mately 9000, implying that the number of individuals peopling the Americas in the first wave corresponds to as much as 1% of the entire East Asian population. The results also show that there could have been substantial levels of migration between Asians and Amerindians in the years after the first wave of migration. Nonetheless, the study clearly describes a picture of demographic events that include strong growth in the population size after the first wave of migration and a very recent migration event.

Some of the parameters of interest could not be estimated with great certainty. For example, the date of the first migration event was associated with much statistical uncertainty, and the relative importance of migration after the first migration event could not be determined. Although this could be seen as a weakness of the study, it really points to the strength of the methodology. The method is based on a statistical method that takes all the relevant information from the genetic data into account. So when some of the parameters are difficult to estimate, it implies that the data does not contain enough information about these parameters. In this way, the methodology significantly helps to quantify the uncertainty in the data. It also raises serious concerns about previous studies which, based on much less data, and without the use of rigorous statistical methods, have made strong claims about human demography using genetic data.

What sets Hey's study apart from other similar studies is the use of complex and more realistic models. While no model can be exactly true, the approach by Hey can help distinguish good models from bad ones. Genetic data in human demographic studies have often been analyzed by interpreting an estimated gene tree or network. As Hey points out, the verbal interpretations are themselves models that often are very simplistic. The method presented by Hey is an important step forward in the field of human genetic demographics, replacing *Ad hoc* story telling with rigorous model testing and statistical inference■

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Biological Clock

Biological clocks may modulate drug addiction

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A recent study by McClung's group (2005),[1] expanding on an earlier report,[2] provides mechanistic insight to the timekeeper gene, *Clock*, which may regulate dopaminergic transmission and cocaine reward. This work provides further evidence that cocaine-induced effects have circadian influences.

McClung and colleagues studied *Clock/Clock* mutant mice,[3] with a single-nucleotide transversion that inactivates the CLOCK protein, and found that they have an increased level of locomotor activity with a circadian activity pattern. Consistent with the observed hyperactivity, *Clock/Clock* mutant mice displayed increased levels of tyrosine hydroxylase (TH; a rate-limiting enzyme of dopamine synthesis) in ventral tegmental area (VTA) cells, as well as increased bursting and firing activity. TH-positive cells in the VTA were also positive for CLOCK protein, indicating potential local regulation of TH by CLOCK. Microarray studies in these mutants revealed that several target genes of CLOCK were downregulated in VTA (notably *Per1* and *Per2*). Intriguingly, other genes involved in excitatory and inhibitory neurotransmission (ie glutamatergic or GABAergic) were also regulated in these mutant mice. Several groups have shown that expression of timekeeper genes in rodents or flies increases after exposure to cocaine, amphetamines, alcohol and morphine.

McClung *et al*[1] found that *Clock/Clock* mutants exhibited robust sensitization to the locomotor-stimulating effects of repeated cocaine, indicating that functional

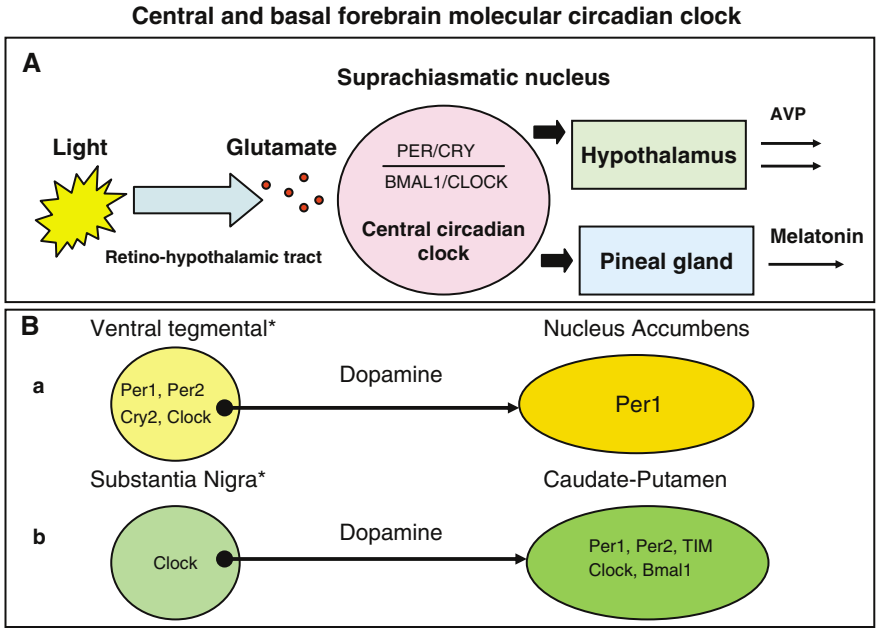

Figure 1 Central and basal forebrain molecular circadian clock. *Timekeeper genes identified to date in four brain regions involved in processes related to drug addiction, including dopaminergic neurons of the mesolimbic/mesocortical (a) and nigrostriatal (b) dopaminergic systems. *Circadian genes*: *Bmal1*, ARNT-like protein 1; *Clock*, Clock; Cry2, Cryptochrome 2; *Per1*, Period 1; *Per2*, Period 2; *TIM*, Timeless.

CLOCK protein is not necessary for this form of cocaine-induced plasticity. These mutant mice also displayed modestly increased cocaine-induced place preference, a model for the rewarding effects of this psychostimulant.

Recent studies clarified the core molecular mechanisms of the circadian clock in the suprachiasmatic nucleus of the hypothalamus, which consists of autoregulatory transcription–translation loops with a periodicity of about 24 h. The positive loop is constituted by transcription factors CLOCK and BMAL1 that activate transcription of *Per1*, *Per2* and *Cry* genes. The PER and CRY proteins assist in the negative feedback by attenuation of the CLOCK/BMAL1 transcription, thus inhibiting their own activation.[4] Timekeeper genes, as transcription factors, may have an impact on the expression of target genes with E-box sequences in their promoter regions, such as dopamine and glutamate transporters, D1 dopamine receptor.

Based on previous studies and this report, it seems that changes in function or expression of different members of the timekeeper gene family may lead to alterations in one or another aspect of drug-induced behaviors. The earliest studies, which were performed by Hirsch and colleagues in *Drosophila*,[5] showed, in sharp contrast to the report herein, that deletion of four different timekeeper genes (*Clock*, *Per*, *Cycle and Doubletime*, but not *Timeless*) resulted in the complete elimination of sensitization to repeated cocaine administration. A study by Abarca's group[6] showed differential roles of *Per1* and *Per2* genes in cocaine-induced behaviors in mice. Our microarray study showed that *Per1* mRNA expression is increased in the caudate–putamen of rats by acute 'binge' cocaine, whereas Per2 mRNA is upregulated only after repeated binge cocaine.[7] *Per1* knockout mice did not exhibit behavioral sensitization to repeated cocaine administration, whereas *Per2* knockout mice displayed more potent cocaine-induced place preference. Also, *Per2* knockout mice showed a higher rate of alcohol consumption.[8] In addition, mice with inactivated *Per1* mRNA did not display morphine-induced place preference.[9] Interestingly, chronic morphine-induced increases in the expression of *Per2* gene in the rat frontal cortex persisted after naloxone-precipitated withdrawal.[10] These data implicate timekeeper genes in common mechanisms of drug abuse-related behaviors (Figure 1).

The various timekeeper genes, which may have different effects in different parts of the brain and periphery, have been studied to a limited extent, with respect to the genetic basis for specific human disorders. In contrast to numerous single-nucleotide polymorphisms (SNPs) found in other human timekeeper genes such as *Per1*, *Per2*, only two variants have been found in the *Clock* gene: one in the 5′-UTR (101 bp upstream of ATG codon) and 3111 T>C in the 3′-UTR regions.[11] A number of studies demonstrated an association of the 3111 T>C SNP with major depression, as well as insomnia and mood disorders. *Per2* gene polymorphisms have been associated with bipolar disorders, and the *Per3* gene has been associated with delayed sleep phase syndrome, and extreme diurnal preference. This may be relevant for patients with addictive diseases, who frequently adopt abnormal sleep–wake patterns with drug self-administration, of especially alcohol, cocaine and other stimulants. Such self-administration occurs primarily in the early and late evening hours (and sometimes through the night). In contrast, heroin (or other short-acting opiate) addicts usually space their self-administration during regular intervals in daytime and evening, although they may shift their sleep period later than normal, and wake up in the morning in opiate withdrawal.

To date, only one of these genes has been studied for an association with addictive diseases. Spanagel and colleagues reported a study of *Per2* gene in 215 alcohol-dependent subjects with low or high alcohol intake, and identified a haplotype of four gene variants associated with low alcohol intake.[8] With rodent studies included in the same report, Spanagel *et al*[8] found that *Per2* mutant mice drank more alcohol than controls. Also, the brain of mutant mice contained excess levels of glutamate, a situation associated with both cocaine and other stimulant exposure, as well as alcoholism. This finding may be related to the reduction in astrocyte-expressed transporter EAAT1, coupled with a modest increase in a second transporter, EAAT2.[8]

Further studies of relationships of polymorphisms or haplotypes in timekeeper-

related genes in specific addictive diseases would be of interest. Studies from our laboratory[12] have identified a functional polymorphism of MOR (mu opioid receptor); we then hypothesized, and other laboratories subsequently have identified, that one copy of this SNP alters critical hypothalamic-pituitary-adrenal (HPA) responsivity to stress. Much earlier, our group and others have shown that the MOR plays a major role in the HPA axis, which is normally under circadian control. We have recently shown a very significant association of this A118G variant of the MOR with both heroin addiction and alcoholism (reviewed in Kreek *et al*, 2005).[13] Therefore, it would be of great interest to determine if polymorphisms of one or more of the timekeeper genes are associated with specific addictive diseases, and possibly with alterations in the stress-responsive circadian HPA axis. This axis has been shown, in laboratory and human studies, to contribute to the acquisition, continuation and relapse to specific addictions■

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Research Network

EuroGentest – a European Network of Excellence aimed at harmonizing genetic testing services

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Genetic services in Europe are based on world-leading scientific expertise. Furthermore, there has been rapid progress from research findings to the many diagnostic genetic tests currently offered in clinics. However, for all these genetic tests scientists, counsellors and doctors have a special responsibility to provide services of the highest quality and to ensure that all the citizens of Europe benefit from the same high standards of genetic care.[1,2] Poor testing and counselling can cause great anxiety among patients and their families. In addition, the annual growth of testing within the EU continues to grow at a staggering rate – between 100 and 300%.[3] An estimated 30 million people now suffer from a genetic disease within the enlarged community. Both new and existing member states find genetics causing an increasing burden upon their healthcare systems, by the latest estimates 500 million Euros. EuroGentest is an EU funded project over 5 years that aims to address these challenges through the creation of a European Network of Excellence (NoE) in genetic testing.

The overall EuroGentest philosophy is summarised in Figure 1. In effect a network of networks, this model works by encouraging a continuous cycle of critical self-examination among the genetic testing community in its widest sense. By involving leading experts from across Europe, EuroGentest will develop the