Effects of the Circadian Rhythm Gene Period 1 (Per1) on Psychosocial Stress-Induced Alcohol Drinking

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Objective: Circadian and stress-response systems mediate environmental changes that affect alcohol drinking. Psychosocial stress is an environmental risk factor for alcohol abuse. Circadian rhythm gene period 1 (Per1) is targeted by stress hormones and is transcriptionally activated in corticotropin releasing factor-expressing cells. The authors hypothesized that Per1 is involved in integrating stress response and circadian rhythmicity and explored its relevance to alcohol drinking.

Method: In mice, the effects of stress on ethanol intake in mPer1-mutant and wild-type mice were assessed. In humans, single nucleotide polymorphisms (SNPs) in hPer1 were tested for association with alcohol drinking behavior in 273 adolescents and an adult case-control sample of 1,006 alcohol-dependent patients and 1,178 comparison subjects. In vitro experiments were conducted to measure genotype-specific expression and transcription factor binding to hPer1.

Results: The mPer1-mutant mice showed enhanced alcohol consumption in response to social defeat stress relative to their wild-type littermates. An association with the frequency of heavy drinking in adolescents with the hPer1 promoter SNP rs3027172 and with psychosocial adversity was found. There was significant interaction between the rs3027172 genotype and psychosocial adversity on this drinking measure. In a confirmatory analysis, association of hPer1 rs3027172 with alcohol dependence was shown. Cortisol-induced transcriptional activation of hPer1 was reduced in human B-lymphoblastoid cells carrying the risk genotype of rs3027172. Binding affinity of the transcription factor Snail1 to the risk allele of the hPer1 SNP rs3027172 was also reduced.

Conclusions: The findings indicate that the hPer1 gene regulates alcohol drinking behavior during stressful conditions and provide evidence for underlying neurobiological mechanisms.

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Although the interaction of stress response and circadian systems and their relevance to alcohol use have been documented at the behavioral and neuroendocrinological levels, the underlying integrative neurobiological mechanisms are not well established.

The molecular basis of circadian rhythmicity (circadian clock) involves a primary loop, with period 1, 2, and 3 (Per1, Per2, and Per3) and cryptochrome 1 and 2 (CRY1 and CRY2) proteins as transcriptional repressors and clock homologue NPAS2 [neuronal periodaryl hydrocarbon receptor nuclear translocator single-minded domain protein 2] and BMAL1 (brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1) proteins as transcriptional activators. This feedback cycle provides near-24-hour timing and drives the rhythmic expression of several clock-controlled and clock-modulated genes, which in turn mediate circadian rhythms in behavior and physiology. The circadian clock synchronizes and dictates the relative phasing of diverse internal physiological processes and molecular pathways. Clock genes also participate in reciprocal regulatory feedback, thereby rendering the circadian clock responsive to the internal environment of the body (11).

There is increasing evidence that the circadian clock gene Per1 is a target gene for glucocorticoids in mice (12) and humans (13) as well as stress-induced transcriptional activation in corticotropin releasing factor-expressing cells (14). We hypothesized that Per1 might be involved in the integration of stress response and circadian rhythmicity and aimed to explore this gene’s relevance to alcohol drinking behavior.

Method

Behavioral Animal Experiments

We examined mice with a mutation of the clock gene (Per1Brdm1). The suppression of this gene results in a behavioral alteration of clock phase. Mice deficient for mPer1 have no circadian rhythms in locomotor activity, nor do they have clock or clock-controlled gene expression (15) (see the data supplement accompanying the online version of this article). The effect of three different stressors on ethanol intake was tested. Once alcohol intake was stable, the mice underwent 3 consecutive days of one stressor, and drinking data were compared with that from the last 3 days of the baseline. The stressors consisted of 6 minutes of swim stress (basin height: 10 cm, 21°C), 5 minutes of unexpected foot shock (0.150 mA; duration: 1–3 seconds; time interval: 1–15 seconds), and a social defeat test in which intruder Per1 mice and wild-type mice were allowed to interact with an aggressive CD-1 mouse for 15 minutes, during which time the Per1 and wild-type mice were attacked (see the data supplement).

Adolescent Sample

Adolescents were recruited via the Mannheim Study of Children at Risk, an epidemiological cohort study examining the outcome of early risk factors from infancy into adulthood (16, 17). The present investigation encompassed 273 adolescents (boys: N=130, girls=143) who participated in the 18-year assessment and for whom genetic data were available (see the data supplement). Adolescents participated in a telephone interview regarding their current drinking behavior. The total amount of alcohol intake during the last 6 months was measured using the Lifetime Drinking History questionnaire. Two drinking variables were derived indicating the average amount of alcohol consumed per drinking day and the frequency of heavy drinking per month, defined as consumption of more than five (four for female adolescents) standard drinks (each with 8–12 g of alcohol) in a row. Data on psychosocial adversity according to an “enriched” family adversity index, as proposed by Rutter and Quinton (18), were derived from a standardized parent interview conducted at the 3-month follow up evaluation, which assessed characteristics of parents, partnership, and family environment during a period of 1 year prior to birth (mean score=1.93 [SD=2.06]; range=0–7) (see Table 1 in the data supplement).

Adult Case-Control Sample

A total of 1,006 adult patients with alcohol dependence were recruited from the Department of Psychiatry at the University of Regensburg (Regensburg, Germany). Patients were admitted consecutively for inpatient treatment and met criteria for alcohol dependence according to DSM-IV. A total of 1,178 individuals were recruited via the University of Bonn (Bonn, Germany) from 2001 to 2003, as part of the German National Genome Research Project, to serve as a comparison sample for genetic studies of several neuropsychiatric phenotypes. For further details on the adult case-control sample, see Table 1 in the data supplement. Informed consent was obtained from all human subjects. All studies were approved by the relevant ethics committees.

Mutation Screening and Identification of Single Nucleotide Polymorphisms (SNPs)

SNPs were identified by sequencing 32 Caucasian DNA samples. Sixteen DNA pools consisting of an equimolar mixture of two DNA samples were prepared and used as polymerase chain reaction (PCR) templates. SNPs were genotyped using the TaqMan system. Probes and primers were created using Assay-on-Demand (Applied Biosystems, Foster City, Calif.).

Statistical and Bioinformatic Analysis of Human Data

Data for minor allele frequencies for SNP makers, Hardy-Weinberg equilibrium tests, and the Cochran-Armitage trend test for single SNP association were calculated using PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/gplink.shtml). To examine gene-by-environment effects on adolescent drinking, linear-regression analyses were performed with sex as a covariate. Models were fitted for the main effects of the hPer1 rs3027172 genotype and psychosocial adversity ratings, with subsequent addition of the interaction term. Significant interactions were further investigated using simple main-effects analyses or simple contrasts. Genotype groups did not differ with regard to sex, age, IQ, and psychosocial adversity (see Table 1 in the data supplement).

Cellular Experiments

Epstein-Barr virus-transformed human B-lymphoblastoid cell lines were established from patients’ blood lymphocytes, genotype-specific for rs3027172 in the hPer1 gene. There were seven cell lines carrying the TT allele and six cell lines with the CC allele. For baseline hPer1 expression analysis, cells were synchronized with 50% horse serum. For detailed information on standard methods for quantitative real-time PCR, cortisol stimulation, electrophoretic mobility shift assay, and western blot, see the data supplement.

Results

Enhanced Stress-Induced Alcohol Consumption in Per1Brdm1-Mutant Mice

We investigated stress-induced alcohol consumption in Per1Brdm1-mutant and wild-type mice. As in our previ-
ous report (19), basal alcohol intake in Per1Brdm1-mutant mice did not differ from that of wild-type mice, except for a disruption of circadian intake patterns (see Figure 1 in the data supplement). When exposed for 3 consecutive days to social defeat stress, which models psychosocial adversity (20), mice with both genotypes showed significantly enhanced alcohol intake following social defeat stress. However, stress-induced alcohol consumption was significantly more pronounced in Per1Brdm1-mutant mice (repeated-measures analysis of variance: stress-by-genotype interaction: F=6.8, df=3, 42, p=0.0008) (Figure 1). This genotype difference became further evident with repeated swim stress (stress-by-genotype interaction: F=4.1, df=3, 140, p=0.004) (see Figure 1 in the data supplement) and foot shock stress (stress-by-genotype interaction: F=10.1, df=3, 117, p=0.000006), demonstrating that different stressors led to enhanced alcohol consumption in the Per1Brdm1-mutant mice. However, social defeat stress produced the most pronounced effect. Since blood corticosterone levels following stress were similar in both wild-type and Per1Brdm1-mutant mice (see Figure 1 in the data supplement), we ruled out a corticosterone-induced increase in alcohol intake (16). Thus, we demonstrated a stress-induced gene-by-environment interaction resulting in increased alcohol consumption, which was not dependent on an altered corticosterone response in the Per1Brdm1-mutant mice.

hPer1 Haplotype Association With Frequency of Heavy Drinking in Adolescents

To examine the possible role of hPer1 in stress-induced gene-by-environment interactions mediating adolescent drinking behavior, we first sequenced regulatory domains, exons, and exon/intron boundaries of this gene in 32 participants from the Centre d’Etude du Polymorphisme Humain reference sample (http://www.cephb.fr/en/cephdb/). We identified 18 genetic variations (two SNPs in the 5’UTR-region; four synonymous SNPs in exons; and 12 variations in introns). Nine of the genetic variations detected were not present in the Single Nucleotide Polymorphism Database (see Table 2 in the data supplement). We selected three haplotype tagging SNPs (rs3027172, rs2304911, and rs2735611) to identify the main haplotypes of heavy drinking per month, a measure of risky drinking. An omnibus test showed significant association with the frequency of heavy drinking (p<0.02, Bonferroni corrected; log likelihood ratio=9.259), and haplotype-specific tests revealed significant association of the TTC haplotype with this phenotype (beta=0.495, p=0.003 [after 10,000 permutations]) (Table 1). The association observed using the omnibus test did not remain significant when we dropped SNP rs3027172, suggesting that haplotypic association is driven by this SNP. Therefore, we selected rs3027172 for subsequent analyses.

Effects of Psychosocial Stress on Heavy Drinking Frequency Moderated by hPer1 SNP rs3027172

We then assessed whether the effect of psychosocial adversity on risky alcohol drinking in the Mannheim Study of Children at Risk sample was moderated by the rs3027172 genotype. In addition to the frequency of heavy drinking (p<0.02, Bonferroni corrected), linear regression revealed a main effect of the rs3027172 genotype on the average amount of alcohol consumed (p=0.008, Bonferroni corrected). Psychosocial adversity had a significant effect on these drinking measures, and there was a significant interaction effect between the rs3027172 genotype and psychosocial adversity on the frequency of heavy, exces-

FIGURE 1. Effects of Social Defeat Stress on Alcohol Drinking Behavior in Wild-Type and Per1Brdm1-Mutant Mice

Drinking data (3-day periods) are depicted as the percentage of increase with respect to the last 3 days of baseline drinking. Exposure to social defeat stress resulted in a significant increase in alcohol intake among both genotypes (p<0.05). The Per1Brdm1-mutant mice showed augmented alcohol intake relative to wild-type mice (Newman-Keuls test, p<0.01). Both genotypes recovered to baseline drinking levels after 3 days following stress application (poststress days 4–6).
Homozygote T | C Allele

Psychosocial Adversity

Frequency of Heavy Drinking (per month)

0.0 1.0 2.0 3.0 4.0

Low Moderate High

87 65 40 29 41 20

rs2253820
A>G

rs3027172
t>C

rs2304911
t>C

A

Stop

FIGURE 2. Human Period 1 (hPer1) Haplotype Single Nucleotide Polymorphisms (SNPs) Associated With Heavy Drinking in Adolescents (N=273)\(^a\)

\(^a\) The top image (A) depicts hPer1 haplotype SNPs. A map of the Per1 gene shows the exons (boxes) and introns (solid lines) and the position of the haplotype SNPs discriminating haplotypes with a frequency >10% (image size is not to scale). The bottom image (B) depicts the frequency of current monthly heavy drinking, adjusted for sex, in adolescents grouped by the hPer1 rs3027172 genotype and exposure to psychosocial adversity. For this analysis, genotypes were classified according to the presence of the minor C allele, and psychosocial adversity was determined using a tripartite split in the psychosocial adversity ratings (low: 0–1, moderate: 2–3, and high: ≥4 adversity factors present).

Catecholamines that carriers of the C allele who had been exposed to severe adversity displayed a higher frequency of heavy drinking than homozygous carriers of the T allele. In contrast, no association between genotype and frequency of heavy drinking was detected among individuals who had been exposed to low or moderate adversity.
Associated Alcohol Dependence in Adults With hPer1 SNP rs3027172

Since we were interested in examining whether Per1 has a more general relevance for adult alcohol dependence, we analyzed 2,184 individuals (adult patients with alcohol dependence, N=1,006; comparison subjects, N=1,178) of German descent who were not assessed for psychosocial adversity (see Table 1 in the data supplement). Because it is unlikely that all alcohol-dependent individuals were drinking alcohol in response to a highly adverse psychosocial load, this sample might have reduced power to detect drinking behavior resulting from gene-by-environment interactions, such as the aforementioned one. Nevertheless, we found a significant association of carriers of the C allele of rs3027172 in more advanced phases of alcohol abuse and dependence.

Cortisol-Induced, Genotype-Specific Transcriptional Activation of hPer1 Involving Binding of the Snail1 Transcription Factor

We next investigated molecular mechanisms, which might underlie the observed Per1-dependent stress response. We selected six hPer1 B-lymphoblastoid cell lines according to the rs3027172 genotype. First, we analyzed genotype-specific expression of hPer1 in synchronized, unstimulated B-lymphoblastoid cell lines and found a significantly higher expression (p<0.05) in CC cell lines relative to TT cell lines (Figure 3). Since a glucocorticoid-induced expression of Per1 was previously reported (12), we investigated the effect of the stress hormone cortisol in a physiologically relevant dose on genotype-specific expression of hPer1 mRNA using quantitative real-time PCR in genotype-specific B-lymphoblastoid cell lines. Following incubation with cortisol (1 μM) for a duration of 4 hours, we observed a fourfold significant increase (p<0.01) in hPer1 mRNA in cell lines carrying the TT genotype of rs3027172, whereas no cortisol-induced stimulation in hPer1 expression could be observed in cells carrying the CC genotype.

To investigate the molecular basis of the observed genotype-specific transcriptional activation, we conducted bioinformatic functional prediction analyses. The rs3027172 SNP is localized in the promoter region of the hPer1 gene, where it is flanked by two putative transcription factor binding sites. One site is a binding site for the c-Rel protein, a member of the NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) family of transcription factors (21); the other is similar to an E2-box binding site for members of the Snail family of transcriptional regulators (22) (Figure 4). Presence of the C allele at rs3027172 destroys all similarity of this DNA sequence with an E2-box.

To experimentally validate the predicted disruption of the E2-box transcription factor binding site by rs3027172, we conducted electrophoretic mobility shift assays and investigated whether there is a DNA binding factor that differentially binds to T and C alleles of this SNP. Extracts of THP-1 (human acute monocytic leukemia cell line) cells, incubated with labeled c-Rel oligonucleotide, produced a specific c-Rel/NF-kB complex (Figure 4). To induce c-Rel protein, these cells were activated with lipopolysaccharide. The T- and C-allele hPer1 oligonucleotides did not form complexes with c-Rel and NF-kB, as evident from competition experiments with a labeled c-Rel probe and from experiments in which T- and C-allele hPer1 oligonucleotides were used as labeled probes. However, incubation with cortisol in cultured B-lymphoblastoid cell lines carrying the TT genotype of rs3027172 resulted in significantly higher expression (p<0.05) than in TT cell lines (Figure 3).
tion of the T-allele hPer1 probe with the extract produced a novel complex, the formation of which was blocked by unlabeled T-allele hPer1 oligonucleotide but not by C-allele hPer1, c-Rel, or NF-xB oligonucleotides. The C-allele hPer1 probe also formed this complex but with lower intensity. Thus, this protein factor had higher affinity for binding to T-allele hPer1 oligonucleotide compared with C-allele hPer1 oligonucleotide. The T-allele hPer1 binding factor demonstrated predominantly nuclear localization in the human brain and was also present at high levels in rat embryonic brain. Oligonucleotide with a canonical E2-box but not a mutated E2-box blocked binding of the factor to the T-allele hPer1 probe, suggesting that T-allele hPer1 binding factor is an E-box binding protein. Furthermore, monoclonal anti-Snail-antibodies, but not unrelated mouse immunoglobulin G antibodies, depleted formation of the novel complex, suggesting that T-allele Per1 binding factor is identical to the Snail1 transcription factor.

Discussion

Gene-by-environment interactions account for substantial heterogeneity in response to environmental factors contributing to psychiatric disorders (23). Circadian and stress-response systems are important physiologic mediators of environmental influences. In research on alcohol dependence, stress-related gene-by-environment interactions have been described (1), and one prominent example relates to the finding that CRHR1 moderates the effect of psychosocial stress on alcohol consumption in rodent animal models (20, 24) as well as in humans (25, 26). In the present article, we show that psychosocial stress moderates the effect of circadian rhythm gene Per1 on excessive alcohol drinking. Mutant mice that lack a functional Per1 gene, as a result of a genetic deletion of the PAS-binding domain, responded to repeated adverse stress exposure with enhanced alcohol consumption. This finding translates to our two human samples (one cohort of adolescents with a high adverse psychosocial load and one adult case-control sample of patients meeting DSM-IV criteria for alcohol dependence and comparison subjects, encompassing 2,457 individuals total).

We further examined underlying molecular mechanisms of the observed gene-by-environment interaction and conclude—from a series of in vitro experiments—that these associations might be caused by cortisol-induced transcripational activation of Per1, which appears

![FIGURE 3. Expression of the Human Period 1 (hPer1) Gene in TT- and CC-Allele Cell Lines](image-url)

**TABLE 3. Alcohol Dependence Associated With the rs3027172 Genotype in Adult Case Patients and Comparison Subjects**

<table>
<thead>
<tr>
<th>Allele</th>
<th>CC (N=104)</th>
<th>CT (N=799)</th>
<th>TT (N=1,281)</th>
<th>Total (N=2,184)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Case patients</td>
<td>56</td>
<td>5.57</td>
<td>381</td>
<td>37.87</td>
</tr>
<tr>
<td>Comparison subjects</td>
<td>48</td>
<td>4.07</td>
<td>418</td>
<td>35.47</td>
</tr>
</tbody>
</table>

*Analysis using the Cochran-Armitage trend test (one-sided) showed statistical significance (χ²=2.1347, df=1, p<0.02 [odds ratio=1.161, 95% confidence interval=1.008–1.336]).

Am J Psychiatry 168:10, October 2011
FIGURE 4. Genotype-Specific Transcriptional Factors Binding to the rs3027172 Single Nucleotide Polymorphism (SNP) of the Human Period 1 (hPer1) Gene

A) A schematic drawing of transcription factors binding to the rs3027172 (SNP2) region in the hPer1 gene is shown. The localization of SNP2 in hPer1 is depicted in red. In the TT allele of hPer1, DNA sequences around SNP2 are identified by the following two transcription factors: Snail and c-Rel. The C allele > T allele substitution of rs3027172 results in the disappearance of the Snail transcription factor binding site in the C allele. B) Results from an electrophoretic mobility shift assay are also shown. Extracts of human lipopolysaccharide-activated mononuclear THP-1 cells (lane 6), incubated with labeled c-Rel oligonucleotide, produced a specific c-Rel/NF-kB complex (lanes 1, 2, 5). T- and C-allele hPer1 (T-hPer1 and C-hPer1) oligonucleotides did not form complexes with c-Rel and NF-kB, as evident from competition experiments with a labeled c-Rel probe (lanes 3, 4) and from experiments in which these oligonucleotides were used as labeled probes (lanes 6–15). A novel complex was produced with the T-hPer1 probe (lanes 11–15) and, with lower intensity, with the C-hPer1 probe (lanes 6–10). T-hPer1 binding factor (BF) showed higher affinity to the T allele (lane 13) relative to the C allele (lane 14) of Per1, as evident from competition experiments. Nonspecific DNA binding complex was formed by Ku protein, which preferentially binds to double-stranded DNA ends and is abundant in human brain tissues. C) Brain expression of T-Per1 BF is shown. T-Per1 BF is present in the adult human brain, where it shows predominantly nuclear localization, as well as in the rat embryonic brain (rEB). Human prefrontal cortex nuclear (hPFCn) extract (lanes 1–3) and human prefrontal cortex cytosolic (hPFCc) fraction (lanes 4–6), prepared from prefrontal cortical tissue obtained from a 56-year-old man, and total extract of rEB tissue (lanes 7–9), obtained from 18-day-old embryos, were analyzed by electrophoretic mobility shift assay. D) Inhibition of T-Per1 BF formation by E-box oligonucleotide is shown. Wild-type E-box (E-box [lane 2]) oligonucleotide but not mutant E-box (mE-box) oligonucleotide (lane 3) inhibited the formation of T-Per1 BF with the T-Per1 probe. E) Inhibition of T-Per1 BF formation by anti-Snail monoclonal antibodies (Sn-MAb) is shown. The Sn-MAb (0.5 μg [lanes 2, 5]), but not the unrelated anti-GAP43 monoclonal antibodies (1.0 μg [lanes 3, 6]), completely depleted complexes formed by T-hPer1 BF from hPFC (lanes 2, 3) and rEB (lanes 5, 6) with the T-hPer1 probe.
rs3027172 with alcohol dependence in adults not only indicates the role of *Per1* as an entry mechanism into addictive behavior but also suggests its relevance for manifestation of alcohol dependence. The modest strength of the observed association may reflect the heterogeneity of the alcohol-dependent sample with respect to environmental exposure to adverse stress.

Cortisol is a main mediator of stress responding and serves as a master switch in the control of neuronal and network responses that underlie behavioral adaptations to stress (2, 4). There is increasing evidence that *Per1* is a target gene for glucocorticoids, including cortisol in mice (12) as well as in humans (13). In line with these observations, we found significant cortisol-induced increase in the levels of *HPer1* transcripts in human B-lymphoblastoid cell lines, which carry the protective genotype, but we did not find increased *HPer1* expression following cortisol exposure in cells carrying the risk genotype (rs3027172). Therefore, we hypothesize that there might be an altered response to stress-induced cortisol increase in carriers of the rs3027172 risk allele (C allele). Our electrophoretic mobility shift assay data further suggest that this altered stress response might be the result of a decreased binding affinity of the zinc finger protein Snail1 (21, 22) to the promoter SNP rs3027172. This finding was further validated in an alcohol-dependent case-control sample. The rs3027172 genotype might be involved in cortisol-induced genotype-specific transcriptional activation of *hPer1*, which is mediated in part by the transcription factor Snail1. Although the interpretation of gene-by-environment interaction in genetic association studies is complicated by the fact that environmental and genetic components are bi-directionally interconnected (i.e., the environment and the individual affect each other) (25, 26), our in vitro and in vivo functional genetic analyses suggest that the effects of exposure to psychosocial stress on risky alcohol consumption are mediated in part by variation in the *Per1* gene.

Drs. Dong and Bilbao contributed equally to this study.

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References

13. Reddy TE, Pauli F, Sprouse RO, Neff NF, Newberry KM, Gara-
bedian MJ, Myers RM: Genomic determination of the gluco-
corticoid response reveals unexpected mechanisms of gene
T, Shibata S: Physical and inflammatory stressors elevate cir-
cadian clock gene mPer1 mRNA levels in the paraventricular
nucleus of the mouse. Endocrinology 2001; 142:4910–4917
of the mPer1 and mPer2 genes in the mammalian circadian
Steigleider P, Stock B, Stoehr RM, Weindrich D, Schmidt MH:
Behavioral sequelae of perinatal insults and early family ad-
versity at 8 years of age. J Am Acad Child Adolesc Psychiatry
2000; 39:1229–1237
17. Laucht M, Esser G, Schmidt MH: Developmental outcome of
infants born with biological and psychosocial risks. J Child Psy-
chol Psychiatry 1997; 38:843–853
18. Rutter M, Quinton D: Psychiatric disorder: ecological factors
and concepts of causation, in Ecological Factors in Human De-
velopment. Edited by McGurk H. Amsterdam, North-Holland
G, Spanagel R: Ethanol self-administration and reinstatement
of ethanol-seeking behavior in Per1(Brdm1) mutant mice. Psy-
chopharmacology (Berl) 2007; 190:13–19
20. Sillaber I, Rammes G, Zimmermann S, Mahal B, Ziegglänsberg-
er W, Wurst W, Holboer F, Spanagel R: Enhanced and delayed
stress-induced alcohol drinking in mice lacking functional
CRHR1 receptors. Science 2002; 296:931–933
21. Parry GC, Mackman N: A set of inducible genes expressed by
activated human monocyotic and endothelial cells contain kapp-
a B-like sites that specifically bind c-Rel/p65 heterodimers. J
Biol Chem 1994; 269:20823–20825
22. Peiró S, Escrivá M, Puig I, Barberá MJ, Dave N, Herranz N, Larr-
ría MJ, Takkunen M, Francé C, Muñoz A, Virtanen I, Baulida J,
García de Herreros A: Snail1 transcriptional repressor binds to
its own promoter and controls its expression. Nucleic Acids Res
2006; 34:2077–2084
23. Gaspi A, Moffitt TE: Gene-environment interactions in psychia-
try: joining forces with neuroscience. Nat Rev Neurosci 2006;
7:583–590
24. Hansson AC, Cippitelli A, Sommer WH, Fedeli A, Björk K, Sove-
chia L, Terasmaa A, Massi M, Heilig M, Ciccioppo R: Variation
at the rat Crhr1 locus and sensitivity to relapse into alcohol
seeking induced by environmental stress. Proc Natl Acad Sci U
SA 2006; 103:15236–15241
M, Saam C, Lascorz J, Soyka M, Preuss UW, Rujescu D, Skow-
ronek MH, Rietschel M, Schumann G: Genetic association of the hu-
m an corticotropin-releasing hormone receptor 1 (CRHR1) with
binge drinking and alcohol intake patterns in two independent samples. Mol
Psychiatry 2006; 11:594–602
tion between CRHR1 gene and stressful life events predicts ado-
lescent heavy alcohol use. Biol Psychiatry 2008; 63:146–151
27. Heilig M, Koob GF: A key role for corticotropin-releasing fac-
28. Desrivières S, Lourdusamy A, Müller C, Ducci F, Wong CP, Kaaki-
en M, Pouta A, Hartikainen AL, Isohanni M, Charoen P, Pel-
ton L, Freimer N, Elliott P, Jarvelin MR, Schumann G: Glu-
corticoid receptor (NR3C1) gene polymorphisms and onset
29. Rose RJ, Dick DM, Viken RJ, Pulkkinen L, Kaprio J: Drinking or
abstaining at age 14? a genetic epidemiological study. Alcohol
30. Buchmann AF, Schmidt B, Blomeyer D, Ziegglänsberger W, Wurst W,
Holsboer F, Spanagel R, Heinz A, Laucht M, Mann K, Schumann G: Genetic asso-
ciation of the human corticotropin-releasing hormone receptor 1 (CRHR1) with
binge drinking and alcohol intake patterns in two independent samples. Mol
Psychiatry 2006; 11:594–602
31. Viner RM, Taylor B: Adult outcomes of binge drinking in ado-
lescence: findings from a UK national birth cohort. J Epidemiol
Comm Health 2007; 61:902–907
32. Vengeliene V, Celerier E, Chaskiel L, Penzo F, Spanagel R: Com-
33. Chambers RA, Taylor JR, Potenza MN: Developmental neurocir-
cuitry of motivation in adolescence: a critical period of addiction