2 DA receptor neurons in the dorsal and ventral striatum express BMAL1 (Fig. 1A). Moreover, using transgenic mice that express Cre and green fluorescent protein (GFP) under control of the *Gpr88* promoter, we confirmed that BMAL1 is expressed in Gpr88-bearing neurons of the striatum (Fig. 1B).

Mice with specific deletion of BMAL1 protein from MSNs of the striatum were generated using the Cre-*lox* recombination strategy. C57BJ/6 mice homozygous for floxed alleles of the *Bmal1* locus ([Bmal1fl/fl], JAX, stock number 7668) were crossed with transgenic mice that express Cre recombinase and GFP under control of the *Gpr88* promoter ([Gpr88Cre/+], JAX, stock number 22510) to yield striatal specific *Bmal1* knockout mice (Gpr88Cre/+; Bmal1fl/fl [Bmal1SKO]), as well as heterozygotes (Gpr88Cre/+; Bmal1fl/+ [Bmal1HET]) and wild type controls (Gpr88+/+; Bmal1fl/fl [Bmal1CTR]). The resulting Bmal1SKO and Bmal1HET male and female mice were similar to their littermate controls in weight. Quantitative real-time polymerase chain reaction (qPCR) analysis of striatal tissue punches (n = 3/genotype) revealed a significant reduction of mRNA levels within the floxed *Bmal1* locus in the dorsal striatum to less than 5% of control levels in Bmal1SKO mice ($P<0.0001$, unpaired two-tailed t test) and less than 50% of control levels in Bmal1HET mice ($P<0.0001$, unpaired two-tailed t test) (Fig. 1C). Using fluorescence immunohistochemistry, we confirmed that striatal brain sections from Bmal1CTR and Bmal1HET mice expressed BMAL1, whereas striatal sections from Bmal1SKO mice lacked BMAL1 immunostaining (Fig. 1D). The reduction of BMAL1 protein in the striatum was further confirmed by western blotting analysis of striatal tissue punches. In both male and female Bmal1SKO mice, the expression of BMAL1 was substantially lower at zeitgeber time (ZT) 2 and ZT14 compared to control animals (Fig. 1E). Moreover, we confirmed that the deletion of *Bmal1* is restricted to the striatum by showing presence of BMAL1 staining in the SCN master clock and hippocampus of Bmal1SKO mice (Fig. 1F). To determine whether deletion of *Bmal1* disrupted the striatal circadian clock we studied the expression of the clock gene, *Per2*, which depends on *Bmal1* for expression and daily cycling (n=3/timepoint) (Fig. 1G). In Bmal1CTR mice, *Per2* mRNA levels in the striatum peaked at night (ZT17). In contrast, in Bmal1SKO mice, the levels of *Per2* mRNA was significantly downregulated at ZT11 (CTR versus SKO, $P<0.005$, unpaired two-tailed t test) and ZT17 ($P<0.005$, unpaired two-tailed t test) and its rhythm was blunted across the light-dark cycle. In addition, the analysis of the mRNA expression of the canonical clock-controlled gene *Dbp* shows that *Bmal1* deletion from MSNs induced a significant downregulation of this gene in the striatum (CTR versus SKO, $P<0.005$ for all timepoints, unpaired two-tailed t test) (Fig. 1H). These results suggest that both *Per2* and *Dbp* expression in MSNs are controlled locally by a *Bmal1* dependent striatal circadian clock mechanism, and that in the absence of *Bmal1* in the MSNs the circadian clock of the striatum is disrupted.

Figure 1. BMAL1 expression is dampened in MSNs of conditional *Bmal1* knockout mice.



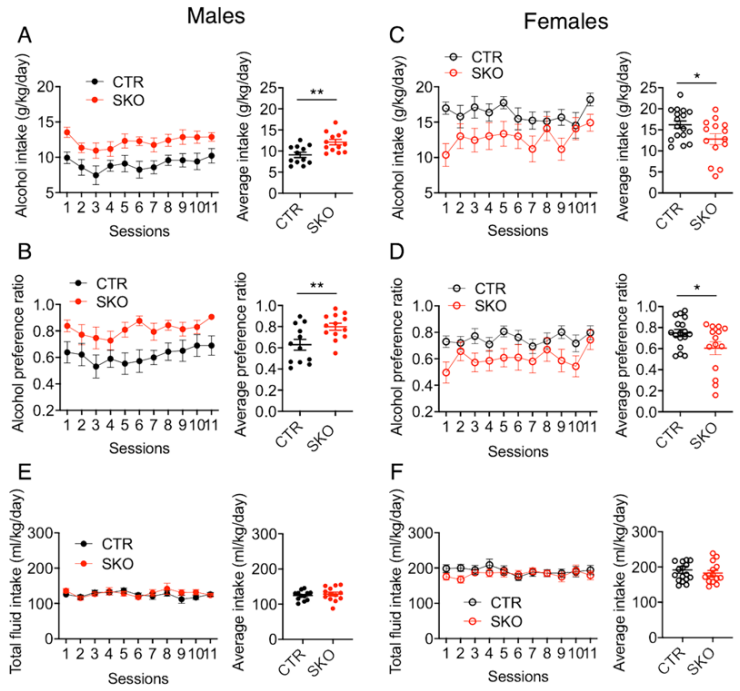
A) Representative images of immunofluorescence staining showing that BMAL1 (blue) is expressed in D1 (red) and D2 (green) dopamine receptor bearing neurons of the dorsal striatum in tdTomato-D1/GFP-D2 double transgenic reporter mice (scale bar = 30 μm).
B) Representative images of immunofluorescence staining showing that BMAL1 (red) is expressed in Gpr88-Cre-GFP (green) positive MSNs of the mouse dorsal striatum (scale bar = 30 μm).
C) Quantitative PCR analysis of dorsal striatal tissue shows a clear effect of gene dosage on *Bmal1* mRNA levels, with *Bmal1* knockout mice displaying almost no expression, and *Bmal1* heterozygote mice exhibiting approximately half of the mRNA levels as control mice in the mouse striatum. There were significant differences between all groups (**P<0.0001, unpaired two-tailed t test).
D) Representative image of immunofluorescence staining showing that *Bmal1* knockout mice which express Cre-GFP in dorsal striatal MSNs, and have two floxed *Bmal1* alleles, display depleted BMAL1 in GFP-positive cells. *Bmal1* heterozygote mice which express Cre, but only possess a single floxed *Bmal1* allele, retain BMAL1 expression. Control mice which contain two floxed *Bmal1* alleles, but lack Cre, retain BMAL1 expression.
E) Representative western blot image confirming BMAL1 depletion in the dorsal striatum of SKO animals at two different times of the day.
F) Representative image of immunohistochemistry staining showing expression of BMAL1 in the hippocampus and SCN, but not in the striatum of *Bmal1* knockout mice.
G) Quantitative PCR analysis showing that *Per2* mRNA levels were downregulated and its rhythm was blunted across the light-dark cycle in the dorsal striatum of conditional *Bmal1* knockout mice. (*P<0.01, **P<0.005, unpaired two-tailed t test).
H) Quantitative PCR analysis of Dbp mRNA expression shows a downregulation of this gene in the dorsal striatum of conditional *Bmal1* knockout mice. (** represent significant difference from control, p<0.005, unpaired two-tailed t test).

Sexually Dimorphic Effect of *Bmal1* Deletion from MSNs on Alcohol Intake and Preference
To study alcohol intake and preference, 12 - 18 weeks old male and female Bmal1CTR mice (males, n = 12; females, n = 17) and Bmal1SKO mice (males, n = 13; females, n = 14), were housed individually under a normal 12:12 h light-dark cycle, with food and water available *ad libitum*. For measurements of voluntary alcohol consumption, mice had free access to one bottle of 15 % ethanol solution in tap water (vl/vl) and one bottle of only tap water every other day, in alternate left-right position, for a total of 11 sessions. Analysis of variance (ANOVA) revealed a significant main effect of genotype on alcohol intake (g/Kg/day) and preference (alcohol

intake/total fluid intake) in both males (Figs. 2A and 2B) and females (Figs. 2C and 2D). Specifically, Bmal1SKO males consumed significantly more alcohol ($P<0.002$, ANOVA) and exhibited significantly greater alcohol preference ($P<0.005$, ANOVA) than Bmal1CTR mice over the 11 alcohol test days. On average, the daily alcohol intake (12.12 ± 0.64 g/Kg) and preference (0.80 ± 0.03) of Bmal1SKO male mice were 33 % and 36 % higher than those of Bmal1CTR mice (average intake, 9.1 ± 0.62g/Kg; average preference, 0.62 ± 0.05; unpaired two-tailed t test, intake: $P<0.003$; preference: $P<0.005$, unpaired two-tailed t test).

In contrast to males, Bmal1SKO females consumed significantly less alcohol than Bmal1CTR mice ($P<0.03$, ANOVA) and exhibited lower alcohol preference ($P<0.05$, ANOVA) over the 11 alcohol test days. The mean daily alcohol intake of Bmal1SKO females was 22 % lower than that of Bmal1CTR females (12.79 ± 1.29 versus 16.21 ± 0.88 g/Kg, $P<0.03$, unpaired two-tailed t test). The mean daily alcohol preference value of Bmal1SKO females was 15 % lower than that of Bmal1CTR females (0.60 ± 0.06 versus 0.75 ± 0.03, $P<0.03$, unpaired two-tailed t test). Contrary to the effects of *Bmal1* deletion on alcohol intake and preference, in both males and females, there were no genotype effects on total daily fluid intake across the 11 test sessions (Figs. 2E and F), suggesting that the deletion of *Bmal1* in the striatum does not alter ethanol intake simply as the result of changes in fluid intake.

Figure 2. Alcohol consumption is altered in *Bmal1* knockout male and female mice



A) Mean ± S.E.M. daily alcohol consumption (left) and average alcohol consumption (right) in control (CTR, n = 12) and *Bmal1* knockout (SKO, n = 13) male mice (**$P<0.01$, unpaired two-tailed t test).

B) Mean ± S.E.M. daily alcohol preference (left) and average alcohol preference (right) in CTR and *Bmal1* SKO male mice (**$P<0.01$, unpaired two-tailed t test).

C) Mean ± S.E.M. of daily alcohol intake (left) and average alcohol intake in CTR (n = 17) and Bmal1 SKO (n = 14) female mice (*$P<0.05$, unpaired two-tailed t test).

D) Mean ± S.E.M. of daily alcohol preference (left) and average alcohol preference in CTR and Bmal1 SKO female mice (**$P<0.05$, unpaired two-tailed t test). SKO male mice drink more alcohol than CTR mice and SKO female mice drink less alcohol than CTR mice. Similarly, SKO male mice show higher alcohol preference than CTR and SKO female mice show lower alcohol preference than CTR mice.

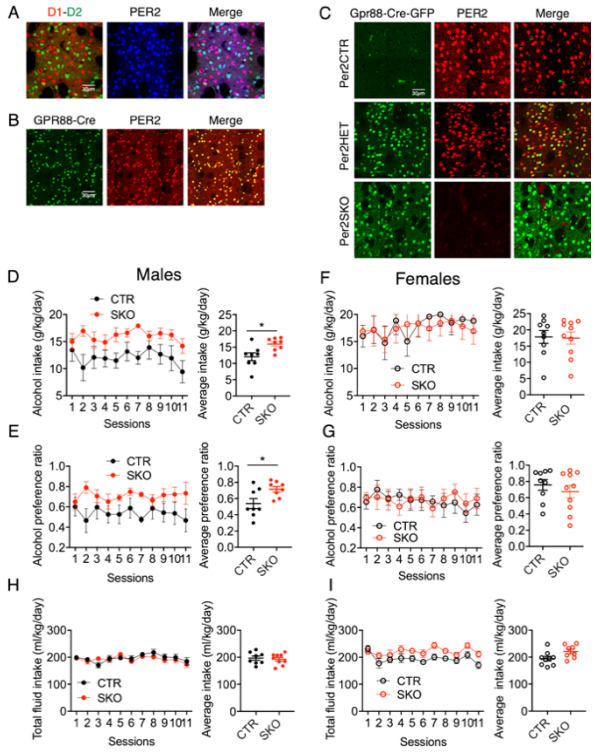
E). Total fluid consumption (left) and average total fluid consumption (right) in CTR and *Bmal1* SKO males.

F) Total fluid consumption (left) and average total fluid consumption (right) in CTR and *Bmal1* SKO female mice.

Striatal Deletion of *Per2* Mimicks the Effect of *Bmal1* Deletion on Alcohol Intake in Males

Bmal1 plays an obligatory role in transcriptional activation of the core clock gene *Per2*, and analysis of *Per2* mRNA in the striatum of Bmal1SKO and Bmal1HET mice revealed gene-dose dependent suppression of *Per2* expression at ZT5 and 17 (Fig. 1G). *Per2* has been associated with alcohol consumption in humans, and global disruption of *Per2* has been shown to augment alcohol intake and preference in male mice [22], raising the possibility that the sexually dimorphic effect of striatal *Bmal1* deletion on alcohol intake and preference involves changes in local expression of *Per2*. MSNs that express D1 or D2 DA receptors and GPR88 also express PER2 (Figs. 3A and 3B). To study the contribution of striatal *Per2* gene expression, we crossed C57BJ/6 mice homozygous for floxed alleles of the *Per2* locus (Per2fl/fl, European Mouse Mutant Archive, Strain ID: EM10599) with mice that express Cre and GFP under control of the *Gpr88* promoter to generate Per2SKO (Gpr88Cre/+; Per2fl/fl), Per2HET (Gpr88Cre/+; Per2fl/+) and Per2CTR (Gpr88+/+; Per2fl/fl) male and female mice. Immunostaining of striatal brain sections from Per2SKO mice revealed complete absence of PER2 immunoreactivity (Fig. 3C). Deletion of *Per2* from MSNs augmented voluntary alcohol intake (Per2SKO versus Per2CTR, $P<0.01$, ANOVA) and preference (Per2SKO versus Per2CTR, $P<0.05$, ANOVA) in males (Figs. 3D and 3E), thus mimicking the enhancing effect of striatal *Bmal1* deletion on male alcohol intake and preference. On average, the daily alcohol intake (15.99 ± 0.7g/Kg) and preference (0.71 ± 0.03) of Per2SKO male mice was ~33 % higher than intake (12.01 ± 1.2g/Kg) and preference (0.53 ± 0.06) in control littermates. In contrast, deletion of *Per2* from MSNs had no effect on alcohol consumption and preference in females (Figs. 3F and 3G) or on total fluid intake in either males or females (Figs. 3H and 3I). Taken together with the results in Bmal1SKO mice, these findings point to a sex dependent dissociation between the effect of *Bmal1* and *Per2* on alcohol consumption in females and a possible association in males.

Figure 3. Alcohol consumption is altered in *Per2* knockout male mice



A) Representative image of immunofluorescence staining showing that PER2 (blue) is expressed in D1 (red) and D2 (green) dopamine receptor bearing neurons of the dorsal striatum in tdTomato-D1/GFP-D2 double transgenic reporter mice (scale bar = 30 μm).

B) Representative image of immunofluorescence staining showing that PER2 (red) is expressed in Gpr88-Cre-GFP (green) positive MSN of the mouse dorsal striatum (scale bar = 30 μm).

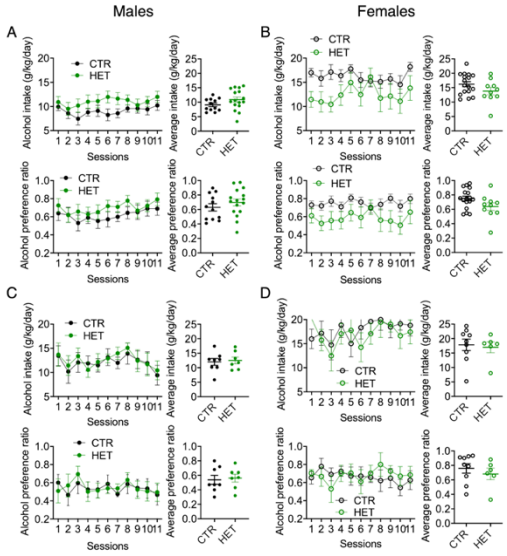
C) Representative image of immunofluorescence staining showing that *Per2* knockout mice which express Cre-GFP in dorsal striatal MSNs and have two floxed *Per2* alleles, display depleted PER2 in GFP-positive cells. *Per2* heterozygote mice which express Cre, but only possess a single floxed *Per2* allele, retain PER2 expression. Control mice which contain two floxed *Per2* alleles, but lack Cre, retain PER2 expression.

D) Mean ± S.E.M. of daily alcohol consumption (left) and average alcohol consumption (right) in control (CTR, n = 8) and *Per2* knockout (SKO, n = 8) males (*P<0.05, unpaired two-tailed t test).
E) Mean ± S.E.M. of daily alcohol preference (left) and average alcohol preference (right) in CTR and *Per2* SKO male mice (*P< 0.05, unpaired two-tailed t test).
F) Mean ± S.E.M of daily alcohol consumption (left) and average alcohol consumption (right) in CTR (n = 9) and *Per2* SKO (n = 10) female mice.
G) Mean ± S.E.M. of daily alcohol preference (left) and average alcohol preference (right) in CTR and Per2 SKO female mice.
H) Total daily fluid consumption (left) and average total fluid consumption (right) in CTR and *Per2* SKO males.
I) Total daily fluid consumption (left) and average total fluid consumption (right) in CTR and *Per2* SKO and female mice.

Effect of Deleting a Single Copy of *Bmal1* or *Per2* from MSNs on Voluntary Alcohol Intake and Preference

For a minority of genes, one functional copy is not sufficient to sustain normal function, and mutations causing the loss of function of one of the copies of such gene can impact behavior [46]. To determine if alcohol drinking behavior is affected when only one copy of *Bmal1* is expressed, we assessed alcohol intake and preference in male and female Bmal1HET mice (males, n = 16; females, n = 8) as described above. Although not statistically significant, Bmal1HET males tended to consume and prefer more alcohol than Bmal1CTR male mice (Fig. 4A). In contrast, Bmal1HET females consumed significantly less alcohol (P<0.02, ANOVA) and had lower preference (P<0.02, ANOVA) than Bmal1CTR females (Fig. 4B). These results indicate that the *Bmal1* gene in the MSNs of the striatum is haploinsufficient regarding normal alcohol consumption and preference in females, whereas in males, deletion of one copy of striatal *Bmal1* appears not to affect alcohol intake and preference. Deletion of one copy of *Per2* (Per2HET) had no effect on alcohol intake and preference in males (n = 7) or females (n = 6), indicating that striatal *Per2* is haplosufficient for alcohol intake (Figs. 4C and 4D).

Figure 4. Alcohol consumption is altered in *Bmal1* heterozygote female mice but not in Per2 heterozygote mice



A) Mean ± S.E.M. of daily alcohol consumption (top left) and preference (bottom left) and of average consumption (top right) and preference (bottom right) in control (CTR, n = 12) and *Bmal1* heterozygote (HET, n = 16) male mice.
B) Mean ± S.E.M. of daily alcohol consumption (top left) and preference (bottom left) and average consumption (top right) and preference (bottom right) in CTR (n = 17) and HET (n = 10) female mice. Alcohol preference ratio is calculated as alcohol / (alcohol + water). Bmal1HET females consumed significantly less alcohol (P<0.03, two-way ANOVA) and had lower preference (P<0.02, two-way ANOVA,) than Bmal1CTR females.

C) Mean ± S.E.M. of daily alcohol consumption (top left) and preference (bottom left) and average consumption (top right) and preference (bottom right) in control (n = 8) and in *Per2* heterozygote (n = 7) male mice.
D) Mean ± S.E.M. of daily alcohol consumption (top left) and preference (bottom left) and average consumption (top right) and preference (bottom right) in control (n = 9) and in *Per2* heterozygote (n = 6) female mice.

Gpr88 Monoallelic Expression Does Not Affect Alcohol Intake

Bmal1SKO and Per2SKO mice and their respective HET counterparts have only one functional copy of *Gpr88* in MSNs since one copy is modified to drive *Cre* and GFP expression. Complete deletion of *Gpr88* has been shown to augment alcohol intake in male mice [47, 48], raising the possibility that the changes in alcohol consumption seen in striatal Bmal1SKO and striatal Per2SKO were due, at least in part, to a contributory effect of *Gpr88* monoallelic expression. To study this possibility, we compared alcohol intake and preference between Gpr88+/+ mice (males, n = 7; females, n = 10) and Gpr88Cre/+ mice (males, n = 7; females, n = 12). As shown in Supplementary Fig. 1, alcohol intake and preference in Gpr88Cre/+ male and female mice were similar to those in Gpr88+/+ control mice. The average daily alcohol consumption of Gpr88+/+ and Gpr88Cre/+ males was 11.01 ± 0.4 g/Kg (n = 7) and 11.03 ± 1.26 g/Kg (n = 7), respectively; whereas, average intake in Gpr88+/+ and Gpr88Cre/+ females was 12.01 ± 1.52 g/Kg (n = 10) and 13.95 ± 1.45 g/Kg (n = 12), respectively. The average daily alcohol preference of Gpr88+/+ and Gpr88Cre/+ males was 0.80 ± 0.03 and 0.73 ± 0.05, and average preference in Gpr88+/+ and Gpr88Cre/+ females was 0.60 ± 0.08 and 0.64 ± 0.07, respectively. These results exclude the possibility that the changes in alcohol intake seen in Bmal1SKO and Bmal1HET mice were due to either *Gpr88* haploinsufficiency or expression of Cre-EGFP in Gpr88 bearing cells.

Striatal *Bmal1* or *Per2* Deletion Has No Effect on Sucrose Intake

Striatal MSNs play an important role in reward processing and in appetitive and consummatory behaviors. To study whether the effect of striatal *Bmal1* or *Per2* deletion on alcohol intake and preference is associated with general changes in appetitive behavior or reward sensitivity, we measured voluntary sucrose consumption between CTR and SKO mice. Mice had free access to 0.25% sucrose solution and water for 3 days and one week later to 2% sucrose solution and water for additional 3 days. As shown in supplementary Figs. 2A and 2B, there were no significant differences in average sucrose intake between Bmal1CTR and Bmal1SKO in males (CTR, n = 9; SKO, n = 10) and in females (CTR, n = 5; SKO, n = 6) across the two sucrose concentrations. Similarly, there were no differences in average sucrose intake between Per2CTR and Per2SKO in males (CTR, n = 9; SKO, n = 9) and in female (CTR, n = 10; SKO, n = 8) mice across the two sucrose concentrations (supplementary Fig. 2C and 2D). These results indicate that the changes in alcohol consumption and preference associated with striatal *Bmal1* or *Per2* deletion are not the result of overall changes in appetitive behavior, taste sensitivity or general reward processing. Also, because in both males and females, sucrose consumption did not differ between genotypes, alterations in alcohol drinking in Bmal1SKO or Per2SKO mice is likely not caused by higher caloric needs in these animals.

Deletion of *Bmal1* from Striatal MSNs Does Not Affect Circadian Wheel-Running Behavior

Global deletion of *Bmal1* or *Per2* or selective deletion of *Bmal1* or *Per2* from the SCN master circadian clock abolish circadian behavioral rhythms in animals [4, 49], and disruption of

circadian rhythms can influence alcohol consumption [50-52]. To determine whether the effects of *Bmal1* or *Per2* deletion in the striatum on alcohol intake and preference was not related to changes in global circadian rhythms induced by ectopic Cre recombinase expression, we monitored wheel-running behavior in alcohol naïve mice (n = 7-9 mice/genotype/sex) housed individually under different lighting conditions. The representative actograms presented in supplementary Fig. 3A show that circadian wheel running rhythms in Bmal1SKO and Bmal1HET male and female mice were indistinguishable from those in respective Bmal1CTR mice. Similarly, wheel running rhythms in Per2SKO and Per2HET male and female mice were indistinguishable from those in respective Per2CTR mice (Supplementary Fig. 3B). In particular, *Bmal1* or *Per2* deletion did not affect entrainment of daily wheel running to a 12:12 h light-dark cycle, adjustments to phase delay and advance shifts in the light cycle, and free running in constant conditions. These results show that Bmal1SKO and Per2SKO male and female mice and their respective HET mice have a functional SCN molecular clock and normal circadian pacemaking. Thus, the changes in alcohol consumption in SKO and HET male and female mice are independent of the SCN clock and not the result of disrupted circadian behavioral rhythms.

Deletion of *Bmal1* or *Per2* from MSNs Eliminates Sex Differences in Alcohol Consumption

Sex differences in alcohol intake and preference are well recognized in animals and humans, with females generally drink more alcohol than males [30-32, 53-55]. Consistent with this, average daily alcohol intake in female Bmal1CTR mice was significantly greater than in Bmal1CTR males ($P<0.0001$, unpaired two-tailed t test) and mean alcohol preference was significantly higher in Bmal1CTR females than Bmal1CTR males ($P<0.05$, unpaired two-tailed t test) (Supplementary Fig. 3A), which corresponds to an 78 % greater intake and 21 % higher preference in females. In contrast, mean alcohol preference in Bmal1SKO male mice was 40 % greater and significantly different compared to Bmal1SKO females ($P<0.005$, unpaired two-tailed t test), whereas no difference was observed in mean alcohol intake between Bmal1SKO males and females (Supplementary Fig. 3A). Similarly, average alcohol intake was 46 % greater and preference was 40 % higher in Per2CTR females compared to males, and both measures differed significantly between sexes (intake: $P<0.001$, unpaired two-tailed t test; preference: $P<0.01$, unpaired two-tailed t test) (Supplementary Fig. 3B). In contrast, there were no significant differences in mean alcohol preference and intake between Per2SKO males and females. These results show that selective deletion of *Bmal1* or *Per2* from MSNs eliminates sex differences in alcohol intake and preference by augmenting consumption and preference in males and suppressing intake and preference in females

Conclusions

Clock genes polymorphism is associated with alcohol consumption and alcohol use disorder. Our work reveals, for the first time, a causal role of *Bmal1* and *Per2* in striatal control of alcohol consumption. The influence of striatal *Bmal1* is sexually dimorphic and specific, associated with repression of alcohol preference and intake in male mice, possibly via effect of *Per2*, and with enhancement of preference and intake in females via a mechanism independent of *Per2*. Strikingly, while wild-type mice exhibit the well-established heightened female-specific alcohol intake and preference, the opposing effects of *Bmal1* and *Per2* deletion on males and females abolish this difference. This suggests that Bmal1-mediated gene expression may play a role in sexually dimorphic alcohol consumption in mice. The mechanisms that mediate this sexually dimorphic influence are as yet unknown but could involve sex-specific interactions of *Bmal1* and *Per2* with sex hormone receptors and dopamine signaling in MSNs [56-67]. Transcriptomic

analysis of differential striatal gene expression in this mouse model could define the downstream outputs of clock gene expression that affect sex differences in alcohol consumption. In summary, our findings uncover a novel *Bmal1*-linked mechanism in striatal MSNs that modulates alcohol intake in mice in a sexually dimorphic manner. We propose that changes in *Bmal1* and/or *Per2* expression in the striatum resulting from circadian disruption and other forms of stress may underlie sex differences in alcohol-related behavioral disorders.

Supplemental Information

Supplemental Information includes four figures and Supplemental Experimental Procedures.

Acknowledgments

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