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Behavioural assessment of drug reinforcement and addictive features in rodents: an overview

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ABSTRACT

Some psychoactive drugs are abused because of their ability to act as reinforcers. As a consequence behavioural patterns (such as drug-seeking/drug-taking behaviours) are promoted that ensure further drug consumption. After prolonged drug self-administration, some individuals lose control over their behaviour so that these drug-seeking/taking behaviours become compulsive, pervading almost all life activities and precipitating the loss of social compatibility. Thus, the syndrome of addictive behaviour is qualitatively different from controlled drug consumption. Drug-induced reinforcement can be assessed directly in laboratory animals by either operant or non-operant self-administration methods, by classical conditioning-based paradigms such as conditioned place preference or sign tracking, by facilitation of intracranial electric self-stimulation, or, alternatively by drug-induced memory enhancement. In contrast, addiction cannot be modelled in animals, at least as a whole, within the constraints of the laboratory. However, various procedures have been proposed as possible rodent analogues of addiction's major elements including compulsive drug seeking, relapse, loss of control/impulsivity, and continued drug consumption despite negative consequences. This review provides an extensive overview and a critical evaluation of the methods currently used for studying drug-induced reinforcement as well as specific features of addictive behaviour. In addition, comic strips that illustrate behavioural methods used in the drug abuse field are provided given for free download under http://www.zi-mannheim/psychopharmacology.de

Keywords Addictive behaviour, drug seeking, drug taking, loss of control, methods, reinforcement, relapse.

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FOREWORD

Addiction is defined as a syndrome in which drug use pervades all facets of the user's life, even precipitating in the loss of social compatibility (e.g. loss of partner and friends, loss of job, crime . . .) [1]. It is obvious that addiction is a genuinely human phenomenon; and one that is therefore no reproducible within the unavoidable constraints imposed by the laboratory setting. However, some of the behavioural characteristics of this syndrome, such as resumption of drug seeking/drug consumption after a protracted abstinence (relapse), can be satisfactorily modelled in laboratory animals. For example, experimental procedures can be designed to be as simple as possible, thereby maximizing internal validity and thus reproducibility. Conversely, procedures can aim to be as holistic as possible, thereby favouring the possible relevance to human situations. Neither one of these two approaches is perfect; each has its respective drawbacks. Indeed, methods designed attending only to internal validity may finally exclude variables of relevance to understanding, explaining or predicting the phenomenon of interest. On the other hand, very complex procedures enhance the difficulty of arriving at conclusions and reduce the capability of both inferring causal relationships between variables and of establishing predictions.

In the following pages, we summarize various methods currently used in the drug addiction field. Methods that evaluate features of addictive behaviour and those measuring the reinforcing properties of drugs are discussed separately. It is assumed here that drug intake (promoted and maintained by the reinforcing properties of those substances) is a requirement for the development of addiction; however, addiction is neither a necessary nor a universal consequence of drug consumption. This review also considers that physical dependence and other consequences derived from long-term drug consumption can be concurrent to the development of addiction, but that they are not aetiologically related and can be dissociated for their study (Li & Volkow 2005). Therefore, procedures measuring phenomena such as tolerance or dependence are not included in the present review. Other methods (i.e. drug discrimination procedures) that provide additional kinds of information relevant to understanding drug consumption and addictive behaviour in all its complexity, but that do not fall in any of these two main categories (reinforcement versus addictive features), are not discussed as well.

It is important to note that in this review the term 'model' will be restricted to those methods that display clear face validity towards human behaviour and phenomena. For example, the rodent drug self-administration procedures currently used in this field are aimed at addressing the main features of the same behaviour in humans, i.e. they show clear face validity. On the other hand, the term 'test' will be applied to describe the experimental methods that do not have a direct resemblance to the human condition (i.e. place conditioning, intracranial self-stimulation-based procedures, etc.).

1 METHODS USED TO ASSESS DRUG-INDUCED REINFORCEMENT

Nowadays, it is assumed that drugs are voluntarily taken and potentially abused because of their 'reinforcing properties', that is because they act as 'reinforcers' of drug-seeking and drug-taking behaviours. While this is probably true, it is also important to consider what the term reinforcer actually means. In the context of drugs of abuse, reinforcers are

[1] The definition of addiction has changed across time and different definitions have been related to the specific characteristics of specific drug classes. Thus, the current definition of addiction fits much better to (and it is mainly studied in) psychostimulants such as cocaine or amphetamine, whereas 10 years ago physical dependence and withdrawal were considered as the landmarks of an alternative view of addictive behaviour more suitable for opiate drugs. Interestingly these changes are not independent of the trends in drug consumption in our society and therefore the consideration of specific drugs as health/social problems. In the clinical language 'dependence' is the most general term to refer to the syndrome that most preclinical researchers define as 'addiction'. This produces some confusion because the term 'dependence' (or less preferably 'physical dependence') is used in preclinical research to define a latent withdrawal state that would be potentially triggered in the absence of the drug. According to the current evidence obtained in animal models, withdrawal symptoms do not seem to be necessary or sufficient for the development of addictive behaviour, but this observation is often ignored in the medical environments. In fact, the DSM-IV (one of the most accepted diagnostic manuals for behavioural disorders) still includes tolerance and dependence as important symptoms of human addiction.

mainly considered in a Skinnerian view and therefore defined as events that follow a response and change the probability of future occurrences of that response. However, this perspective often ignores that the reinforcement process also accounts for changes in behaviour when the 'reinforced' response appears in the absence of the reinforcer or when the reinforcer is non-contingently administered. In addition, any comprehensive definition of reinforcement should account for the generalization and pre-eminence of previously reinforced behavioural outcomes in novel situations. Therefore, the actions of reinforcers should be understood in a broader context intimately related to learning and memory processes (White & Milner 1992; White 1996; Hyman 2005).

At this point it is necessary to highlight that very often the term 'reward' is misused and confused with the term 'reinforcer' (or the 'reinforcement process'). Reward, as a scientific concept, was coined within the field of experimental psychology and has three possible meanings. First, reward can be used as it would be used in a non-scientific context to describe stimuli with appetitive (desirable) consequences. Second, reward can be used (as opposed to 'punishment') to refer the learning contingency in which the emission of a response brings such an appetitive stimulus. This kind of contingency is often referred as 'positive reinforcement'. Third, reward is also used to refer to a hypothetical pleasurable internal state (hedonia), which derives from the acquisition, use or consumption of appetitive stimuli. In this regard, as summarized by Everitt & Robbins (2005), reward (or related concepts, such as 'liking') refers to the subjective responses associated with the post-presentational consequences of reinforcers, becoming later on important characteristics of the internal representation of these stimuli (and those others surrounding their occurrence). It is also important for the purpose of this review to clarify that so far these hedonic attributive processes are simply hypothetical and that their measurability in rodents is an open debate. Regardless of which one of these three meanings is ascribed to reward, this concept cannot be equated to 'reinforcement'. Reinforcement is a broader concept that refers to the ability of some stimuli (reinforcers) to change the probability of specific behavioural repertoires in different learning contingencies ('positive' versus 'negative' reinforcement [2]). This concept includes 'reward-related processes' but also 'reward-independent mechanisms' which lead to an increase of the emission probability of a particular response.

Acting as reinforcers, drugs can promote changes in the probability of emission of specific responses in three ways: first, reinforcers can reduce specific needs or drives (negative reinforcement). This perspective is perhaps not essential to understand the reinforcing properties of drugs of abuse in initial states of its consumption but the importance of this phenomenon is likely to grow after prolonged drug exposure and/or when introducing deprivation/abstinence phases. Second, drugs of abuse can act as primary motivators in positive reinforcement contingencies (positive reinforcement). In those situations, other stimuli associated with the presence of the drugs can also acquire incentive-motivational properties becoming 'conditioned reinforcers'. Finally, and referring to the initial meaning attributed to this concept by Pavlov, Thorndicke or Hull, reinforcers can enhance the storage of information about situations in which they occur, via a process that does not involve learning about the reinforcer itself. Thus, reinforcers by promoting an increased associative strength of specific stimuli-response contingencies can bias the choice of particular responses and increase their probability of emission.

These different dimensions of reinforcers can be used to study drug-induced reinforcement. Thus, as mentioned before, the traditional view of reinforcers in this field considers them as primary appetitive stimuli, and their motivational properties are often evaluated through consumption/preference-based measures. However, methods that measure the strengthening action of drugs on learning/memory processes are briefly introduced together with their possible usefulness in the context of drug abuse research.

1.1 Self-administration models

Self-administration-based methods are widely used in basic/preclinical drug abuse research. This is because these procedures have a good construct as well as appealing face validity towards drug consumption in humans. Of course, how the drug is obtained or even why the drug is consumed varies notably between both a human being and his/her social environment and a non-human experimental subject in a constricted laboratory set-up. However, it is assumed that the neural chemistry and anatomical circuitry involved generating, selecting and setting in motion these behavioural patterns is similar in both situations. Consequently, these procedures appear to be adequate models in unravelling

[2] Positive and negative reinforcement result in an identical outcome: the increase of probability of a particular response. The difference between both phenomena refers to the underlying process. Thus, the term 'positive reinforcement' refers to situations in which the emission of a response leads to the presentation of an appetitive consequence. Conversely, in a 'negative reinforcement' contingency the probability of emission of a particular response is increased because of its ability to suppress an undesirable event. Drugs of abuse can promote both kinds of reinforcement, and self-administration behaviours can be elicited and sustained to obtain the drug but also to suppress the effects of its absence (i.e. prevent withdrawal).

common neural mechanisms and therefore help to identify strategies useful in the intervention regarding human drug consumption.

The self-administration procedures can be classified according to different criteria. Thus, from a pharmacological perspective they can be classified according to the route of administration via which the drug is ultimately delivered to the organism. This is not a trivial consideration because by determining the latency between the response and the perceived effects as well as the amount of the drug, the route of administration partially determines several drug effects, including those that allow a substance to act as a reinforcer.

From a behavioural perspective self-administration methods can be classified as operant and non-operant procedures. When using operant procedures the dependent variables analysed refer to the response itself (frequency, rate, etc.) whereas the most commonly reported dependent variables in non-operant procedures are centred in the amount consumed. Thus, methods based on operant and non-operant responses differ in procedural characteristics, but also may differ in their sensitivity to the manipulation of specific brain substrates and may require a differential framework in the experimental design and in the results' interpretation.

1.1.1 Non-operant methods

In rodents, non-operant procedures are restricted to oral self-administration procedures. These kinds of methods are very common in the context of alcohol research but they have been also, although less frequently, used with other drugs of abuse such as nicotine (Slifer 1983), cocaine (Falk & Lau 1997), amphetamine (Meliska *et al.* 1995) or morphine (Schuster, Smith & Jaffe 1971). The most obvious reason for choosing a non-operant self-administration procedure is that rodents hardly consume alcohol via other routes of administration. Conversely, when drugs such as morphine or amphetamine are orally self-administered, they show reduced motivational efficacy (Meisch 2001), and therefore other administration routes are preferred to study their psychopharmacological actions. Indeed, most of the studies involving oral self-administration of drugs other than ethanol use very specific procedures (i.e. schedule-induced adjunctive behaviour) to induce their consumption/preference. Consequently, these behavioural indexes may not necessarily reflect the psychopharmacological (i.e. motivational) properties of these drugs. In addition, and because a similar selection pattern is also observed in humans, the use of the appropriate route of self-administration for each drug of abuse provides an additional source of validity to these rodent models. Therefore, the following paragraphs will be solely focusing on oral ethanol self-administration procedures.

Oral ethanol self-administration methods present clear face and construct validity as models of human alcohol consumption. Indeed, in both cases, subjects can choose to drink alcohol or not, and to do it as much and at the moment that suits them. In addition, these methods have proven to be useful in the identification of pharmacological treatments to prevent excessive alcohol drinking and then confirming their predictive validity (Spanagel & Zieglgänsberger 1997). Furthermore, because of its technical simplicity, these methods are often sound and produce reproducible results.

Oral ethanol self-administration is usually implemented by simultaneously making available two bottles, one containing an aqueous solution of alcohol and another containing tap water. However, different studies have explored factors that can affect alcohol consumption, including the number and kind of available bottles and/or other reinforcers (Tordoff & Bachmanov 2003), the temporal accessibility to alcohol (Files, Lewis & Samson 1994), the incorporation of a previous operant response (Samson, Czachowski & Slawecki 2000), etc. In general, it has been shown that alcohol consumption increases when a higher number of alternative alcohol solutions are presented or when subjects are given restricted access to them. The alcohol concentration is a critical issue in these procedures, because low or overly high concentrations can be orally consumed or rejected because of their mild-sweet or aversive tasting properties, respectively. Moreover, as the amount of ingested fluid is limited by physiological constraints, too low, an ethanol concentration may result in negligible brain alcohol levels. Thus, it is usually considered that ethanol concentrations below 4% (v/v) are not pharmacologically relevant and that a concentration in the range of 8–12% is a suitable standard for consumption by the rodents. On the other hand, when initially offered, most rodent strains will most likely not drink from a so highly concentrated ethanol solution. Consequently several procedures have been developed to 'train' rodents to orally self-administer pharmacologically relevant amounts of alcohol, including the presentation of ascending concentrations of ethanol, the addition of a sweet flavour agent (i.e. sucrose), which can progressively be faded out or not, or the inclusion of a time period of forced exposure to ethanol (a review of different ethanol initiation processes can be found at Boyle *et al.* 1994). The use of a 'beer drinking model' also results in high alcohol intake and pharmacologically relevant blood alcohol levels despite a low ethanol concentration of beer (McGregor *et al.* 1999). The problem of taste preference for beer *per se* can be well controlled for by using alcohol-free beer. An alternative strategy to obtain high alcohol consumption/preference is by using genetically selected rodent strains with an inborn preference for alcohol (McBride & Li 1998) but this strategy may result in reduced generalization potential.

The most common dependent variables in these non-operant oral models of ethanol self-administration are the amount of pure ethanol consumed (expressed as g/kg of body weight/time unit), its relative preference over water and the total fluid intake. In this regard, these models provide information about consumption of a reward (i.e. ethanol), without distinguishing among the different factors that could contribute to it. One additional problem is that preference/self-administration changes do not have a unidirectional interpretation. Thus, enhanced preference could be understood as a reflection of enhanced, but also decreased, reinforcing properties of this reward (see *reinforcing efficacy* in the next section), and the same interpretations would hold also for a decrease in preference. Furthermore, different procedural aspects can affect and must be considered when using this kind of measures. Thus, the values of ethanol preference over water in a restricted access paradigm (i.e. 1 hour/day) are expected to be higher and probably less sensitive to changes in the concentration of the alcohol solution than those observed in equivalent time periods in an uninterrupted access model. Conversely, in this kind of unrestricted access procedure and when using a genetically heterogeneous population, preferences values are expected to be 'low'. In such a situation, an increase in the alcohol concentration might lead to a decrease in preference, which (in the absence of a change in the amount of total alcohol consumed) should not be interpreted as indicative of reduced reinforcing properties of the alcohol solution. Actually, this 'titration' of the reinforcer is a clear index of stimulus-controlled behaviour (Bickel, Marsch & Caroll 2000; Sugrue, Corrado & Newsome 2005), which is equivalent to saying that the animal's behaviour is governed by the alcohol solution content and, by implication, its psychopharmacological effects. Therefore, preference values cannot be just evaluated in comparison with a theoretical value of indifference (e.g. 50%), as much as consumption values (at least, when low) should not be considered independently of their temporal distribution (i.e. existence of significant 'drinking bouts'; Marcucella, Munro & MacDonall 1984; Samson *et al.* 2000).

In an attempt to provide less ambiguous information about the 'reasons' underlying consumption/preference differences, further refinement of the classical two bottles model may be needed. In this regard, the 'Matching Law' described by Herrnstein (1970), which predicts that animals allocate their time or responses in direct proportion to the fraction of total reinforcers earned from two alternative sources of reinforcement, provides an interesting framework for developing alternative preference-based measures. Such an experimental situation can be implemented in a series of trials in which two sources of reinforcement that offer different payoffs are made simultaneously available at the same cost. Although 'matching behaviour' has been mostly documented in the context of concurrent operant (e.g. variable interval) schedules, it can be also studied in a two bottles paradigm (Martinetti *et al.* 2000; Sanchis-Segura *et al.* 2004, 2005). Thus, by making temporarily available across a large number of trials several pairs of bottles containing solutions with different concentrations of the reward, it can be evaluated to which extent the individual's preference among those paired conditions is sensitive to the statistics governing reward availability. This 'sensitivity to reward' can be estimated by using the 'generalized matching equation' (for practical applications see Martinetti *et al.* 2000; Sanchis-Segura *et al.* 2004, 2005). The information provided by such an index is not appropriate when trying to predict the individual choice in the face of a particular contingency (i.e. outcome of next trial) nor the neurophysiological correlates of those valuation/decision processes, but may provide a quantitative index of the steady (i.e. molar) internal evaluation of a particular reward (Sugrue *et al.* 2005) and has additional advantages over classical measures of preference (Sanchis-Segura *et al.* 2005). Because this kind of procedure requires a large number of trials, they can be less useful when assessing short-lasting interventions (i.e. acute stress, most pharmacological treatments) and should be rather used to study innate or irreversible differences between individuals or groups (genotypes, lesions, etc.).

Other developments concerning alcohol non-operant models of self-administration require more complex experimental set-ups. Thus, there are some reports in which the investigators utilized lickometers or similar devices coupled to bottles to analyse the microstructural pattern of alcohol consumption under different experimental conditions (for an example see Marcucella *et al.* 1984; Marcucella & Munro 1986). These studies are still scarce but it has already been shown that specific changes in the pattern, e.g. temporal dynamics, but not necessarily in the amount, of alcohol consumption can be linked to the activity of specific neural systems (Gill, Amit & Smith 1996) or be associated to the development of features such as dependence (Kampov-Polevoy *et al.* 2000) or compulsive behaviour (Hölter *et al.* 1998). Following the experience with other reinforcers (Davis & Levine 1977; Davis 1996), these systems could also be helpful in differentiating the effects of any experimental manipulation on ethanol reinforcement from those produced by other determinants of ingestion such as taste, satiation, etc.

In summary, non-operant self-administration procedures have been mainly used in the context of alcohol research and they present adequate psychometric guaranties. However, although this kind of method does not require sophisticated equipments or major technical expertise, the obtained results may be clearly influenced by a priori methodological decisions. Furthermore, their easy implementation does not mean that the interpretation of the obtained data is straightforward or that these procedures can only provide rough behavioural information as that summarized in the

total amount of reward consumed. Indeed, further developments of this kind of procedures, incorporating aspects of the temporal distribution of the reward consumption (i.e. drinking patterns) or more detailed indexes of preference, may provide a qualitative impulse to our understanding of orally self-administered drugs.

1.1.2 Operant drug self-administration procedures

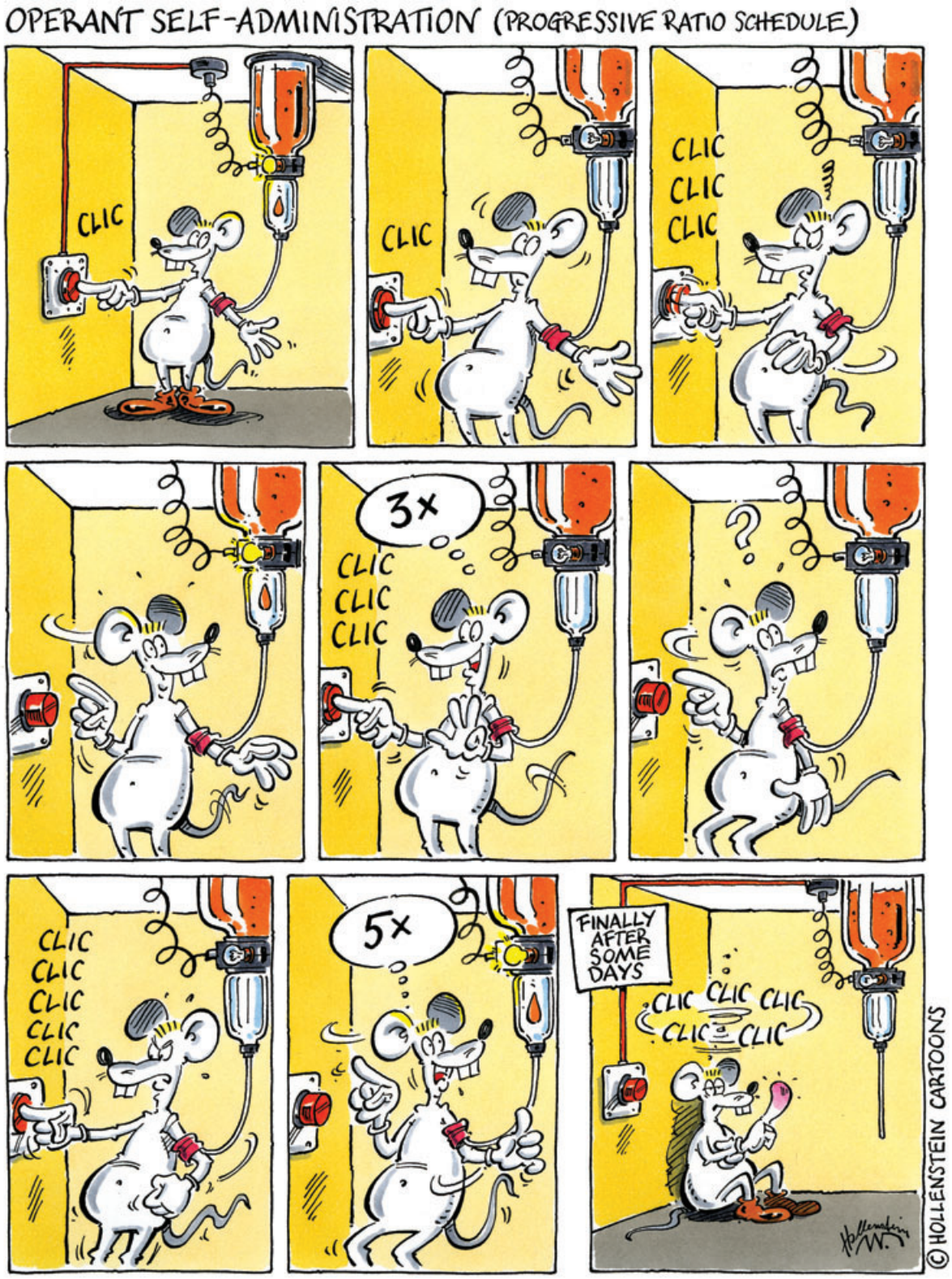
The term *operant* or, more precisely *operant conditioning*, was coined by B.F. Skinner in the 1930s. Although usually associated with his work, the view that behaviour is controlled by its consequences is not an original idea by Skinner. Indeed the concept of operant conditioning is not essentially different from the earlier termed 'law of effect' (Thorndike 1911) or 'instrumental learning'. However, what Skinner really developed was the study of the reinforcement schedules and their control over behaviour. Under the term 'reinforcement schedule' (or reinforcement programmes), any procedure that determines reinforcer delivery under operationally well-defined rules is included. A concise review of these schedules and their effect on subject's performance can be found in almost any experimental psychology textbook (e.g. Domjan 2004). In this section, the use of operant methods based on the learning contingency defined as 'positive reinforcement' (i.e. those in which a positive reinforcer is delivered contingently to the completion of the schedule requirements) as models of drug-taking behaviour is described. Nevertheless, it should be noted that similar experimental arrangements have been used from a 'negative reinforcement' perspective assuming that the delivered reward attenuates a previous negative state such as drug-withdrawal or stimulus deprivation (Ahmed & Koob 2005).

The most common apparatus used in conducting an operant drug self-administration procedure is the so-called 'operant box' (or 'skinner box'). These chambers consist of a box, equipped with one or more *manipulandi*, transmitting the operant response and one or several devices delivering the reinforcers. These boxes are commercially available and their configurations can be adapted to specific needs including additional modules, which allow the programmed occurrence of other events (lights, tones, etc.) as discriminative stimuli and/or secondary reinforcers. Considering the manipulandi, these are usually levers, although systems based on more 'natural' responses (i.e. nose-poking for mice or disk-pecking for pigeons) have also been developed. Usually there is a manipulandum defined as 'active', meaning that it is linked to a reinforcer delivery, and another one referred to as 'inactive', which respectively result in the delivery of the drug vehicle or lacks any programmed consequences.

When analysing the drug self-administration literature, it is clear that only a short number of operant schedules are routinely used in these studies. Indeed, there is a clear predominance of studies using single versus complex, ratio versus interval, as well as fixed (or progressive) versus variable schedules. In fact, the most common programme of reinforcement in these studies is the fixed ratio (FR), followed far behind by the progressive ratio (PR) schedule. Under an FR schedule, the reinforcer is delivered every time that a pre-selected number of responses are completed. Here it should be noted that, especially when using some routes of administration (e.g. intravenous) that result in a fast drug delivery, a time out after receiving each reinforcer is usually incorporated. This inclusion, although often needed to prevent drug overdose, supposes a violation of the theoretical rule defining the operant programme and, consequently, it affects the subject's performance as well as the impact of some co-adjuvant treatments (for an excellent description of this phenomenon and other aspects of intravenous drug self-administration see Caine, Lintz & Koob 1993). Therefore, this parameter should be carefully chosen. On the other hand, in the studies using drugs of abuse as reinforcers, the number of responses is generally kept low, the FR1 (also referred as continuous reinforcement) being the most used ratio requirement, whereas the number of reinforced responses (equated to the number of delivered reinforcers when using an FR1 schedule) is the reported dependent variable. However, under a PR schedule, the required ratio increases following a predefined progression, which usually is an arithmetic one. This progressive increase is introduced across different sessions or within a single session. When using a within session PR schedule, the most common index of performance is the so-called 'breaking point', defined as the highest response rate accomplished to obtain a single reinforcer.

Regarding the reinforcer delivery systems, the use of operant procedures is not particularly associated with any one route of drug administration. Indeed, intravenous, intraventricular, intracranial, intragastric, and oral delivery of drugs, sustain operant behaviour. However, as mentioned before, that does not mean that all drugs are equally self-administered irrespective of the programmed route of administration. In addition, when the operant programme results in access to an oral reinforcer (a common situation in alcohol related studies), neither the number of responses or delivered reinforcers should be equated to the number of consumed reinforcers. In these situations, it is highly recommendable to incorporate additional measures that provide effective information on the amount of drug effectively consumed (i.e. blood drug concentrations).

Even when knowing the amount of drug effectively consumed, the interpretation of data obtained using operant procedures can be less straightforward than what might be believed at first sight. The concept of *reinforcing efficacy* was



initially introduced as a phenomenon underlying the ability of a reinforcer to maintain operant behaviour regardless of the method used to evaluate it. This concept was readily accepted in the context of drugs of abuse, and in this context, there appeared one of its first formal definitions (Griffiths, Brady & Bradford 1979). From this perspective, reinforcing efficacy was considered as a stable property of the reinforcer, linearly related to its magnitude (or dose). It was assumed that this property could be apprehended by outcome measures coming from different schedules and procedures. Therefore, measures such as peak response rates or breaking point should be mutually interchangeable as all of them reflect, through response rate changes, the reinforcing efficacy of the delivered reinforcer. However, several sources of evidence have challenged this proposal. First, it has been shown that procedural factors (i.e. route of or duration of drug administration) modify reinforcing efficacy, which is also dependent on the internal state of the organism (i.e. food restriction, dependence) and of subjects' reaction to environmental influences (i.e. stress). These observations show that reinforcing efficacy is neither a stable property of the reinforcer nor exclusively derived from its magnitude. In addition, the relationship between dose and measures such as peak rate is not linear but rather tends to show an inverted U-shaped distribution. A review of this evidence as well as of the theoretical implications for the reinforcing efficacy concept can be found in Bickel *et al.* (2000).

As mentioned for non-operant procedures, the challenge to the traditional view of reinforcing efficacy has important consequences for the development of drug self-administration models. First, it reflects the importance of some procedural factors (i.e. drug dose) in the design of an experiment and the interpretation of results of concurrent treatments (Caine *et al.* 1993). On the other hand, if reinforcing efficacy is not a unitary concept, the idea that measures coming from different operant procedures are mutually interchangeable is no longer tenable. Indeed, the opposite seems to be true. Data obtained using different schedules have different theoretical implications. Thus, it is generally accepted that FR1 performance is less affected by motivational (i.e. incentive value) factors than other schedules of reinforcement. However, by increasing ratio requirements, the subject's performance becomes more influenced by these anticipatory phenomena, a fact that can be easily summarized by the breaking point index obtained with PR schedules or by analysing the elasticity of an animal's behaviour across different ratios, which increase across successive sessions (Rowlett 2000). Supporting this dissociation, it has been shown that the activities of some neural systems (Salamone & Correa 2002) or experimental conditions (Morgan *et al.* 2002) do not modify the number of reinforcers obtained under an FR1 schedule. However, the same manipulations can result in significant behavioural changes in the breaking points observed, when using progressive increases in ratio (i.e. PR schedules). Therefore, when the objective is to assess the potential liability of a drug or to assess its initial consumption as a result of its unconditioned psychopharmacological effects, the FR1 procedure may be a first choice. However, when considering changes in later stages of drug consumption such as the development of addictive behaviour, FR1 response rate-based measures may be less informative.

In summary, compared with the non-operant models, the use of operant schedules provides more flexibility in the experimental design and they can be more informative as information of the temporal distribution of the responses can be analysed. In addition, operant drug self-administration procedures are considered to be valid and reliable models of human drug consumption. Thus, the automation in these methods reduces human intervention and results in a good reproducibility index. On the other hand, the construct validity of these methods is analogous to that of non-operant methods, with the exception of predictive validity. In this regard, as medications to cope with excessive drug consumption are at the present time almost restricted to alcohol or cigarette smoking, the predictive validity of these methods using other drugs of abuse is not yet satisfactorily proven.

Finally, before finishing this section, it should be mentioned that, in the context of drug abuse research, more complex operant procedures than those described above are used, although they are not primarily intended to be self-administration models. Among these, a few which have been used to study some specific features of addictive behaviour will be described at a later stage in this review. However, it should be taken into account that complex operant procedures require learning abilities that are not always acquirable by all species. Indeed, learning abilities can be a determinant factor in operant-based procedures and therefore a confounding factor, if they are not properly controlled. In a similar way, considering the number of responses by the inactive lever, mice and rats should be viewed differently, because in the former, lever pressing seems to be *per se* a reinforcing activity (Spanagel & Sanchis-Segura 2003).

1.2 Tests used to measure the reinforcing properties of drugs of abuse

In this section, different procedures aimed at measuring the ability of drugs to act as reinforcers are summarized. One major characteristic all of them share is the fact that the drug is administered by the experimenter, at a dosage and temporal distribution independent of the subject's will and behaviour. This and other characteristics separate these methods from the human situation and classify them as tests and not as a model with clear face validity towards human behaviour.

1.2.1 Tests based on conditioned preference

In the conditioned preference procedures, the drug's effects (which is presumed to act mainly as the unconditioned stimulus; US) and a previously neutral stimulus are repeatedly paired. Thus, as mentioned before, the US (drug) is administered regardless subject's behaviour but contingent to the occurrence of an event (i.e. presence/absence of a stimuli) also controlled by the experimenter. Through this process, which is thought to be Pavlovian in nature, this stimulus acquires the ability to act as a conditioned stimulus (CS). Thereafter, this CS will be able to elicit approach/avoidance behaviour depending on the nature of the used unconditioned stimuli. By measuring these approach/avoidance behaviours, further insight about the drug acting as US can be gained.

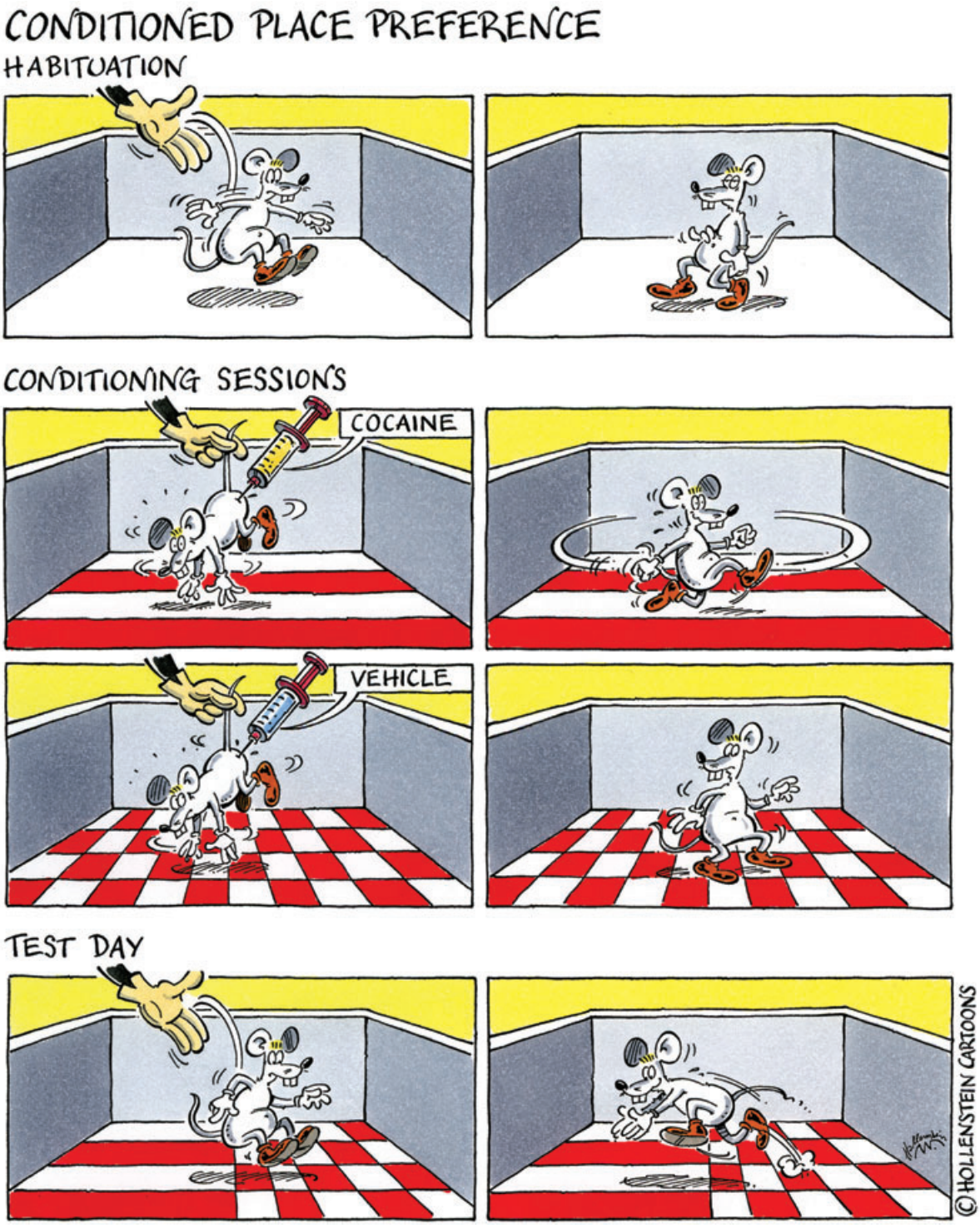
1.2.1.1 Place conditioning procedures

The most popular methods used to study conditioned preference apply an environmental stimulus as a CS. These procedures are referred to as conditioned place preference (CPP) or conditioned place aversion (CPA) when using US with appetitive and aversive properties, respectively. Although conditioned approach/avoidance towards specific stimuli can occur in humans as a result of drug consumption, CPP and CPA are not primarily intended to model any feature of human behaviour. Indeed, there is not a single controlled study designed to explore the occurrence of such a phenomenon in humans (Bardo & Bevins 2000) and it is rather supposed that these procedures provide more information about the drug than about the individual's behaviour. This section will mainly focus on CPP procedures because of the major interest in it regarding drug abuse. It should be also noted that there are excellent published reviews about this topic (Bardo, Rowlett & Harris 1995; Tzschentke 1998; Bardo & Bevins 2000), discussing this test at a level of detail that cannot be incorporated here.

The most commonly used apparatus to carry out the CPP test has been either the two- or three-compartment 'conditioning boxes'. In these boxes one compartment will become associated with drug injections, whereas other will be accessed only after vehicle administration. Following repeated pairings of this drug/vehicle administration regime, on the test day, the animal will be allowed to freely move across both compartments, usually under a drug-free state. The increase on the time spent in the drug-associated compartment is considered as a measure of conditioned preference (see below). In the procedures involving three compartments, the third one of them is only accessible during the test day. This additional compartment provides a start-box but can also be used as a control for novelty-related interferences (Tzschentke 1998). Nevertheless, there is no agreement about the possible advantages this procedure holds in comparison with the more classical one using two compartments. On the other hand, the conditioning compartments can be designed to prevent any innate preference (although this needs to be empirically tested) or to the contrary. This decision determines the rest of the protocol, leading to the so-called *unbiased* and *biased* CPP procedures. With respect to the former, the drug injection is associated with one arbitrarily chosen compartment (and is usually counterbalanced across the subjects), whereas regarding the latter, the drug is paired with the non-preferred compartment and CPP is measured as overcoming the initial aversion for that environment. In general, there is a wider use and higher appreciation for the *unbiased* procedure, although the theoretical or empirical advantages of either one of the methods are not clear. An excellent review of the functional consequences of these and other protocol differences in the establishment of CPP can be found in Bardo *et al.* (1995) and Cunningham, Ferree & Howard (2003).

Drugs of abuse display a differential ability to produce CPP. Thus, whereas opiates and psychostimulants produce robust CPPs over a wide range of experimental conditions, other drugs such as ethanol, nicotine or cannabinoids produce more inconsistent results (Cunningham, Niehus & Noble 1993; Tzschentke 1998). In general, a clearer preference is achieved when drugs are administered just before context exposure and when the route of administration ensures fast and high brain concentrations of the drug. Indeed, when the drug administration occurs after context exposure, CPA rather than CPP is observed (Fudala & Iwamoto 1990; Cunningham, Okorn & Howard 1997; Font, Miquel & Aragon 2006). The number of CS–US pairings also influences CPP; thus in general the higher the number of pairings, the higher/the more persistent the observed conditioned preference. Conversely, exposure to the environment in the absence of the drug may result in the presentation of *Pavlovian latent inhibition*—a behavioural phenomenon that describes the reduction of conditioning caused by previous experience with the conditioned stimuli in absence of the unconditioned stimuli—or *extinction* of a previously acquired conditioned preference. Finally, drug administration long after context exposure or association of a context to the descending limb of the pharmacokinetic curve of the drug also results in weak preferences or in CPA. These considerations are especially notable in drugs that show weaker ability to establish CPP.

The theoretical interpretation of CPP/CPA results is also a matter of controversy. Thus, CPP and CPA procedures have been proposed as useful in isolating/inferring the hedonic value ('rewarding properties') of a drug, but also as a



measure of drug-seeking behaviour useful to identify 'anticraving drugs', as a model of state-dependent learning and as a drug discrimination test. A more recent interpretation of CPP, which seems to more accurately describe the individual's behaviour in this paradigm, is its consideration as an index of 'conditioned approach' (Mead *et al.* 2005), similar to those observed in autoshaping-based procedures (see section 1.2.2) although the conditioned response is defined as a less complex one. Part of this plurality of interpretations is due to variations in the use of this test. Thus, when a co-adjuvant treatment alters the ability of a drug to *induce* CPP or CPA, it is generally assumed that this is because of an interference with its primary motivational properties (although some controls to rule out an effect over the associative process itself should be included). On the other hand, drugs that are administered only on the test day and

reduce the so-called *expression* of CPP have been considered as being potential candidates in the treatment of human drug craving. Finally, the use of CPP/CPA to investigate state-dependent learning/discrimination is only possible, when two test days are included and compared, one under the effects of the paired drug and the other in a drug-free state (the paired drug or a 'substitutive' one, respectively). Similar testing conditions after a previous extinction (achieved by repeated saline injections in both compartments) are required, when using CPP as a possible test of cue-induced reinstatement of drug-seeking behaviour (see section 2.2.3).

The interpretation underlying most of the published studies concerning CPP/CPA is that these procedures can be useful in determining if a drug produces reward/aversion as inferred from its ability to produce approach/avoidance. However, CPP is cumbersome for providing the graded dose-effect curves needed for answering some pharmacological questions. Thus, CPP data allow us to classify drugs according to their emotional valence but provide less information about their potency. However, even this classificatory capacity of CPP/CPA procedures can be questioned, as the emotional valence of drugs is not unequivocal (Ettenberg 2004) and conditioned preference procedures produce sometimes surprising results. Thus, for example, ethanol, which acts as a powerful positive reinforcer across a wide range of animal species including humans, usually produces CPP in mice but CPA or indifference in rats (Cunningham *et al*. 1993). Even more confusing, ethanol self-administration can result in CPA (Stewart & Grupp 1986) but intragastric administration of similar amounts of ethanol may result in CPP (Ciccocioppo *et al*. 1999). Conflicting results have also been found when using other drugs such as nicotine. However, probably the major source of conflict with this interpretation of CPP is that the same drugs, which are able to produce CPP, also produce, at the same doses, conditioned taste aversion (CTA, Turenne *et al*. 1996), a topic that will be extensively presented in the following section. On the other hand, the predictive value of this test in identifying pharmacological treatments for human craving is based on the perhaps too simplistic identification between this phenomenon and cues-induced behaviour, but the evidence obtained with the currently available anticraving compounds is not conclusive (McGeehan & Olive 2003; Herzig & Schmidt 2005). This possibility is further discussed in section 2.2.3.

As a final note, it is noteworthy to mention that there is an implicit asymmetry between the terms 'conditioned place preference' and 'conditioned place aversion'. Thus, whereas the term 'preference' is perhaps closer to be an operational concept (although not completely devoid of subjective implications) that arises of the dependent variable measured, the term 'aversion' presumes a negative emotional affective state that is not actually measured. In this regard, the term 'conditioned place avoidance' could probably be more appropriate for this procedure. Indeed, although for different reasons, the distinction between 'aversion' and 'avoidance' has been introduced in other tests of conditioned preference (see below).

1.2.1.2 Other measures of conditioned preference

The development of conditioned preference/aversion is not restricted to CPP and CPA procedures. Indeed, according to the concept of 'biological preparedness' introduced by Seligman (1970), rodents should more readily associate internal subjective states such as those produced by drugs of abuse with gustatory (or olfactory) stimuli than with visual or auditory. The most commonly used procedures developed accordingly to this rationale are usually referred to as CTA and conditioned taste preference (CTP). In this case, and contrary to place conditioning, CTA has been used more often than CTP.

Conditioned taste aversion was initially described by Garcia, Hankins & Rusiniak (1974) and it has been largely studied as a singular type of learning that can firmly establish behavioural avoidance and is described as the emotional aversion for a taste after a single illness episode associated to it. This learning is also extremely resistant to extinction and is readily generalized to similar flavours or other dimensions (odour, colour, etc.) of the initial stimuli. However, more recent research has demonstrated that most of those aforementioned CTA characteristics are reproduced in many other Pavlovian procedures, when choosing conditioned stimuli ecologically related to the US (for a recent review of this functional perspective of classical conditioning, see Domjan 2005).

Conditioned taste aversion experimental procedures often use oral sucrose (or saccharin) consumption as a dependent variable. In some cases, this solution is offered with concurrent access to water but it is only available for a restricted length of time during the day. Procedures widely differ in the level of fluid deprivation and the schedule of sucrose/saccharin presentation (i.e. alternate days). Once a reliable baseline is established, this solution is paired with the US subject to study (i.e. a drug injection is administered immediately following completion of access to sucrose/saccharin). Typical US include emetic drugs such as lithium chloride or other forms of induced malaise (i.e. forced rotation). The decrease in the consumption/preference of this fluid after the pairing procedure is understood as the result of an emotional rejection (aversion) due to a Pavlovian CS–US association. A more extensive description of a standard protocol as well as of the current state of knowledge about its physiological basis can be found in Bures, Bermudez-Rattoni & Yamamoto (1998) or by accessing the article database available at the following webpage: http://www.ctalearning.com.

Interestingly, almost all drugs of abuse, at the same dose range that produces CPP or those that are self-administered, are able to produce CTA (Hunt & Amit 1987). Theoretical refinements of the initial CTA concept have provided a new framework to understand this paradox. Thus, it has been proposed that drugs of abuse generate *conditioned taste avoidance* rather than *conditioned taste aversion* (Parker 1993, 2003). The concept *conditioned taste avoidance* refers to a behavioural avoidance (reduced fluid consumption) that not necessarily implies an aversive (emotional) reaction. Indeed, facial disgust reactions have only been observed in CTA procedures involving emetic agents but not drugs of abuse. Furthermore, the c-fos expression pattern observed in CTA procedures involving drugs of abuse such as amphetamine differs from that observed, when lithium chloride is used as a US (Swank, Schafe & Bernstein 1995). From a theoretical perspective, it has been suggested (Sorge, Fudge & Parker 2002; Parker 2003) that conditioned taste avoidance could be an expression of the phenomenon called 'anticipatory contrast'. This concept refers to the fact that the anticipation of an about-to-come more potent reinforcer outweighs the perceived value of the available one (i.e. sucrose/saccharin solution) and, consequently, suppresses its consumption (Flaherty & Checke 1982; Sorge *et al.* 2002). However, it should be noted that regardless of the current state of knowledge about this topic from a more basic perspective, in the context of drugs of abuse, confusion between aversion and avoidance is frequently found. Indeed, most of the CTA data involving drugs of abuse are still interpreted from an 'aversion perspective' or as a reflection of the simultaneous activation of several, and sometimes, opposing neural systems after drug administration (White 1996).

Conditioned taste preference has been less used than CTA in the context of drugs of abuse. The CTP procedures are very similar to those used in CTA experiments and also involve the use of the consumption of a flavoured solution as the dependent variable. In this case, US are obviously restricted to appetitive stimuli such as drugs of abuse and caloric or nutritionally relevant agents (i.e. restoration of specific diet deficiencies). The main difference with a CTA procedure is the pairing sequence, between the CS and US. Thus, and in close homology to the CPP/CPA procedures, CTP is observed when the CS and US are simultaneously presented. Indeed, it has been shown that the same dose of morphine produces CTP, when administered simultaneously with the CS solution, but results in conditioned taste avoidance, if it is administered after the termination of CS availability (Lett & Grant 1989). Moreover, it should be noted that CTP is usually established using flavoured but not highly palatable solutions (Lett & Grant 1989; Cunningham & Niehus 1997; Ackroff, Rozental & Sclafani 2004; for similar evidence in humans, see Yeomans *et al.* 2000), which would prevent the appearance of the phenomenon called 'anticipatory contrast', previously introduced.

In summary, CTP and CTA (conditioned taste aversion) could be of potential interest as tests to study the rewarding/motivational effects of drugs of abuse. Indeed, these tests can have some advantages over other methods assessing conditioned preference. Thus, CTP and CTA rely on an evolutionary selected trend that primes the association between taste and internal states. This can be especially convenient, when using drugs, such as ethanol or caffeine, that do not easily establish other conditioned preferences (i.e. in the CPP paradigm) but which have been proven to be effective when using CTP and CTA procedures (Cunningham & Niehus 1997; Yeomans *et al.* 2000; Ackroff *et al.* 2004). In addition, and in contrast to the CPP or CPA, the occurrence of taste conditioning has been experimentally confirmed in humans (Richardson, Rogers & Elliman 1996).

1.2.2 Other procedures based on Pavlovian conditioning: autoshaping (sign tracking)

Autoshaping also known as sign tracking was initially described by Brown & Jenkins (1968) and refers to the emergence of a conditioned approach response that appears when a CS reliably predicts an appetitive US. This initial approach response is often followed by the emergence of other responses that imply contact/and or manipulation of the CS. Thus, for example, a pigeon will peck the key light that indicates food delivery, although doing so does not change the probability or the delay to obtain it.

As recently summarized in Kearns & Weiss (2004) autoshaping and human drug abuse have some similarities because both imply (1) the existence of discrete cues which reliably predict the reinforcer; (2) enhanced approach/attention responses towards the CS; and (3) the behaviour shows an important degree of independence from its functional consequences. This later statement can be questioned when referring to the initial stages of drug consumption, which are probably sustained by response–outcome contingencies. However, after long-term drug consumption and especially in those individuals that transit to addiction, self-administration responses could be more independent of their outcomes (Tiffany 1999; Everitt & Robbins 2005), then better fulfilling this third feature. Despite these similarities between both phenomena, there are few studies in the drug addiction field involving this methodology. One reason for the scarce use of autoshaping procedures is that there is also little agreement about what autoshaping could model/measure in the context of drug abuse research. Thus, autoshaping-based procedures have been proposed as a method to facilitate the initiation of drug self-administration (Carroll & Lac 1997; Tomie 2001; Tomie *et al.* 2002), but also to

investigate impulsive behaviour (Monterosso & Ainslie 1999) as well as to measure approach behaviours towards drug-associated stimuli (Everitt, Dickinson & Robbins 2001).

Alternatively, it can be considered that similar to CPP, autoshaping provides a suitable measure of Pavlovian conditioned approach that can be informative on the ability of certain substances to act as US. Thus, in both cases (autoshaping and CPP), an exteroceptive stimuli is paired through a Pavlovian contingency with an appetitive US and promotes a conditioned response that involves an approach towards the CS. However, there are important differences between both procedures that must be considered (Newlin 1992; Kearns & Weiss 2004). These differences relate to the CS (discrete cues versus diffuse contexts), the pairing procedure (short and distributed over a large number of trials versus long and single daily pairings) and the nature of the response evaluated (preference versus complex patterns of approach and contact with the CS) used in CPP and autoshaping procedures, respectively. The latter of these differences is likely to be related to the nature of the US and requires a special comment. Autoshaping was initially described as a phenomenon that appears only when food is used as US (Brown & Jenkins 1968), although later on it was extended to other natural reinforcers (Jenkins & Moore 1973; Burns & Domjan 2000). It should be noted that stimuli predicting any of these natural reinforcers set in motion complex behavioural patterns that are largely innate and that resemble the behaviour involved in the consumption of these rewards. This is a major difference with drugs of abuse, which consumption is not associated to any innate behavioural act (except perhaps those orally self-administered). In consequence, drugs of abuse might fail to 'shape' complex behavioural patterns of approach towards CS predicting them, providing misleading results about the motivational capabilities of these substances. Indeed, the ability of drugs such as cocaine, which exhibit reinforcing properties in other experimental set-ups (i.e. self-administration or CPP procedures), to act as US in autoshaping procedures has been questioned (Kearns & Weiss 2004).

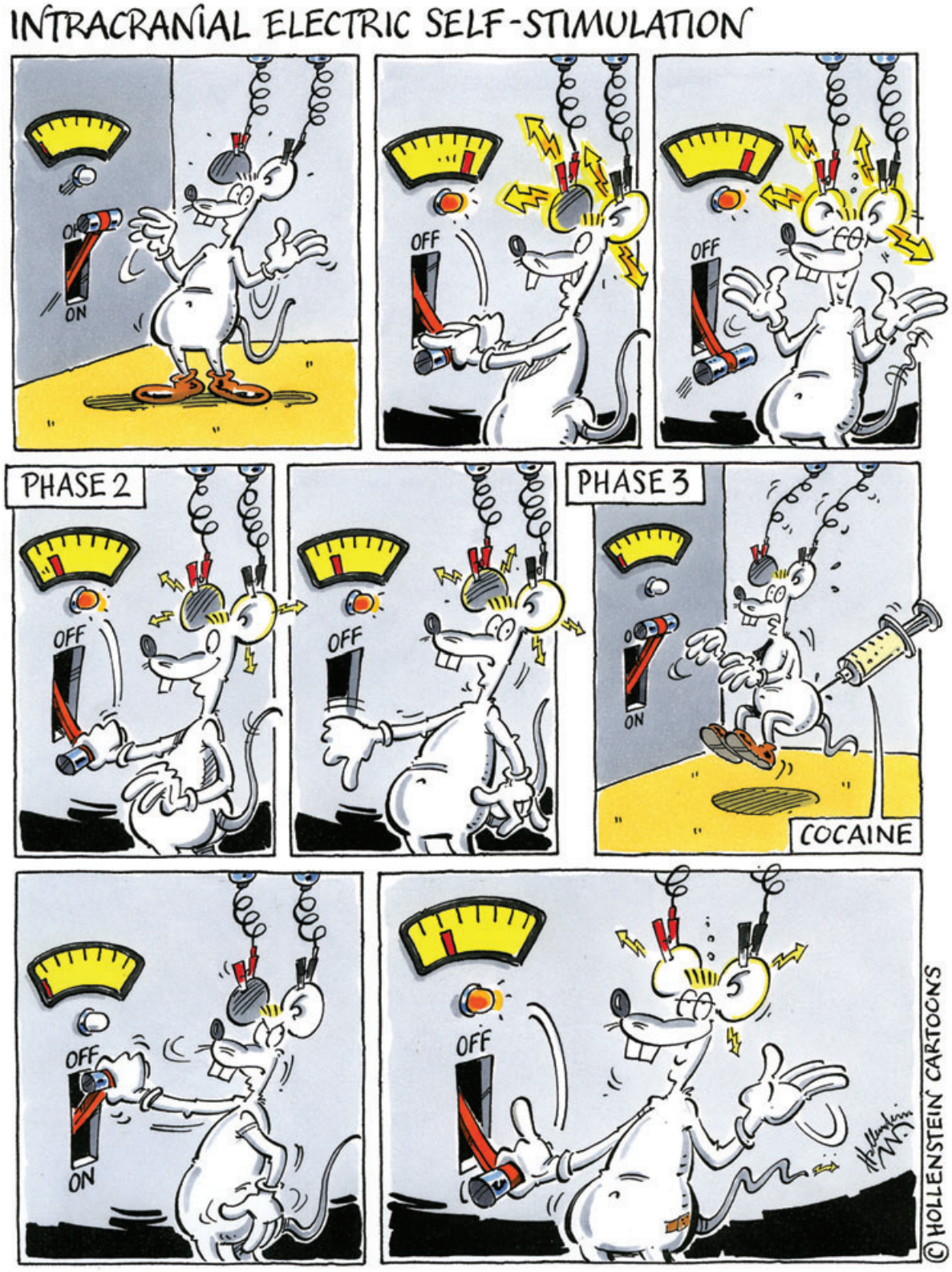
In summary, autoshaping-based procedures have not been extensively used in the drug addiction field. Some of the problems associated to the use of this kind of procedures are intimately related to predefined characteristics of the expected conditioned response but also to some specific features of drugs of abuse acting as US. Nevertheless, autoshaping-based procedures may still provide relevant information in the drug addiction field. In addition to explore the suitability of these methods with drugs that are orally self-administered (e.g. ethanol, etonitazene), more subtle approach indexes that do not imply complex patterns of approach (i.e. attentional responses) towards the CS could be assessed. Yet another possibility is to explore the disturbing effects of drug-associated CS in the approach/operant responding for a reinforcer (Krank 2003). Finally, using natural reinforcers as US, autoshaping procedures can still be informative when trying to dissect the processes affected in more complex experimental procedures involving drug-associated cues (Everitt *et al*. 2001).

1.2.3 Facilitation of intracranial electric self-stimulation

Intracranial electric self-stimulation (ICSS) experiments were fundamental in the establishment of the reward concept and its application to the current views of drug consumption and addictive behaviour (Olds & Milner 1954). Indeed, these data led to the hypothesis that ICSS produces the direct activation of brain circuits usually activated by natural reinforcers or 'hijacked' by drugs of abuse. Therefore, ICSS-based procedures are used in psychopharmacological research to study the cerebral circuits mediating the 'rewarding' effects of drugs of abuse (Esposito *et al*. 1984; Porrino *et al*. 1984) as well as to identify other pharmacological agents aimed at blocking them. This goal can be pursued with the additional advantage of bypassing a large part of the input systems.

It should be noted that a wide variety of ICSS-based procedures have been developed (Stellar & Stellar 1985), but at least two ICSS-related procedures have been extensively validated and used to explore the possible effects of drugs of abuse: the discrete-trial current-intensity (DT-CI) and the rate-frequency curve shift (R-FCS). An excellent description of both methods with very detailed practical information can be found in Markou & Koob (1993). In the DT-CI, at the beginning of each trial, the subject receives a non-contingent electric pulse in a 'reward-related brain site' (e.g. lateral hypothalamic area) and it should perform a response (e.g. press a lever) within an arbitrary time window (usually 5–10 seconds) to obtain an identical stimulus in each separate trial. As the intensity of the initial electrical stimulus is modified in the form of an ascending/descending series, a threshold of the minimal current needed to promote the ICSS response can be estimated.

This measure can be an indication of the subject's 'reward threshold', whereas changes in the latency to respond are indicative of performance-related contamination effects. On the other hand, the R-FCS procedure is also aimed at estimating this threshold but in this case, it is based on the frequency relationship between input and output. This method generates sigmoidal curves from which the asymptotic point and the locus of rise (inflexion point) can be calculated. As this procedure has been extensively validated, we now know that this locus of rise is a measure of 'reward' and it is



defined as the stimulus frequency that sustains an arbitrary criterion of performance, whereas the asymptotic point reflects performance-related artifacts.

Interestingly, the 'reward threshold' estimated through ICSS-based procedures is very stable over time and therefore it can be used as a baseline value to determine its possible change in response to specific treatments. In the context of the drugs of abuse, it has been shown that the administration of several drugs of abuse including cocaine,

amphetamine, morphine, ethanol or nicotine results in a reduction of the ICSS reward threshold in some brain areas (Kornetsky & Bain 1992; Wise 1996). On the other hand, as a reverse validation, an elevation of the same threshold has been observed in drug-dependent animals during withdrawal (Schulteis *et al.* 1995; Epping-Jordan *et al.* 1998; Cryan, Hoyer & Markou 2003) or when administering drugs with aversive effects (Todtenkopf *et al.* 2004). From these data, it seems that the ICSS-based procedures may be of potential interest in identifying substances that could reduce drug 'reward'-related processes and, consequently, their consumption. However, very few studies have used this paradigm to identify potential treatments for excessive drug consumption.

1.2.4 Drug-induced memory enhancement as an alternative assessing the reinforcing effects of drugs

As mentioned before, the notion of reinforcement was initially defined in its non-technical sense, suggesting strength. More specifically, this concept referred to as a presumed synaptic strengthening process that results from the presentation of certain stimuli (reinforcers), leading to an increase of the storage information about the situation in which those stimuli are encountered. This effect of the presentation of a reinforcer can also be used to study the ability of drugs to establish habits, trends and behavioural preferences and it can be done using procedures that are independent of their motivational properties (White & Milner 1992). Indeed, this memory enhancement has been demonstrated using drugs devoid of rewarding properties such as strychnine (McGaugh 1989). Therefore, and although most researchers have been less attracted by this possibility, procedures based on this 'memory-enhancing function' of reinforcers, could be very valuable in the drug-addiction field, especially when studying drugs, such as nicotine or caffeine, which might have a low 'hedonic' value but which potently establish/maintain self-administration in humans (for a recent review of the special significance of these processes in nicotine-induced reinforcement see Chaudhri *et al.* 2006).

A clear method to illustrate this enhancing function is the ability of a reinforcer (B) to improve the learning of a task sustained by a reinforcer (A). A main characteristic of these procedures is the absence of contingence between reinforcer B and the subject's behaviour. This prevents any learning based on the motivational properties of this reinforcer (i.e. any reinforcement in the Skinnerian sense). Thus, it has been shown that non-contingently administered nicotine can increase lever-pressing behaviour contingently reinforced by a visual stimuli (Donny *et al.* 2003; Palmatier *et al.* 2006). To further separate both components, usually a reinforcer with appetitive properties is used to boost aversive (or emotionally neutral) learning. By using this kind of procedure, it has been demonstrated that the administration of drugs of abuse such as amphetamine (Evangelista & Izquierdo 1971) or morphine (although with more conflictive results) improves performance in avoidance procedures (McGaugh 1989). Similarly, ethanol injections improve short-term social memory in rodents (Prediger & Takahashi 2003; Manrique, Miquel & Aragon 2005), an effect similar to the enhanced verbal recall observed in human social drinkers (Bruce & Pihl 1997). Finally, the ability of caffeine (Kopf *et al.* 1999) and nicotine (Levin & Simon 1998; Uzum *et al.* 2004; Chaudhri *et al.* 2006) to enhance information storage has also been largely demonstrated. For an more conceptual view of this topic see White (1996).

From a theoretical perspective, the strengthening of memory observed after the non-contingent administration of drugs has been suggested to be a result of an improvement of the acquisition and/or consolidation processes (White & Milner 1992; Chaudhri *et al.* 2006). Interestingly, it has been proposed that this could be related to the ability of almost all drugs of abuse to enhance striatal dopamine levels (White & Milner 1992). Indeed, some of the late conceptualizations about the possible role of the Medial Forebrain Bundle, identify dopamine release as a 'learning/gating signal' (Spanagel & Weiss 1999; Waelti, Dickinson & Schultz 2001; Montague, Hyman & Cohen 2004; Salamone *et al.* 2005), which coupled with reward value information (processed in other brain areas) would lead to the determination of 'incentive value' of each stimulus and 'envigorize' the organism's behaviour consequently to obtain it. Regarding the capital importance of incentive motivation within some of the current views of drug addiction (Robinson & Berridge 1998, 2003; Everitt & Robbins 2005), it seems clear that these methods can be very valuable, especially when assessing this gating role of dopamine without interference from the putative drug's reward value.

2 BEYOND REINFORCEMENT: ADDICTIVE BEHAVIOUR

It would almost be impossible to find an adult human, who had not used psychoactive drugs at least once in his/her life time. In some cases, our contact with these substances is so frequent and socially accepted that most people have difficulties recognizing certain substances as 'drugs', e.g. caffeine and, until very recently, alcohol or nicotine. Addiction, however, is a much more restricted phenomenon with a prevalence of not higher than 10% of the total population (Bechara 2005; Li & Volkow 2005). These data reveal that addiction is not a necessary consequence of drug consumption, although of course, the latter is a requirement for the development of the former. Indeed, assuming a purely statistical criterion of 'normal behaviour', drug consumption should be considered as a normal behaviour, whereas

addiction would be a behavioural disorder. The main symptom of this disorder is a progressive loss of control over the amount and the circumstances accompanying drug use. This loss of control results not only in higher drug consumption, but also in a compulsive search and inability to refrain from its use even after long periods of abstinence (relapse), especially when exposed to stimuli previously associated with the drug or a stress source. As a consequence of this compulsion, there is a reduction in the goals and repertoires of the individual's behaviour with the resulting loss of social compatibility. Thus, addiction is a behavioural disorder that seems to occur exclusively in humans. Indeed, there is to date not even one description of addictive behaviour for any other animal species living in its habitual ecosystem [3]. A discussion of the possible reasons for this discrepancy is beyond the scope of this review; however, it raises a very important question: if addiction does not appear in animals living in their naturalistic environments, are these animals appropriate subjects to recapitulate addiction in a laboratory setting? While this question may be unanswerable, the position held in this review is that addiction, in contrast to drug consumption, can hardly be reproduced in its entirety in an experimental situation (although some remarkable attempts have been made). Yet, specific elements of addictive behaviour could be adequately modelled. Further, this strategy might be a valuable one to identify both the basic mechanisms governing addiction as well as therapeutic agents to improve the coping strategies of the addicted patient [4]; perhaps, though, it may never provide us with a complete understanding of this phenomenon.

Among the attempts to develop a holistic model of drug addiction in rodents, the work of Heyne and Wolffgramm should be highlighted (Wolffgramm & Heyne 1995; Heyne 1996; Heyne & Wolffgramm 1998). In their experiments, after long periods of voluntary drug consumption, some rats developed the principal behavioural features included in our current definition of addiction. Thus, these rats showed an increased preference for the drug, even when the drug (but not the water concurrently available) was adulterated with bitter quinine flavour. This observation demonstrates persistence in drug consumption in this subgroup of animals regardless of the adverse consequences associated with it. In addition, the intake in these rats was no longer sensitive to certain environmental (i.e. housing conditions) and social (i.e. social rank) factors that had an important influence during previous stages of the experiment; this observation indicates 'loss of control'. Thus, in accordance with current definitions of addiction, these authors showed that addictive behaviour can develop independently of physical dependence; moreover, the presence of the latter does not accompany or predict the appearance of the former. An extensive comparison of these studies from an integrative approach can be found in Heyne *et al.* (2000).

Other researchers, in the alcohol field (McBride & Li 1998; Spanagel & Hölter 1999; for a recent adaptation in mice see Fachin-Scheit *et al.* 2006), have partially reproduced the methods and findings used by Heyne and Wolffgramm; however, when studying other drugs, most of the current addiction research utilizes procedures that model specific features of addictive behaviour (or tests aimed at isolating specific related processes). In the following sections we review the most common of these procedures. However, it should be noted that the methods trying to model addiction features have only recently been developed; not all of them have been clearly standardized and their description is relatively more vague and conceptual than for the reinforcement procedures. Finally, regarding the growing use of transgenic mice to explore the neurobiological mechanisms governing drug consumption and/or addiction, it should be kept in mind that most of these methods have been primarily established and tested in rats. Indeed, the complexity of some of them has made the adaptation for mice rather difficult (Spanagel & Sanchis-Segura 2003).

2.1 Modelling features of addictive behaviour

2.1.1 Modelling drug seeking and relapse

Drug-seeking behaviour is a common expression that refers to those behavioural patterns aimed to search, acquire and forage for the drug when this is not readily available. Some authors refer to this phenomenon as the behavioural expression of a hypothesized craving. Compulsive drug seeking and relapse to drug use is considered as one of the central

[3] There are descriptions of occasional and even chronic drug consumption in several non-human species in their naturalistic environment. However, none of these reports describe the identification of individuals that have a compulsive drug-seeking or -taking behaviour, or individuals that show disruption of their normal circadian patterns (i.e. feeding), or individuals that become isolated of their congeners.

[4] We strongly believe that this kind of research can result in the identification of therapeutic agents that will increase the probability of success of the addicted patient to remain abstinent or to increase the control over drug consumption. However, note that we avoid the use of terms such as 'cure' because we consider that the neuroadaptations sustaining the transition from drug consumption to drug addiction could be very long lasting or even irreversible. In addition, by this formula we provide room to the evidence showing the importance of social factors as contributing factors for drug addiction as well as for success in the achievement and maintenance of the therapeutic objectives.

features distinguishing addictive behaviour from controlled drug use. Indeed, at the clinical level, craving and relapse is now considered as the main problems of human drug addiction, substituting the former focus on dependence/withdrawal. Although according to negative reinforcement theories withdrawal seems a logical trigger of relapse, there is not a single controlled study showing such a link (Shaham & Miczek 2003). In fact, the present clinical concerns are not so much related to the detoxification and initial abstinence periods as to the apparition of relapse after a long interruption in drug consumption (Hyman 2005). Therefore, it has been necessary to develop new methods to model drug seeking and relapse that appear without a withdrawal syndrome or long after it has passed. In these attempts it is assumed that relapse can be triggered by three main types of events: stress, environmental stimuli (i.e. cues) and internal stimuli (i.e. priming). The most common procedure to study drug-seeking and relapse-like behaviour has been the so-called reinstatement model.

2.1.1.1 Assessing drug seeking by the reinstatement model

The origin of the 'reinstatement model' can be traced to some findings of Pavlov and Skinner, but its application in the context of drug abuse research did not appear until 1971 (Strecht, Gerber & Wood 1971). The first report using this procedure as it is now actually understood was published in the classical paper by De Wit & Stewart (1981) and, since then, the number of studies referring to the use of this paradigm has grown exponentially over the last 10 years (Shaham *et al.* 2003).

Under this term different procedures are included, assessing all of them the resumption of a previously extinguished drug-reinforced behaviour in response to non-contingent drug delivery (i.e. priming), environmental stimuli previously associated to it or stressful stimuli. The reinstatement model is currently used in many laboratories to investigate mechanisms underlying '*relapse*' or relapse-like behaviour. However, it should be noted that the reinstatement test is performed under drug-free conditions. In contrast, a typical relapse in drug addicts and alcoholic patients is defined as enhanced drug/alcohol consumption following a period of abstinence; a relapse can therefore not happen under drug-free conditions. Having said this it remains unclear to the authors how the reinstatement model was put forward by many researchers as a model of relapse. This is *per definition* wrong but does not diminish the value of this model to measure augmented drug-seeking behaviour.

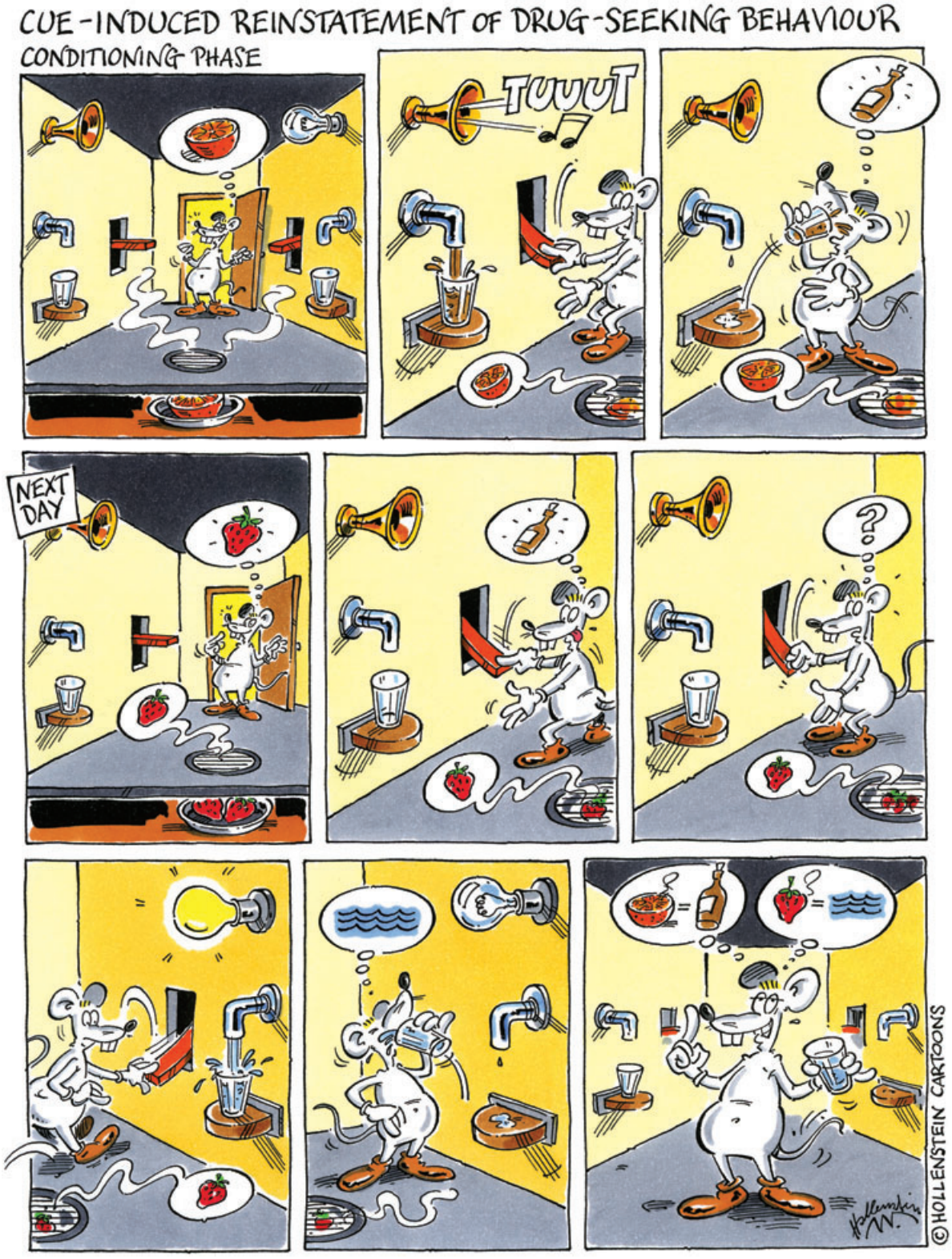
From a procedural point of view, it is clear that any drug reinstatement assessment needs to first 'instate' the self-administration behaviour to an adequate level, followed by a drug-free period (i.e. extinction) after which the resumption of the extinguished behaviour in response to a specific trigger will be tested. These three phases can be implemented in a single or, more often, several experimental sessions (for an excellent review on operant reinstatement procedures see Shaham *et al.* 2003). The drug self-administration procedure usually follows the generalities described in the section 1.1.2.

After this self-administration period, reinstatement procedures usually include an extinction phase (i.e. allowing the subject to perform the operant response, without programmed consequences) rather than an abstinence process. This supposes a reduction of the face validity of these procedures, since rarely do humans undergo extinction (for a more detailed discussion of this topic see Conklin & Tiffany 2002 and the correspondence generated by this review). Finally, the re-emergence of this behaviour in response to a specific trigger is conducted under a drug-free condition. This allows for studying the recovery of the extinguished behaviour without the interference produced by the psychoactive effects of the drug (i.e. a drug that increases locomotor behaviour could produce spurious increases in lever pressing), and the increase in the number of operant responses (compared with that observed during extinction) is understood as an enhancement of the subject's drug-seeking behaviour. However, this factor also distinguishes preclinical reinstatement procedures of human relapse episodes. In fact, in these methods, the operant response is reinstated, but again the subjects, *sensu strictu*, do not relapse because they actually do not resume drug consumption.

Three main sources of reinstatement triggers are used: stress, cues and priming drug injections. These three kinds of stimuli classify a wide number of procedural alternatives. It should be noted that the neurobiological mechanisms underlying the ability of these different kinds of stimuli (stress, cue or priming injections) to initiate and sustain lever-pressing behaviour after extinction show only a partial overlap (Sutton *et al.* 2003) and indeed they can show additive efficacy when combined (Liu & Weiss 2002).

Stress-induced reinstatement is usually implemented by the use of foot shock; however, other methodological possibilities have also been explored. Among them, food deprivation is of special interest because its effects on reinstatement can be abolished by leptin administration (Shalev, Yap & Shaham 2001). This finding resembles the human situation as reduced circulating levels of leptin are associated with self-reported craving (Kiefer & Wiedemann 2004).

Cue-induced reinstatement can be also implemented in several ways. Thus, cues can be introduced as a contingent consequence of operant responding. In this case, it is generally assumed that these stimuli act as 'conditioned reinforcers'



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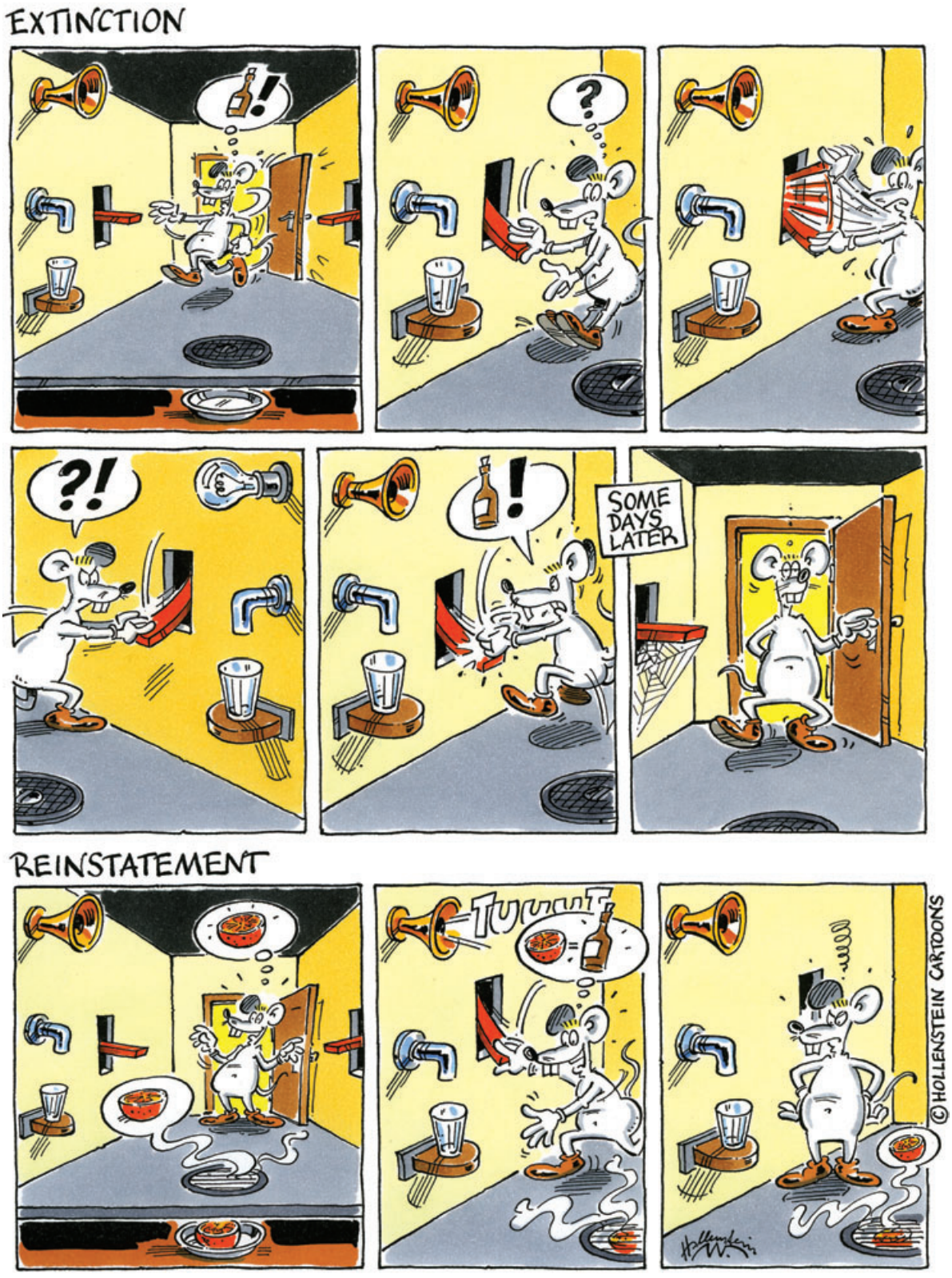
which boost lever-pressing behaviour. Furthermore, contextual stimuli or responding independent cues can also be implemented during the conditioning phase or non-contingently introduced during the reinstatement test sessions. In such a situation, these stimuli would rather act as 'occasion setters', i.e. with an 'informative' rather than 'incentive' value. Both kinds of cues can be also combined in the same procedure (e.g. see Bachteler *et al.* 2005) and separate tests



assessing the ability of each cue to trigger reinstatement could provide additional information of the psychological processes underlying a similar behavioural outcome (i.e. increased lever presses). At this point it is necessary to mention that in most of the cue-induced reinstatement studies have used cues that are contingently presented upon the completion of the operant requirements. However, according to some authors, a non-contingent introduction of drug-associated cues is a more valid strategy when assessing drug-seeking/relapse-like behaviour (Le & Shaham 2002).

It has been shown that cues associated to an alternative reinforcer can trigger the reinstatement of operant responding in the manipulandum previously associated to drug delivery (for a recent example, see Glasner, Overmier & Balleine 2005). This fact has been understood as a reflection of a change in the mechanism controlling operant performance, which would be initially dependent of the response–outcome contingency but would become rather governed by the ability of cues to set in motion-specific responses. In other words, this phenomenon suggests that, as proposed to other forms of cue-related behaviour (Holland 2004), extended drug self-administration may progress from an initial goal-directed performance to the formation of habits in which behaviour is mainly controlled by the 'activation'-related properties of conditioned stimuli (Everitt & Robbins 2005). However, information in this respect is still scarce and its interpretation is an open issue (Hyman & Malenka 2001).

Finally, reinstatement can also be triggered by priming injections. These priming injections are usually higher than the unit injection dose used in the self-administration procedure, but this parameter has not been clearly standardized. On the other hand, although priming injections could be considered as a particular case of cue-induced reinstatement (in which drug administration acts as an interoceptive cue), priming doses trigger other mechanisms that could also contribute to the observed enhancement of operant responding (i.e. non-specific activity increases). It is also important to note that when priming injections are used this procedure cannot be longer considered as a 'drug-free' test.

From this brief description it is clear that the reinstatement procedure, although usually referred to as a 'model', display only limited face validity. However, as mentioned before, face validity is not a critical aspect of the heuristic value of a procedure. Indeed, the key point is whether data obtained in the reinstatement model can be of relevance for understanding human drug-seeking behaviour or relapse. Thus, the question should be re-addressed towards the construct and predictive validity of this method. Considering construct validity, a direct comparison with human studies is difficult. As mentioned above, this procedure provides more a 'drug-seeking' than a 'relapse' model. However, clinical studies focus on the concept of 'craving', which seems to have a poor correlation with 'relapse' and 'drug seeking' (Tiffany & Conklin 2000). The predictive validity of the reinstatement model is also undetermined at present. In this regard, very few medications have been approved for relapse prevention and all of them are designed for the management of alcoholics or smokers. This contrasts again with preclinical studies, which have screened a high number of compounds, mainly with respect to cocaine reinstatement. Further, even when restricting this comparison to the closest points of overlap between clinical and preclinical studies, the findings are still inconclusive. Thus, some compounds such as naltrexone or acamprosate (Katner, Magalong & Weiss 1999; Bachteler *et al.* 2005) prevent ethanol reinstatement in rodents and diminish craving and relapse incidence in alcoholics. However, other compounds (i.e. fluoxetine) only seem to show a positive effect on animal models of reinstatement (Epstein & Preston 2003) or are used in the clinics without being tested in this procedure (i.e. bupropion; LeFoll & Goldberg 2006).

In summary, although this procedure is probably one of the more widely used methods for studying preclinical analogues of human drug-seeking behaviour, more research is needed to validate and improve its use. For a better overview of the present state of this question, we recommend reading the reviews by Epstein & Preston (2003) and Shaham *et al.* (2003).

2.1.1.2 Assessing relapse by drug deprivation

An alternative approach to assess relapse-like behaviour is the introduction of deprivation periods in animals that had voluntary long-term access to a drug. Thus, drug-experienced animals show a transient increase in drug intake after a period of forced abstinence, which is termed the 'deprivation effect'. The deprivation effect has so far mainly been applied to alcohol and it needs more systematic work to examine whether a deprivation effect might also occur with other drugs of abuse (for an example with cocaine, see Morgan *et al.* 2002). The alcohol deprivation effect (ADE) refers to the observed transient increase of ethanol consumption, when resumed after a period of forced abstinence. Although the first description of this phenomenon was made in the 1960s (Le Magnen 1960; Sinclair & Senter 1968), the use of ADE in the study of relapse-like behaviour is more recent. However, over the last few years, an increasing number of studies have explored the possible relevance of this method as an alternative to evaluating some aspects of relapse episodes that are not accounted for with the reinstatement model (Le & Shaham 2002).

The ADE can be considered as a model of relapse because it actually allows the individual to self-administer ethanol after a period of protracted abstinence. In this regard, it is not clear what the trigger exactly is, concerning this resumption, although ethanol itself may be acting as a cue (i.e. smell, taste), as a priming stimulus or both. Regarding face and construct validity, it should be noted that the ADE has also been observed in humans. Thus, when alcoholics violate a period of abstinence, they undergo a transient episode of heavy drinking, which eventually dissipates (Larimer, Palmer & Marlatt 1999). Furthermore, an ADE can be also reliably observed in heavy social drinkers. Clinical reports in smokers suggest a similar phenomenon. Furthermore, naltrexone and acamprosate, two medications currently used in the

treatment of alcoholic patients (Spanagel & Zieglgänsberger 1997), effectively reduce the ADE in rodents. Therefore, it seems that ADE in rodents relates to a similar phenomenon in humans and that it is useful in identifying possible therapeutic compounds. However, as a model of relapse, ADE presents also some disadvantages. First, although the length of the deprivation is an important factor in determining its appearance, the size of the ADE is rather constant. Second, although it is a very robust phenomenon in most rat strains, it is more inconsistent in mice and an opposite

ADE has been described in hamsters (Sinclair & Sheaff 1973; Spanagel & Sanchis-Segura 2003). Third, as there is not any direct assessment of the individual's behaviour, it is not clear which psychological processes underlie this phenomenon. However, more information can be obtained by the use of either a lickometer system or a drinkometer system in combination with telemetric devices. The future use of these combined apparatuses may help to study temporal drinking patterns and other behavioural responses during the ADE.

The repeated use of deprivation phases (i.e. repeated ADE) has been proven to be a useful method in inducing addictive features in long-term alcohol drinking rodents (Spanagel 2000). Thus, the introduction of repeated deprivation periods intercalated in long-term drinking models supposes something other than just a replication of the ADE. Indeed, this kind of manipulation should be rather considered as general strategy in holistic models of alcoholism and not as a series of repeated testing. In this regard, we have developed a procedure, in which after several months of voluntary alcohol consumption in a four-bottle free choice paradigm (0, 5%, 10%, 20% ethanol solutions) animals undergo repeated deprivation (2/3 weeks) phases (Spanagel *et al.* 1996; Spanagel & Holter 2000). From a methodological point of view, some aspects should be highlighted here. First, long-term self-administration is a major requirement of such a model. Indeed, increasing evidence has demonstrated that addictive features are only apparent in experimental subjects that have extensive drug experience (Deroche-Gamonet, Belin & Piazza 2004; Vanderschuren & Everitt 2004, 2005; Ahmed 2005). Second, the repeated deprivation phases should occur and have a duration that cannot be predicted by the subjects. Third, the use of a four-bottle design is not essential; however, it provides specific information to identify individuals that have transited from controlled drinking to an alcohol addiction-like state. Nevertheless, it has to be noted that due to the limitations of this particular experimental arrangement, a proper evaluation of addictive behaviour in those subjects requires additional testing, which is usually conducted during the deprivation phases. Thus, some animals undergoing such a procedure develop behavioural changes that resemble some of the core features of drug addiction. In these subjects there is an increase in the intake of alcohol, which arises from consuming larger amounts of more highly concentrated (i.e. 20%) alcohol solutions (Spanagel 2000) but also of an alteration of the temporal pattern of drinking (i.e. drinking activity during the daily dark and light phases no longer differs). These observations are reminiscent of the drug escalation and circadian disturbances (Danel & Toitou 2004) reported in human addicts. Further, drug escalation (Ahmed 2005) but also the alteration of the circadian-related machinery (Spanagel *et al.* 2005) might be correlates or part of the mechanisms that lead to other major features of addiction.

In addition, after repeated deprivation phases, alcohol intake remains unchanged after several manipulations (i.e. inelastic drug consumption). Thus, adulteration of alcohol solutions with quinine did not modify the ADE in those animals showing the aforementioned features of addictive behaviour (an aspect further discussed in section 2.1.3). Similarly, the concurrent presentation of a highly palatable sucrose solution did not reduce the enhanced ethanol preference in those animals. This observation seems to indicate a loss of interest for other rewards other than the drug, which is another of the DSM-IV criteria for 'dependence' (or, in the terms used in this review, addiction) [5]. To obtain more information about the functional consequences of this procedure, some additional tests can be easily incorporated. These tests usually require the use of separate equipment. Thus, using an operant procedure similar to those described in section 1.1.2), it was confirmed that after repeatedly experiencing deprivation phases, rats exhibit a higher motivation for alcohol, as illustrated by higher breakpoints under a PR schedule.

In summary, alcohol drinking after repeated deprivation phases seems to differ from that observed in the same subjects at earlier stages of their alcohol history. Thus, in some subjects, repeated deprivation phases lead to the development of core characteristics of addictive behaviour such as intake escalation, insensitivity to the negative consequences or alternative reinforcers to alcohol consumption as well as the interference of this behaviour on the

[5] This DSM-IV criteria of 'drug dependence' has been as controversial because an apparent disparity between preclinical and clinical studies. Thus, although in human addicts a lack of interest in alternative rewards and activities is often self-reported, preclinical research has accumulated data indicating that other reinforcers (i.e. food, sex, etc.) can gain salience after chronic drug exposure (for a general comment see Ahmed 2005; Vanderschuren & Everitt 2005) and that the availability of an alternative reinforcer (i.e. sucrose) reduces drug self-administration (Campbell & Carroll 2000). However, these two separate sets of observations do not necessarily contradict each other but rather seem to highlight the differences between controlled and 'compulsive' drug consumption. Thus, at least for some authors (Heyman 2003), the term 'compulsive' should be used as synonymous 'incontrollable' or 'insensitive to the impact of new information, incentive and values'. In this regard, we agree with this author when asserting that the term 'compulsive' has been used too laxly in the drug abuse research, becoming almost as a synonymous of 'enhanced preference'. However, we do not subscribe that using the label 'compulsive' to characterize addiction supposes to ignore how much is known about drug use. Rather, we think that the use of this term should be restricted to describe behavioural patterns that remain insensitive to the negative consequences associated to drug consumption and/or the possibility to access to alternative reinforcers.

normal behavioural and physiological patterns (i.e. circadian rhythms of activity/rest). From this perspective, this kind of manipulation provides a suitable experimental strategy to induce some characteristics of alcoholism in rodents, in which the ADE can be used as a measure of relapse behaviour. However, it is important to note that in this model, only one behavioural outcome can be assessed (consumption/preference) which does not provide all necessary information about the underlying processes to these behavioural shifts. Therefore, it needs to be complemented with other additional testing procedures (for a more complete discussion of this procedure see Spanagel & Hölter 1999; Spanagel 2000).

2.1.2 Modelling loss of control/impulsivity

Addictive behaviour is usually characterized as 'habitual', 'compulsive' or 'inelastic' meaning that, in the transition from drug consumption to drug addiction, there is a progressive loss in the ability to refrain from drug-related behaviours. This loss of control by the subject over its own behaviour results in increased drug seeking and consumption, including its extension to new contexts and situations, in spite of the appearance of harmful consequences. Therefore, loss of control is considered to be a core feature of drug addiction. However, the psychological processes underlying 'loss of control' are not fully understood and, consequently, there is no general agreement how to measure this phenomenon in laboratory animals in the context of drug abuse.

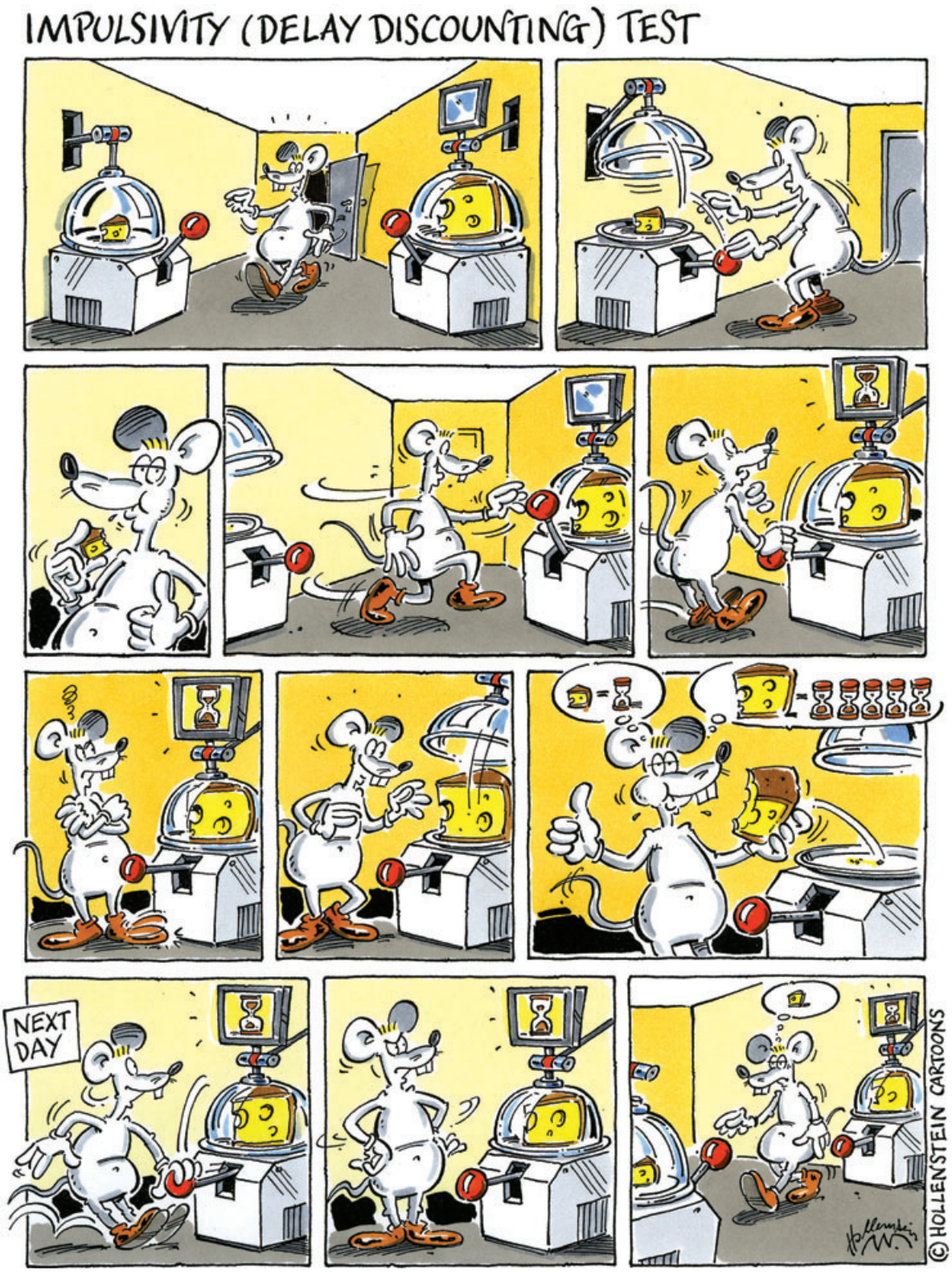
Some researchers assume that this phenomenon can be considered as a consequence of other features involved in the development of addiction (i.e. craving) and therefore it may be inferred from increased drug taking (Koob 2000). Thus, data obtained in some of the aforementioned models and tests to measure drug reinforcement can be reinterpreted from an 'addiction perspective', especially when drug taking/seeking increases (or persists) after the devaluation of its rewarding properties (Ahmed *et al.* 2002). Indeed, one of the current theories of addiction has widely used this kind of methods to measure its central concept, namely allodynia (Koob 2000; Ahmed *et al.* 2002; Koob *et al.* 2004; Markou *et al.* 1993)—the concept of allodynia refers to the establishment of a new 'set-point' of the reward system. Others have proposed that loss of control could derive from enhanced (i.e. sensitized) drug-seeking behaviour (Robinson & Berridge 2003). At the methodological level, this addiction theory emphasizes an increased effort invested to obtain the drug (e.g. increased breakpoints in PR schedules) as the principal landmark of addiction, but still using drug reinforcement-related methods (Salamone & Correa 2002; Robinson & Berridge 2003; Salamone *et al.* 2003). A third current theory of addiction proposes that loss of control and the main features of addictive behaviour arise from wrong decision-making processes and/or impulsive behaviour (Bickel & Marsch 2001; Bechara 2003, 2005). However, it should be noted that most of the evidence about the role of decision-making processes in addictive behaviour comes from studies in human and non-human primates, although more recently an effort to study these processes in rodents has been made. Such an attempt has required the development of a specific methodological approach able to capture complex behavioural features such as 'impulsivity' or 'willpower'.

Willpower is defined as the combination of determination and self-discipline that enables somebody to do something despite the difficulties involved, including enduring sacrifices now to obtain gratifications later (Bechara 2005). Thus, willpower seems to provide the reverse side of a general definition of the 'loss of control' that characterizes drug addiction [6]. From this theoretical perspective, behavioural choices as those involving the decision to consume a drug or not are derived from the relative preponderance of at least two interacting dynamic systems with opposing roles, one producing immediate urges and other pondering them through the use of non-actual (i.e. memory recall, prevision) information. These systems are usually referred as the 'impulsive' and the 'reflective' system, respectively (Bechara 2003, 2005). Increased activity of the impulsive system leads to behavioural hyperactivity as well as to hypersensitivity and biased attention towards immediate rewards. Such a behavioural pattern presents clear similarities with the notion of impulsivity, which is considered an important temperamental [7] factor in the vulnerability towards drug addiction (Kelley, Schochet & Landry 2004; Kreek *et al.* 2005) and that underlies to several criteria of the DSM-IV diagnostic criteria for 'substance abuse' (Evenden 1999).

Different procedures have been proposed as 'rodent models of impulsivity' but not all of them seem to be measuring the same phenomenon (Evenden 1999; Winstanley *et al.* 2004). To understand loss of control and addictive behaviour, an important impulsivity component is the so-called 'impulsive choice', which is often operationally defined as an

[6] The lack of 'willpower' is also commonly invoked in non-scientific views of addiction. However, in these cases the lack of 'willpower' is considered as a moral weakness, whereas the scientific use of this concept refers to a cognitive impairment similar to that observed in patients with ventromedial prefrontal lesions.

[7] The concept of 'temperament' is here referred to 'personality' to emphasize its existence in mammals other than humans. In this regard, it should be noted that 'impulsivity' definitions vary across fields (psychiatry, human psychology, etc.), probably as a result of the different weight assigned to different dimensions of this concept.



abnormally high preference for small but immediate rewards over larger delayed rewards (Monterosso & Ainslie 1999). Although theoretically it could emerge from an abnormal processing in the magnitude of rewards, empirical findings suggest that impulsive choice results from a specific devaluation factor associated to reward-access delays. Interestingly, some methods to assess this cognitive bias have already been developed. Most of these methods rely or can be reinterpreted from the principles of temporal discounting (Monterosso & Ainslie 1999). Temporal discounting may be

defined as the temporal weakening of consequence effects, due to delay and therefore it is opposed to self-controlled behaviour (Monterosso & Ainslie 1999; Critchfield & Kollins 2001). Thus, these methods are usually designed so as to present the subject two behavioural alternatives, one offering access to a small reinforcer after a short delay and another that results in a bigger reinforcer after a larger but variable lapse. Choosing a bigger but delayed reinforcer is then understood as a reflection of self-controlled behaviour, whereas a higher degree of temporal discounting indicates a predominance of immediacy when valuating different behavioural options, a process that can be extended to the cues that predict those rewards.

Operant boxes are ideal devices for establishing temporal discounting-based tasks in rodent models (Monterosso & Ainslie 1999). Among them, probably the most common is the procedure called 'delay of reward' (for an example, see Cardinal *et al.* 2004). In such a procedure two different levers are used, each one associated with a different reinforcement alternative. It is fundamental that the levers representing each alternative are simultaneously presented and that the operant requirements are identical (usually an FR1). Therefore, the two available options differ only in the delay (which can be cued or not) and magnitude of the obtained reinforcer. The time between the response and the reinforcer delivery for the delayed reinforcer is then changed across discrete trials. The obtained data are used to generate the so-called *indifference curves* that permit to shape the rate at which the delayed rewards are discounted. In this regard, it was initially proposed that this weakening followed an exponential decay; however, empirical data rather support a hyperbolic (or very similar) discount function. The discounting rate can then be calculated by specific equations (Mazur 1986, 2001; Ho *et al.* 1999; Bickel & Marsch 2001).

Considering drug consumption as a choice between the 'immediate' rewarding properties of the drug and the deferred benefits of abstinence (i.e. health), delay discount procedures seem to represent a suitable method for the exploration of some aspects of loss of control. In this regard, an important source of construct validity of rodent models based on this rationale comes from the higher rate of discount displayed by drug addicts in similar tasks (Bickel & Marsch 2001; Kirby & Petry 2004). Interestingly, temporal discounting seems to be related to the nature of the delayed reinforcer. Thus, although temporal discounting is a general bias, in addicts to tobacco the discount index is bigger for cigarettes than for other reinforcers (with the possible exception of money). Furthermore, neuroimaging studies in humans as well as lesion studies in rodents have shown that several brain areas and neurobiological systems are involved in both, temporal discounting and drug addiction (Cardinal *et al.* 2004; Bechara 2005).

In summary, the application of the principles of temporal discounting to explore loss of control in the context of drug addiction has produced an initial set of encouraging results (for a recent review of this and other application of the behavioral economics in the context of addition can be found Heather & Vuchinich 2003). However, this conceptualization of loss of control has also some drawbacks. Thus, it should be noted that enhanced delay discount functions is not an exclusive feature of drug addicts, but is also present in other psychiatric disorders (i.e. attention hyperactivity disorder). Furthermore, although temporal discounting aims to provide an explanation of loss of control in drug addicts, the neuropsychological processes responsible for this phenomenon are not clear and need further research.

Before ending this section, it should be highlighted that the construct of impulsivity, or better its possible antonyms 'self-control' or 'willpower', provide a wider framework that goes beyond temporal discounting-based methods. These concepts can be useful to understand the selection of non-adaptive behavioural choices that characterize loss of control in drug addicts as well as in the search of therapeutic interventions over them. Thus, for example, the exaggerated influence that drug-associated cues generate over addicts' behaviour can arise from a decreased activity of the 'reflective' and other inhibitory systems. This phenomenon has been related to the so-called 'motor-impulsivity' (Patton, Standford & Barratt 1995; Brunner & Hen 1997; but see also Evenden 1999), which can be an innate behavioural style but also a consequence of extended reinforcement of the responses leading to drug acquisition and consumption (i.e. habit formation). This kind of behaviour implies the existence of pre-potent responses and a disconnection between responses and the obtained outcome and it can be analysed in a large number of behavioural tests. Thus, and just referring to procedures described in this review, cue disruption in delay discounting tasks as well as the resumption of previously extinguished operant responses (e.g. reinstatement) or Pavlovian approach can be understood as impulsive behaviour derived of a poor inhibitory control. In this regard, drugs able to enhance inhibitory control over cues-induced behaviour might provide a more fruitful therapeutic approach than trying to modify the internal valuation of drugs as rewards (i.e. extinction-, devaluation-based strategies), which has been the objective pursuit in most of the current 'anti-addictive' medications (i.e. naltrexone, antabus). Interestingly, different mechanisms (e.g. control of attention) contributing to the maintenance/enhancement of self-controlled behaviour have already been identified (Monterosso & Ainslie 1999) and, consequently, the development of preclinical models that allow their exploration might be a promising avenue in the field of the psychopharmacology of drugs of abuse.

2.1.3 Modelling drug consumption despite negative consequences

One of the criteria applied in any definition of drug addiction is the use of the drug despite its negative consequences. Thus, despite being aware of the deleterious effects of drug consumption, addicts display enormous difficulties in maintaining abstinence or exhibiting controlled drug intake. This situation relates to the previously introduced notion of loss of control in the sense that it implies biased 'decision-making' strategies as well as the extension of time and resources devoted to obtaining and consuming the drug. However, both phenomena can be dissociated, because some aspects of the 'loss of control' may occur regardless any aversive event. Indeed, separating these two concepts seems to be in agreement with some theoretical models widely accepted in the fields of psychology ('impulsivity' versus 'venturesomeness'; Eysenck 1993) and psychiatry (i.e. 'reward dependency' versus 'harm avoidance'; Cloninger 1987).

Any attempt to model this feature of addictive behaviour should provide a scenario in which drug seeking and/or drug taking ought to persist regardless of adverse consequences. One approach is the Iowa Gambling Task (Grant, Contoreggi & London 2000; Bolla *et al.* 2003) that has been developed to study erroneous decision-making processes implying aversive consequences in humans and other primates. These studies have shown that drug addicts persist in making risky choices that lead to important reward losses (i.e. money). An adapted version of this task for rodents is currently under development (Van der Boss, Lasthuis & Spruijt 2005) although so far it has been not explored in the context of drug addiction research.

Other attempts to model this feature of addictive behaviour provide a scenario in which the impact of concurrent aversive events (or stimuli that signal them) on drug-seeking and/or drug-taking behaviours are studied. In this regard, a few studies have developed adaptations of traditional 'conflict paradigms' to reproduce such a situation by the simultaneous delivery of a self-administered drug and an aversive/painful stimulus. Thus, for example, Deroche-Gamonet *et al.* (2004) assessed the resistance to punishment during cocaine self-administration by simultaneously delivering both the drug and an electrical shock. In this study, the suppression of cocaine self-administration was reduced in rats with a longer history of drug consumption and a higher sensitivity to cocaine-induced reinstatement. Interestingly, this sensitivity to punishment also displayed a good correspondence with other proposed measures of addictive behaviour such as drug seeking in the absence of drug delivery and motivation for drug consumption as measured by a PR schedule. Indeed, all three tests saturated in only one factor (termed 'compulsive drug intake') separated from other phenomena underlying addictive behaviour identified in this study as 'impulsivity/disinhibition'. However, the interpretation of data obtained in conflict-based procedures can be cumbersome, at least when using drugs of abuse which modify pain and/or anxiety thresholds (i.e. morphine or ethanol). Indeed, it is well known that ethanol reduces drinking suppression in different conflict tests such as the 'Vogel test' or the Geller-Seifer procedure (Baldwin *et al.* 1991; Millan & Brocco 2003) and that morphine administration modifies pain thresholds, then reducing the impact of these 'negative consequences'.

Some alternatives have been explored to surmount the problems created by the interaction between the pharmacological actions of drugs and the aversive/harmful events used in these conflict-based procedures. Thus, some authors (Vanderschuren & Everitt 2004) have proposed the substitution of the harmful event (i.e. foot shock) with a CS associated to it (i.e. tone). This procedure seems to be embedded in the principles of the conditioned emotional response and it is somehow similar to the Pavlovian instrumental transfer (PIT), and it demonstrates that CS associated to harmful events produce a relative suppression of responding for cocaine. Interestingly this effect is not apparent in long-term cocaine self-administering rats, an observation that seems to be in accordance with the current views on addiction as a disorder that develops only after prolonged self-administration. However, this procedure also presents some inconveniences: first, the suppressant effect of the CS is rather small (although this could also be due to the 'group-based' statistical treatment of the data chosen in this study). Second, in this model, the aversive consequences are not proportional nor contingent to the self-administration behaviour, a fact that clearly separates it from the human situation and that could have important consequences in the subject's behavioural choices.

In the context of alcohol research, the inelasticity in alcohol consumption after the addition of a bitter flavour such as quinine (Wolffgramm & Heyne 1995; Spanagel *et al.* 1996) has been understood as a rodent equivalent to human drug consumption despite harmful/aversive consequences. This procedure has the advantage that the psychopharmacological effects of the drug do not seem to interfere with the aversive properties of quinine. In addition, conversely to other similar reward devalution-based procedures (Dickinson, Wood & Smith 2000; Miles, Everitt & Dickinson 2003), in this case the magnitude of the aversive event is contingent and proportional to the self-administration behaviour. Futher, the suppressant effect of quinine addition is not observed in long-term experienced rats. However, this procedure may be more difficult to implement for other drugs of abuse because, as described in section 1.1.1, not all drugs

are easily orally self-administered, although it should be noted that its suitability for amphetamine and etonitazene has been proven (Heyne 1996; Heyne & Wolffgramm 1998).

In summary, nowadays some attempts to model drug consumption despite aversive/harmful consequences have been proposed. However, as for almost all procedures evaluating addictive behaviour features, none of them have been properly validated. In addition, from an operational perspective, it should be noted that most of these animal methods are based on punishment-related contingencies, whereas human studies use suppression of rewards (i.e. money in the Iowa Gambling Task) to model 'negative consequences' of wrong decision-making processes. These methodological differences might have an impact in understanding this phenomenon in addicted patients. In this regard, given its importance in the current criteria to identify/diagnose a subject as an 'addict', the development of animal models for this phenomenon (and the related 'loss of control') should be a primary objective within the drug abuse field.

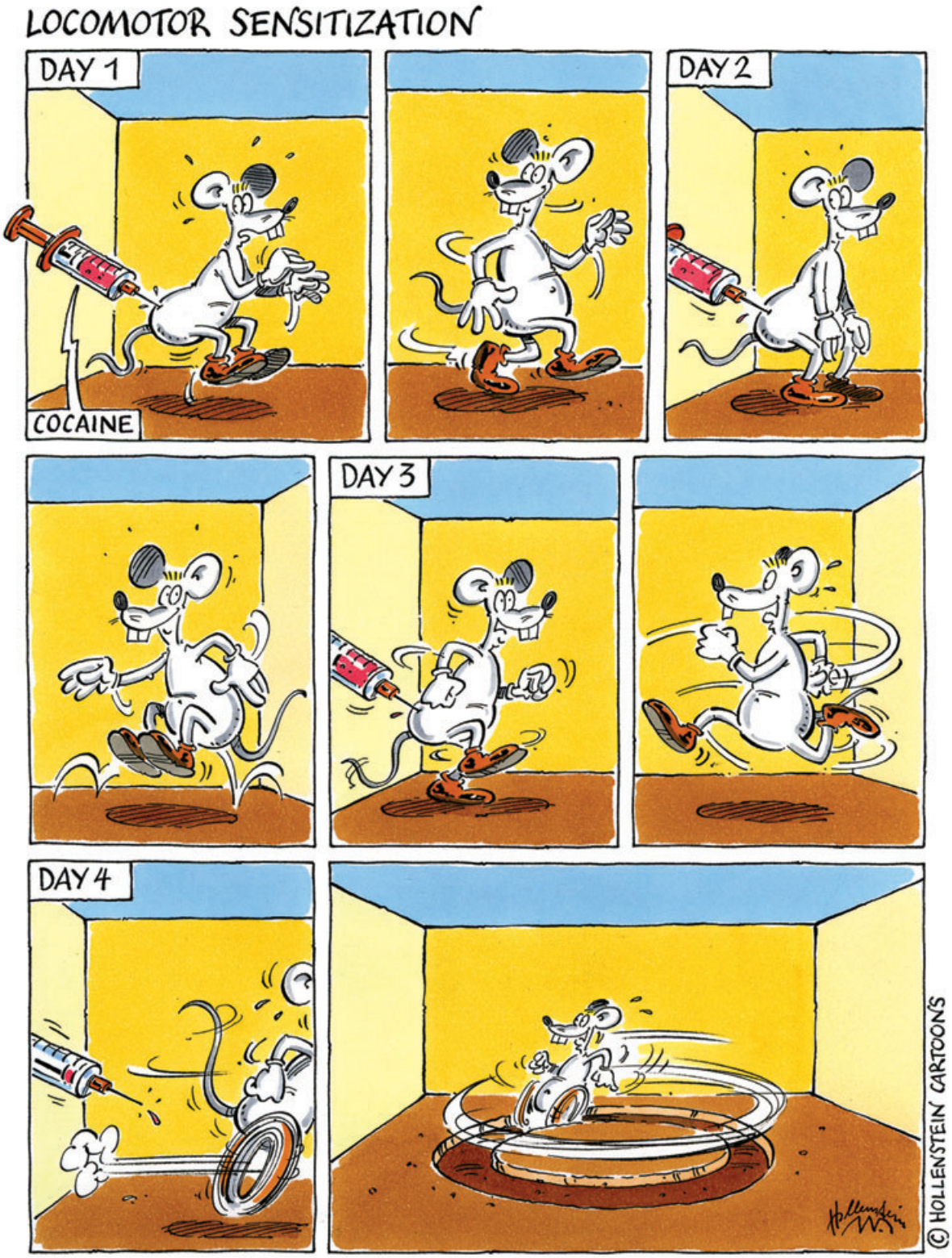
2.2 Tests currently used in the study of addictive behaviour

2.2.1 Behavioural sensitization

The term sensitization refers to an increase in a response after the repeated occurrence of the stimulus that promoted the aforementioned response. Sensitization in drug abuse research has been mainly studied with respect to locomotor activity. Thus, the ability of addictive drugs to increase locomotion after an acute administration is progressively enhanced, when drug exposure is repeated. This so-called *psychomotor sensitization* is a very robust phenomenon that has been observed across several species.

From a procedural perspective, and although there are variations among the published locomotor sensitization protocols, to achieve this effect, any sensitization protocol should use a route of administration with a fast onset of drug effect (usually intraperitoneal or intravenous injections are used) and the drug has to be given intermittently (Samaha & Robinson 2005). Moreover, sensitization is stronger, when the dose is higher or when escalating doses are administered (Badiani, Oates & Robinson 2000; Michel & Tirelli 2002). Some drugs such as psychostimulants (Jackson & Nutt 1993) and morphine (Vanderschuren *et al*. 2001) can even trigger a sensitized response after a single pre-exposure if the dose used was high enough. Finally, although sensitization is in essence a non-associative learning process, the context plays a major role in the development and expression of locomotor sensitization (Robinson & Berridge 2003). Thus, a higher degree of sensitization is usually observed when drug injections are administered in a context different from that of the home cage and very often subjects may fail to express sensitization, when they are challenged in a different context, where they had never experienced the drug.

The interest of this phenomenon in the context of drug addiction comes from its central role in one of the most currently assumed theories of addiction (Robinson & Berridge 1998, 2003). According to this proposal not only psychomotor stimulation but also the incentive salience attributed to an initially neutral stimulus is progressively increased, when this stimulus is contingent and repeatedly associated to the drug administration, therefore leading to an *incentive sensitization* (for a comprehensive description see Robinson & Berridge 1998, 2003). This 'incentive sensitization' has been proposed as a possible mechanism in explaining the transition from a regular pattern of voluntary drug intake to compulsive drug-seeking and -taking behaviour (Robinson & Berridge 1998, 2003). Thus, in the terms often used by these authors, drug addiction can be seen as a product of the sensitization in the drug 'wanting' with no change (or a decrease) of the drug 'liking'. However, to test the occurrence of this phenomenon, but ruling out other confounding processes (i.e. changes in the hedonic value of the drug), a very sophisticated experimental approach is required (Wyvel & Berridge 2000, 2001). For this reason, no standard protocols to test incentive sensitization are currently available. Considering the practical difficulties of directly assessing incentive sensitization, an alternative has been to explore the differences between 'sensitized' and 'non-sensitized' animals in different model/tests related to addictive behaviour. These investigations have shown that repeated drug exposure results in enhanced responsiveness to Pavlovian conditioned stimuli and conditioned reinforcers as well as in increased breaking points in PR drug administration schedules (Vezina 2004) as well as enhanced drug-seeking behaviour in priming- but not stress-induced reinstatement (for a critical review of these findings, check Cardinal & Everitt 2004; Shaham & Hope 2005). However, because most of this evidence has been obtained in cocaine-related studies, the generality of these findings needs to be specifically addressed. Furthermore, it should be noted that continued drug exposure results in neuroplastic changes other than those related to sensitization that also could promote addictive behaviour through sensitization-independent processes (Kalivas 2005; Koob & Le Moal 2005; Nestler 2005). Finally, the possible relevance of drug-induced sensitization in humans is still unclear (Sax & Strakowski 2001) and it is generally agreed that more research is needed about the exact significance of sensitization and the



neuroplastic alterations underlying this phenomenon (Cardinal & Everitt 2004; Kalivas 2005; Shaham & Hope 2005; Vanderschuren & Everitt 2005).

2.2.2 Second order schedules in drug-seeking behaviour assessment

Although the concept of second-order schedules had a broader meaning initially, in the context of drug addiction research it is almost restricted to refer the use of chained schedules. In such a situation, the subject has to complete two

different trains of responding before obtaining the reinforcer. Thus, the completion of the requirements of a first operant schedule results in the apparition of an initially neutral stimulus. Satisfying the requirements of the second schedule (in the presence of the neutral stimulus) will result in the delivery of a reinforcer. Therefore, while the behaviour for the final schedule would still be paired with a primary reinforcer, the behaviour in the earlier component of the chain is maintained by the acquired motivational properties of a previously neutral event. That is, by a Pavlovian association, this stimulus acts as a conditioned reinforcer.

Second-order schedules of drug injection maintain high rates of responding. From this perspective, they can be a useful method for investigating the reinforcing effects of drugs and some aspects of addictive behaviour. However, it is difficult to maintain behaviour separated by more than a few links from the primary reinforcer. Because of this, although the second-order schedules were introduced in the context of primate drug self-administration in the 1970s, its use is rather scarce, especially in mice. However, and of major importance for the study of addictive behaviour, second-order schedules of reinforcement can, if used appropriately, separate responses that are affected by the self-administered drug from those that are not. Thus, in second-order schedules, 'drug-seeking' behaviour, can be equated to responding in the first link of the chain, distinguishing it from the more familiar 'drug-taking' behaviour, which is related to the second train of responding of this method (Everitt & Robbins 2000). In addition, the influence of drug-paired stimuli can also be assessed. Nevertheless, it should be noted that until present, these schedules have been mainly used for cocaine and heroin studies, while its possible viability when studying other drugs is more doubtful (for a review, see Schindler, Panlilio & Goldberg 2002).

As mentioned above, one of the major 'problems', when using second-order schedules, is that they require extensive training. Thus, it is usually recommended to train the animals in a simple (i.e. FR1) schedule, in which drug delivery is accompanied by the apparition of a neutral stimulus (i.e. light). When this response has been established, the second-order procedure can be started. Both chained schedules may involve fixed (rather low) ratio requirements, although the use of fixed interval schedules in the first link could provide some advantages (Everitt & Robbins 2000). It is noteworthy that responding in second-order schedules usually shows higher variability than that observed in other operant procedures. This is not necessarily an inconvenience as it could rather be reflecting individual differences. Indeed, such an individualized analysis can be preferred when studying conditioned processes (Gallistel, Fairhurst & Balsam 2004). Furthermore, assuming that addiction only occurs in a subset of individuals that consume drugs, studies accounting for individual differences can be more relevant than those providing group averages. In this regard second-order schedules may be able to capture those individual variations better than other procedural alternatives.

On the other hand, second-order schedule performance captures different learning processes (both Pavlovian and instrumental) underlying the global phenomenon of drug-seeking behaviour. These different processes can be further characterized by using other experimental methods directly focusing on each of them, such as autoshaping and PIT (Everitt *et al.* 2001). From a more general perspective, the current interpretation of data obtained under second-order schedules implies a separate treatment of the performance under a 'drug-free condition' (i.e. until the first drug injection). The construct validity of this separation seems to be supported by the fact that it is differentially influenced by several procedural variables. Thus, rate response in this initial phase presents a linear relationship with the drug dose finally delivered. This fact provides a clear advantage for statistical treatment than the usual u-inverted relationship often found in drug self-administration under operant conditions (including the subsequent cycles in a second-order schedule). In addition, this first train of responses is exclusively under control of the CS, thus providing an experimental situation to explore the influence of cues without introducing an extinction procedure. A detailed discussion of this topic can be found in a recent review by Everitt & Robbins (2000).

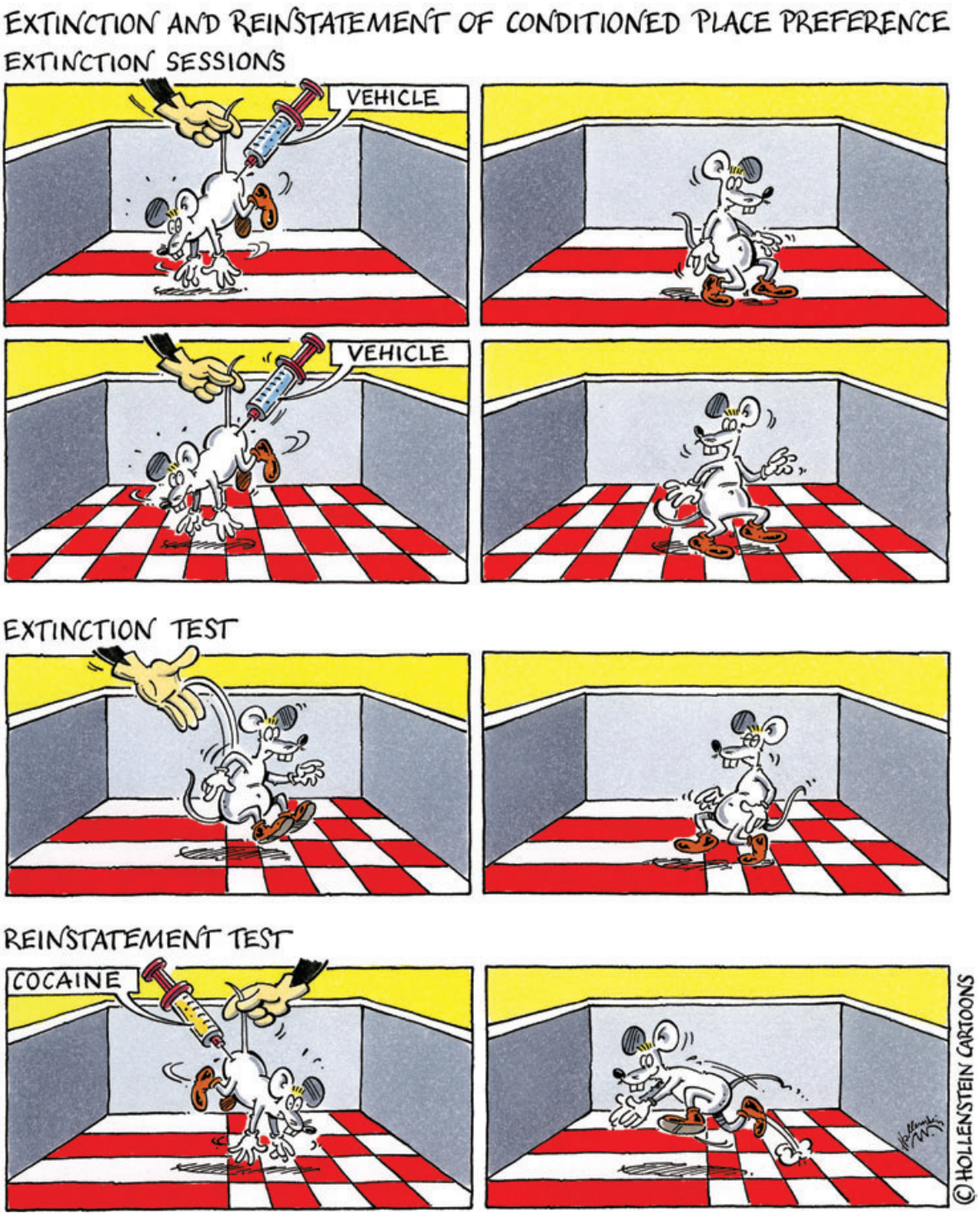
In summary, the main advantages of second-order schedules of drug injection in the study of addictive behaviour are that they maintain high rates of responding, allowing an assessment of the impact of drug-paired stimuli. In addition, for the duration of the first cycle, drug-free behaviour (i.e. drug seeking) can be measured. Further, this kind of procedures could be specially sensitive to isolate individual differences, which may be of major interest in the study of addictive behaviour. However, it is clear that further development and a more extensive validation of these procedures is needed. In addition, second-order schedules involve complex learning, which translates into long training but also giving rise to possible concerns and limitations, when using mice (Spanagel & Sanchis-Segura 2003).

2.2.3 The use of conditioned place preference to measure the reinstatement of drug-seeking behaviour

In the context of reinstatement behaviour, some efforts have been made to study reinstatement of drug-induced CPP in rodents. As explained above, in a typical CPP experiment, subjects are injected daily with the drug and paired with a specific compartment. On alternating days, the animals receive saline injections and are then paired with a

distinguishable compartment in a conditioning box. After several days of conditioning, a drug-induced CPP is achieved. Then, and contrary to the ordinary CPP procedure, this acquired preference is extinguished with repeated saline injections in both the previously drug-paired compartment and the saline-paired compartment. Following the extinction phase, the reinstatement of CPP is initiated by drug priming. Thus, the ability of a drug priming injection in renewing a marked preference for the previously drug-paired compartment is understood as indicative of reinstatement *drug-seeking* behaviour.

This procedure has already been used in rodents' and it has been demonstrated that drug priming injections (Itzhak & Martin 2002; Kuzmin *et al.* 2003) can reinstate extinguished drug-seeking behaviour in a CPP paradigm. In



addition, there is evidence that, at least to a certain extent, relapse can be triggered by drugs which share a similar mechanism of action with that use to establish the CPP (Itzhak & Martin 2002). However, what is required in the future is a more rigorous standardization of the test protocol. For example, there is a trend towards high doses as the priming stimulus (i.e. Itzhak and Martin used a dose representing a 75% of the conditioning dose) but no systematic studies have been conducted to optimize this critical variable. Although this procedure seems straightforward, more systematic work is still necessary in order to fully understand the conceptual background of this paradigm. Thus, understanding the results of this test in the context of drug-seeking behaviour is still a controversial issue (see section 1.2.1.1). Furthermore, in a recent study it has been shown that conditioned as well as unconditioned factors may contribute to the reinstatement of cocaine place conditioning in C57BL/6J mice (Szumlinski *et al*. 2002). Finally, it should be mentioned that the extinction phase of this paradigm has not received proper consideration. More specifically, we think that differences in the resistance to extinction could provide avaluable index of drug seeking behavior, which interpretation seems more straight forward than that of the reinstatement of CPP after a priming injection.

2.2.4 Other attempts to measure drug-seeking behaviour

Runway-based procedures have been also used to study the reinstatement of drug-seeking behaviour after an extinction phase (for a representative example check McFarland & Ettenberg 1997). In this method, animals are trained to run, after a cue is presented, from a start box to a goal box, where they receive drug injections. In alternate trials, another cue is associated to saline injections. Over trials, the latency to reach the goal box is selectively reduced in those trials signalled by the cue predicting drug injection, reflecting an enhanced motivational state (drug seeking). At this point, an extinction procedure is introduced by programming trials in which no cues are presented and no drug is delivered. This produces an increase on the latency to reach the goal box, achieving values similar to those observed in the 'saline-trials'. Reinstatement of drug-seeking behaviour can then be assessed by reintroducing the cues or by a priming injection in the goal box during the first trial of the reinstatement session.

Clearly, this method has some similarities with the use of place preference in measuring the reinstatement drug-seeking behaviour, although perhaps, with the advantages of demanding a more 'active' demonstration of this phenomenon and incorporating the possibility of assessing cue-driven behaviour. A drawback of this procedure (also shared by CPP-based reinstatement tests) is that drug exposure is not a consequence of voluntary self-administration and confined to a reduced number of injections. This fact seems to reduce the interest of this procedure to mimic the processes derived from 'compulsive' drug use, but a definitive evaluation of its usefulness should wait until more studies have determined its validity.

3 CONCLUDING REMARKS

From the overview provided, a series of conclusions can be derived. These conclusions cannot be completely objective and also include personal opinions about the current state of the behavioural models and tests used in studying drug reinforcement and addictive behaviour.

1 Models for self-administration and reinforcing efficacy are more or less currently established and some of them are properly validated. However, other experimental alternatives, such as CTP, with a clear potential utility are virtually unexplored in this field. Furthermore, there exists a pervasive confusion about what exactly is being measured by some of the currently available tests. A better use of the theoretical framework provided by experimental psychology and affiliated disciplines (i.e. behavioural economics) is required for future improvements.
2 Although we have tried to classify models with respect to specific elements of addictive behaviour, these methods are not clearly established and most lack a clear validation. Furthermore, depending on the author, it is claimed that one procedure measures one or another aspect of addictive behaviour. Therefore, the unification in the terminology and conceptual framework is needed. This unification is not only needed at the preclinical level but should also provide the interface for a fruitful exchange with clinical researchers.
3 The field of addictive behaviour requires further development of both existing and new methods and tests. A new methodological perspective is also probably needed. Thus, if one assumes that addiction does not occur in more than just a limited subset of drug consumers, the search for addictive features common to all animals of a group may be pointless. In other words, studying addictive behaviour may require focusing on the study of individual differences rather than examining group-based averages; the former strategy is already producing very interesting alternative views of other behavioural phenomena (Gallistel, Fairhurst and Balsam 2004).
4 Another major issue that emerges from this review is that not all methods reviewed above are equally suitable for all drugs of abuse. For example, autoshaping and non-operant self-administration seem appropriated for the study of

orally self-administered drugs (such as alcohol, etonitazene and caffeine). However, some of these drugs can produce more conflicting results in tests such as CPP. This fact does not indicate that these drugs have lower reinforcing capabilities or abuse/addiction-liability than psychostimulants. Indeed, cocaine fails to establish autoshaped behaviours and can result in inconsistent self-administration when orally administered, although it produces robust CPP. The implications of these observations go beyond the experimental design of any single study, but rather must be integrated into our understanding of what different procedures tell us about drugs and their abilities to reinforce behaviour and, potentially, to override its control and precipitate addiction.

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