The Varied Uses of Conditioned Place Preference in Behavioral Neuroscience Research: An Investigation of Alcohol Administration in Model Organisms

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Place conditioning procedures have been used to study human addiction to alcohol for the past several years. This experimental resource has been utilized successfully due to the fact that investigators can carefully manipulate the experimental design in order to explore specific hypotheses. Only three choices exist regarding animal response to place conditioning: aversion, preference, or no change. This review provides an in-depth analysis of five variables commonly adjusted or changed in place conditioning experiments with ethanol. These include: apparatus design, administration methods, choice of model organism, age of model organism, and model paradigms. It is suggested that the two-chamber design, the intragastric administration, the mouse model, the adolescent age group, and the pre-exposure to stress paradigm are the best current options available in place conditioning experiments with ethanol. The basis for evaluation used throughout this review is that investigators should adjust the variables employed in place conditioning experiments in a manner that most accurately represents and models complex human addiction to alcohol.

Abbreviations: Unconditioned Stimulus – US; Conditioned Stimulus – CS; Conditioned Response – CR; Conditioned Place Preference – CPP; Conditioned Place Aversion – CPA; Tetrahydrocannabinol – THC; Intragastric – IG; Intraperitoneal – IP; Gastrointestinal – GI; Knock Out – KO; Neuronal Nitric Oxide Synthase – nNOS; N-methyl-D-aspartate - NMDA

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Introduction

To understand and treat dependence disorders, researchers have utilized place conditioning to develop appropriate models of addiction (Carboni and Vacca, 2003). Addiction is a physical and psychological dependence that develops over a period of time for a given substance. Place conditioning is broadly defined as a pairing between an unconditioned stimulus (US) and a conditioned stimulus (CS) where the US is the administration of the drug or other reward to the model organism and the CS is the distinct environment in which the organism is placed after administration of the drug or reward (Tzschentke, 2007). The pairing often develops into an association between the US and the CS that results in a future conditioned response (CR). The CR can take the form of preference or aversion displayed during the testing period. While in the test period, the organism has free access to enter a chamber previously associated with the drug or to enter a chamber not associated with the drug. Conditioned Place Preference (CPP) is based on a motivational aspect of the investigated drug and can be defined as an inclination for the model organism to choose the location paired with the drug. Conditioned Place Aversion (CPA) is based on a repulsive effect of the drug and can be defined

as an inclination for the model organism to choose the location not paired with the drug. A vehicle (most often saline) is administered instead of the drug whenever an organism is chosen for the control group. Depending on the chemical properties of the drug, the dose, the number of trials, and the administration method, either CPP, CPA, or no change can be observed. The place conditioning procedure has been used successfully to show CPP with opiates (Spiteri et al., 2000; Vekovischeva et al., 2004; Rizos et al., 2005), cocaine (Russo et al., 2003a; Stromberg and Mackler, 2005; Sellings et al., 2006b; Thanos et al., 2010), nicotine (Tapper et al., 2004; Walters et al., 2005a; Ji et al., 2006), and tetrahydrocannabinol (THC) (Braida et al., 2004; Soria et al., 2004; Le Foll et al., 2006). CPP has also been seen with ethanol in a mouse model (Hill et al., 2003; Houchi et al., 2005; Cunningham et al., 2006), rat model (Bagrov et al., 1999; Matsuzawa et al., 2000b; Haleem et al., 2005), and a zebrafish model (Mathur et al., 2010).

To narrow the scope, this review will analyze the literature that uses ethanol as an US to develop CPP and will not discuss instances of CPA. Previous reviews of CPP and ethanol were conducted with the focus of understanding the motivational aspects, historical framework, and neuropharmacology associated with CPP (Risinger et al, 2002) and to also present the advantages and disadvantages of using place preference procedures (Bardo and Bevins, 2000). Further still, other reviews have highlighted the significant results obtained using ethanol in place conditioning procedures without offering an analysis of conflicting results and designs (Tzschentke 1998, 2007). This review will provide a unique evaluation by analyzing place conditioning approaches based on the route of drug administration, the choice and age of the model organism, the design of the conditioning chamber, and the applicability to human addiction. It will be argued that a two-chambered conditioning apparatus is better than a three-chambered apparatus (Cunningham et al., 2006), that intragastric (ig) administration as compared to intraperitoneal (IP) administration is the preferred choice for drug delivery (Fidler et al., 2004), that the mouse model over the rat and zebrafish models is the most ideal to use in place conditioning experiments with ethanol (Cunningham et al., 1992), that an adolescent model is more accurately representative than an adult model (Song et al., 2007), and that pre-exposure to stress before place conditioning provides the best available animal model for alcoholism in humans (Matsuzawa et al., 1998a).

Review

Apparatus Design

The apparatus used in rodent place conditioning comes in two basic formats: the two-chamber design and the three-chamber design. The two-chamber design uses two compartments separated by a sliding guillotine door (Cunningham et al., 2006). The compartments are made vastly different from each other by adjusting the lighting, tactile sensations, or smell of each compartment. On adaptation day, the animal is placed in the apparatus and the guillotine door is opened to allow free movement between the chambers. The investigator must choose to make the chambers biased or unbiased. Biased design means the individual animal prefers one chamber more than the other chamber during adaptation. Unbiased design means the animal prefers each chamber equally during adaptation. The unbiased design is more ideal because on test day it allows investigators to fully determine significant CPP for the chamber paired with drug administration. When analyzing the data, the investigator simply notes and records differences in chamber preference as compared to the animal's initial level of equal preference for both chambers during adaptation.

For the period of conditioning with the two-chamber design, drug is administered to the animal and the animal is placed in one of the compartments with the guillotine door shut. On test day, no drug is administered and the guillotine door is again lifted to allow the animal free access to both compartments. With this two-chamber design, the animal must enter a chamber, and therefore the time spent in each chamber can be measured and compared to the initial level of time spent in the chambers on acquisition day. The two-chamber design is also

used with the zebrafish model by using aquariums filled with water or a water/drug combination and a sliding plate. During the acquisition and testing days, freshwater filled aquariums are used. One side is visually different from the other side. Two plates that initially keep the fish in the middle of the aquarium are removed. This occurs very quickly after the fish is initially placed in the water. During the conditioning day, a fixed liquid-permeable plate separates the two sides of the aquarium and the aquarium is filled with an ethanol/freshwater mixture. The zebrafish is placed on one side of the aquarium and the plate prevents it from swimming to the other side (Mathur et al., 2010). The apparatus for rodents is more adaptable because additional stimuli can be adjusted to make the appropriate differences between the chambers. The stimuli that can be altered in the rodent model but not the zebrafish model include tactile, olfactory, and auditory stimuli. The apparatus for the zebrafish model is limited to changes only in visual stimuli. Visual stimuli can also be manipulated in the rodent apparatus.

The two-chamber rodent design offers distinct advantages over the three-chamber design. In the three-chamber design, one small compartment separates two larger compartments that are distinctly different from one another (Torrella et al., 2006; Song et al., 2007). The central chamber is designed to be neutral and it connects to both of the large chambers. The three-chamber design includes two guillotine doors with a single door separating each large chamber from the small connecting chamber. Throughout acquisition and testing, the animal is placed in the small central chamber and the guillotine doors are opened. This allows the animal to move freely between chambers. For the duration of conditioning, the drug is administered and then the animal is placed in the small chamber while only one guillotine door is opened. The problem with this design is that the animal might make an association with the central chamber instead of, or in conjunction with, an association to the large chamber. Therefore, during testing day, the results could be skewed since the animal might be showing avoidance or preference for the central chamber instead of for the large chambers. The two-chamber design avoids this pitfall because the animal merely has a single choice between the two distinctly different chambers and is not forced to cross a theoretically neutral, small chamber. The one benefit of the three-chamber design is the ease with which the experimenter can place the animal in the center of the apparatus. Although it might be more difficult to place an animal exactly in the middle of the two-chamber apparatus, the disadvantages of the three-chambered design do not outweigh this solitary gain.

Ethanol Administration Methods

The two drug-delivery methods commonly used in place conditioning experiments with ethanol are IP andIG administration. With IP administration, ethanol is injected into the abdominal-pelvic cavity using a syringe and needle. The syringe is gauged to allow for precise measurement and administration of the drug. Using 2g/kg IP administration of ethanol, CPP was observed in mice (Ozburn et al., 2008; Itzhak et al., 2009; Bhutada et al., 2010) and rats (Cole et al., 2003). Other doses such as 1g/kgIP and 3g/kg IP have also produced CPP in both mice (Szumlinski et al., 2005b; Marchand et al., 2006) and rats (Matsuzawa et al., 2000a) depending on the timing of administration and number of conditioning trials.

Two types of IG administration used in CPP experiments with ethanol are gavage and catheter administration. A gavage is a removable gastric feeding-tube, and it must be placed directly into the stomach via a surgical technique. A catheter is a small solid tube that is placed permanently in a body cavity by means of surgical implantation. The catheter is either placed in the stomach or small intestine. CPP has been observed in rats with 2g/kg IG gavage administration of ethanol (Peana et al., 2008) and 1g/kg IG catheter administration of ethanol (Fidler et al., 2004). A few groups have tried per os (by mouth) self-administration (Bedingfield et al., 2009; Lardeux and Baunez, 2007), but this method is highly unreliable. Since place conditioning depends on the timing and amount of drug administered, it is impractical to assume that the animal will self-administer a sufficient and appropriately timed

amount of ethanol to induce CPP. Even if CPP is observed, it would be hard if not impossible for another group to replicate the results seen in the original study due to individual differences in animal consumption.

The IG administration method is superior to the IP administration method because it allows for the digestion and processing of ethanol directly from the gastrointestinal (GI) tract instead of indirectly from the surrounding body cavity. This is critically important because human ethanol consumption involves drug delivery through the GI tract and not from the space surrounding body cavities. The advantage of IP administration is the speed and efficiency of drug delivery. Since the IP administration method requires little set-up and involves simple injection techniques, many investigators have chosen to use this method instead of the more complicated IG administration method (Groblewski et al., 2009; Gremel and Cunningham, 2010). The IG method characteristically involves surgical implantation of the gavage or catheter, an extended period of recovery time for the animal, and the careful monitoring of the animal by the investigators to prevent unnecessary infection. Although more difficult, the IG administration is more representative of human ethanol consumption. In a comparison study (Krishnamra et al., 1987), 3 or 5g/kg quantities of ethanol were administered to different groups of female Wistar rats by either IP or IG methods. Plasma $45Ca^{2+}$ activity was measured for each animal, and a significant difference between groups was reported 30 minutes after initial administration. The group given IP administration of ethanol had increased tissue content of $45Ca^{2+}$ by 40% in the duodenum, 38% in the jejunum, and 39% in the colon. The group given IG administration of ethanol had increased tissue content of $45Ca^{2+}$ by 31% in the duodenum, 27% in the jejunum, and 33% in the colon. These differences cannot be overlooked. For the sake of developing the most accurate model of human drug addiction, it is necessary to use the more complicated yet more accurate IG administration method. Even if the differences are simply due to a change in timing for drug metabolism, this small change in timing could call into question the validity of CPP observed with specific doses of ethanol administered via the IP method.

The administration method for the zebrafish model is unique in that ethanol can be mixed with the aquarium water to titrate the dose of drug delivery (Mathur et al., 2010). Although the method is reliable, the zebrafish model is less pertinent than the mouse model in the study of human alcohol addiction for reasons that will be discussed later.

Choice of Model Organism

The choice of model organism is often the most important decision in designing an experiment. The chosen species can limit the accuracy of the model or help it become more representative of actual human disease, addiction, or a physiological process. The specific model organisms used for CPP procedures with ethanol include the rat, mouse, and zebrafish. CPP has been observed in each of these species, but the mouse model has been most successful in developing CPP (Cunningham et al., 1992; Newton et al., 2008). One reason for this success might include the ability to create transgenic and knockout (KO) animals (Thanos et al., 2005). Transgenic refers to adding genes where KO refers to deleting or blocking the expression of genes. The use of transgenic and KO animals allows investigators to look at individual physiological components of alcohol addiction such as the influence of ghrelin levels (Jerlhag et al., 2009), the importance of the neuronal nitric oxide synthase (nNOS) gene (Itzhak et al., 2009), and the effects of the histamine H3 receptor ligands (Nuutineen et al., 2010). All three of these components have been implemented in the development of CPP with mice. These genetic manipulation techniques are especially beneficial when investigators create high-ethanol consuming or low-ethanol consuming animals (Short et al., 2006; Lardeux and Baunez, 2008). The modified animals can be used to model different preferences for ethanol in the human population, and therefore provide a more comprehensive population focused approach for studying addiction.

Rats are the second best animal models to use in place conditioning experiments with ethanol because of larger surface areas for

surgery, limited differences between the few rat strains in contrast to the many differences between the multiple mice strains, and similar consumption patterns of ethanol during free choice paradigms as those observed in humans (Pautassi et al., 2008). But, since it is more difficult to genetically modify rats due to the complexity and time required to develop transgenic models, the use of these animals is limited to specific experimental designs with specific rat strains. The three common strains of rats used in place conditioning experiments with ethanol are Long-Evans, Sprague-Dawley, and Wistar. Long-Evans rats are often used in experiments requiring surgical implantation or lesions delivered prior to place conditioning due to their size (Lardeux and Baunez, 2007), and in experiments using multi-drug place conditioning procedures due to the strain's toleration of administration (Jones et al., 2010). Sprague-Dawley rats are useful for experiments requiring graded drug administration (Philpot et al., 2003) and for experiments that involve pre-conditioning stress procedures (Matsuzawa et al., 2000a) because of the strain's adaptability to novel training paradigms. Wistar rats are useful in studying the biochemistry associated with ethanol addiction due to the vast knowledge already obtained from other fields of study (Kotlinska et al., 2007). If the rat model is being used for standard place conditioning procedures where only one drug is needed and no pre-surgery requirements are necessary, the Sprague-Dawley strain is the best to use due to the mild temperament of the animals and the ease of handling during conditioning experiments (Allan et al., 1998).

The least viable organism used in CPP procedures with ethanol is the zebrafish. Although CPP has been observed in the zebrafish (Blaser et al., 2010; Mathur et al., 2010, 2011b), there are limitations that will prevent the continued use of this model unless proper and thorough solutions are developed. First, zebrafish are phylogenetically further removed from humans than rodents (Patil et al., 2004). This phylogentic separation is important because zebrafish process and respond to ethanol in a different manner than humans (Dlugos and Rabin, 2003). In addition, different administration methods and apparatus designs are necessary for place conditioning with zebrafish. As mentioned previously, visual stimuli alone can be manipulated in the apparatus used for zebrafish, and currently only one administration method of ethanol is available for this organism. Processing ethanol from the water through the gills is not similar to ethanol consumption in humans. The zebrafish are exposed to both an internal and external milieu of ethanol via its aquarium water which rodent models and, more importantly, humans only experience internal administration methods through GI processing. Despite such limitations, a few advantages do exist with the zebrafish model such as the ability to genetically modify the organism (Tanguay and Reimers, 2008), the relatively inexpensive cost of housing the fish compared to housing rodents, and the ability to observe CPP after a single pairing with ethanol (Mathur et al., 2011a).

Age of model organism

Alcohol addiction in the human population is primarily a process that begins in adolescence. If an individual begins consuming alcohol before the age of fourteen, he or she is four times more likely to become an addict than an individual who waits to begin drinking until the legal age of twenty-one (Philpot et al., 2009). It is therefore important to use animals that are in the adolescent phase of development when conducting CPP trials with ethanol. Unfortunately, a separate line of thought has consistently plagued the field of ethanol CPP research until recently. Many investigators have rightly noted that human addiction to alcohol is frequently observed in adults (Bie et al., 2009; Zarrindast et al., 2010; Wrobel, 2011, but they overlooked the fact that adulthood is not where addiction often begins to develop. The faulty line of reasoning has led to a plethora of studies CPP with adult animals and relatively few studies that have investigated CPP with adolescent animals. Since ethanol administration in adolescent rats alters the development of N-methyl-D-aspartate (NMDA) receptors (Sircar and Sircar, 2006), it is likely that the adult models do not accurately represent the process of addiction as it occurs in some humans.

In a comparison study, adult and adolescent rats have shown markedly different preferences to the same doses of ethanol (Dickinson et al., 2009). The investigators found that adolescent animals required significantly more ethanol to develop CPP than the adult animals. This might explain why human addiction begins in adolescence, since increased levels of ethanol are needed to experience reward at this stage of development (Philpot et al., 2003). Furthermore, in another comparison study (Chin et al., 2011) a team examined differences in spatial learning between adult and adolescent rats exposed to ethanol. Interestingly, adolescent rats required higher doses of ethanol to develop preference, but once injected with enough ethanol, spatial learning deficits measured via the Morris Water Maze test were consistent between adult and adolescent animals. It has thus been proposed that the physiological responses to ethanol are no different in adolescent animals but that the mechanisms of reward processing are dissimilar to those seen in adult animals (Pautassi et al., 2011). Therefore, in future studies it will be necessary to reconfigure the current approach used to study the rewarding properties of ethanol in light of adolescent brain differences.

Stress Pre-Exposure Paradigm

In humans, social and environmental complexity often leads to increased consumption of alcohol for those who are prone to alleviate stress by drinking (Pohorecky, 1981). Since those who suffer from alcoholism often consume ethanol during stressful periods, it would behoove investigators to develop models where animals have been pre-exposed to stress (Matsuzawa et al., 1998a). In recent years, many investigators have used pre-exposure to stress in the form of animal foot or tail shock as a more indicative model of human alcoholism (Matsuzawa et al., 2000, Der-Avakian et al., 2007, Sperling et al., 2010). Not surprisingly, these investigators have found a more robust CPP in animals pre-exposed to stress than in the control animals not exposed to stress.

A few reasons exist regarding why more investigators do not use this approach. First, not all encounters with ethanol involve stressful situations. In some instances alcohol is used to enhance social situations or increase perceived sexual success (Prauss et al., 2006). Moreover, the stress paradigm requires a more complex apparatus and it increases the time necessary to conduct the experiment. Finally, many investigators have focused a great deal on individual components of addiction without looking at the larger picture of the disease and the way it develops in humans. Thus, they have limited their explorations to understanding individual mechanisms of addiction while not reevaluating the overall correlation to human alcoholism (El-Ghundi et al., 1998; Houchi et al., 2005; and Brown et al., 2010). It is necessary to redirect the field towards broader approaches that will incorporate all the individual components. Stress pre-exposure is an experimental design that simulates these broader approaches, and it offers researchers a resource by which to utilize CPP procedures in a manner consistent with understanding human addiction.

Discussion

Despite the advantages of the five ideal variables presented in this review, no single study possesses all of them for many different reasons. Often an investigator must pick and choose the variables to use in the CPP experiments based on the available resources, the specific aims, and according to the work previously conducted in the field. It is a challenge to incorporate new components while trying to build upon an already established baseline. For example, the ability to create transgenic and KO mice, which can be carefully manipulated for specific research focuses, (Thanos et al., 2005) has been a boon for CPP experiments. However, the pre-exposure to stress paradigm has only recently been proposed for use with mice. Therefore, investigators must either spend a lot of time and resources to develop a model, that incorporates a pre-exposure to stress paradigm with transgenic and KO mice, or they can continue in the line of previous work and simply use the pre-exposure to stress rat model that is already developed, but which lacks transgenic and KO manipulation (Philpot et al., 2003).

Some variables are easier to choose, such as the two-chamber apparatus (Cunningham et al., 2006) instead of the three-chamber apparatus. But, it still requires time and effort to transition to something new. The investigator will either have to build the device or have a machine and technology expert create it. Another variable that investigators could change is making the switch from an IP to an IG administration method (Fidler et al., 2004). The two-chamber apparatus and IG administration method should become the standard in the future, but it is important to note that it will take time to change a field with certain fixed techniques. For example, the time spent training the lab personnel in IG surgical techniques would take longer than the training required for the IP technique.

Although some people develop addiction to ethanol later in life, the vast majority of addicts start consuming ethanol during adolescence (Philpot et al., 2009). Since CPP develops at higher doses in adolescent animals (Dickinson et al., 2009) and ethanol alters brain development at this stage (Sircar and Sircar, 2006), it is critical that further work be done with adolescent animals. All five variables highlighted in this review reflect upon judgments about the best representation of human addiction to alcohol. As knowledge continues to expand regarding alcoholism, it will be necessary and beneficial to revise and expand CPP experiments.

Conclusion

CPP is just one technique of many that has been used to study ethanol's rewarding and aversive properties (Correia et al., 2009). Other tests include the free-choice paradigm, Loss of Righting Reflex, Morris Water Maze, and Elevated Plus Maze. What is unique about CPP is that it gives an animal a choice which can be easily measured. Unlike the free-choice paradigm, the experimental animal in CPPis force exposed to ethanol. CPP might better represent early alcohol exposure in humans because the forced exposure to ethanol overcomes the initial taste aversion. In human adolescents taste aversion to ethanol is overcome by peer pressure and unwholesome role models (Pautassi et al., 2011).

Another advantage of place conditioning is that many variables can be adjusted or redesigned. Five ideal variables were highlighted in this review, but there will be ways to improve upon these variables in the future. This section will highlight the author's suggestions about potential improvements. With advanced technology, it will eventually be possible to have a three-chamber design that converts into a two-chamber design once the animal initially enters the large chamber. This will alleviate the problem of placing the animal exactly in the center of the device. It will also abolish an animal's possible association with the small chamber because the small chamber will instantly disappear after the animal moves into a large chamber. Also, new injection techniques might be designed where IG administration will no longer involve a surgical procedure. This will make it more appealing for investigators to switch from IP to IG administration. Additionally, as progress continues in the development of more reliable transgenic and KO rats, the ideal model organism might become the rat instead of the mouse due to the larger surface areas for surgery and less variability between strains. Moreover, it might be possible to build upon the pre-exposure to stress paradigm. It could be beneficial for researchers to examine place conditioning after an animal has been exposed to isolation stress early in development (Lopez et al., 2010). Many people who become addicted to alcohol and other drugs of abuse were exposed to stressors in utero (Shor et al., 2010). Still further, it would be valuable for future studies to investigate CPP with ethanol in animals as they age from adolescence to adulthood. This progressive longitudinal approach would be an even more representative model of lifetime addiction often seen in humans. The place conditioning technique will continue to remain a staple in the study of alcohol addiction and as new variables are presented better models will continue to be developed.

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