Current Biology *18*, 678–683, May 6, 2008 ©2008 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2008.04.012

Report

# Regulation of Monoamine Oxidase A by Circadian-Clock Components Implies Clock Influence on Mood

**Gabriele Hampp,[1] Jürgen A. Ripperger,[1] Thijs Houben,[2] Isabelle Schmutz,[1] Christian Blex,[3] Stéphanie Perreau-Lenz,[4] Irene Brunk,[3] Rainer Spanagel,[4] Gudrun Ahnert-Hilger,[3] Johanna H. Meijer,[2] and Urs Albrecht[1,*]**

[1]Department of Medicine
Division of Biochemistry
University of Fribourg
1700 Fribourg
Switzerland
[2]Department of Molecular Cell Biology
Laboratory of Neurophysiology
Leiden University Medical Center
2300 RC Leiden
The Netherlands
[3]AG Functional Cell Biology
Center for Anatomy
Charité-Universitätsmedizin Berlin
10115 Berlin
Germany
[4]Department of Psychopharmacology
Central Institute of Mental Health
68159 Mannheim
Germany

## Summary

**The circadian clock has been implicated in addiction and several forms of depression [1, 2], indicating interactions between the circadian and the reward systems in the brain [3–5]. Rewards such as food, sex, and drugs influence this system in part by modulating dopamine neurotransmission in the mesolimbic dopamine reward circuit, including the ventral tegmental area (VTA) and the ventral striatum (NAc). Hence, changes in dopamine levels in these brain areas are proposed to influence mood in humans and mice [6–10]. To establish a molecular link between the circadian-clock mechanism and dopamine metabolism, we analyzed the murine promoters of genes encoding key enzymes important in dopamine metabolism. We find that transcription of the monoamine oxidase A (*Maoa*) promoter is regulated by the clock components BMAL1, NPAS2, and PER2. A mutation in the clock gene *Per2* in mice leads to reduced expression and activity of MAOA in the mesolimbic dopaminergic system. Furthermore, we observe increased levels of dopamine and altered neuronal activity in the striatum, and these results probably lead to behavioral alterations observed in *Per2* mutant mice in despair-based tests. These findings suggest a role of circadian-clock components in dopamine metabolism highlighting a role of the clock in regulating mood-related behaviors.**

## Results and Discussion

### The Murine *Maoa* Promoter Is Regulated by Clock Components In Vitro

We analyzed the promoter of *Maoa* for presence of E-box elements. These elements serve as potential binding sites for heterodimers of CLOCK/BMAL1 or NPAS2/BMAL1, key components of the circadian-clock mechanism [11]. In the promoter of *Maoa*, we found E-box elements, which are conserved among mouse, rat, and human, suggesting comparable regulation of this gene in these species (Figure 1A). To determine whether the *Maoa* promoter is regulated by clock components, we cloned a 1.1 kb promoter fragment of the murine *Maoa* (*mMaoa*) gene containing one canonical and two noncanonical E-boxes into a luciferase reporter vector. Cotransfection with clock components of this reporter construct into the neuroblastoma cell line NG108-15 revealed regulatory effects of clock proteins on the *mMaoa* promoter (Figure 1B) in a concentration-dependent manner (Figure S1A available online). Surprisingly, CLOCK/BMAL1 does not activate the *mMaoa* promoter in the neuroblastoma cell line (Figure 1B) but in COS-7 monkey kidney cells (Table S1), suggesting a possible involvement of cell-type-specific cofactors in this process. Cotransfection of *Cry1*, a clock component of the negative limb of the clock regulatory mechanism [12], dampened the activation by NPAS2/BMAL1 in neuroblastoma cells. Cotransfection of *Per2* resulted in increased activation (Figure 1B) as observed previously for the activation of the *aminolevulinate synthase 1* promoter [13]. To test whether the conserved classical E-box is of importance in the *mMaoa* promoter, we deleted it. This resulted in a shortened 0.7 kb promoter that was still activated by NPAS2/BMAL1, however in a strongly reduced manner, indicating functional importance of the most 5′ E-box in the 1.1 kb construct (Figures 1A and 1B). In contrast to *mMaoa*, neither a 1.2 kb fragment of the murine *monoamine oxidase B* (*mMaob*) promoter (Figure S1B) nor a 3.3 kb promoter fragment of the tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis (data not shown), displayed comparable effects in our assays. Taken together, our experiments indicate that the *mMaoa* promoter is prone to specific regulation by clock components in vitro.

### The *MaoA* Gene Is Hardwired Directly to the Circadian Oscillator

In order to test circadian functionality of the *mMaoa* promoter, we transfected the *mMaoa*-luciferase reporter construct into NG108-15 neuroblastoma cells and followed its expression by using real-time bioluminescence monitoring. After synchronization with dexamethasone [14], we monitored luciferase activity in the cell population over 4 days (Figure 1C and Figure S1C). We observed an ~24 hr oscillation of luciferase activity with the same phase as a control reporter construct containing four E-box elements derived from the clock-controlled *Dbp* gene [15]. Similar results were also obtained upon transfection of the construct into NIH 3T3 fibroblasts cells (Figure S1C). This indicates that the *mMaoa* promoter is capable of oscillating in a circadian fashion.

In a next step, we investigated the regulation of the *mMaoa* promoter in vivo. We wanted to know whether BMAL1 directly interacts with the *mMaoa* promoter in brain regions that express this gene. Chromatin immunoprecipitation with antibodies against BMAL1 revealed binding of this protein to the promoter of *mMaoa* in brain tissue containing the VTA (Figure 1D). This binding was also time dependent with

*Correspondence: urs.albrecht@unifr.ch

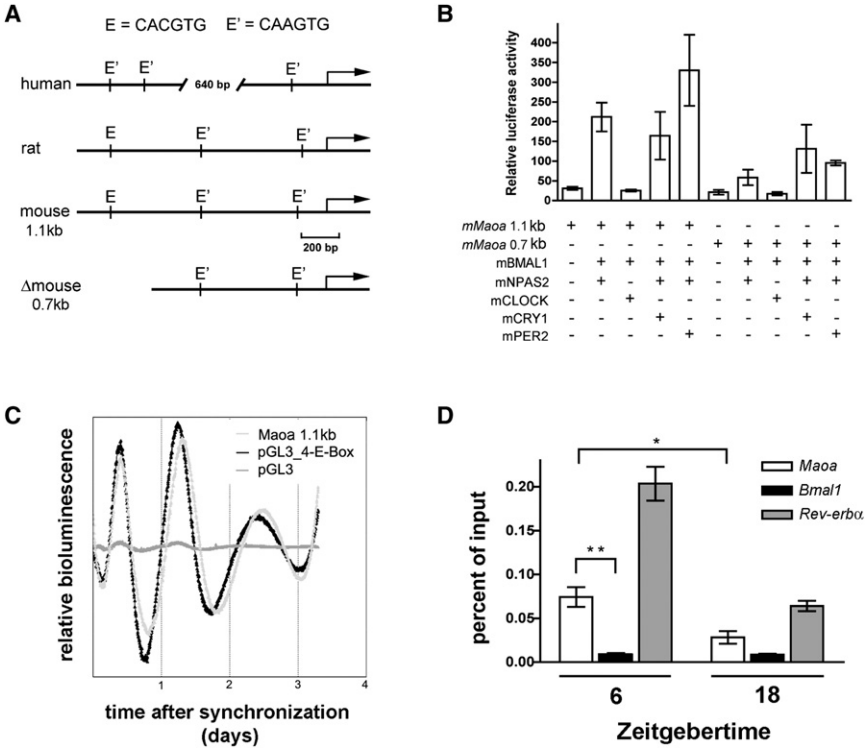


Figure 1. Regulation of the *mMaoa* Promoter by Clock Components

(A) Schematic representation of E-boxes conserved in human, rat, and mouse *Maoa* genes. Letters E and E' refer to sites of canonical and noncanonical E-box elements, respectively, serving as potential binding sites for the BMAL1-NPAS2 heterodimer. Arrows indicate the transcription start site.

(B) Transcriptional regulation of the *mMaoa* gene by clock components in NG108-15 cells. Luciferase reporter plasmids containing either a 1.1 kb *mMaoa* 5′ upstream region, including the three E-boxes (*Maoa* 1.1 kb) or a deletion of the canonical E-box (*Maoa* 0.7 kb) was used for the transcriptional assays. Presence (+) or absence (−) of the reporter and expression plasmids is shown. Each value represents the mean ± SD of three independent experiments with three replicates for each experiment.

(C) Circadian oscillations of luciferase reporter activity in dexamethasone synchronized NG108-15 cells. Detrended, normalized time series, each derived by averaging the bioluminescence profiles of two independent cultures (representative experiment out of three independent experiments), are shown. "pGL3" refers to a luciferase reporter (gray, negative control), "pGL3_4-E-box" refers to a pGL3 reporter containing four E-boxes of the *Dbp* promoter (black, positive control), "Maoa 1.1kb" refers to a pGL3 reporter containing a 1.1 kb promoter fragment of the mouse *Maoa* promoter (light-gray line).

(D) Binding of BMAL1 to the *mMaoa* promoter in mouse brain tissue collected at ZT 6 and ZT 18 as revealed by chromatin immunoprecipitation (ChIP). BMAL1 does not bind to its own promoter (black bars, negative control, $p > 0.05$, ZT 6 versus ZT 18) but binds in a time-dependent fashion to the *mRevErbα* promoter (gray bars, positive control, $p < 0.05$, ZT 6 versus ZT 18) and to the *mMaoa* promoter (white bars, *$p < 0.05$ and **$p < 0.01$). Each value represents the mean ± SEM of three independent experiments with the p values determined by the Student's t test.

significantly more BMAL1 binding at Zeitgeber time (ZT) 6 compared to ZT 18 ($p < 0.05$, t test) comparable to the time-dependent binding of BMAL1 to the promoter of *Rev-erbα*, a circadian-clock component (Figure 1D). The lower signal of BMAL1 binding at the *mMaoa* promoter in vivo probably reflects the fact that fewer cells in the analyzed brain region express *mMaoa* in a circadian manner as compared to the *Rev-erbα* gene. Binding of BMAL1 is not observed in the promoter region of the *mBmal1* gene, a circadian gene that does not regulate itself. BMAL1 binding at the *mMaoa* promoter was also not observed in the cortex region or the liver of the same animals (data not shown). We conclude that the *mMaoa* promoter can be regulated by BMAL1 in a time-dependent fashion in brain tissue containing the VTA.

### Expression of *Maoa* Is Reduced in *Per2* Mutant Mice

*Per2*$^{Brdm1}$ mutant mice display altered responses to drugs of abuse [2, 16], implying abnormal signaling in the mesolimbic dopaminergic system of these animals. Therefore, we investigated region-specific and time-dependent expression of *mMaoa* and *mMaob* in the mesolimbic system including the striatum and the VTA. We found cycling diurnal expression of *mMaoa* mRNA in the VTA of wild-type animals ($p < 0.01$, one-way ANOVA) with a maximum at ZT 6, whereas *mMaob* expression was not cycling diurnally (Figure 2A). No diurnal variation for both *mMaoa* and *mMaob* could be detected in *Per2*$^{Brdm1}$ mutant mice having a defective circadian clock ($p > 0.05$, one-way ANOVA). Significantly lower mRNA levels of *Maoa* were observed in these animals at ZT 6 ($p < 0.05$, two-way ANOVA) (Figure 2A; for micrographs, see Figure S2). Diurnal expression for *mMaoa* was also observed in the ventral striatum (NAc) for both genotypes with reduced expression in *Per2*$^{Brdm1}$ mutants at ZT 6 and ZT 12 ($p < 0.05$, two-way ANOVA), whereas *mMaob* expression was not diurnal (Figure 2B). However, *mMaob* expression was lower in *Per2*$^{Brdm1}$ mutants at ZT 18 ($p < 0.01$, two-way ANOVA). These observations support our finding that the *mMaoa* promoter can be regulated by clock components and that PER2 probably plays a positive role in this mechanism by increasing the amplitude (Figures 1B, 2A, and 2B). The expression analyses presented above indicate that *mMaoa* mRNA is stronger expressed than *mMaob* in parts of the mesolimbic dopaminergic system, but we do not know whether this translates to the protein level.

### MAOA Activity Is Reduced and Dopamine Levels Are Elevated in *Per2* Mutant Mice

Alterations in expression of *Maoa* mRNA in *Per2*$^{Brdm1}$ mutant mice lead us to determine the total activity of MAO (MAOA and MAOB) in the VTA. We find that it follows the mRNA expression pattern of *mMaoa* with a maximum of MAO activity at ZT 6 and a significantly cycling diurnal variation in wild-type mice ($p < 0.05$, one-way ANOVA) but no variation ($p > 0.05$, one-way ANOVA) and reduced activity in *Per2*$^{Brdm1}$ mutants ($p < 0.0001$, two-way ANOVA) (Figure 2C). In the striatum (composed of the caudate Putamen [CPu] and the NAc), to which the VTA projects, MAO activity was diurnal in wild-type mice ($p < 0.05$, one-way ANOVA). This activity was constant in *Per2*$^{Brdm1}$ mutant animals ($p > 0.05$, one-way ANOVA). The maximum of activity was delayed to ZT 12 in wild-type animals (Figure 2D) compared to the maximal activity in the VTA (Figure 2C), and activity was significantly reduced at this

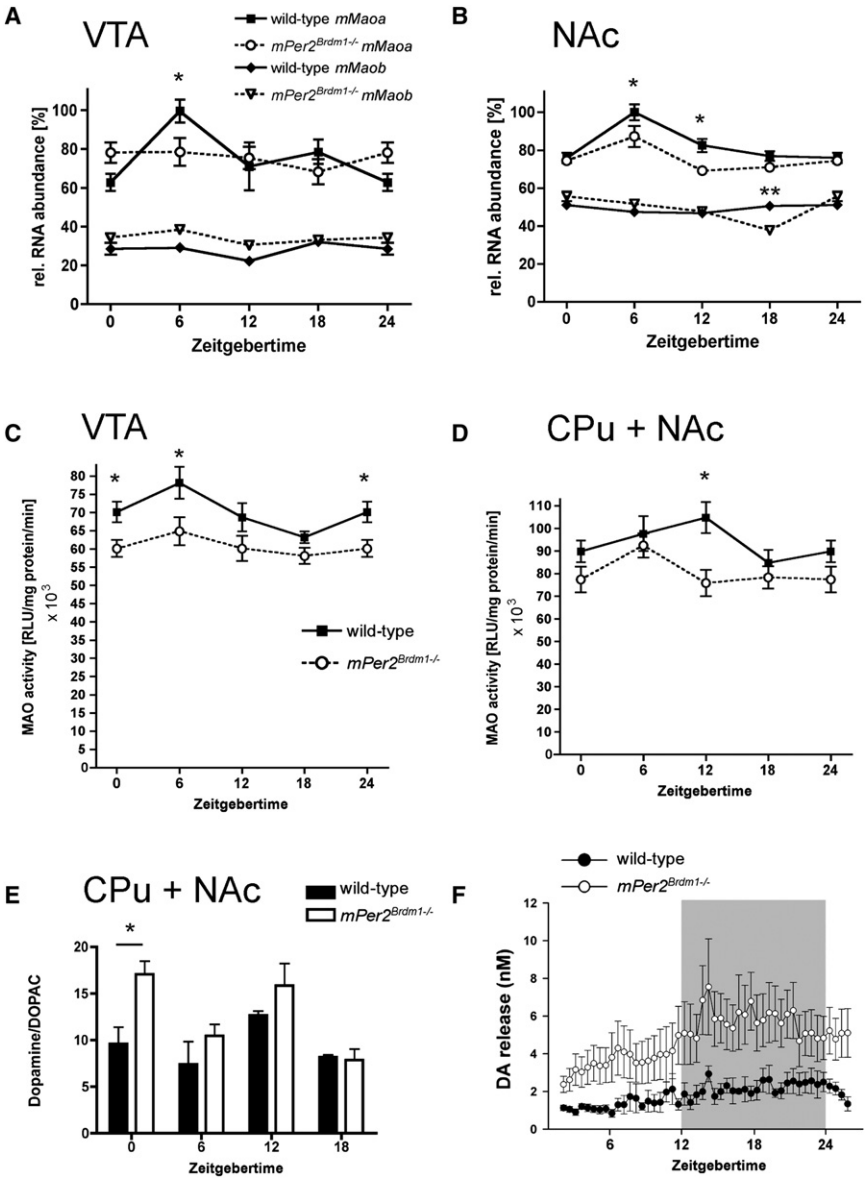


Figure 2. Expression of *mMaoa* and *mMaob*, MAO Activity, and Striatal Dopamine Levels in Wild-Type and *Per2*$^{Brdm1}$ Mutant Mice

(A) mRNA expression of *mMaoa* (wild-type, black squares; *Per2*$^{Brdm1}$ mutant, open circles) and *mMaob* (wild-type, black diamonds; *Per2*$^{Brdm1}$ mutant, open triangles) in the VTA (n = 3 per genotype each). Two-way ANOVA with Bonferroni post test revealed for *mMaoa* a significant effect between the genotypes at ZT 6 (*p < 0.05) but no difference between genotypes for *mMaob* (p > 0.05). One-way ANOVA shows a significant variation of expression over time for *mMaoa* in wild-type mice (p < 0.01) and no variation in *Per2*$^{Brdm1}$ animals (p > 0.05). No variation in time was observed in both genotypes for *mMaob* (p > 0.05). *mMaoa* was significantly more highly expressed than *mMaob* in both genotypes (p < 0.001).

(B) mRNA expression of *mMaoa* and *mMaob* in the ventral striatum (NAc) (n = 3 per genotype each). Two-way ANOVA with Bonferroni post test revealed for *mMaoa* a significant effect on genotype (p < 0.01) and time (p < 0.01) but no interaction between the two (p > 0.05). Significant differences between the genotypes at ZT 6 (*p < 0.05) and ZT 12 (*p < 0.05) were observed. For *mMaob*, a significant difference between genotypes was observed at ZT 18 (**p < 0.01). *mMaoa* was significantly more highly expressed than *mMaob* in both genotypes (p < 0.001).

(C) Enzymatic activity of MAO in the VTA (n = 3 per genotype). Two-way ANOVA revealed a significant effect on genotype (p < 0.0001) and time (p < 0.05) and no interaction between the two factors (p > 0.05). Bonferroni post test shows a significant difference between genotypes at ZT 6 (*p < 0.01) and ZT 0/24 (*p < 0.05). One-way ANOVA shows significant variation of enzyme activity over time in wild-type mice (p < 0.05) and no variation in *Per2*$^{Brdm1}$ animals (p > 0.05).

(D) Enzymatic activity of MAO in the striatum (CPu + NAc) (n = 3 per genotype). Two-way ANOVA revealed a significant difference in genotype (p < 0.001) and time (p < 0.05) but no interaction between the two (p > 0.05). Bonferroni post test shows a significant difference between genotypes at ZT 12 (*p < 0.001). One-way ANOVA shows significant variation of enzyme activity over time in wild-type mice (p < 0.05) and no variation in *Per2*$^{Brdm1}$ animals (p > 0.05).

(E) Dopamine/DOPAC ratio in the striatum (CPu + NAc). Two-way ANOVA revealed a significant difference in genotype (p < 0.05) and time (p < 0.01) but no interaction between the two (p > 0.05). Bonferroni post test shows a significant difference between genotypes at ZT 0/24 (*p < 0.05). Data for ZT 0 and ZT 24 are double plotted. Values represent the mean ± SEM.

(F) Extracellular levels of dopamine in the ventral striatum (NAc). *Per2*$^{Brdm1}$ animals showed higher basal levels of dopamine release compared to wild-types (p < 0,05; two-way ANOVA for repeated-measures; genotype $F_{1, 9} = 5.4$). A diurnal rhythm was observed in *Per2*$^{Brdm1}$ mice (p < 0.0001; one-way ANOVA; time $F_{47, 235} = 2.6$) as well as in wild-type littermates (p < 0.0001; one-way ANOVA; time $F_{47, 188} = 2.5$). Values represent the mean ± SEM (n = 5–6 per genotype).

time point in *Per2*$^{Brdm1}$ mutant mice (Figure 2D) (p < 0.001, two-way ANOVA). The delay of maximal MAO activity in the striatum compared to maximal *mMaoa* mRNA expression in the ventral striatum (NAc) might be the result of MAO activity in the CPu contributing, besides the NAc, to the total activity in the striatum. However, it appears that the reduced expression levels of *mMaoa* in *Per2*$^{Brdm1}$ mutants are reflected in the total MAO activity, indicating that in the mouse striatum, dopamine is metabolized predominantly by MAOA under basal conditions. This is consistent with previous findings in *Mao*-deficient mice [17, 18]. Taken together, our observations indicate that loss of functional PER2 lowers activity of MAO, which appears to be the result of reduced expression of *mMaoa*. Because dopamine is the most prominent neurotransmitter in the NAc of the striatum, we expected an increase in the dopamine to DOPAC ratio in this region of the brain. We found that this ratio was significantly elevated in the striatum (CPu and NAc) of *Per2*$^{Brdm1}$ mutant mice (p < 0.05, two-way ANOVA) (Figure 2E). This is consistent with our finding that MAO activity is reduced. To investigate whether this increase can be observed extracellularly, we performed microdialysis in the ventral striatum (NAc). We find that under basal conditions, dopamine release is significantly increased in *Per2*$^{Brdm1}$ mutant animals compared to wild-type littermates (p < 0.05; two-way ANOVA). Furthermore, we observed in both genotypes diurnal changes in the levels of this neurotransmitter;

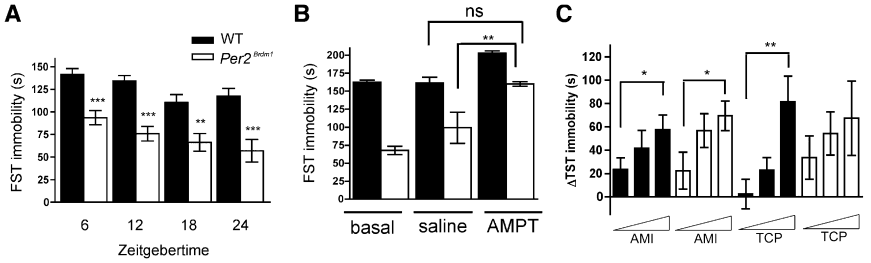


Figure 3. Depression-Resistant-like Phenotype in *Per2*$^{Brdm1}$ Mutant Mice and Relation to MAO

(A) Comparison of immobility in the forced-swim test (FST) between wild-type (black bars) and *Per2*$^{Brdm1}$ mutant mice (white bars) at different times (n = 6–14 per genotype). Two-way ANOVA shows a significant effect on genotype ($p < 0.0001$) but not on time ($p > 0.05$) and no interaction between these two factors ($p > 0.05$). One-way ANOVA with Bonferroni's multiple-comparison test reveals significant differences between the genotypes at all time points (**$p < 0.01$, ***$p < 0.001$).

(B) Rescue of the depression-resistant-like phenotype assessed by FST in *Per2*$^{Brdm1}$ mutant mice by blocking tyrosine hydroxylase activity with alpha-methyl-p-tyrosine (AMPT) at ZT 6. A Student's t test reveals significant differences after AMPT treatment (200 mg/kg) in *Per2*$^{Brdm1}$ mutant mice compared to basal levels and saline treatment (**$p < 0.01$, n = 9–12) but no significant difference (ns) to saline-treated wild-type animals.

(C) Decrease in immobility at ZT 6. Tail-suspension test (TST) performed 30 min after saline injection and the subsequent day 30 min after drug treatment with amitriptyline (AMI) and tranylcypromine (TCP). Concentrations used for AMI were 3, 6, and 9 mg/kg body weight and for TCP 6, 9, and 12 mg/kg body weight (n = 11–18). A Student's t test revealed significant differences between the lowest and the highest dose for the AMI treatment in both genotypes (*$p < 0.05$). For TCP treatment, only wild-type mice show a significant difference between the lowest and the highest dose (**$p < 0.01$). This is not the case for *Per2*$^{Brdm1}$ mutant mice, indicating the higher sensitivity of these animals to TCP. Values represent the mean ± SEM.

these changes are in opposite phases as compared to MAO activity in the same brain region. We conclude that loss of functional PER2 is likely to lower MAOA activity in the striatum, contributing to increased dopamine levels in *Per2*$^{Brdm1}$ mutant mice in this brain area. In contrast to *Maoa*-deficient mice that show aggressive behavior and elevated serotonin levels, we did not make these observations in *Per2*$^{Brdm1}$ mutants (data not shown), probably because *Maoa* expression is not completely eliminated in our mutants. However, studies that associate human *MAOA* with alcoholism [19, 20] highlight a possible correlation between reduced expression of *mMaoa* in *Per2*$^{Brdm1}$ mutants and increased ethanol intake in these animals [16].

### Differences in Despair-Based Behavioral Tests between *Per2*$^{Brdm1}$ Mutant and Wild-Type Mice

In humans, dopamine levels are related to mood [6]. Because *Per2*$^{Brdm1}$ mutant mice display increased levels of this neurotransmitter in the striatum, we wanted to probe for behavioral alterations by applying despair-based behavioral tests believed to correlate with human mood disorders [21]. We examined wild-type and *Per2*$^{Brdm1}$ mutant mice in the Porsolt forced-swim test (FST) and the tail-suspension test (TST). They measure the duration of immobility occurring after exposure of mice to an inescapable situation. However, they appear to be regulated by different sets of genes and hence may result in different outcomes [22]. These tests could be used because basal locomotor activity in the two genotypes is not different [2, 23]. The FST shows that *Per2*$^{Brdm1}$ mutant mice display significantly less immobility compared to wild-type animals ($p < 0.0001$, two-way ANOVA, Figure 3A). This indicates an increase in neurotransmitter levels in *Per2*$^{Brdm1}$ mutants. Because the response to cocaine [2] as well as expression and activity levels of *mMaoa* was highest at ZT 6 in the VTA, we performed all subsequent behavioral tests at ZT 6. To examine whether the lower immobility in *Per2*$^{Brdm1}$ mutants is due to their elevated dopamine levels (Figure 2F), we aimed to diminish the amount of dopamine in these animals. We treated the mice with alpha-methyl-p-tyrosine (AMPT), a potent inhibitor of tyrosine-hydroxylase (TH), the rate-limiting enzyme of catecholamine synthesis, to reduce dopamine levels. We find that AMPT increased immobility in *Per2*$^{Brdm1}$ mutants compared to saline-treated mutants ($p < 0.01$, t test), and immobility became comparable to saline-treated wild-type mice (nonsignificant difference [ns]; Figure 3B). We conclude that inhibition of TH leads to a behavioral rescue of *Per2*$^{Brdm1}$ mutants in the FST (Figure 3B) indicating an involvement of dopamine (and/or other catecholamines) in this phenotype.

In TST, we find that immobility times are not different between the genotypes (Figure S3). Different outcomes in these despair-based behavioral tests for mice are not unusual [22]. However, because of the comparable behavioral baselines of the two genotypes, we could use the TST to titrate MAO activity with an inhibitor in the two genotypes. Because *Per2*$^{Brdm1}$ mutants show less MAO activity (Figures 2C and 2D), we expected these animals to respond to lower doses of tranylcypromine (TCP), a MAO inhibitor. In comparison amitriptyline (AMI), a nonselective monoamine reuptake inhibitor mainly influencing serotonin and noradrenaline levels in the synaptic cleft should be effective at similar doses for both genotypes. We found these predictions to be met by intraperitoneal injections of AMI and TCP (Figure 3C). Both genotypes show a similar dose-dependent decrease in immobility for AMI, whereas *Per2*$^{Brdm1}$ mutant mice are more sensitive to TCP. These experiments are in agreement with the observation that *mMaoa* expression and MAO activity is reduced in *Per2*$^{Brdm1}$ mutants and therefore less inhibitor is necessary to abolish MAO function. Taken together, these findings indicate that *Per2*$^{Brdm1}$ mutants react differently compared to wild-types in tests believed to correlate with human mood disorders.

### Electrical Neuronal Activity Is Altered in *Per2* Mutant Mice in Response to MAO Inhibitors

To test how electrical activity is affected after treatment with AMI and TCP in wild-type and *Per2* mutant mice, we measured neuronal activity in the ventral striatum (NAc) in vivo. Multiunit activity recordings show that wild-type and *Per2*$^{Brdm1}$ mutant animals react similarly to AMI (Figures 4A, 4B, and 4E). Interestingly, wild-type mice do not show altered activity traces after injection of 6 mg/kg TCP at ZT 6. In contrast, *Per2* mutant mice display a strong response visible in the change of the activity trace after TCP injection at ZT6 (Figures 4C and 4D). It appears that neuronal activity in *Per2* mutant mice is significantly affected compared to that in wild-type animals ($p < 0.05$, t test, Figure 4E). This result indicates that *Per2* mutants are more sensitive to TCP than wild-type mice. This might be the result of lower amounts of MAOA enzyme due to a reduced expression of the *mMaoa* gene (Figure 2). Hence,

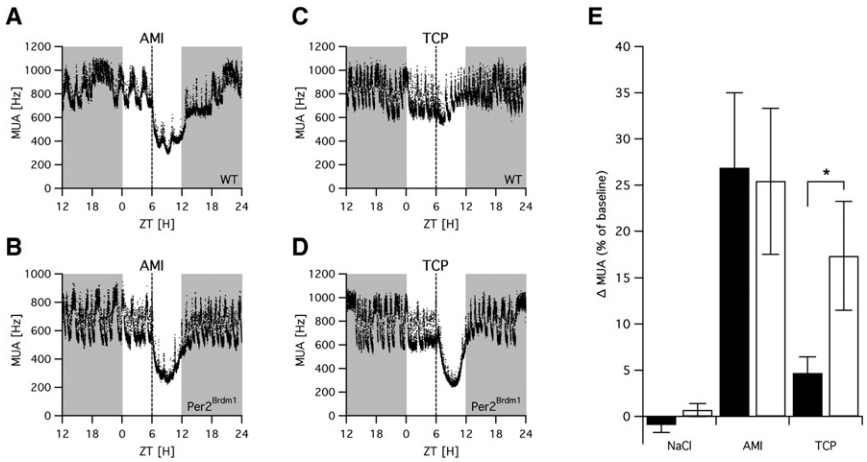


Figure 4. NAc Electrical Activity Responses to AMI and TCP Injections in Wild-Type and *Per2*$^{Brdm1}$ Mutant Mice

(A–D) Raw data traces show the effect of AMI or TCP injection on multiple unit activity (MUA) in the NAc of wild-type (WT) and *Per2*$^{Brdm1}$ mutant mice per 10 s. The gray background represents lights off, whereas the white background is lights on. Dotted lines indicate time of injection of 9 mg/kg body AMI or 6 mg/kg TCP. (A) shows the response to AMI in a WT animal. (B) shows the response to AMI in a *Per2*$^{Brdm1}$ mutant mouse. (C) shows the response to TCP in a WT animal. (D) shows the response to TCP in a *Per2*$^{Brdm1}$ mutant mouse.

(E) Comparison of the reduction of NAc firing rate in response to saline, AMI, and TCP injections between WT (black bars) and *Per2*$^{Brdm1}$ mutant (white bars) mice (n = 8–11). TCP responses were significantly different between WT and *Per2*$^{Brdm1}$ mutant mice (Student's t test, *p < 0.05). Values represent the mean ± SEM.

these mice are potentially useful to screen for drugs targeting MAOA to readjust intracerebral dopamine levels.

The behavioral and neuronal activity measurements for *Per2*$^{Brdm1}$ mutant mice (Figures 3 and 4 and [2]) could also be explained by a change in the expression of the dopamine transporter (DAT) and changes in dopamine receptors (DR1 and DR2). This seems to be unlikely because expression of DAT is not significantly altered in *Per2*$^{Brdm1}$ mutants (Figure S4). Furthermore, expression of the excitatory dopamine receptor DR1 is reduced, and expression of the inhibitory dopamine receptor DR2 is elevated (Figure S4). This indicates a compensatory response of the mutant organism to the elevated dopamine concentrations at the level of its receptors.

## Conclusions

Taken together, our results indicate a direct influence of circadian-clock components on *mMaoa* expression and activity in the mesolimbic dopaminergic system. In particular, PER2 appears to act as a positive factor, and its absence leads to reduced *Maoa* expression and activity resulting in elevated dopamine levels in the ventral striatum (NAc). The behavioral alterations that are observed in *Per2* mutant mice with tests modeling human mood disorders are probably due to the elevated dopamine levels. This implies that alterations in the clock, as they occur in shift workers, pilots, and people suffering from jet-lag, may have profound consequences for brain function including mood regulation by the mesolimbic dopaminergic system.

### Supplemental Data

Experimental Procedures, four figures, and three tables are available at http://www.current-biology.com/cgi/content/full/18/9/678/DC1/.

### Acknowledgments

We would like to thank Dr. S. McKnight and Dr. U. Schibler for reagents, Dr. John Reinhard, S. Baeriswyl-Aebischer, A. Hayoz, and G. Bulgarelli for technical assistance, and Dr. J.L. Dreyer, Dr. C. deVirgilio, and Dr. P. Lavenex for suggestions on the manuscript. This research was supported by the DFG SP383 (R.S.), the Velux Foundation (U.A.), the Swiss National Science Foundation (U.A.), and EUCLOCK (J.M. and U.A.).

Received: January 29, 2008
Revised: April 1, 2008
Accepted: April 3, 2008
Published online: April 24, 2008

### References

1. Baird, T.J., and Gauvin, D. (2000). Characterization of cocaine self-administration and pharmacokinetics as a function of time of day in the rat. Pharmacol. Biochem. Behav. *65*, 289–299.
2. Abarca, C., Albrecht, U., and Spanagel, R. (2002). Cocaine sensitization and reward are under the influence of circadian genes and rhythm. Proc. Natl. Acad. Sci. USA *99*, 9026–9030.
3. Andretic, R., Chaney, S., and Hirsh, J. (1999). Requirement of circadian genes for cocaine sensitization in Drosophila. Science *285*, 1066–1068.
4. Yuferov, V., Kroslak, T., Laforge, K.S., Zhou, Y., Ho, A., and Kreek, M.J. (2003). Differential gene expression in the rat caudate putamen after "binge" cocaine administration: Advantage of triplicate microarray analysis. Synapse *48*, 157–169.
5. Sanchis-Segura, C., and Spanagel, R. (2006). Behavioural assessment of drug reinforcement and addictive features in rodents: An overview. Addict. Biol. *11*, 2–38.
6. Nestler, E.J., and Carlezon, W.A., Jr. (2006). The mesolimbic dopamine reward circuit in depression. Biol. Psychiatry *59*, 1151–1159.
7. Lüscher, C. (2007). Drugs of abuse. In Basic and Clinical Pharmacology, 10th Edition, B.G. Katzung, ed. (New York: McGraw Hill), pp. 511–525.
8. Andretic, R., and Hirsh, J. (2000). Circadian modulation of dopamine receptor responsiveness in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA *97*, 1873–1878.
9. McClung, C.A., Sidiropoulou, K., Vitaterna, M., Takahashi, J.S., White, F.J., Cooper, D.C., and Nestler, E.J. (2005). Regulation of dopaminergic transmission and cocaine reward by the Clock gene. Proc. Natl. Acad. Sci. USA *102*, 9377–9381.
10. Roybal, K., Theobold, D., Graham, A., Dinieri, J.A., Russo, S.J., Krishnan, V., Chakravarty, S., Peevey, J., Oehrlein, N., Birnbaum, S., et al. (2007). Mania-like behavior induced by disruption of CLOCK. Proc. Natl. Acad. Sci. USA *104*, 6097–6098.
11. Liu, A.C., Lewis, W.G., and Kay, S.A. (2007). Mammalian circadian signaling networks and therapeutic targets. Nat. Chem. Biol. *3*, 630–639.
12. Wijnen, H., and Young, M.W. (2006). Interplay of circadian clocks and metabolic rhythms. Annu. Rev. Genet. *40*, 409–448.
13. Kaasik, K., and Lee, C.C. (2004). Reciprocal regulation of haem biosynthesis and the circadian clock in mammals. Nature *430*, 467–471.
14. Balsalobre, A., Brown, S.A., Marcacci, L., Tronche, F., Kellendonk, C., Reichardt, H.M., Schutz, G., and Schibler, U. (2000). Resetting of circadian time in peripheral tissues by glucocorticoid signaling. Science *289*, 2344–2347.
15. Ripperger, J.A., Shearman, L.P., Reppert, S.M., and Schibler, U. (2000). CLOCK, an essential pacemaker component, controls expression of the circadian transcription factor DBP. Genes Dev. *14*, 679–689.
16. Spanagel, R., Pendyala, G., Abarca, C., Zghoul, T., Sanchis-Segura, C., Magnone, M.C., Lascorz, J., Depner, M., Holzberg, D., Soyka, M., et al. (2005). The clock gene Per2 influences the glutamatergic system and modulates alcohol consumption. Nat. Med. *11*, 35–42.

17. Fornai, F., Chen, K., Giorgi, F.S., Gesi, M., Alessandri, M.G., and Shih, J.C. (1999). Striatal dopamine metabolism in monoamine oxidase B-deficient mice: A brain dialysis study. J. Neurochem. *73*, 2434–2440.
18. Cases, O., Seif, I., Grimsby, J., Gaspar, P., Chen, K., Pournin, S., Muller, U., Aguet, M., Babinet, C., Shih, J.C., et al. (1995). Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Science *268*, 1763–1766.
19. Vanyukov, M.M., Moss, H.B., Yu, L.M., Tarter, R.E., and Deka, R. (1995). Preliminary evidence for an association of a dinucleotide repeat polymorphism at the MAOA gene with early onset alcoholism/substance abuse. Am. J. Med. Genet. *60*, 122–126.
20. Hsu, Y.-P., Loh, E., Chen, W., Chen, C.-C., Yu, J.-M., and Cheng, A.T. (1996). Association of monoamine oxidase A alleles with alcoholism among male Chinese in Taiwan. Am. J. Psychiatry *153*, 1209–1211.
21. Castagné, V., Moser, P., Roux, S., and Porsolt, R.D. (2007). Rodent models of depression: Forced swim and tail suspension behavioral despair tests in rats and mice. Current Protocols in Pharmacology (Supplement 38).
22. Renard, C.E., Dailly, E., David, D.J., Hascoet, M., and Bourin, M. (2003). Monoamine metabolism changes following the mouse forced swimming test but not the tail suspension test. Fundam. Clin. Pharmacol. *17*, 449–455.
23. Zheng, B., Larkin, D.W., Albrecht, U., Sun, Z.S., Sage, M., Eichele, G., Lee, C.C., and Bradley, A. (1999). The *mPer2* gene encodes a functional component of the mammalian circadian clock. Nature *400*, 169–173.