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Review

Individual differences in drug dependence in rats: The role of genetic factors and life events

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Abstract

Drug dependence and addiction is a chronic mental illness that has far reaching consequences for society in terms of economic loss, health costs and judicial problems. A crucial question in drug addiction, is what factors are involved in its aetiology, and especially what mediates the shit from use to abuse. As in most other mental illnesses, addiction can best be described using the so-called three hit model, which states that a disease results from an interaction between genetic factors, early lie events and late environmental factors. However, the precise nature of these factors still remains to be elucidated.

This present review discusses the results from an animal model in which these three different hit are currently being investigated. The apomorphine susceptible (APO-SUS) and apomorphine unsusceptible (APO-UNSUS) rats, originally selected on the basis of their behavioural response to the dopaminergic agonist apomorphine, were recently found to be genetically different in the number of gene copies of a component of the γ-secretase complex called *Aph-1b*. Whereas APO-UNSUS rats have three copies of the gene, APO-SUS rats have either 1 or 2 copies. In addition we have shown that these rats show differences in cocaine and alcohol self-administration, and that both early life events and late environmental factors can alter this self-administration behaviour. Thus, the data so far support the hypothesis that the APO-SUS and APO-UNSUS rats offer an interesting animal model for drug dependence in which genes and environment interact. We finally propose a theoretical model which can explain this gene—environment interaction.

Keywords: Alcohol; *Aph-1b*; APO-SUS; APO-UNSUS; Cocaine; γ-secretase; Self-administration; Tyrosine hydroxylase

Contents

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1. Introduction

Addiction is one of the most severe mental disorders, with many medical, social, judicial and political implications. Although recognised as a mental disorder by most clinicians and scientists (APA, 2000), it is often equated with a weak character or a lack of self-control by the general public. Estimations from the National Survey of Drug Use and Health showed that in the USA approximately 19.5 millions Americans (8.2% of the general population age 12 and above) were considered current users of illicit drugs in the year 2003 (http://www.oas.samhsa.gov/nhsda/2k3nsduh/2k3Results.htm), with marihuana being the most commonly taken illicit drug (14.4 million users). Although the addictive potential of marihuana is still doubtful, a further 2.3 million Americans (1% of the population age 12 and above) used the much more addictive drug cocaine. Additionally, the incidence of addiction to legal drugs is much higher. Thus, 16.1 million Americans were considered heavy drinkers (6.8% of the population age 12 and above) and about 70.8 million Americans were current tobacco users (29.8% of the population age 12 and above). Comparing these numbers to other countries shows comparable incidences in Western Europe (see Table 1), in spite of very different governmental policies. For instance, although cannabis possession, use and sale is severely prosecuted in USA, incidences are not lower than in the Netherlands, were it use and sale (in limited quantities). Likewise, although it has often been suggested that legalizing so-called soft drugs (like marihuana and hashish) would increase the incidence for using hard drugs, the epidemiological figures seem to speak against this, as there is no higher incidence of cocaine (or opiate, data not shown) addiction in the Netherlands as compared to other countries. On the other hand, it is clear from the incidence of tobacco and alcohol addiction that availability and/or social acceptance does have an influence on the selection of the drugs of abuse. Finally, over the years, these figures are remarkably stable with incidences neither increasing nor declining significantly (data not shown). One exception to this is the use of tobacco, which has declined (in the Netherlands) from 59% in 1970 to 31% in 2002.

An intriguing issue in addiction research is the question of why certain people develop an addiction and others do not. Although the self-administration of the drug of abuse is an important factor in this, it is well known that only a certain percentage of people that have been exposed to addictive drugs develop an addiction. In Table 2, we have compared the incidence of one time users with the current users. Although one might speculate that the ratio between these two measures may give an indication of the relatively addictive potential of the drug, it is important to realise that it is difficult to compare drug use and drug abuse across drugs. Thus, in the case of nicotine, current use is defined as 'smoking daily', whereas in the case of opiates, current use is defined as having used the drug within the last month. Nonetheless, the data clearly show that 'only' between 10% and 50% of the people that come into contact with a drug of abuse actually develop an addiction. Identifying the factors that are involved in this susceptibility for developing an addiction can help us identify people at risk at an early stage, making it possible to develop prevention strategies. Given the relative high levels of relapse seen with current treatments (Lingford-Hughes, 2002), and the mounting evidence that drugs of abuse can permanently alter the central nervous system (Crombag et al., 2004; Robinson et al., 2001), such preventive therapies might offer a much better alternative to the treatment of already existing addiction.

Table 1
Incidence of current drug use in the USA and Europe

Country	Marihuana (%)	Cocaine (%)	Alcohol (%)	Tobacco (%)
USA	6	1	7	30
Netherlands	6	1	11	30
France	8	0.3	6	27
Spain	10	2.6		32
Sweden	1	0.0	14	19

Data are based on the drugs monitor published by the Trimbos Institute (http://www.trimbos.nl/default5256.html).

Table 2
Incidence of one time and current drug users in the Netherlands

Drug	Used once (%)	Current user (%)	Ratio (%)
Tobacco	60	30	50
Alcohol	92	11	12
Cannabis	17	6	36
Cocaine	2.9	1	35
Opiates	0.7	0.2	43

Data are based on the Drugs Monitor published by the Trimbos Institute (http://www.trimbos.nl/default5256.html).

2. Identifying risk factors in addiction

Epidemiological research in the past decades has shown that addiction is a psychiatric disease similar to most others in terms of etiological factors involved. As discussed elsewhere, most major psychiatric diseases are best described by the so-called 'three hit model of psychopathology' (Ellenbroek, 2003). This model states that a psychiatric disease results from an interaction between three different factors: [1] Genetic factors; [2] Early life events; and [3] Late environmental factors. We will briefly go through some of the evidence that has accumulated in favour of this interaction model in the last decade.

2.1. Genetic factors

It has long been recognised that drug addiction runs in the family. Most research in this field has focussed on alcoholism, but results from family histories, and studies in first degree relatives of substance abusers have consistently demonstrated that substance-use disorders is elevated among relatives of drug abusers, with an eight fold increased risk of drug disorders among relatives having been reported (Merikangas et al., 1998). For a recent review see (Merikangas, 2002). High risk and twin studies have further underlined the significance of genetic factors in addiction. Thus, in twin studies for cocaine,

marihuana, opiates, sedatives and stimulants, heritability of liability ranged from 50% to 80% in both male and female twins (Kendler et al., 2000; Kendler and Prescott, 1998a,b). Very strong evidence for the genetic vulnerability for drug abuse comes from studies with monozygotic twins that were separated from birth. In a very famous study by Grove and colleagues, the heritability for drug abuse was estimated to be 45% (Grove et al., 1990), far exceeding that for alcoholism (11%). Adoption studies represent a final source of information relevant to establishing a genetic factor, for instance the classical adoption studies of Cadoret and colleagues (Cadoret et al., 1996, 1995, 1986).

In spite of these strong indications of the involvement of genetic factors in the etiology of addiction, the molecular nature of these factors is still not fully understood, though the involvement of changes in genes related to the dopaminergic system has often been reported. Various aspects of addiction (to several different drugs of abuse) have been found to be linked to changes in dopamine synthesis, metabolism and re-uptake, as well as to various dopamine receptors (Rossing, 1998). The single most often reported association is between substance abuse disorder and the *TaqI* A1 allele of the dopamine D2 receptor. Following the original positive report for alcoholism, many positive reports followed, including associations with cocaine addiction and tobacco smoking (Noble, 2000; Rossing, 1998). However, association studies have not yielded consistent results, and many negative studies have also been reported (see (Merikangas, 2002). An association between polymorphisms in the D_4 receptor and addiction have also been reported, which may prove interesting (Li et al., 2000; Comings et al., 1999), given the association between this polymorphism and the character trait of 'novelty seeking' and the proposed relation of novelty seeking and addiction (see also below). Apart from associations with dopamine related genes, other associations between addiction and for instance metabolizing genes (Rossing, 1998; Batra et al., 2003), opioid receptors (Franke et al., 1999; Compton et al., 2003) or the serotonergic neurotransmission (Gerra et al., 2005; Garwood et al., 2004) have also been reported.

2.2. Early life events

The family and twin studies have not only shown that genetic factors are of great importance in the liability to develop addiction, but also that non-genetic factors play an important role. Thus, concordance studies in monozygotic twins have consistently shown rates well below 100% (Kendler and Prescott, 1998b), a clear indication that non-genetic factors are also important in determining drug addiction. Unfortunately, even less human epidemiological research has been performed to identify such environmental factors. Several papers, however, have reported an increase risk of developing addictive behaviour after early childhood abuse (Marcenko et al., 2000; Wilsnack et al., 1997). This is corroborated by other studies that found that addiction is strongly related to several categories of adverse experiences during childhood, such as emotional or physical neglect, mental illness in the household, battered mothers and other household dysfunction (Dube et al., 2003). In agreement with this, it was found that parental loss through separation, but not death, increased the risk for alcoholism significantly (Kendler et al., 1996).

2.3. Late environmental factors

The idea that stressful life events can contribute to the development of addictive disorders is fairly well-accepted within both clinical and research communities, though it has not been investigated in great detail so far. Nonetheless, addiction and trauma-based mental health problems often co-occur (Freeman and Kimbrell, 2004; Miller, 2002; Saxon et al., 2001). Moreover, several reports have recently pointed to a gene * environment interaction. Thus, it was recently found that the occurrence of alcoholism could best be predicted by low socio-economical status combined with the occurrence of the *TaqI* allele of the D2 receptor, but not by either of these factors alone (Madrid et al., 2001).

3. An animal model for addiction: the APO-SUS/UNSUS rats

Above we discussed the evidence that addiction results from an interaction between genetic factors, early life events and late environmental factors. However, in spite of the evidence in favour of this so-called three hit model, the nature of these three hits, as well as how their interaction affects the normal functioning on the brain is still largely unknown. This is largely due to the fact that clinical and epidemiological studies are not able to answer such questions. Animal research on the other hand, allows for a good control over the environmental factors while at the same time allowing a detailed investigation of the brain. In addition, addictive behaviour can be measured in animals using the self-administration procedure. Moreover, there is strong evidence that rats from the same strain show a lot of variety in drug self-administration. Indeed several groups have shown that the individual's response to a novel environment can be used to predict the susceptibility to drug self-administration. Thus, rats with a high response to novelty show a larger propensity to self-administer cocaine (Kabbaj et al., 2001) or amphetamine (Piazza et al., 1990). On the other hand, a reduced consumption of alcohol was reported in high responders to novelty (Gingras and Cools, 1995), though this may be dependent on the specific set-up to measure self-administration (Nadal et al., 2002). This is interesting in view of the fact that in humans the novelty seeking character trait has been linked to the occurrence of addiction (Le Bon et al., 2004; Franques et al., 2003). Interestingly, to our knowledge no research has been done to investigate whether the high response to novelty is a genetic trait than can be inherited. However, in order to investigate the three hit model, a genetic component is essential. Most of the genetic models that have been developed so far are related to alcohol addiction, and are based on the selection of behavioural variables such as preference or avoidance of alcohol, followed by subsequent selective breeding (Ritz et al., 1994; Aalto and Hilakivi, 1986; Tampier et

al., 1984; Eriksson, 1968). So far, however, comparable models for other addictions have not been developed.

In order to investigate individual differences in addictive behaviour, we decided to use a different approach, by focussing on the underlying mechanisms of addiction. Although many aspects of addiction are still far from elucidated, it is well known, from both clinical and preclinical studies that dopamine plays a crucial role in the acquisition of drug-taking behaviour and in the craving for drugs of abuse (Volkow et al., 2004; Koob, 1992; Carboni et al., 1989). We therefore started to select Wistar rats on the basis of their response to the dopamine D1/D2 receptor agonist apomorphine, injected subcutaneously in a standard dose of 1.5 mg/kg. Using the modified Ungerstedt box (Ljungberg and Ungerstedt, 1978), we found a bimodal shape of variation with approximately 40–45% showing a weak gnawing response and 40–45% showing an intense gnawing response (Cools et al., 1990; Ellenbroek et al., 2000). This bimodal variation was also seen in (outbred) Sprague Dawley rats, but not in inbred strains, such as the Brown Norway, F344 or Lewis rats (unpublished data). Using a specific breeding scheme, which prevents inbreeding to a large degree, we were able to create two lines of rats, termed APO-SUS (apomorphine susceptible, i.e. rats that show a strong gnawing response to apomorphine) and APO-UNSUS (apomorphine unsusceptible, i.e. rats that show a weak gnawing response to apomorphine) rats (Cools et al., 1990). Moreover, 10 years after the original selection we replicated the selection procedure and found virtually the same separation in APO-SUS and APO-UNSUS rats (Ellenbroek et al., 2000).

In the years following our original description of the APO-SUS and APO-UNSUS rats, much research was devoted to the phenotypical characterization, and we could show that the APO-SUS and APO-UNSUS rats differed in many aspects of the central nervous system, the neuro-endocrine system as well as the immunological system. It would be beyond the scope of the present paper to discuss or even summarize these data and the reader is referred to the other reviews on the topic (Cools et al., 1993; Cools and Ellenbroek, in press; Ellenbroek and Cools, 2002; Cools and Gingras, 1998).

In recent years we have investigated whether the APO-SUS and APO-UNSUS rats also differ in drug self-administration. We first focussed on alcohol consumption and found that when rats were given a free choice of either an ethanol solution of water in their home cage, the APO-UNSUS rats drank significantly more of the ethanol solution than APO-SUS rats (Sluyter et al., 2000). Moreover, this increased consumption of the APO-UNSUS rats was seen at alcohol concentration from 4% up to 10% (higher concentrations were not tested). In addition, we could recently show that in a habituated situation, the APO-UNSUS rats also more readily self-administered cocaine than the APO-SUS rats (van der Kam et al., 2005b). Thus, under normal conditions, the APO-UNSUS rats seem to be more prone to develop addiction, which is reminiscent of the data on alcohol addiction in humans, since patients with alcoholism have a reduced growth hormone response to apomorphine (Wiesbeck et al., 1998).

4. Gene * environment interactions in the APO-SUS/UNSUS model

The finding that the APO-SUS and APO-UNSUS rats differ in self-administration of alcohol and cocaine offers the possibility to investigate how genetic and environmental factors interact to determine the drug abuse liability Although we are currently still in the process of evaluating the three hit model for addiction, the data obtained so far seem to be in agreement with this general scheme (Cools and Gingras, 1998).

4.1. Genetic factors

We have previously shown that the apomorphine susceptibility in our APO-SUS and APO-UNSUS rats has a strong genetic component. First of all, there was a clear-cut separation in apomorphine response in APO-SUS and APO-UNSUS rats after only a few generations (Cools et al., 1990) and we could replicate this in a second independent cohort of Wistar rats (Ellenbroek et al., 2000). Moreover, we found that cross breeding APO-SUS with APO-UNSUS rats leads to intermediate gnawing scores (Ellenbroek et al., 2000). Although all these data are strongly indicative of a genetic component, it was not until recently that we were able to identify the major genetic difference between the APO-SUS and APO-UNSUS rats. Using a cDNA/oligonucleotide microarray approach with subsequent detailed genomic DNA analyses, we could show that whereas the APO-UNSUS rats have three copies of the gene *Aph-1b*, the APO-SUS rats have only one or two copies (Coolen et al., in press). This so-called dosage imbalance was caused by an unequal crossing-over event and, interestingly, the site of recombination concerned a segmental duplication within the *Aph-1b* locus. Moreover, we found that this dosage imbalance was seen in both the original and the replicate line. *Aph-1b* is a small peptide which forms an integral part of the γ-secretase complex which plays an important role in cleaving type I transmembrane peptides (Kopan and Ilagan, 2004). The minimal molecular composition of the γ-secretase complex has recently been resolved, namely, presenilin (either PS1 or PS2), nicastrin, presenilin enhancer 2 (PEN-2) and anterior pharynx defective 1 protein (Aph-1aS, Aph-1aL or Aph1b). Thus, at least six different configurations of the γ-secretase complex are possible. Although the exact function of the Aph-1 component is not yet established, it may play a role in stabilizing the γ-secretase complex and possibly in determining substrate specificity. The γ-secretase complex has been shown to cleave at least 14 different transmembrane peptides, including amyloid precursor protein, notch, neuregulin and the neuregulin receptor ErbB4 (Kopan and Ilagan, 2004). This variety of substrates, many of which are critically involved in various aspects of development may explain why APO-SUS and APO-UNSUS rats show such fundamentally different properties in the nervous, endocrine and immune system. However, to what extent the genetic deficit in *Aph-1b* is related to addiction remains to be investigated. To that extent, we have now selectively bred rats with 1, 2 or 3 copies of the *Aph-1b* gene. Moreover, by backcrossing with the

original strains, we have obtained animals that are genetically identical, except for the *Aph-1b* locus. So far these rats have only been tested in their gnawing response to apomorphine, and we found that, as with the original APO-SUS and APO-UNSUS line, rats with 1 or 2 copies had a significantly higher gnawing response than animals with 3 copies of the *Aph-1b* gene (unpublished data).

4.2. Early life events

Previous research from our department has shown that the phenotypical expression of apomorphine susceptibility is also determined by early life events. Thus, a single 24 h period of maternal deprivation at postnatal day 9 (birth being postnatal day 0) significantly enhanced the apomorphine-induced gnawing response in the APO-UNSUS, but not in the APO-SUS rats. Conversely, cross fostering APO-SUS pups to APO-UNSUS rats decreased the apomorphine-induced gnawing response in these pups. Cross fostering APO-UNSUS rats to APO-SUS mothers did not affect the apomorphine-induced gnawing response. Likewise, cross fostering APO-SUS pups to other APO-SUS mothers (a procedure generally referred to as in-fostering) did not affect the apomorphine-induced gnawing response in the pups (Ellenbroek et al., 2000).

With respect to addiction, we recently found that maternal deprivation in APO-UNSUS rats also affected cocaine self-administration. In fact maternally deprived APO-UNSUS rats showed a self-administration profile very similar to that of APO-SUS rats, namely a reduction in cocaine self-administration under normal, non-challenged conditions (unpublished data). So far we have not yet investigated the effects of cross fostering on self-administration.

4.3. Late environmental factors

The third element in the three hit-model of psychopathology is the late environmental factors. We had already shown in the past that APO-SUS and APO-UNSUS rats show a different response to mild stressors, often opposite to each other. Thus, whereas the basal levels of corticosterone are lower in APO-SUS rats, after stress, the levels are higher compared to APO-UNSUS rats (Rots et al., 1995). We have also recently found that the self-administration of cocaine and alcohol is strongly dependent on environmental stressors. As discussed above, under non-challenged conditions APO-SUS rats drink significantly less alcohol than APO-UNSUS rats. However, after a single stressful experience, the APO-SUS rats showed a strong and prolonged increase in drinking behaviour, whereas the APO-UNSUS rats only showed a small and short-lasting increase (van der Kam et al., 2005a). The situation is even more interesting in the case of cocaine self-administration. Thus, whereas under baseline non-challenged conditions, the APO-UNSUS rats self-administered more cocaine than the APO-SUS rats, the reverse was true under challenged conditions (van der Kam et al., 2005b). An interesting pattern occurred when rats were first allowed to self-administer during non-challenging conditions, after which a challenge was introduced (turning off the light during self-administration). Using this protocol, APO-UNSUS rats rapidly learned to self-administer cocaine, after these animals were shaped (by giving them one forced nose-poke upon placing the animals in the cage, see Fig. 1). When subsequently the light stressor was applied, the APO-SUS rats also readily learned to self-administer cocaine. Interestingly, APO-UNSUS rats did not change their intake pattern in the last phase of the experiment, when the lights were turned off. This suggests that stress plays a role during acquisition, but not during the maintenance phase. Interestingly, when maternally deprived APO-UNSUS rats were subjected to this protocol, they showed exactly the same pattern as APO-SUS rats, namely, an increase in cocaine self-administration only during challenged conditions, when the lights were turned off (Van der Kam et al., in preparation).

5. General discussion

The data obtained so far indicate that the alcohol and cocaine self-administration behaviour in APO-SUS and APO-UNSUS

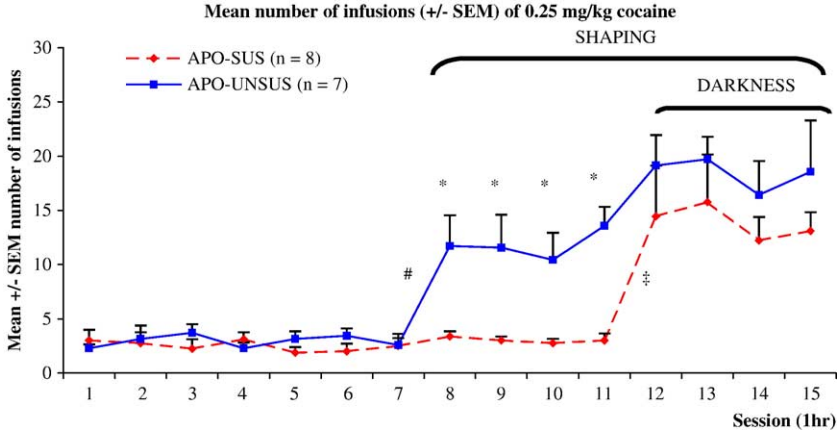

Fig. 1. Pattern of self-administration of cocaine by APO-SUS and APO-UNSUS rats. The results show that whereas APO-UNSUS rats readily self-administer cocaine after shaping, APO-SUS rats require a stressor to start (see also text). * indicates a significant difference between APO-SUS and APO-UNSUS rats; # indicates a significant increase in self-administration in the APO-UNSUS rats after shaping started; ‡ indicates a significant increase in self-administration in the APO-SUS rats after lights were turned off during the test.

rats results from an interaction between genetic factors, early life events and late environmental factors. The important question is of course, how these factors interact to shape the brain (and body) of these animals. So far, we have found a large number of differences between APO-SUS and APO-UNSUS rats, some of which may be of relevance for the control of addictive behaviour. Especially in relation to dopamine, it has been shown that APO-SUS rats have an increased mRNA activity for tyrosine hydroxylase, and an increase in tyrosine hydroxylase-immunoreactivity in the nucleus accumbens when compared to APO-UNSUS rats (Rots et al., 1996; van der Elst et al., 2005a). APO-SUS rats also have more D2 receptors (Rots et al., 1996) and dopamine transporters (Van der Kam et al., in preparation) in the dorsal striatum. When challenged with a mild stressor, APO-SUS rats show a stronger and more prolonged release of dopamine in the nucleus accumbens (van der Elst et al., 2005b). Moreover, this challenge leads to a stronger increase in tyrosine hydroxylase-immunoreactivity in the APO-SUS rats, which may represent a rebound response to the increased dopamine synthesis. We recently investigated the effects of repeated cocaine administration on in tyrosine hydroxylase-immunoreactivity in APO-SUS and APO-UNSUS rats and found some intriguing results. Thus whereas cocaine increased in tyrosine hydroxylase-immunoreactivity in the nucleus accumbens of the APO-UNSUS rats, it increased it in the medial prefrontal cortex of the APO-SUS rats, indicating that cocaine affected different brain circuitries in both rat types (van der Elst et al., in preparation). Close inspection of the data showed that the basal levels of in tyrosine hydroxylase-immunoreactivity were already very high in the nucleus accumbens of the APO-SUS rats and that after cocaine treatment the levels in the APO-UNSUS were similar to the basal levels of the APO-SUS rats. In other words the non-treated APO-SUS rats resemble the cocaine-treated APO-UNSUS rats. In this respect it is interesting to note that other psychostimulants like amphetamine also affect different brain regions in sensitized versus non-sensitized rats (Ferguson and Robinson, 2004). It remains to be clarified to what extent this differential involvement of the nucleus accumbens and the medial prefrontal cortex is also related to the observed behavioural differences between APO-SUS and APO-UNSUS rats. Moreover, we are currently evaluating how in tyrosine hydroxylase-immunoreactivity changes after early life events such as maternal deprivation and cross-fostering.

Another important question relates to the nature of the interaction between the genetic factors and the early life events. As discussed above, maternal deprivation affects only the APO-UNSUS rats, whereas cross fostering affects only the APO-SUS rats. It is important to realise that not only the nature of these early life events is different, but also the timing. Thus, whereas maternal deprivation is done on postnatal day 9, cross fostering is performed within 24 h after birth. In this respect we have recently provided evidence that the timing of the early life event is a crucial determinant of its long term effect. Using systemic injections of clonidine and saline as early life events, we found that injections in the first few days after birth affected the apomorphine induced gnawing response only in the APO-SUS rats, whereas injections around postnatal day 9 affected the apomorphine-induced gnawing response only in the APO-UNSUS rats (Degen et al., 2004). It is well known that early stressful life events lead to rapid, and often long lasting changes in the hypothalamic pituitary adrenal axis (Levine, 1994), which can have a detrimental effect of the developing nervous system, as high corticosteroid levels affect programmed cell death, neurogenesis and gliogenesis (Gould, 1994). In this respect it is interesting that we recently found that APO-SUS and APO-UNSUS rats have different speeds of development, with the APO-UNSUS rats developing faster than the APO-SUS rats (Degen et al., 2005). This has led us to propose a more general model explaining the gene * early environmental interaction (see Fig. 2). This model proposes that (1) the general speed of development is primarily determined by the genetic background of rat and (2) that an early life event primarily affects brain structures (and thus function) that are currently developing. This explains why manipulation A in Fig. 2 affect rat strain I but not II or III, whereas manipulation C affect strain III, but not strain I. Given the importance of γ-secretase in development, and the fact that our rat lines with I, II, or III copies show a strong reduction in γ-secretase cleavage activity during development (Coolen et al., in press), it is tempting to speculate that indeed the difference in Aph-1b activity is causally related to the difference in developmental speed of these rats. However, with our current knowledge this is only a working hypothesis which needs to be (in)validated.

In summary, our present data suggest that the acquisition of cocaine and alcohol self-administration in APO-SUS and APO-UNSUS rats is the result of a complex interaction between genetic factors, early life events and late environmental factors. Although the precise interaction between these factors remains to be investigated, we have proposed a testable hypothesis that the genetic difference in Aph-1b activity determines the speed of development, and thereby when an early stressful life event will have a long lasting effect. Although most of the data discussed in the present paper are related to drugs of abuse and the development of addictive behaviour, the proposed model may have far reaching implication for other diseases as well,

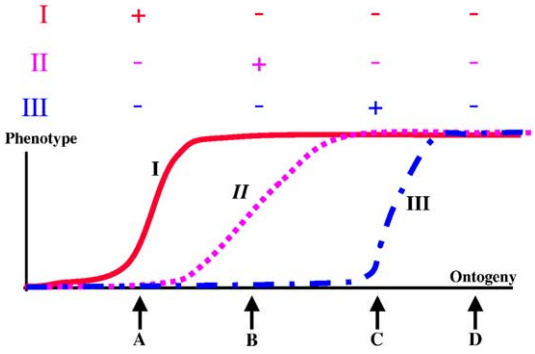

Fig. 2. A hypothesis regarding the long-term consequences of gene–environment interactions. This hypothesis states that the developmental speed is determined by genetic factors, and thus differs between strain I, II and III. Early environmental factors (A–D) are supposed to change the functioning only of those brain structures that are developing at the time of impact (see also text).

since the three hit model seems to be fit the aetiology of most psychiatric disorders (Ellenbroek, 2003).

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