D1 RECEPTORS IN MAOI AND NICOTINE SELF-ADMINISTRATION

Introduction/ Objective.

Tobacco smoking is the leading cause of preventable death in the United States, taking the lives of about 440,000 people (Centers for Disease Control, 2004). It is commonly believed that nicotine is the key agent in the addictive properties of tobacco smoking. Nicotine is a direct agonist of nicotinic acetylcholine receptors, which are largely expressed through the brain. At the present time, nicotine replacement therapy (NRT) is used as a substitute treatment to help smokers to stop tobacco consumption (Silagy et al., 1994). However, NRT seems to only improve tobacco withdrawal partially. Moreover other studies have shown that denicotinized cigarettes were also efficient to decrease withdrawal and craving signs (Pickworth et al., 1999). Finally, tobacco smoke contains about 3000 different compounds and it is possible that some of them are involved, as nicotine, in the addictive processes induced by tobacco (Pickworth et al., 1999).

Among the different compounds contained in tobacco smoke, monoamine oxidase inhibitors (MAOI) have recently been the focus of a particular interest (Berlin and Anthenelli, 2001). Monoamines are neurotransmitters found in the central nervous system. They are involved in sending signals to the brain which regulate sleep, arousal and pleasure ("Dopamine"). The three main monoamines studied by researchers are dopamine (DA), norepinephrine and serotonin. Monoamine oxidase is one enzyme responsible for the degradation of the monoamines. MAOIs are molecules that inhibit monoamine oxidase, and as a result increase the levels of monoamines found in the brain. According to newly developed research, a synergy between nicotine and MAOIs could be of importance for tobacco addiction to set up (Berlin and Anthenelli, 2001). Then, the association between nicotine and MAOI seems to be an appropriate model to study mechanisms underlying tobacco addiction.

Among the three types of monoamines, DA plays a key role in the addictive processes because it is known as the "reward chemical". Dopamine prolongs pleasurable experiences of the brain. DA acts by binding and stimulating specific receptors. DA can activate several receptors belonging to two different classes called the D1 and D2. D1 and D5 belong to the D1 receptors sub-class and D2, D3 and D4 belong to the D2 receptors sub-class (Strange 2000). D1 receptors are found in some brain areas with a high level of DA, such as neostriatum, substantia nigra, nucleus accumbens (Strange 2000). D1-dopaminergic receptors are "G-protein coupled receptors" (metabotropic receptors) that interact with stimulatory G-protein (figure 1). The binding of an agonist, such as dopamine, to the D1-

dopaminergic receptors causes a stimulatory effect in the brain. On the other hand, the binding of a specific D1-receptor antagonist, such as SCH 23390, blocks the activation induced by DA binding to the D1 receptor.

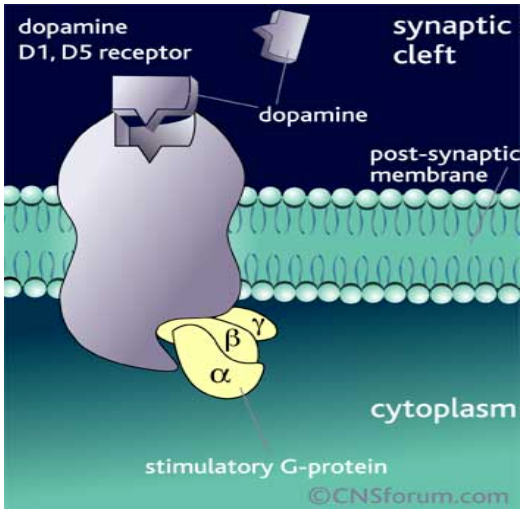


Figure 1. Functioning of D1 and D5 dopamine receptors.
DA released in the synaptic cleft recognizes and binds dopaminergic receptors. Following the binding of DA on receptors, it activates the stimulatory G-protein linked to the receptor, activating an intracellular cascade resulting in the activation of the cell. <http://www.cnsforum.com>.

The D1-dopaminergic receptor has been implicated in cognition and addictive processes (Strange 2000). Several studies have shown that systemic injection of low doses of SCH23390, a specific D1-dopaminergic receptor antagonist, increases responding for IV injections of cocaine in the paradigm of self-administration in rats (Koob et al., 1987; Maldonado et al., 1993). The authors of these studies explain the increase in self-administration behavior by considering it as “a compensatory mechanism for decreases in the magnitude of the reinforcer, similar to the increase observed when the dose of the self-administered stimulant is reduced” (Maldonado et al., 1992). These results suggest that the D1 receptor activation may be an important component of cocaine reward.

In the case of tobacco addiction, it is possible that MAOIs, which are present in tobacco, increase the levels of DA, causing nicotine to be more addictive. High levels of DA allow tobacco use to be a more pleasurable and rewarding experience (Villégier et al., 2003). Thus, I propose to test the hypothesis that DA plays an essential role in nicotine self-administration in rats pre-treated with MAOI. As the role

of D1 receptors in addictive processes has been demonstrated in the case of cocaine self-administration, I propose to test the role of this particular receptor by using SCH23390, which is a selective D1 receptor antagonist.

Approach.

Self Administration: The addictive properties of nicotine will be tested using the paradigm of self-administration. Self-administration is a behavioral model of addiction. In this model rats are implanted with an intra-jugular catheter and are able to inject themselves drugs. Self-administration will be conducted in Plexiglas chambers (28×25×30 cm) with individual enclosed ventilation and sound-resistant boxes. The chambers will have two holes for the rats to place their heads in to initiate self-administration (Manzardo et al., 2002). The reinforced hole (R) will trigger the self-administration of nicotine, while the non reinforced hole (NR) will not inject anything (figure 2). The rewarding effect of nicotine will be determined by the number of self-injections.

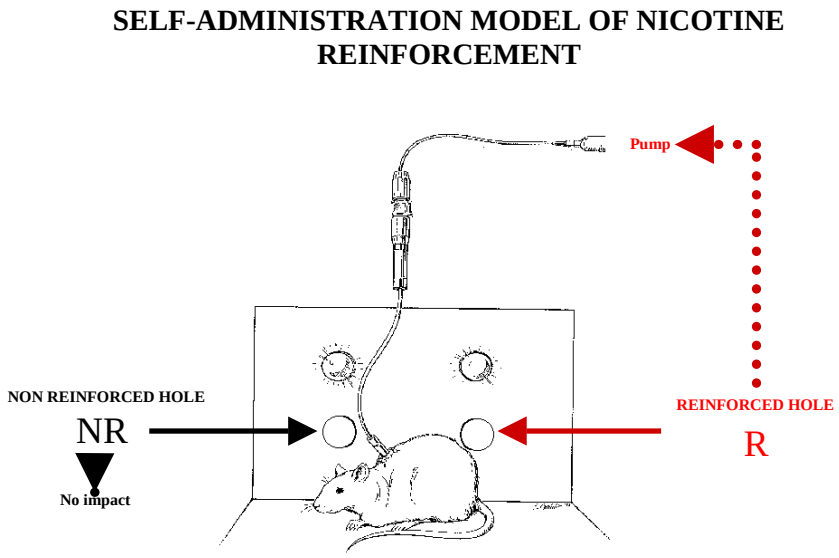
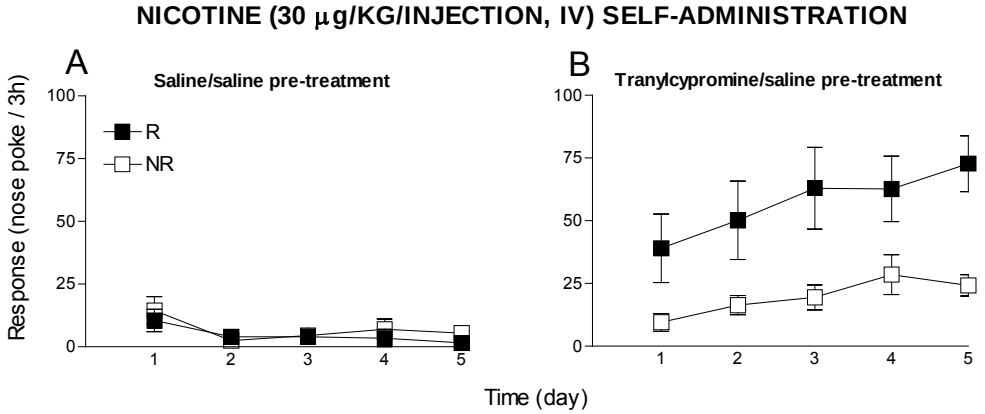


Figure 2. Model of a self administration box.
The self-administration box is characterized by the presence of two holes. When the rat pokes his nose in the non reinforced hole (NR), it has no impact on the environment or on the rat itself. When the rat pokes his nose in the reinforced hole (R), it activates the pump, then the rat receive one injection of the solution contained in the syringe.

D1 Receptor Antagonist: To test the involvement of D1 dopaminergic receptors in nicotine self-administration a pharmacological approach has been chosen, by using a specific D1 receptor antagonist, SCH23390.

Control and Variables: The control for the experiment will be nicotine self-administration in rats pre-treated with tranylcypromine (pre-treatment) /saline (treatment) solution (Group1). Tranylcypromine is an MAOI that has been shown to increase nicotine self-administration (figure 3). Saline (pre-treatment)/saline (treatment) (Group2) will test the addictive properties of nicotine alone (without MAOIs). Finally saline (iv) self-administration will also be tested in rats injected with tranylcypromine (pre-treatment)/saline (treatment) (Group3) and saline (pre-treatment)/saline (treatment) (Group4) as a negative control.



R=Reinforced hole NR= Non-reinforced hole

Figure 3. Graph A presents the relationship between Saline pretreatment and Nicotine IV injections. The graph shows that saline pretreatments do not cause the rats to develop a nicotine addiction. Graph B on the other hand presents the relationship between Tranylcypromine pretreatment and Nicotine Iv injections. The graph shows the Tranylcypromine pretreatments have caused the rats to develop an addiction to nicotine. (Preliminary data)

The experimental groups will be the same groups as 1 to 4, except that saline treatment will be replaced by a D1 antagonist, SCH23390 (0.02 mg/kg). I want to see how D1 antagonist affects nicotine self-administration in rats. Different groups are summarized in the following table:

Table 1.	Groups	Pre-treatment	Treatment	Self-administered solution
Controls	1	Tranylcypromine	Saline	Nicotine
	2	Saline	Saline	Nicotine
	3	Tranylcypromine	Saline	Saline
	4	Saline	Saline	Saline
Experimental	5	Tranylcypromine	SCH23390	Nicotine
	6	Saline	SCH23390	Nicotine
	7	Tranylcypromine	SCH23390	Saline
	8	Saline	SCH23390	saline

Methods.

Catheter Construction: The catheter will require 12 cm of Silastic tubing (0.012 in. I.D.×0.025 in. O.D.) and a 22-gauge guide cannula (Plastics One, Roanoke, VA). The Silastic tubing will be placed in HEMO-De (Fisher Scientific, Pittsburgh, PA) causing the tubing to expand. Afterwards the tubing will be glide over the 22- gauge cannula at a 90 degree angle. The cannula with the tubing will be flushed with water and air to remove and HEMO-De trapped in the tubing. The next day 2 cm of large tubing (64 m I.D.×120 m O.D.) will be placed in HEMO-De for expansion then will be glided over the smaller tubing. The bigger tubing will protect the smaller tubing in catheter molding because the smaller tubing is very fragile and can easily tear. The cannula will be flushed with water and air once again to remove any blockages of HEMO-De in the tubing. The next day cranioplastic cement will be used to mold the catheter and the back of the catheter will have a 2 cm Marlex mesh (Bard Inc., Billericay, MA) attached. The tubing at the end will have a 1cm patch of Mersiline mesh (Ethicon, Somerville, NJ) 2cm from the end of the tubing to used in the implantation of the catheter in the jugular vein. (Manzardo et al., 2002).

Preparation of Animals: Experiment will last 5 days and for each group height male albino Sprague–Dawley rats will be used, weighing about 90 gram and 30 days old. Prior to and during the course of the experiment the rats will each have their own cage equipped with a water bottle and food. The rats will be anesthetized to undergo sugary where a 12 cm tubing catheter will be placed in the right jugular vein. The catheter tubing will stretch under the skin across the shoulder allowing the catheter to be placed on the back on the rat. Stylet will be used to seal the rats after surgery and antibacterial ointment will be applied to the area where incisions were made on the rat to prevent infection. Then the rat will have 3 days to recover before undergoing self administration. Each day the catheter will be flushed with 0.1 ml of a heparinized saline solution to prevent and check for blockages. The catheter is an effective tool directly injecting nicotine dosages the blood. (Manzardo et al., 2002).

Preparation of solutions: Each iv injection delivers 30μg/kg of nicotine in 20μl (groups 1, 2, 5 and 6) or 20μl of saline solution (groups 3, 4, 7 and 8). Nicotine is dissolved in saline and pH is adjusted to 7.4 with NaOH solution. Before the experiment begins 3 mg/kg of tranylcypromine in 0.5 ml will be given to the rats as pretreatment an hour before the rats are placed in the self-administration boxes (groups 1, 3, 5 and 7) while other groups receive saline as a pre-treatment (groups 2, 4, 6 and 8). Tranylcypromine is dissolved in saline. Rats from groups 5, 6, 7 and 8 will receive 0.02 mg/kg of SCH23390 as a treatment, half an hour before they are placed in the self-administration boxes, while groups 1, 2, 3 and 4 receive saline as a treatment. SCH23390 HCl will be dissolved in water.

Protocol: Each group will consist of six rats and Rats from groups 1 to 4 will receive treatment described in table 1 during the five days of the experiment. Rats from group 5 to 8 will receive the same treatment as group 1 to 4, respectively, during the three first days of the experiment. The purpose of this procedure is to establish nicotine self-administration in group 5. At day four and five, rats from groups 5 to 8 receive SCH23390 instead of saline as a treatment. The introduction of the antagonist will illustrate the mechanism between dopamine and nicotine and its ability in the addiction of tobacco use.

Self- Administration boxes: The experiment will be conducted for three hours each day for five consecutive days and afterwards the animals will be flushed with .1ml of heparinized saline solution and will be returned to their home cages. The control of all experimental parameters and the collection of all data will be controlled by a multichannel computer system (MED Associates Inc., St Albans, VT).

Time line.

Week	
1	Make catheters and perform surgery for the first set of rats
2	Run the first self-administration experiment. The last 3 days of the week will be used to perform surgery on the next set of rats.
3	Run the next self-administration experiment. Make catheters.
4	Perform surgery for the next set of rats. Make all the catheters for the all the experiments
5	Run the next self-administration experiment. The last 3 days of the week perform surgery on the next set of rats.
6	Run the next self-administration experiment. The last 3 days of the week perform surgery on the next set of rats.
7	Run the next self-administration experiment. The last 3 days of the week perform surgery on the next set of rats.
8	Analyze Data. Perform another self-administration experiment if needed if the data is not adequate. Perform Surgery.
9	Run the self-administration experiment.
10	Analyze data.

Expected results.

There are two outcomes of the nicotine self-administration experiment. One is that the blockade of D1 receptor will decrease the intake of nicotine causing addiction levels to decrease. The second is that the blockade of D1 receptor will cause the increase of nicotine intake. In fact, studies using SCH 23390 in self-administration of other drugs, such as cocaine, ecstasy or methamphetamine, have shown both

outcomes. The fist outcome will result from the rat not receiving pleasure from nicotine due to the absence to dopamine. This will cause the rat to stop using nicotine (Sziraki et al,. 1998). The other outcome illustrates the rat may no longer feel pleasure from nicotine but will try to find that pleasure point through the consumption of more nicotine. Therefore the rat will consume more nicotine until realizing it can no longer feel pleasure. (Daniela et al., 2004)

Responsibility and Level of Involvement.

During winter quarter I became familiar with the lab by constructing catheters, learning how to do surgeries with the guidance of my mentor, setting up the self administration experiments and handling animals. This quarter I will be performing surgeries on my own without the guidance of my mentor, handling animals on a regular basis, giving pretreatments to animals, flushing the animals with saline after experiments and assembling surgery kits. These tasks will help prepare me for the summer when I will conduct my own experiment involving the self-administration of nicotine with D1 receptor antagonist.

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