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THE ROLE OF ENDOGENOUS D2 RECEPTOR LEVELS IN MORPHINE ADDICTION: A CORRELATIVE STUDY OF MORPHINE PLACE CONDITIONING AND IN VIVO [3H]-RACLOPRIDE BINDING

Naqi Khan, Samuel Gatley

ABSTRACT

Dopamine is a neurotransmitter that has a wide array of effects on an individual's mental state. It is vital in the regulation of motor skills and in generating the effects of substance abuse. This study examined the dopamine D2 receptors found in the striatum of the brain. The impetus for investigating this receptor lies in the perception that it plays an influential role in drug addiction. It has been conjectured on the basis of human PET studies that possession of low levels of D2 receptors will heighten an individual's susceptibility to drug addiction. However, an alternative explanation of low D2 receptor levels in drug dependent individuals is that these levels are a consequence of drug abuse. To understand this phenomenon, the present study employed the paradigm of conditioned place preference (CPP). In CPP, individuals of an out-bred mouse strain are observed to spend time in environments where they had previously been exposed to a drug that is abused by humans. The drug chosen for our studies was morphine because it has been previously shown to generate a robust place preference in mice and is a prototypic abused drug in humans. D2 receptor levels were quantified using an in vivo binding study involving [3H]raclopride, a radioactive compound that binds to D2 receptors. The results showed a significant place preference for morphine following the conditioning procedure. Additionally, data from the binding analysis agreed with previous studies that the striatum contains high levels of D2 receptors. However, there was no consistent relationship between the extent of morphine CPP and D2 receptor levels as revealed by [3H]-RAC binding. This finding does not support the hypothesis that low levels of D2 receptors predispose a mouse to easy morphine conditioning. Further experiments are required to determine the ability to generalize our findings to other species and other drugs of abuse.

INTRODUCTION

Neurotransmitters can act either directly or indirectly upon the gated ion channels housed within the membrane of a postsynaptic neuron. In the direct case, the receptor that the neurotransmitter binds to is itself a part of the ion channel protein. The indirect method is more involved, requiring the interaction of second messengers and/or G-proteins to bring about a desired effect [1]. The overall effect can either be excitatory or inhibitory to the postsynaptic cell, and is dependent on the neurotransmitter in play.

Dopamine (DA), considered an inhibitory neurotransmitter, is vital to awareness, judgment, controlling motor skills, and motivation. It has also been discovered to play a role in substance abuse and addiction. Found primarily in the mesencephalon, the DA cells are distinctly organized into the substantia nigra (SN) group, the retrobulbar group, and the ventral tegmental area (VTA), with projections into the striatum (ST). The DA is manufactured by DA cells and is enveloped by a vesicle that shields it from monoamine oxidase (MAO). The DA is flooded into the synapse once the cell receives an action potential. The concentration of the neurotransmitter is then maintained by DA transporters. The actions of these transporters are significant in determining the amount of DA that actually binds to and activates DA receptors [2].

	BOX	N	Mean	Std. Deviation	Std. Error Mean
TIME_MOR 8 mg/kg	1	6	427.17	56.96	23.25
	2	6	294.67	69.43	28.34
TIME_SAL	1	6	379.17	96.31	39.32
	2	6	374.33	78.64	32.11
TIME_MOR 6 mg/kg	1	16	477.94	75.10	18.78
	2	16	261.88	63.39	15.85

Table 1: Shows the performance of all three groups of mice .
BOX 1 = Morphine-paired chamber for morphine conditioned mice, or black chamber for control mice;
BOX 2 = Vehicle-paired chamber for morphine conditioned mice, or white chamber for control mice.

Raclopride (RAC) is a drug that binds to DA D2 receptors. It is frequently used, labeled with the short-lived positron emitting isotope carbon-11, in PET experiments to measure levels of these receptors in the striatum of human and monkey subjects. Since DA and RAC compete for binding to D2 receptors, PET experiments can also be designed to detect changes in DA [3, 4]. However, we were interested primarily in D2 receptor levels, and we injected the mice we used as experimental subjects with tritiated raclopride ([^{3}H]RAC). Radioactivity was subsequently measured in brain tissue using a liquid scintillation counter. Previous human PET studies have indicated that individuals who are dependent on drugs of abuse tend to have low D2 receptor levels than non-dependent individuals. Our aim was to determine whether mice with fewer D2 receptors behaved as though they had a greater "liking" for an abused drug than mice with higher receptor levels.

Swiss-Webster mice were chosen as the subjects of this study, for they are out-bred mice and each individual is expected to have a varying level of endogenous D2 receptors. Morphine place conditioning was chosen as it is known to be rapidly acquired by mice [5].

Place conditioning involves the association of a rewarding drug with stimuli originating from the environment. With repetitive administration of the rewarding drug in a specific environment, the mouse will develop a preference for the environment [6]. When given free access to the place conditioning apparatus, the mouse will choose the rewarding environment over other environments by using the visual and textual cues with which it has been repeatedly presented [5].

Naturally, each mouse will develop a preference for a rewarding substance at a different rate and to varying extents. It is proposed that increased levels of D2 receptors will reflect a lowered tendency to develop a preference for morphine. This study was intended to examine this possibility by coupling conditioned place preference to morphine with an in vivo binding study of [3H]raclopride to striatal D2 receptors.

MATERIALS AND METHODS

Drugs and Compounds

Morphine was obtained from Sigma Chemical Co. (St. Louis, MO) and raclopride methoxy-[^{3}H] (78 Ci/mmol) was acquired from Dupont New England Nuclear (Boston, MA).

Subjects

Male Swiss-Webster mice, weighing 25 – 30 g, were obtained from Taconic Farms (Germantown, NY). They were allowed free access to food and water. The subsequent morphine injections were performed intraperitoneally (i.p.), with saline as a vehicle. Either a 6 mg/kg or 8mg/kg dosage of morphine was administered to the mice, as these doses have been shown to generate a robust place preference [5, 6].

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig	T	df	Sig (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
TIME_MOR 8 mg/kg	Equal variances assumed	.110	.747	3.61	10	.005	132.50	36.66	50.81	214.19
	Equal variances not assumed			3.61	9.63	.005	132.50	36.66	50.39	214.61
TIME_SAL	Equal variances assumed	.420	.532	.095	10	.926	4.83	50.76	-108.27	117.94
	Equal variances not assumed			.095	9.62	.926	4.83	50.76	-108.89	118.55
TIME_MOR 6 mg/kg	Equal variances assumed	.219	.643	8.79	30	.000	216.06	24.57	165.89	266.24
	Equal variances not assumed			8.79	29.18	.000	216.06	24.57	165.83	266.30

Table 2. A collection of Student t-tests. TIME_SAL = control mice;
TIME_MOR = mice conditioned with either 6 mg/kg morphine or 8 mg/kg morphine.

Place Conditioning

Place preference boxes obtained from MED Associates, Inc. were used as the conditioning environment. Each of these boxes was composed of three distinct chambers. The chambers were separated by sliding, opaque partitions. The end compartments were designated for conditioning. One was white with a stainless steel mesh floor, and the other was black with a stainless steel rod-type grid floor. The middle compartment was designated as the choice chamber. It was gray with a smooth PVC floor.

The place conditioning procedure was conducted over a period of eight successive days. The mice were randomly assigned into one of the following three groups: controls,

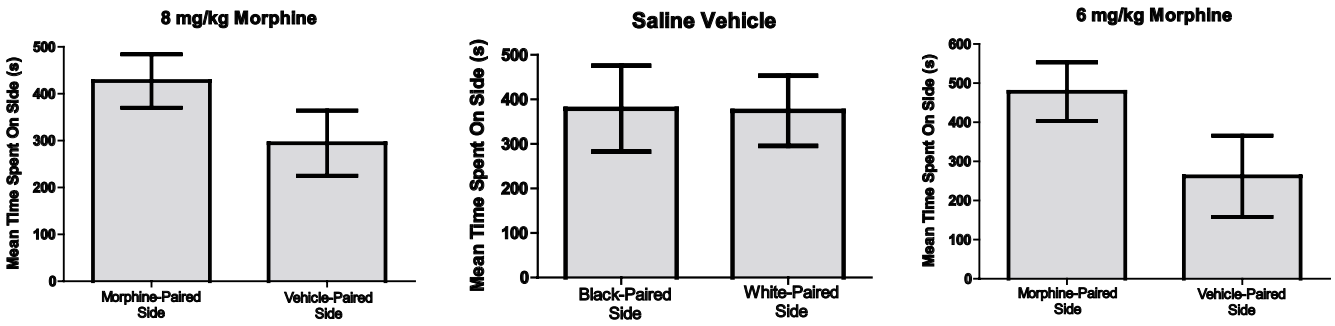

Figure 1. The average time spent □
mice) in the place preference box. (a) Mice conditioned with 8 mg/kg morphine (significant, P<0.005, n=6). (b) Control mice that received saline in both chambers (not significant, n=6). (c) Mice conditioned with 6 mg/kg morphine (significant, P<0.0001, n=16). All data are means +/- S.E.M.

drug-paired with the white side, or drug-paired with the black side. The first three days were used for preconditioning. During the first two days of this phase, the mice were brought to the testing room and handled. This was done in order to have the mice acclimate to the handler and to the environment. On the third day, the mice were placed into the choice chamber and were given 15 minutes to explore all three compartments.

The next four days were used for drug conditioning. During each conditioning day, the mouse received an injection of saline vehicle in one of the end compartments, dependent upon its group designation, and then received a morphine injection five hours later in the other end compartment. The mice remained in the chamber for 30 minutes after receiving an injection. A control group which received saline vehicle on both sides was also present in the study.

On the eighth day, the mice were placed into the choice chambers without any injections. They were allowed to roam in the three compartments freely for 15 minutes while their behavior was recorded with video cameras.

In Vivo Binding

Three days after the place conditioning procedure, each mouse was given a 1.0 μCi tail vein injection of [^{3}H]-RAC. The mouse was then allowed to rest for 30 minutes before it was sacrificed by cervical dislocation.

The brain was then immediately removed from the skull, and placed on its ventral surface. First the cerebellum was removed and placed into a 7-mL glass vial. The two cerebral hemispheres were then separated with a midsagittal cut along the corpus callosum. This allowed the cortical manticles to be folded back laterally, exposing the striatal tissue. The striata were then removed and placed into a different 7-mL glass vial. The tissue samples were then weighed and allowed to dissolve overnight in tissue solubilizer (0.5 mL/vial). Scintillation fluid (UltimaGold XR) was added the following morning and the vials were placed in a Packard Model 1600 TR liquid scintillation counter in order to measure the radioactivity in the tissue samples.

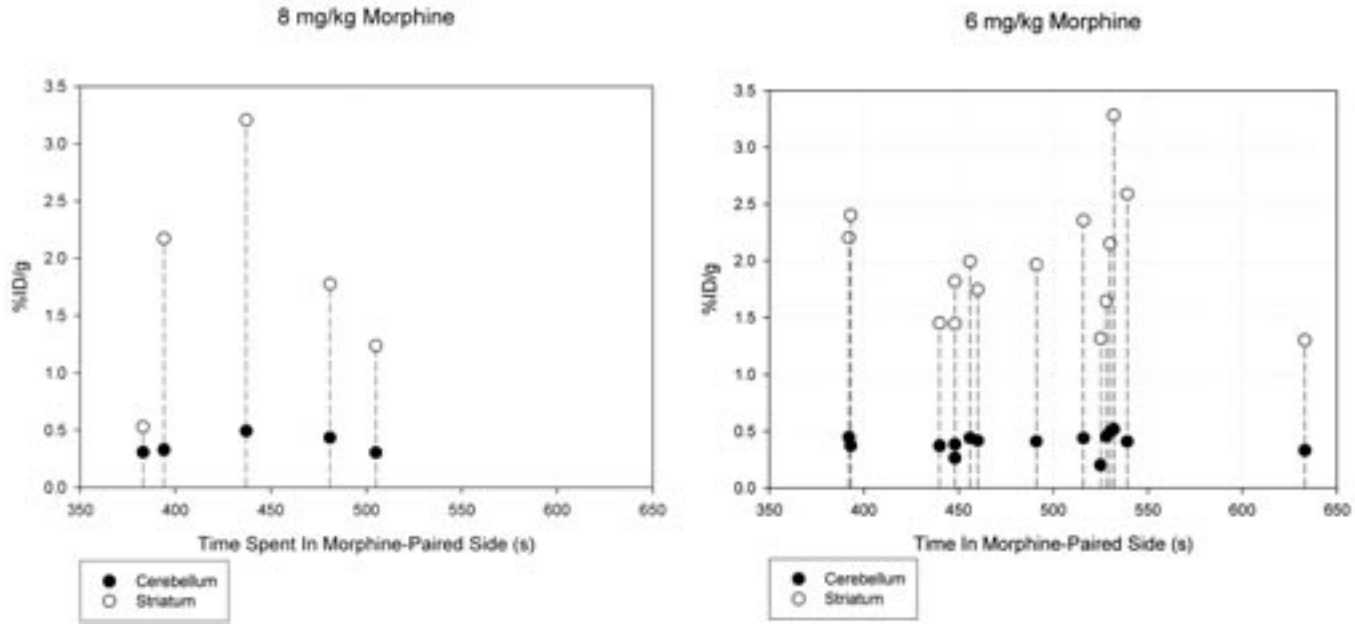

Figure 2. The relationship b□
Mice condi□

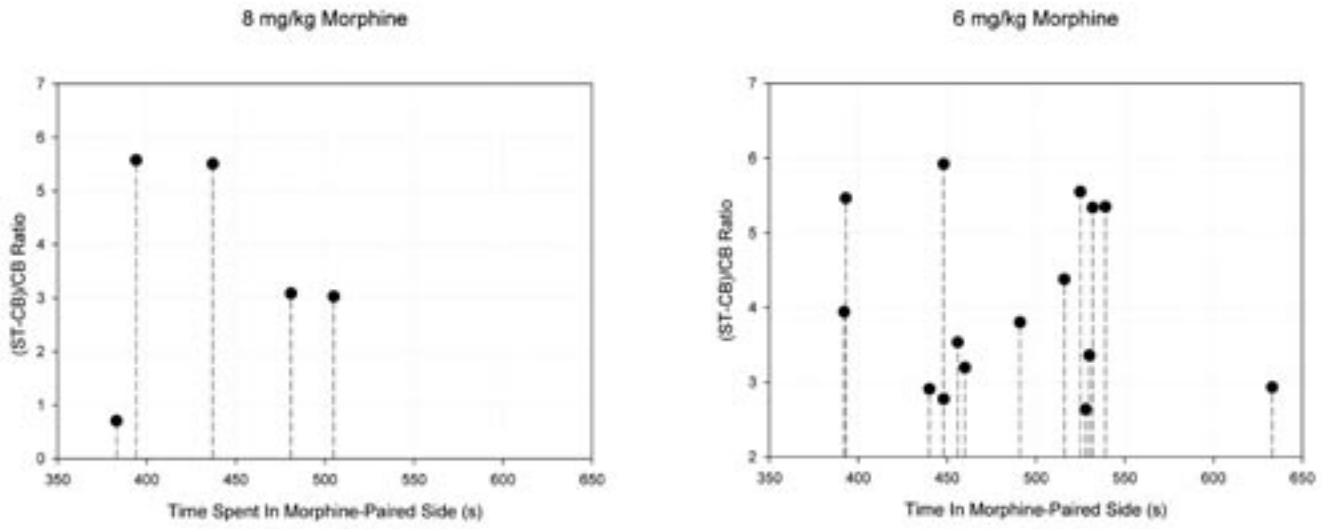

Fig. 3. The relationship between the seconds spent in the morphine-conditioned chamber and the (ST-CB)/CB ratio of [3H]-RAC binding. (a) Mice conditioned with 8 mg/kg morphine. (b) Mice conditioned with 6 mg/kg morphine.

Data Analysis

A t-test was performed on the total time each mouse spent in the saline vehicle paired compartment compared to the time spent in the morphine-paired compartment. Successful place conditioning to morphine was indicated by a significantly greater mean time spent in the morphine-paired chamber than in the saline vehicle-paired chamber.

For the in vivo binding element of this study, the primary ratio of comparison between mice was:

$$\frac{Striata(DPM/mg) - Cerebellum(DPM/mg)}{Cerebellum(DPM/mg)}$$

The percent-injected dose of [^{3}H]-RAC per gram (%ID/g) of tissue was also examined.

RESULTS

A preference for the morphine-paired compartment was induced as a result of the place conditioning regime. This was apparent in both dosage groups (Fig. 1). The 6 mg/kg dose of morphine caused a significant preference ($P<0.0001$) as did the 8 mg/kg dose ($P<0.0050$), as seen in Fig. 2. The group receiving 8 mg/kg of morphine for place conditioning spent on average 427 seconds (SD=57 seconds) in the drug-conditioned box (Fig. 1a, Table 1a). They spent only an average of 295 seconds (SD=69 seconds) in the saline vehicle-paired box (Fig. 1, Table 1a). Similarly, the 6 mg/kg group spent an average of 478 seconds (SD=75 seconds) in the drug-conditioned compartment. This group spent a mean time of 262 seconds (SD=104 seconds) in the vehicle-paired box (Fig. 1c, Table 1b). Meanwhile, the control group, which received saline vehicle in both compartments, did not show a preference for either box.

The raclopride concentration in striatal tissue was significantly greater than the raclopride concentration in cerebellum tissue that was collected, agreeing with previous studies (Fig. 2) Plotting the data obtained from the in vivo study against the place conditioning data did not depicted a trend that was favorable to our hypothesis. An inverse correlation between [3H]-RAC binding and the time spent in the morphine-conditioned box was not observed. Instead, a stochastic pattern surfaced as time in drug-conditioned compartment was compared to (ST-CB)/CB ratios (Fig. 3). As is observed, the ratio fluctuates randomly as the amount of time spent in the drug-conditioned compartment increases. This is true for both the 6 mg/kg and 8 mg/kg groups. The lowest observed ratio for the 8 mg/kg group occurs at a time of 383 seconds, with a value of 0.71. The highest ratio in this group occurs at 394 seconds with a value of 5.57. For the 6 mg/kg group, the lowest ratio of 2.45 occurs at a time of 316 seconds. The greatest ratio is observed at 448 seconds, with a value of 5.92.

The percent injected dose of [^{3}H]-RAC for striatal tissue fluctuates in the same manner as the (ST-CB)/CB ratios (Fig. 2). A low of 0.53% is detected at 383 seconds and a high of 3.21% is identified at 394 seconds for the 8 mg/kg group. The 6 mg/kg group had a peak of 3.28% at 532 seconds, and displayed its nadir of 1.30% at 633 seconds. The cerebellum's %ID/g stayed relatively constant for both 6 mg/kg and 8 mg/kg groups.

DISCUSSION AND CONCLUSION

The behavioral results obtained in this study allowed us to conclude that morphine can generate a place preference in Swiss Webster mice at administrations of both 6 mg/kg and 8 mg/kg (Fig. 1, Table 1). This can be attributed to morphine's known addictive properties and its ability to trigger the brain's reward center [7]. There is clearly something addictive and even pleasurable about the drug if the mice independently

sought out the environment in which they had received it [6]. This ability of the mouse to differentiate between compartments rested in its ability to associate the visual and textual cues of the compartments with its original drug experience.

The calculated (ST-CB)/CB ratios and %ID/g values both attest to an increased presence of [^{3}H]-RAC in the striatum, when they are compared with similar data from the cerebellum (Figs. 2, 3). This is due to the presence of D2 receptors in the striatal region, which readily bind [^{3}H]-RAC. These findings complement previous studies that have shown [^{3}H]-RAC to preferentially accumulate in the striatum of the mouse brain [8].

When data gathered from the place conditioning study was correlated with the [^{3}H]-RAC binding analysis, no sensible relationship emerged. This varied set of data suggests that the extent of addiction displayed by a mouse is not as related to the binding of [^{3}H]-RAC to D2 receptors as was previously surmised. More significantly, the findings imply that D2 receptor level is not associated with a mouse's susceptibility to morphine place preference. This raises the possibility that the low D2 receptor status observed in humans with substance abuse disorders is an effect rather than a cause of their illnesses.

Future studies could extend and improve these studies in terms of a number of technical issues relating to choice of radioligand, animal species, behavioral paradigm and conditioning drug. One critical question is the extent to which the development of conditioned place preference in rodents is a reasonable proxy for development of a substance dependence or addiction in humans.

ACKNOWLEDGEMENTS

Mr. Khan thanks the United States Department of Energy for giving him the opportunity to participate in the SULI program at Brookhaven National Laboratory. Additionally, the authors would like to thank Dr. Andrew Gifford and Onarae Rice for their constant support. Special thanks go out to Sara Gonzalez, Sandra Saint Victor, and Beatrice Pyatt for their assistance in the lab.

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