Animal models of drug addiction

Modelos animales de adicción a las drogas

María Pilar García-Pardo*,**; Concepción Roger-Sánchez*; José Enrique de la Rubia Ortí**;
María Asunción Aguilar Calpe*.

*Universidad de Valencia. Facultad de Psicología. Avenida Blasco Ibáñez, 21. 46010. Valencia.
**Universidad Europea de Valencia. c/ General Elio, 8, 46010. Valencia.

Abstract

The development of animal models of drug reward and addiction is an essential factor for progress in understanding the biological basis of this disorder and for the identification of new therapeutic targets. Depending on the component of reward to be studied, one type of animal model or another may be used. There are models of reinforcement based on the primary hedonic effect produced by the consumption of the addictive substance, such as the self-administration (SA) and intracranial self-stimulation (ICSS) paradigms, and there are models based on the component of reward related to associative learning and cognitive ability to make predictions about obtaining reward in the future, such as the conditioned place preference (CPP) paradigm. In recent years these models have incorporated methodological modifications to study extinction, reinstatement and reconsolidation processes, or to model specific aspects of addictive behavior such as motivation to consume drugs, compulsive consumption or drug seeking under punishment situations. There are also models that link different reinforcement components or model voluntary motivation to consume (two-bottle choice, or drinking in the dark tests). In short, innovations in these models allow progress in scientific knowledge regarding the different aspects that lead individuals to consume a drug and develop compulsive consumption, providing a target for future treatments of addiction.
Keywords: Reward; Addiction; Animal models; Drugs of abuse.

Resumen

El desarrollo de modelos animales de refuerzo y adicción a las drogas es imprescindible para el avance en el conocimiento de las bases biológicas de este trastorno y la identificación de nuevas dianas terapéuticas. En función del componente del refuerzo que deseemos estudiar podemos servirnos de un tipo de modelos animales u otros. Podemos utilizar modelos de refuerzo basados en el efecto hedónico primario que produce el consumo de la sustancia adictiva, como los modelos de autoadministración (AA) y autoestimulación eléctrica intracraneal (AEIC), o modelos basados en el componente relacionado con el aprendizaje asociativo y la capacidad cognitiva de realizar predicciones sobre la obtención del refuerzo en el futuro, como el modelo de condicionamiento de preferencia de lugar (CPL). En los últimos años los modelos han incorporado modificaciones metodológicas para incluir el estudio de los procesos de extinción, reinstauración y reconsolidación o para modelar aspectos concretos de la conducta adictiva como puede ser la motivación para consumir la droga, el consumo compulsivo o la búsqueda de la droga bajo situaciones de castigo. Otros modelos interrelacionan diferentes componentes del refuerzo o modelan la motivación voluntaria por consumir (modelos de "two-bottle choice" o "drinking in the dark"). En definitiva, las innovaciones en estos modelos contribuyen al avance en el conocimiento científico de los diferentes factores que llevan a tomar una droga y a desarrollar un consumo compulsivo, ofreciendo una vía para identificar futuros tratamientos para la adicción.
Palabras clave: Refuerzo; Adicción; Modelos animales; Drogas de abuso.

1. Introduction

Studying the hedonic or pleasurable effects of a drug is essential for understanding the mechanisms that cause the development of drug addiction. However, it is a complex matter for which we need to use animal models that allow us to investigate the components involved and identify new therapeutic targets (Koob, Arends & Le Moal, 2014). The use of these animal models has the advantage of allowing great control of experimental variables such as the age at which the animals are exposed to the drug, the dose, duration or exposure time, among others.

A drug itself causes reinforcement which can lead to the development of substance abuse, dependence or addiction in vulnerable persons. Addiction to drugs is a neuropsychiatric disorder characterized by loss of control when seeking and consuming a drug, the appearance of negative emotional states and an intense craving for the drug when consumption ceases, alongside a high propensity to relapse, even after long periods of abstinence. According to Koob & Volkow (2016), drug addiction represents a profound disruption of motivational circuits in the brain caused by a combination of several factors. Firstly, there is a reinforcement deficit and increased stress reactivity due to the desensitization of the brain's reward system and over-activation of stress systems. Secondly, there is an exaggerated incentive salience relating to stimuli or contexts associated with the drug, and rigid stimulus-response habits are established which cause the subject to seek and consume the drug when it is present or when there are signs of its availability. These changes have been associated with a transition from the ventral to the dorsal striatum in controlling the behavior of drug use. Thirdly, there is a deterioration of executive functions such as decision-making, inhibitory control and self-regulation (Romero-Martínez & Moya-Albiol, 2015) due to the dysfunction of the prefrontal cortex, resulting in a lack of control and the inability to inhibit drug-taking behavior despite the negative consequences it entails. Therefore, a transition occurs in some vulnerable persons from the initially controlled consumption of a drug for recreational purposes to compulsive consumption. The presence of a stimulus associated with the drug thus triggers the urge to consume it, and this habit that the subject cannot control (more so than the reinforcing effect of the drug itself which is essential in its recreational use) is one of the main causes of persistent consumption and relapse (Everitt, 2014; Everitt & Robbins, 2013).

It is precisely for the study of these processes and their different phases (such as the acquisition, extinction, and reinstatement of a motivated behavior) that we use animal models. However, although many of the components of addictive behavior can be studied in experimental animals (for example, the primary reinforcing effect of drugs, cognitive aspects such as the processing of drug-context associations, phenomena of sensitization or tolerance to the different ef-

fects of the substance, etc.), it is necessary to emphasize that the addiction disorder (or disorders of drug use, according to DSM-5 criteria) is impossible to model in animals. Therefore, paradigms which are explained throughout this review only model specific aspects of addiction.

Among the major animal models used to study reinforcement produced by drugs and addictive behaviors we find the self-administration (SA), conditioned place preference (CPP) or intracranial self-stimulation (ICSS) paradigms; there are other models, nevertheless, which also offer relevant information and are therefore widely used, each of which will be discussed in the following sections.

2. Reinforcement and addiction

To understand the neurobiological mechanisms involved in addiction through animal models, we need to study the initial element of the addictive process, i.e. the reinforcing effect induced by the drug. This is the element that causes a loss of control over drug use to develop in some consumers, as only those substances which are capable of producing reinforcement can increase the likelihood of the drug-taking behavior reoccurring in the future, resulting in the progressive onset in vulnerable persons of symptoms that characterize addiction. It should be noted that the loss of control over consumption only occurs in "vulnerable" individuals; when they are exposed to the drug, the addictive disorder is triggered. Clearly not all individuals who consume a drug and experience reinforcement become addicted to it. An obvious example of this is alcohol. Furthermore, studies in humans and experimental animals indicate that impulsivity is a vulnerability trait predictive of abuse and addiction to psychostimulants (Everitt, 2014). The environment or social context, stage of life development and genetic factors also modulate vulnerability to addiction (Volkow, Koob & McLellan, 2016).

In the psychology of learning, reinforcement is defined as the process responsible for strengthening a response, increasing the rate or probability of occurrence. This strengthening is due to this response being contingently followed by a stimulus or event, known as a reinforcer. In the case of positive reinforcement, the reinforcer (food, for example) appears when the subject performs the response, whereas in the case of negative reinforcement the reinforcer (of an aversive nature, for example a painful stimulus) is made to disappear when the subject performs the response. Therefore, reinforcement is the term referring to the situation or experimental procedure in which a reinforcer is presented or removed on a certain response or behavior, while the reinforcer is the appetitive or aversive stimulus that appears or disappears when an operant behavior is performed, and results in an increase in the likelihood of occurrence or learning of such behavior (Skinner, 1938; Thorndike, 1932). Moreover, one can distinguish between

primary reinforcers, those who have a positive or negative motivational value in themselves (for example, stimuli necessary for survival as food and drink or the avoidance of pain or a predator) and conditioned reinforcers, initially of a neutral nature but which acquire a motivational value by association with the primary reinforcer.

Drugs are considered primary reinforcers because they are able to activate the brain reward system. The main anatomical substrate of this system is the mesolimbic pathway that originates in the ventral tegmental area (VTA) and projects into the nucleus accumbens (NA) and different cortical areas including the anterior cingulate cortex (ACC) and orbitofrontal and prefrontal cortex (Berridge & Kringelbach, 2015; Carlezon & Thomas, 2009; Dalley & Everitt, 2009; Wise, 2008). In these areas dopamine, the main neurotransmitter related to strengthening and pleasure is released (Lammel, Lim & Malenka, 2014), as are other neurotransmitters such as serotonin (Müller & Homberg, 2015). As we mentioned above, there are different components to reinforcement, such as the subjective experience of pleasure or a component related to learning or the ability to generate cognitive representations with predictions of reward in the future. Hence each animal model focuses on a different aspect; for example, models such as intravenous SA or ICSS reflect the primary hedonic reinforcing effect of the drug, while CPP emphasizes the aspect of learning such reinforcement (Berridge & Kringelbach, 2008).

The use of animal models not only allows the evaluation of the reinforcing effects of drugs but have in recent years been redesigned to study the extinction, reinstatement and reconsolidation processes and to more accurately reflect the behavioral characteristics of addiction. The extinction-reinstatement paradigm permits the study of the extinction process (through training sessions in which the animal is exposed to the same conditions as in the acquisition phase but where the reinforcing substance is not present) and the modeling of relapse in drug seeking, which is the principal clinical problem in the treatment of addiction (Ghitza, 2015). Similarly, it has been proposed that manipulation of the memories associated with a drug could be an effective method of removing these memories and so prevent relapse. The memory of the effects produced by drugs or the associative learning between its reinforcing properties and environmental cues associated with its consumption sustain drug consumption, triggering the desire to consume and thus the relapse (Yan et al., 2014). It has been widely demonstrated that after being recovered and becoming accessible, memory undergoes an unstable transitional stage and needs to be consolidated again in order to prevail, a process known as reconsolidation (Alberini, 2011; Inda, Muravieva & Alberini, 2011; Lee, 2010; Muravieva & Alberini, 2011). Updating drug-related memories is an important part of subsequent reconsolidation

and has been linked to the persistence of drug addiction (Sorg, 2012; Taylor, Olausson, Quinn & Torregrossa, 2009). Therefore, destabilizing the memory of drug-related learning through behavioral or pharmacological procedures can be a means of promoting abstinence and preventing relapse (Everitt, 2014).

Similarly, animal models have led to increased knowledge of the neurobiological processes underlying the development of dependency and addiction (Koob et al., 2014; Volkow et al., 2016.). Thus, there are ever more studies evaluating the effects of different lesions in specific brain regions, or on pharmaceuticals with specific effects on different neurotransmission systems and how these in turn affect the reinforcing properties of drugs observed in these models. As an example, in our laboratory we have demonstrated the essential role of NMDA glutamate receptors in the reinforcing effects conditioned by different drugs such as morphine, cocaine or ecstasy (Do Couto, Aguilar, Rodríguez-Arias & Miñarro, 2005; García-Pardo, Escobar-Valero, Rodríguez-Arias, Miñarro & Aguilar, 2015a; Maldonado, Cauli, Rodríguez-Arias, Aguilar & Miñarro, 2003). Some studies even evaluate the effects of drug administration in specific brain areas on the reinforcement induced by chronic administration of the drug, which could clarify the involvement of a certain system of neurotransmission in a specific brain area in the reinforcing properties of the drug concerned and/or its ability to induce dependency. The description of advances in the neurobiology of drug addiction obtained by using these models is beyond the scope of this review. In this respect the contribution made recently by Koob and Volkow (2016) is recommended.

3. Animal models of reinforcement

3.1. Models of self-administration

In general, all substances with a high addiction potential for humans are self-administered by animals voluntarily, although it has been difficult to demonstrate this with some drugs. Moreover, this relationship is so strong that SA models are considered to have high predictive power, and different classifications of these models exist. Firstly, we have those paradigms where the animal must perform an operant behavior, such as pressing a lever to receive a dose of the substance, usually orally or intravenously. Secondly, there are the paradigms in which the animal has free access to the substance and can readily consume it orally (Teruel, 2008).

3.1.1. Models of operant learning.

This section includes both intravenous and oral SA since the learning model is similar, regardless of the way in which the substance is ingested.

The intravenous SA paradigm is the most important procedure and the most commonly used in animals, mainly

rodents, to assess the primary intrinsic reinforcing effect of drugs (Moser, Wolinsky, Duxon & Porsolt, 2011; Yahyavi-Firouz-Abadi & See, 2009). In this paradigm animals are trained to obtain the drug by performing an operant response, for example pressing a lever or the inserting its snout into a hole. Thus, when the animal responds, its behavior is reinforced with the injection of the drug and it consequently acquires a new operant response by learning that the behavior in question is associated with obtaining the reinforcer (Yahyavi-Firouz-Abadi & See, 2009). Most SA procedures use a fixed response program (FR) in which the animal must perform a fixed number of responses in order to obtain the dose of the drug (Moser et al., 2011), although a variable response program is used by other studies.

There are different factors, both pharmacological and environmental, to consider when using the SA paradigm, such as the dose of the substance administered, the rate of infusion of the drug, the sex of the animal or the stage of its evolutionary development. However, other factors such as the duration of the SA session or the level of challenge involved in the response required for the drug have proved more decisive (Moser et al., 2011).

3.1.1.1. Advantages and disadvantages of SA. SA is so frequently used because the model has excellent predictive validity, given the great similarity between the results obtained with the model in animals and for human addictive behaviors (Koob et al., 2014. Mead, 2014; Schenk, 2009; Soria, Barbano, Maldonado & Valverde, 2008). Compared to other models of addiction, the SA paradigm is closely related to drug abuse in humans in terms of how the substance is administered and the behavioral response that is generated in order to obtain the administration (O'Connor, Chapman, Butler & Mead, 2011). Moreover, since this paradigm measures how animals behave when seeking drugs, the technique can be used to study the neurobiological mechanisms involved in this process (Fuchs, Feltenstein & See, 2006).

Another important advantage of this paradigm is that it makes it possible to analyze the motivation for drug seeking by using a progressive reinforcement schedule in which the animal has to perform a progressively greater number of responses to obtain it (Richardson & Roberts, 1996). As the SA session progresses, procuring the following reinforcer requires a greater effort by the animal. The maximum number of operant responses that the animal is able to perform to obtain a reinforcer is called "breaking point" and measures the limit of an animal's motivation to seek the drug.

Although intravenous SA is the most commonly used paradigm, it also has some drawbacks, the principal of which is the complexity of the technique. In order to measure the reinforcing effect, it is necessary to surgically implant an intravenous catheter (Graf et al., 2011). One solution to this handicap is to use an alternative SA paradigm, such as oral SA, where the animal is freely able to consume the addictive substance orally, following the same procedure described for intravenous SA (Pautassi, Miranda-Morales & Nizhnikov, 2015). Oral SA is not as reinforcing for animals and has other limitations such as the fact that the animal has to be previously familiarized with the addictive substance in order to drink it voluntarily (it is therefore not used with certain drugs that animals do not usually find reinforcing orally, such as cocaine). Another disadvantage of the SA model is that for the proper application of this technique it is necessary to train animals to learn to acquire operant behavior. This drawback is most pronounced with drugs whose initial reinforcing strength is not particularly high, such as 3,4-methylenedioxymethamphetamine (MDMA) (Trigo, Panayi, Soria, Maldonado & Robledo, 2006; Schenk, 2009). In these cases, the animal is previously trained with a more reinforcing substance such as cocaine (Schenk, 2009), or a priming dose is administered previously (Trigo et al., 2006). In some cases, a food restriction pattern is even used before the SA acquisition phase (Soria et al., 2005).

3.1.1.2. Extinction, reinstatement and reconsolidation processes in SA. SA studies allow us to investigate different processes such as acquisition, maintenance, extinction and reinstatement of the operant response. To this end a procedure comprising several stages is used. The acquisition phase is defined as the time period necessary to achieve a stable rate of drug SA (Soria et al., 2005). This is followed by the maintenance phase which may take days or weeks. Extinction refers to a progressive decrease in the operant response associated with the drug when the substance is not present (Epstein, Preston, Stewart & Shaham, 2006; Shaham, Shalev, Lu, de Wit & Stewart, 2003; Stewart, 2000). After extinction, the restoration phase of the behavior takes place in which the ability is measured of certain stimuli called "primers" (pharmacological, physical or environmental) to restore initially learned operant responses (Soria et al., 2008). Currently, the extinction-reinstatement model in SA is very popular for modeling relapse in drug seeking (Bossert, Marchant, Calu & Shaham, 2013; Epstein et al., 2006; Shaham et al., 2003; Sinha et al., 2011; Soria et al., 2008; Steketee & Kalivas, 2011; Yahyavi-Firouz-Abadi & See, 2009; Yan & Nabeshima 2009). This paradigm has been used with different types of drugs, including MDMA or ecstasy and cocaine (Colussi-Mas, Wise, Howard & Schenk, 2010; Schenk, Gittings & Colussi-Mas, 2011; Schenk, Hely, Gittings, Lake & Daniela, 2008; Trigo, Orejarena, Maldonado & Robledo, 2009). However, it should be noted that although the extinction model has produced a large amount of research, it is not the most clinically relevant. Paradigms involving longer periods of abstinence (whether forced, imposed by punishment, or chosen) are more useful in terms of their ecological validity.

María Pilar García Pardo, Concepción Roger Sánchez, José Enrique de la Rubia Ortí, María Asunción Aguilar Calpe

Recently, some studies have evaluated the effects of destabilizing the memory of what animals learned during the acquisition of SA through behavioral (extinction) or pharmacological procedures. In these studies, after achieving stable acquisition of SA, memory is reactivated by briefly exposing the animals to the SA chamber, and a short time afterwards (ranging from minutes to a few hours) the animals are given extinction sessions or receive an injection of an amnesiac drug. Both treatments quickly and effectively reduce drug seeking (accelerating the extinction of the SA response) and block reinstatement (Hellemans, Everitt & Lee, 2006; Lee, Milton & Everitt, 2006; Lee, Platt, Rowlett, Adewale & Spealman, 2005; Sánchez, Quinn, Torregrossa & Taylor, 2010; Yan et al., 2014. Yan, Kong, Wu, Newman & Xu, 2013).

3.1.1.3. Variations on the SA model. Over the last decade, different variations of the SA model have been developed to study the main characteristics of addiction by manipulating the type of reinforcer or the waiting time to obtain it, drug seeking in situations involving punishment, or the development of compulsive drug seeking models. In relation to these variations it is worth mentioning the experiments that allow the animal the choice between a drug and a natural reinforcer in order to study alternative reinforcement caused by sugar and sweet food (Ahmed, 2014; Ahmed, Guillem & Vandaele, 2013). Lenoir, Cantin, Vanhille, Serre and Ahmed (2013) showed that, after a period of training in SA of both cocaine and water sweetened with sucrose, most rats chose to leave cocaine and seek out the alternative reward. These experiments demonstrate that sugar and sweet foods can not only replace drugs but also be even more attractive and reinforcing (Ahmed et al., 2013).

A further variation is the extended access model of SA. This is a model of operant conditioning of excessive drug consumption that provides an approach to studying why some animals make a transition from initially low/moderate to abusive or excessive consumption (Edwards & Koob, 2013). While the consumption of animals whose access to the drug is limited in terms of time (for example, one or two hours daily) is stable over time, animals with extended access to the drug (for example, six hours per day) show an escalation in their SA behavior (Koob et al., 2014). This phenomenon has been observed with the use of different drugs, including cocaine (Roberts, Morgan & Liu, 2007) and heroin (Lenoir, Cantin, Vanhille, Serre & Ahmed, 2013). Escalating SA is a more complete model of addiction because animals subjected to prolonged access to substances of abuse have various symptoms related to the criteria for substance dependence in humans, such as the emergence of uncontrollable drug use despite the negative consequences this causes, compulsive behaviors linked to drug seeking, and increased vulnerability to re-

lapse or reinstatement of the behavior after exposure to different stimuli. Similarly, when animals return for testing after a period of abstinence subsequent to chronic administration of the drug, they display a greater response under a progressive schedule, suggesting that the value of the reward or the effectiveness of the drug is enhanced when subjects are dependent (Koob et al., 2014). Moreover, in the SA paradigm the search for cocaine intensifies after the withdrawal of extended access. This effect is related to the phenomenon called incubation of cocaine craving, in which cocaine seeking induced by the re-exposure to the cues associated with the drug increases progressively during the first two months of cocaine abstinence (Lu , Grimm, Dempsey & Shaham, 2004).

Other researchers have used the SA paradigm in order to model the main features of addiction in humans based on the DSM-IV/5 criteria. In these experiments, the daily SA sessions typically consist of periods of access to the drug (indicated to animals by a light above the active lever or hole and during which the operant response is accompanied by the presence of the reinforcer), and drug-free periods (in which the whole SA box is lit and operant responses have no reinforcing outcome for the animal). Three behaviors linked to addiction criteria in humans are evaluated: loss of control or persistence in drug seeking (by active counting lever/hole responses during periods in which the reinforcer is not available), high motivation for the drug (using a progressive reinforcement schedule where the appearance of the reinforcer increasingly requires the performance of a greater number of operant responses by the animal) and maintenance in consumption despite the negative outcomes arising from it (performance of the operant response despite the existing association between reinforcement and an electric shock in the animal's legs) (Deroche-Gamonet & Piazza, 2014). Such studies allow further research into the pathological transition to addiction that takes place in some drug users as a result of the interaction between individual vulnerability (related to behavioral and personality variables), the degree of exposure to the drug and loss control (Belin, Balado, Piazza & Deroche-Gamonet, 2009, Belin & Deroche-Gamonet, 2012; Deroche-Gamonet, Belin & Piazza, 2004; Deroche-Gamonet & Piazza, 2014; Piazza & Deroche-Gamonet, 2013).

Another variant of operant SA models are those known as second-order schedules. This type of program was introduced and developed in an impressive series of experiments carried out by Bergman, Goldberg, Katz and colleagues in the early 1970s (Goldberg & Gardner, 1981; Goldberg & Tang, 1977; Spear & Katz, 1991) which have become a paradigm of reference in current animals studies to assess reinforcement (Everitt & Robbins, 2000; Giuliano et al., 2015; Giuliano, Robbins, Nathan, Bullmore & Everitt, 2012; Giuliano, Robbins, Wille, Bullmore & Everitt, 2013).

This is an operant SA paradigm in which, as its name suggests, animals (usually rats) are trained to perform an operant behavior to get the reinforcer (such as food or any type of drug) using a continuous reinforcement schedule. During this period each self-administered drug infusion is associated with a stimulus (such as a light or sound) which is contingently presented in the training sessions (thus turning into a conditioned stimulus). Once the animals are on a stable SA schedule, the second-order paradigm is introduced with a fixed response rate, so that each time the animal responds, the conditioned stimulus and the drug infusion are produced, and the rate of responses and intervals is increased (Everitt & Robbins, 2000). Therefore, second-order protocols are more complex, as they include two different schedules at the same time: one of fixed interval (FI) and the other fixed rate (FR). For example, in an FI5min-(FR1:S) schedule, the first response after the end of a 5-minute interval obtains the reinforcer, while in an FI5min-(FR5:S) five responses are required before the reinforcer can be obtained.

Essentially, under second-order schedules the reinforcer is presented in accordance with a program in which a sequence of (more or less extended) responses is intermittently reinforced. The completion of each fixed response schedule is accompanied by the response contingent to the presentation of the conditioned stimulus. For example, a typical second-order schedule would consist of a 60-minute fixed interval schedule with a 30:S (FI60min-FR30:S) response pattern, where the conditioned stimulus is presented after each 30 responses, while the animal must perform a further 30 responses after completion of the fixed interval of 60 minutes before the conditioned stimulus is served alongside the reinforcer, for example, an intravenous infusion of heroin or cocaine, or access to food, depending on the aims of the study (Everitt & Robbins, 2000; Giuliano et al., 2012; Giuliano et al., 2015; Giuliano et al., 2013).

Therefore, in a second-order schedule the behavior specified by a contingency program is treated as a single response that is reinforced according to a given program. Thus, the performance of a number of behaviors by the animal involves the presentation of a stimulus previously linked to an infusion of the reinforcing substance. In order to obtain the drug, the animals must perform a number of responses which are also preset to make the conditioned stimulus appear. In this paradigm long sequences of behavior can be observed before any drug is administered. This is seen as a measure of the strength of the conditioned stimuli which triggers drug seeking (Teruel, 2008) or, in other words, a measure of the effort the animal is willing to make in order to receive the drug (motivation). This model is thus predictive of the behavior generated in humans where the appetitive phase of consumption behavior (collection and preparation of the drug) precedes the act of taking the drug.

3.1.2. Voluntary consumption models.

This section, rather than provide a thorough explanation of models of voluntary consumption, will instead discuss two prominent models, the two-bottle choice and drinking in the dark (DID). These models differ from previous ones in that the substance is readily available, which means that the memory/learning component is much smaller. Thus, given that access to the substance requires very little effort by the animal, it is more difficult to assess the motivational component in these models (i.e., the amount of effort or the number of behaviors or responses the animal is willing to make in order to obtain the drug).

A.- Two-bottle choice. Among the animal models of reinforcement used to evaluate the preference that leads a subject to consume, we find the two-bottle choice paradigm of voluntary consumption. There are different modalities or varieties within this procedure but the main objective is to measure the preference of the animal for the substance of abuse (usually alcohol) and oral consumption thereof compared to an alternative non-addictive substance (such as water) (Bahi, 2012, 2013a, 2013b). To measure this preference, animals are exposed to both substances for days and have the possibility of drinking only water, only alcohol or both substances simultaneously.

In the most commonly applied method, animals initially have access to two bottles of water, and then one of them is replaced by an alcohol solution at a certain percentage % v/v, which is increased progressively (every 3-4 days) to achieve the desired concentration (typically 8-10%). The animals are thus permanently exposed to alcohol with no periods of abstinence. In our laboratory we follow the protocol used by the group around Professor Everitt of Cambridge University, where access to alcohol is available on alternate days. In this case, the animals have three sessions per week (typically Monday, Wednesday and Friday) of 24 hours with unrestricted access to both bottles (one with alcohol and the other with water). There is a period of abstinence lasting 24 and 48 hours on the other weekdays and at the weekend respectively, during which time two bottles of water (and no alcohol) are presented. To counteract the possible learning of place, the bottles are moved for each alcohol exposure session (Barak, Carnicella, Yowell & Ron, 2011; Carnicella, Amamoto & Ron, 2009; Giuliano et al., 2015). To quantify the animal's preference for the substance of abuse, the volume of alcohol and water are measured every day. Similarly, to estimate the amount evaporation that may exist in the bottles, a bottle containing water and another with alcohol are placed in an empty cage (Giuliano et al., 2015).

B.- Drinking in the dark. This paradigm was originally described by Rhodes, Best, Belknap, Finn & Crabbe (2005). The most common variation of this model uses

María Pilar García Pardo, Concepción Roger Sánchez, José Enrique de la Rubia Ortí, María Asunción Aguilar Calpe

isolated mice whose water bottle is replaced by a bottle containing ethanol at different percentages depending on the object under investigation. Access to the substance of abuse is made available in this way starting during the animal's dark cycle (hence the name) and usually with a duration of between 2 and 4 hours, thus simulating the binge drinking pattern of alcohol consumption common among teenagers at the weekend. Using this procedure, the animals can normally reach high blood concentrations of ethanol, so it is very interesting for the study of alcohol addiction (Thiele & Navarro, 2014).

The basic idea behind this paradigm is that the substance should be consumed in a cycle of darkness in which intake levels are higher. The animals have to choose between freely consuming or avoiding alcohol without being subjected to any injection, thus minimizing various stress conditions to which animals are subjected in other animal models of binge alcohol consumption. Another advantage of this paradigm is that it requires no prior training for animals nor prior inclusion of other components, such as for example, sweet substances to increase the motivation of the animal to consume ethanol. It follows that the drinking in the dark model is widely used and very productive in the study of the neurobiological and genetic factors involved in alcohol consumption.

3.2. Conditioned place preference (CPP)

CPP is a paradigm that evaluates the conditioned reinforcing effect of substances of abuse, given that the contextual stimuli (such as the color or texture of the floor or the compartment where the drug is received) can acquire appetitive properties when associated with the primary reinforcing stimulus, in this case the substance of abuse (Aguilar, Rodríguez-Arias & Miñarro, 2009; Bardo & Bevins, 2000; Tzschentke, 1998, 2007). To achieve this goal, researchers use a box with two or three compartments which are clearly distinct in terms of the stimuli present in the different models of boxes available. For example, in our laboratory (García-Pardo et al., 2014, 2015a, 2015b; Roger-Sánchez, Rodríguez-Arias, Miñarro & Aguilar, 2013a, 2013b) two compartments are used with different color and texture. One of them has a rough floor which is white, while the other has a smooth black floor, both being separated by a neutral central platform. The animals thus receive the drug in an environment with specific characteristics in order to evaluate later if they have learned to associate the environmental cues of the place where the drug is received and the reinforcing effect it produces. Similarly, using this paradigm can evaluate the reverse process, conditioned place aversion (CPA), which has been observed with high doses or abstinence of certain drugs. For example, opiate dependent animals develop CPA through the compartment associated with the administration of an opioid antagonist such as naloxone or naltrexone (García-Carmona,

Baroja-Mazo, Milanés & Laorden, 2015; Maldonado, Cauli, Rodríguez-Arias, Aguilar & Miñarro, 2003).

Unlike other more complex paradigms such those described above, CPP is characterized by its great methodological simplicity, resulting in very frequent use, in conjunction with the fact that under the right conditions, CPP may be sensitive to a wide range of substances (Aguilar et al., 2009; Aguilar, Roger-Sánchez, Rodríguez-Arias & Miñarro, 2015; Tzschentke, 1998, 2007). Thus, in our laboratory we have demonstrated CPP with different types of drugs such as cocaine (Montagud-Romero et al., 2015.), MDMA (Daza-Losada et al., 2007; Daza-Losada, Miñarro, Aguilar, Valverde & Rodríguez-Arias, 2011; Daza-Losada, Rodríguez-Arias, Aguilar & Miñarro, 2009; Do Couto et al., 2011; 2012; García-Pardo et al., 2014, 2015a, 2015b; Roger-Sánchez et al., 2013a, 2013b; Roger-Sánchez, Aguilar, Manzanedo, Miñarro & Rodríguez-Arias, 2013c; Vidal-Infer et al., 2012), opioids (Manzanedo, Aguilar, Rodríguez-Arias & Miñarro, 2001b; Manzanedo, Aguilar, Rodríguez-Arias, Navarro & Miñarro, 2004; Manzanedo, Serrano, Aguilar, Rodríguez-Arias & Miñarro, 2001a), alcohol (Roger-Sánchez, Aguilar, Rodríguez-Arias, Aragón & Miñarro, 2012) and nicotine (Navarrete et al., 2013).

Generally, the CPP paradigm consists of three phases: a first phase known as "pre-conditioning" that serves to confirm that there is no innate preference of the animal to one of the compartments. In the second phase, called acquisition or conditioning in which the association between the reinforcing effect of the drug and environmental cues is established through the administration of the drug in one of the compartments and the administration of a control substance in the other compartment with different environment. This combination is repeated over several sessions separated by a time interval which differs depending on the characteristics of the drug being tested. For example, with the protocols we follow in our laboratory, in the case of cocaine the time interval between the drug injection and the injection of saline is 4 hours, with the process being repeated for 4 days. In the case of MDMA, the animal receives the drug or control substance alternately every 24 hours over 8 days. In the final phase, called "post-conditioning" the existence of conditioning is assessed. If the animal has associated the reinforcing value of the drug and the environmental stimuli, it will spend more time in the compartment where it has received the substance and is therefore considered to have acquired CPP (Aguilar et al., 2009; Parker & McDonald, 2000; Wang, Luo Ge, Fu & Han, 2002; Wang, Luo, Zhang & Han, 2000).

Furthermore, it is worth noting that the CPP procedure can also be used to evaluate other processes such as the extinction of motivated behavior and its reinstatement (Aguilar et al., 2009; García-Pardo et al., 2014, 2015a, 2015b.). For this purpose, after CPP acquisition the animals are subjected to a process of extinction, defined as a decrease

in the frequency or intensity of the learned response after removal of the unconditioned stimulus (in this case the drug) that reinforced learning (Pavlov, 1927). Here, the animal is continuously exposed to the conditioned stimulus (the compartment which is linked to the drug) without the presence of the unconditioned stimulus (the drug) so that the association between the reinforcing value and environmental cues weakens, and the conditioned preference finally disappears. While there are variations of the process of extinction, for example, forced extinction (confinement in the compartment associated with the drug after administration of the control substance) or spontaneous extinction (unrestricted movement through both compartments without any treatment), the underlying objective is always to decrease the CPP originally induced by the drug (Yahyavi-Firouz-Abadi & See, 2009). An important detail is that the period needed for the preference to be extinguished is influenced by different factors such as the motivational properties of the drug (Pulvirenti, 2003), previous exposure to drugs (Daza-Losada et al., 2009; Do Couto et al., 2011), the dose with which acquisition was produced (García-Pardo et al., 2015a) or exposure to aversive events in the acquisition phase or even before, such as acute (García-Pardo et al., 2014) or repeated stress (García-Pardo et al., 2015b).

The CPP paradigm is also useful for studying reinstatement brought about by the re-exposure to drugs or stress. Reinstatement refers to the recovery of a learned or conditioned response and involves renewed learning of the association between the environmental reinforcing effect of the substance and the cues once extinction has taken place (Aguilar et al., 2009; Do Couto et al., 2006; Do Couto, Aguilar, Lluch, Rodríguez-Arias & Miñarro, 2009; García-Pardo et al., 2014, 2015a, 2015b). Reinstatement can be induced by different factors, either pharmacological, physical or social, or in experimental contexts such as dose priming, which involves re-exposure to a low dose of the drug with which conditioning took place (Cruz, Marín & Planeta, 2008; Daza-Losada et al., 2007), exposure to a stressful situation like an electric shock to the legs (Bossert, Marchant, Calu & Shaham, 2013) or defeat in an agonistic encounter (García-Pardo et al., 2014; 2015b; Shaham et al., 2003; Shalev, Grimm & Shaham, 2002; Tzschentke, 2007).

There are different variations of the CPP paradigm based on research objectives. For example, similar to the procedures of extended-access SA, the number of conditioning sessions can be increased in order to assess whether the vulnerability to develop addiction-related symptoms or susceptibility to relapse also rises (Rodríguez-Arias, Castillo, Daza-Losada, Aguilar & Miñarro, 2009). In this study from our laboratory we note that there is an inverted U-shaped relationship between the number of sessions of conditioning and vulnerability to reinstatement. More specifically, mice exposed to 12 sessions of conditioning with 25 mg/kg

of cocaine had increased vulnerability to reinstatement induced by a priming dose of cocaine, compared to animals exposed to a greater or smaller number of conditioning sessions. Likewise, in other as yet unpublished studies we have seen that these animals with prolonged conditioning exhibit behaviors similar to those seen in cocaine users, such as continuity in the use of the drug despite adverse consequences, or search for the substance when it is not available (Aguilar et al., in preparation).

Finally, the CPP paradigm may also be used to study the effects of certain pharmacological and behavioral manipulations of the reconsolidation process. As discussed above, the memories of the effects produced by the drugs, and by signals associated with their consumption trigger the desire to consume, as well as relapse. In these studies, animals acquire the CPP in the normal way, after which the memories of the drug are reactivated (by exposing the animals to the compartment associated with the drug during conditioning for a short period of time). After a short interval (10 minutes to 2 hours) they are subjected to CPP extinction sessions or an amnesic treatment. These techniques cause a rapid extinction of the conditioned response and prevent the reinstatement of CPP after exposure to a priming drug (Liu et al., 2015; Lv, Sun, Cui & Han, 2015; Miller & Marshall, 2005; Slaker et al., 2015).

3.3. Intracranial self-stimulation (ICSS)

The ICSS model is linked to the classic experiments designed by Olds and Milner in 1954, which led to the discovery of the brain's reinforcement system.

In this type of paradigm, animals perform an operant response that allows them to self-administer short electric pulses in different brain areas related to the reinforcer (Koob et al., 2014; Negus & Miller, 2014), because the electrodes are generally placed in the medial forebrain bundle at the level of the lateral hypothalamus or in the nucleus accumbens, areas belonging to the brain's reward system. The frequency or amplitude of the stimulation of these structures is manipulated to generate a wide range of response rates (Negus & Miller, 2014). It is known that acute administration of drugs lowers the ICSS threshold so that the animal needs less stimulation to perceive the reinforcing sensation, while withdrawal increases it (Koob et al., 2014; Negus & Miller, 2014). This means that if a drug lowers the ICSS threshold it is because the drug has high reinforcing power, thus animals do not need as much stimulation to feel reinforcement. As a result, the lower the ICSS threshold, the greater the reinforcing power of the drug.

It is a complex procedure since it requires stereotactic surgery and the intensity of stimulation must be manipulated in order to identify the appropriate value for each animal. This paradigm has shown that the release of dopamine is stimulated ahead of serotonin, which influences the expression of the effects obtained. This is what can be

María Pilar García Pardo, Concepción Roger Sánchez, José Enrique de la Rubia Ortí, María Asunción Aguilar Calpe

observed, for example, in the case of MDMA, a nonselective substance of abuse for dopamine producing mixed effects: on the one hand it lowers the ICSS threshold but also decreases the maximum response rate, which can be interpreted as a reduction in the ability to induce drug abuse of this drug in comparison to drugs that induce greater releases of dopamine, such as cocaine or methamphetamine (Bauer, Banks, Blough & Negus, 2013).

4. Conclusion

As discussed throughout this review, animal models of drug addiction provide a very useful tool for studying the neurobiological and behavioral processes involved in addiction, contributing to the identification of new therapeutic targets for the treatment of this disease. Within this global aim, each of the animal models employed focuses on a different component of reinforcement (for example, motivation or learning).

Among the main animal models used to evaluate the reinforcing effects of drugs we find CPP, the SA and the ICSS, although we have seen that depending on the objectives pursued, the substances of abuse in question or the parameters of the addiction we are studying other animal models may also be very useful. Thus, for example for research on alcohol the two-bottle choice and the drinking in the dark paradigms are highly relevant. On the other hand, if we wish to study the motivation of an animal to obtain the drug (similar to what happens in humans) progressive or second order SA programs can offer more relevant results. Finally, if we are interested in the role played by drug-conditioned stimuli in the maintenance of addictive behavior, the CPP paradigm may be the most appropriate.

However, as previously mentioned all paradigms that make use of animals have a number of limitations and even though they try to model the different aspects of drug addiction in the best possible way, the results obtained cannot be extrapolated directly to humans. Although there are a great many similarities in behavioral, pharmacological and neurobiological terms, it is clear that the correlation is not always perfect. Nevertheless, the use of such models has led to important research, and great progress has been made in the field of drug addiction. The goal now should be to improve and perfect the different animal models in order to increase their face and predictive validity.

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Conflict of interests

The authors have no conflicts of interest to disclose.

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