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Effects of estrogen in a place preference conditioning paradigm using non-sexual social interaction to condition female rats

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Effects of Estrogen in a Place Preference Conditioning Paradigm Using Non-Sexual Social Interaction to Condition Female Rats

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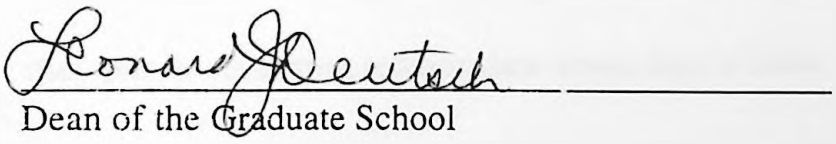

Dean of the Graduate School

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Abstract

Female Sprauge Dawley rats were used to determine if non-sexual social interaction could be used as a positive reinforcing stimulus in a place preference conditioning (PPC) paradigm. PPC is a commonly used paradigm to test the addictive properties of abused drugs, but has also been used to determine the positive effect of sexual activity and play in juvenile rats. Individually housed female rats were paired with group housed female rats or left isolated in a chamber for 15 minutes for positive or negative conditioning, respectively. Three different groups of rats were tested: intact, bilateral ovariectomized (OVX), and bilateral ovariectomized with estrogen benzoate replacement (OVX + EB; 0.2 ml/day) to determine the effects of social conditioning due to estrogen. All three groups were conditioned by social interaction and there was a tendency for the OVX group to have a stronger conditioning than the intact or OVX + EB group. Our data indicate that the social interaction can be used to condition female rats as shown in the PPC test and that ovarian hormones may play a role in that conditioning.

Introduction

There are three classes of mechanisms that govern the internal environment of the body: autonomic, endocrine, and reward system of the brain (Kandel *et al.* 1991). The autonomic mechanism refers to the division of the nervous system that controls glands and internal organs of the body and causes involuntary responses, such as heart rate, dilation of arteries, pupillary movements, and activity of the gastrointestinal system (Beatty, 1995). Endocrine control is accomplished either directly by the release of neuroendocrine products into the bloodstream or indirectly by releasing regulatory hormones, which control the synthesis and release of hormones in the anterior pituitary (Kandel *et al.* 1991). The reward system of the brain refers to an internal environment that cause a voluntary behavior (Kandel *et al.* 1991).

Kandel *et al.* (1991) describe reward systems as inferred mechanisms postulated to explain the extent and direction of a variety of complex behaviors, such as temperature regulation, feeding, thirst, and sex drive. The reward system or "drive" is urges or impulses based on needs that impel humans and animals into action. For example, a temperature regulating drive controls behaviors that directly affects body temperature, such as shivering or rubbing the hands together. These behaviors are caused by a change in the internal environment and evoke rewarding qualities or feelings of pleasure.

Olds and Milner (1954) found similar rewarding qualities to intracranial electrical brain stimulation. They observed that rats will press a lever as rapidly as 2000 times per hour to obtain electrical brain stimulation. Rats will continue to respond at this rate for twenty-four hours or longer; they will ignore other rewards, such as food and water, to continue receiving the electrical brain stimulation. Intracranial self-stimulation (ICSS) via electrical stimulation to the brain can

be achieved in a wide variety of different brain regions, but the highest rates of lever pressing was observed in the septal area, the amygdala, and the anterior hypothalamus (Olds and Milner, 1954).

The midbrain dopamine system plays a key role in mediating the ICSS phenomena as well as the reward system in general (Wise and Bozarth, 1987). The dopamine system is affected by a number of different drugs, including opiates, cocaine, amphetamine, nicotine, and alcohol (Beatty, 1995). Opiates appear to exert a powerful effects on the dopaminergic neurons of the nervous system's reward system (Beatty, 1995). For this reason, both endogenous and exogenous, opioids appear to have rewarding or reinforcing properties (Beatty, 1995).

To test rewarding or reinforcing properties of different stimuli the place preference conditioning (PPC) paradigm is commonly used. Traditionally, PPC had been used to study the addictive properties of drugs (Crowder and Hutto, 1992). But, in other cases, had also been used to determine the rewarding aspects of reinforcers such as food, water, and sexual encounters. Before PPC, addictive drugs were tested by self-administration test, where the test animal would depress a lever and in return would receive a dose of the drug being tested. The problem with these types of tests were at the higher dose range a rate change in the lever pressing in either direction is not a true indication of reinforcement. With the development of PPC, such distortions could be prevented by using distinct trials, each which begins with a drug administration and which are spaced apart enough to have a complete recovery of previously administered drugs before administration of more drugs (Crowder and Hutto, 1992).

The principles underlying PPC are based on classical conditioning where a test animal prefers a camber of the apparatus that is associated with a positive or rewarding stimuli and reject a chamber of the apparatus that is associated with negative or aversive stimuli. Two chambers to

the right and left are the conditioning chambers and a smaller box in the middle is an intermediate compartment that has access to both lateral compartments. The two conditioning chambers are not meant to develop a preference in the test species, but are meant to be markedly different by a number of environmental cues to enable the test species to readily discriminate between the two compartments. For example, the two conditioning chambers are visually different by color, have distinguishing olfactory scents, and may even have a different textured floor. A place preference occurs when the test animal spends more time in the chamber of the box associated with the stimulus.

The rewarding properties of endogenous opioids have been linked to the reinforcement of sexual behavior (Agmo and Berenfeld, 1990). Sexual behavior is a form of social behavior. Social behavior is a broad term that can represent a range of different activities, including sexual activities, maternal behavior, pair bonding, social interaction, and group formation. These fundamental behaviors are necessary for reproductive success and species survival.

Objectives

As sexual behavior may be reinforced by opiate systems, it is of interest to determine if all types of social behavior is reinforced by opiate systems. Using the place preference conditioning paradigm, social interaction in female rats will be evaluated for rewarding behavior. In addition, what effects the endocrine system plays in this reward system, specifically the ovarian hormones are analyzed.

Literature Review

Opioids

The level of endogenous opioids in the body's internal environment is constantly fluctuating in response to the body's needs (Panksepp 1980, Stolerman 1985). Opioids have been linked to a number of biological pathways, including pain, reproduction, and parental behavior (Kinsley *et al.* 1993, Panksepp *et al.* 1978). In place preference experiments with opioids, rats have been found to develop a place preference to a conditioning chamber of the place preference box associated with the infusion of exogenous opioids (Ågmo and Gomez 1991, Bals-Kubik *et al.* 1993, Blander *et al.* 1984, Hoffman 1989, Mucha and Herz 1985, Mucha *et al.* 1982, Shippenberg *et al.* 1987). In addition to the place preference caused by opioid agonists, naloxone, an opioid antagonist, has been shown to block the effects opioids on conditioned preferences. In some cases, naloxone has caused an aversion to the chamber associated with the naloxone infusions (Mucha *et al.* 1982). Two characteristics of the opioid involvement in place preference are: opioids cause a place preference and these affects are reversible with the addition of antagonist, such as naloxone. Mucha *et al.* (1982) found the infusions of opioids and their antagonist to be dose dependent. Morphine for example had no effect until 0.08 mg/kg (iv) was infused. In the same manner, naloxone was found ineffective until the doses reached 0.1mg/kg (iv).

Specific regions of the brain respond differently to opioids. Van Der Kooy *et al.* 1982 studied the reinforcing effects of microinjections of morphine into different regions of the brain. Place preference conditioning tests revealed that the major reinforcing areas of the brain due to

opioids was the lateral ventricles, lateral hypothalamus, the nucleus accumbens (NAc), and the periaqueductal gray.

Opioid Receptors

There are three types of opioid receptors; μ, δ, and κ is each specific to certain ligand agonist and antagonist. The overall effect of opioid receptors is to stop or slow down neural transmission (Knapp *et al.* 1995). All three opioid receptor types can mediate the inhibition of cAMP formation consistent with the activation of the G-protein effector system. Activation of the μ and δ opioid receptors increases potassium ion conductance which inhibits action potential generation. Activation of the κ opioid receptors typically results in the inhibition of calcium ion conductance, which inhibits synaptic transmission.

Molecular Effects of Opioids

Terwilliger *et al.* (1992) studied the molecular effect of chronic morphine and cocaine on different regions of the brain. Chronic effects of morphine were surveyed on the G-protein/cyclic AMP system in several brain regions to determine how widespread the regulation of the G-protein/cyclic AMP system might be. In response to morphine, the (NAc) and amygdala showed increases in adenylate cyclase and cyclic AMP dependent protein kinase activity while the thalamus showed an increase in cyclic AMP-dependent protein kinase activity only. Morphine regulation of G-proteins was variable, with decreased levels of $G_{i\alpha}$ seen in the NAc, increased levels of $G_{i\alpha}$ and $G_{o\alpha}$ in the amygdala, and no change in the thalamus or the other brain regions studied. Chronic treatment of rats with cocaine produced similar changes compared to morphine in G-proteins, adenylate cyclase, and cyclic AMP-dependent protein kinase in the NAc, but not in

other brain regions. Regulation of the G-protein/cyclic AMP system in NAc represents a mechanism by which a number of opiate sensitive neurons adapt to chronic morphine and thereby develop aspects of opiate tolerance or dependence.

Panksepp *et al.* (1979) conducted a study that tested the capacity of 18 different drugs, including those which modify brain opioid, serotonin, norephinephrine, dopamine, and acetylocholine activity to determine the rates of different biological pathway in social isolation. Young chicks were isolated from their mother and the frequency of distress vocalizations (DV) were measured. The capability of the drug to diminish or lessen the DV was interpreted as elimination of the neural effects of social isolation. Only morphine was able to alleviate DV and this was antagonized by the administration of naloxone.

Opioids have been linked to the distress calls that infants let out when socially isolated (Winslow and Insel, 1991). In the case of rat pups, two models have been proposed as to the roles opioids play in social isolation. The comforting effects of social interaction is result of endogenous opioids and the distress calls is analogous to opioid withdraw. The second model suggest that social isolation is a stressor that ultimately recruits endogenous opioids to suppress the isolated feeling. Winslow and Insel (1991) tested each of these models. In their first experiment they investigated what effects morphine and naloxone had on distress calls. Morphine decreased the amount of calls in a dose dependent manner and naloxone reversed this effect. However, the doses that caused call reduction were so high that there was also a reduction in the rat pups' locomotor activity. Naloxone by itself had no affect on calls, only reversing the effects of morphine treated rat pups. In another experiment, rats were given an irreversible opioid antagonist, β-funaltrexamine. Again, the opioid antagonist alone had no effect, although sensitivity to morphine was significantly decreased. Winslow and Insel concluded that due to the

inability of the opioid antagonist to have any effect on distress calls, the opioid system is not an essential part of the rat pup's distress call in response to social isolation.

One system that seems to be closely linked to the opioid system is the dopamine system which may be activated by opioid systems (Kandel *et al.* 1991). Furthermore, the dopamine system has the ability to block the affect of the opioid system and motivation (Mark et al 1991). In place preference conditioning tests a dopamine antagonist, SCH23390, blocked the effect of morphine. In addition, SCH23390 was aversive in place preference conditioning tests (Shippenberg and Herz, 1987).

Sexual Interaction

Sexual activity has also been shown to be able to produce a place preference to the chamber associated with sexual activity. In both male (Ågmo and Berenfeld 1990) and female (Broekman *et al.* 1988, Oldenburger *et al.* 1992) rats, a place preference was observed in chambers of the place preference apparatus paired with sexual activity. Male rats were allowed to have one ejaculation with a female rat then promptly placed in the conditioning chamber of the place preference apparatus (Agmo 1990). When naloxone was administered the reinforcing affect of the sexual activity was lost. The fact that naloxone was able to block the effect of sexual reinforcement linked the opioid pathway with the pathway that reinforces sexual activities. It is proposed then that the release of endogenous opioids renders sexual activity rewarding; making reproduction rewarding.

Ågmo and Gomez (1993) tested what brain regions was responsible for the place preference due to male ejaculation. Bilateral infusion of methylnaloxonium (opiate antagonist; 5μ/g) in to the medial preoptic area (MPOA) or NAc of sexually experienced male rats blocked

place preference produced by ejaculation without affecting sexual behaviors (Agmo and Gomez 1993). The MPOA was chosen because it is important for sexual behavior and the NAc was selected because it appears to be a critical structure for drug-induced reward. Infusion of the antagonist into the NAc did not reduce the reinforcing properties of ejaculation. Shippenberg and Herz (1987) showed that the dopamine and opioid systems were linked, however, Ågmo and Berenfeld (1990) found a dopamine antagonist to have little to no affect on ejaculation induced reward. It could be suggested that dopamine has little importance to the reinforcing effect of copulation. The release of endogenous opioids during the course of sexual activity may serve two functions: to facilitate ejaculation and to afford the reinforcing properties of the event of ejaculation or reproduction.

Aggressive Encounters

Meisel and Joppa (1994) conducted a series of experiments to determine if female Syrian hamsters would develop a place preference for aggressive or sexual encounters. In the first group females were allowed to have sexual activity with a male hamster and then placed in a conditioning compartment of the place preference apparatus. The second group of females had aggressive encounters with a male hamster and placed in a conditioning compartment of the place preference apparatus. After the conditioning sessions, all females were given free access to the entire place preference apparatus. Females that had been conditioned with sexual or aggressive encounters spent a significant more amount of time in the conditioning chamber suggesting that female hamsters preferred the compartment associated with previous sexual or aggressive encounters. Therefore, aggressive and sexual encounters must be rewarding.

Non-sexual Social Interaction

Non-sexual social activity has also been shown to have reinforcing properties. Taylor (1981) found that male rats actively sought the opportunity to interact with other male rats, in order to alleviate fear. The presence of another male rat reduced the behavioral signs of fear in rats when loud noises or quick threatening movements occurred.

Several studies have shown that, in juvenile rats, social interaction in the form of play is reinforcing. For example, Calcagnetti and Schechter (1992) showed that juvenile rats will readily learn to traverse a T-maze for the opportunity to play (quantified by rough and tumble pinning) with another similarly aged rat. The pairing partner was significant to the amount of conditioning, a non-playing partner would decrease the time spent in the preferred side suggesting a conditioned place aversion.

Normansell and Panksepp (1990) tested the affect of morphine and naloxone on play reward in juvenile rats. Neither morphine or naloxone affected choice or running time during the acquisition of the task (T-maze), even though in the goal box the morphine treated animals played more. They concluded that brain opioid systems seem to influence the expression of play without affecting the apparent appetitive strength of play motivation. In the absence of play reward the animals treated with morphine continued to complete the task more often and in less time than the naloxone treated animals. This supports the conclusion that opioid systems are important in the maintenance of social habits. Observation of juvenile rats during play indicated that morphine would increase the aggression of playing and naloxone would decrease the level of play. Panksepp *et al.* (1985) found that by injecting naloxone in a rat that was previously labeled as a dominant rat, that rat would become more submissive when playing. Similarly, injecting

morphine into a submissive rat would make that rat a dominant play partner. They concluded that brain opioids are important in controlling the vigor of social relations. Siviy and Panksepp. (1985) found that lesions to certain parts of the thalamus could reduce "rough-and-tumble" social play and play solicitation behaviors in the juvenile rats. Rat pups with lesions to the parafascicular region of the thalamus were also insensitive to the play modulating effects of both naloxone and morphine. Suggesting that the thalamus has a role in the modulation of social play by opioids.

Juvenile rats could be readily trained or reinforced in a PPC paradigm when paired with adult female rats (Amsel et al, 1977). Initially, the preference was believed to be as a result of the young rats objection to weaning. But later was considered to be result of the juvenile rats objection to isolation and search for companionship.

Not all the data collected agrees that social interaction is rewarding or that it can be a assessed using the PPC. Crowder and Hutto (1992) found that male rats could not be conditioned by social interaction. The social interaction they used was what was earlier referred to as "rough-and -tumble" play. The only difference is that they used the play with adults instead of with juveniles as previously mentioned. Using a similar testing apparatus to PPC, operant place conditioning (OPC) testing apparatus was able to detect the reward of water drinking to a thirsty rat, but did not find a significant rewarding aspect to social interaction.

Hormones and Behavior

Hormones have a role in behavior. As a female rat cycles through it's four day estrous cycle it's behavior will change towards other rats (Erskine 1989). When a female rat in estrous encounters a male rat, the female rat will increase receptive and proceptive (ear wiggling and

darting) behavior (Edwards and Pfeifle 1982, De Jonge and Van de Poll 1986, Erskine 1989, Gorzalka and Moe 1992). This same behavior is also exhibited in the hamster (Carter 1972). Estrogen is the key hormone in determining sexual motivation (Edwards and Pfeifle, 1982). A non-estrous female exhibits aggression toward other rats of either sex. The inhibition of fighting on estrous day depends on the presence of both estrogen and progesterone (Floody and Pfaff 1977).

Progesterone plays a role in the social response a female rat displays during estrous (De Jonge and Van De Poll, 1986; Rubin and Barfield, 1983). The amount of time ovariectomized female rats spent with another rat (both male and female) or in a different chamber of a conditioning box was measured. Females that received estrogen benzoate (EB) or testosterone propionate (TP) spent less time with a partner and more time alone. Females that received EB or TP followed by the treatment with progesterone showed a significant amount of more time in the chamber with the partner (De Jonge and Van De Poll, 1986)

Meisel *et al.* (1988) tested hormonal control of aggression and sexual behavior in female hamsters. Isolated ovariectomized female hamster's response to group housed ovariectomized female hamsters (aggression) and a group housed intact male hamster (sexual) was assessed. Following the baseline test, the experimental females were implanted with silastic capsule containing different concentrations of estradiol and then retested. In the baseline test (females rats with ovariectomies) high levels of aggression were observed towards other female rats, with no changes in aggression toward an intruder female observed for any implant group on subsequent tests. Despite these high levels of aggression toward another female, most of the estradiol treated females were sexually responsive in the presence of a male. Where as the baseline female did show aggression toward a male, but was not sexually responsive.

Behavioral estrous can be induced by acute or chronic estrogen administration (Carter 1972, Gorzalka and Moe 1992). Gorzalka and Moe (1992) found chronic administered estrogen via implants caused a more reliable behavioral change, due to the fact that acute injections of estrogen generally produce estrogen levels much higher than the normal blood levels in the estrous rat. Zelman and Adler (1977) conducted a dose-response experiment (2, 4, 8, 14, 37, 56, 131, and 151 μg/kg) and found the effect of estrogen to be dose dependent up to a certain threshold. The smallest doses of estrogen benzoate could induce vaginal estrous, but only doses of 131 and 151 μg/kg could cause behavioral estrous. Induction of estrous behavior can also be achieved by injecting estrogen and progesterone directly to the ventromedial hypothalamus (VMH, Rubin and Barfield, 1983). By injecting estrogen and progesterone via a cannulae into specific regions of the VMH that estrous behavior of proceptive and receptive behavior could be induced.

Conclusion of Literature Review

Place preference conditioning is a valid test to prove the reinforcing or rewarding properties. Opioids, sexual encounters, and social interaction all have rewarding properties. The estrous cycle has an effect on behavior and social interaction in the rat.

Material and Methods

Subjects

All studies employed female Sprague-Dawley rats (Hls:(SD)BR Hilltop Labs, Scottsdale, PA) weighing 200-300 g at time of testing. The rats selected as experimental subjects were individually housed in stainless steel hanging cages equipped to provide *ad lib* access to food and water, others were caged in similar cages in groups of 4-6. The rats were maintained in an isolated room with a 14:10 h light dark cycle (Light on at 5:00am and off at 7:00pm).

Surgical Procedures

Only rats that displayed sequential 4 day estrous cycle, as determined by daily vaginal smears, were selected for these studies. This initial screening was necessary to reduce variability in the experimental results and therefore, reduced the number of animals needed to achieve adequate data interpretation. Rats were anesthetized by placing them individually in a 4L glass beaker containing cotton batten soaked with liquid anesthesia grade ether. A wire grid sat above the saturated cotton batten to prevent the rats from coming in direct contact with the liquid ether. An ether cone was used for subsequent administration of ether during the surgical procedure.

Apparatus

A baseline test is then run prior to any conditioning, in order to establish an initial preferred side. The test animal is allowed free access to all the chambers of the test box. The test species will spend more time in one chamber than the other and this is the preferred side or the non-conditioning side (CS-). Then the test animal is conditioned to the initial non-preferred

chamber (CS+). How the conditioning is done is dependent on what your conditioning with, but generally there is three to five conditioning and non-conditioning trials per chamber are done before the condition is completed. Once the conditioning days are over a post test is run where the rat is allowed to roam the box freely. If the test animal significantly increase the time spent in the CS+ compartment or decrease time spent in the CS- compartment then the conditioning stimulus is considered reinforcing or rewarding.

The place preference cages consisted of three compartment boxes made of wood, with the lateral compartments measuring 27 x 37 x 32 cm and the middle compartment 22 x 24 x 32 cm. One lateral compartment was painted white, and the floor was covered with cedar wood chips. The shavings were removed and changed before each new experimental session. The opposite lateral compartment was painted black and the floor was swiped with acetic acid (2% **vol:vol**) immediately before an animal was placed within it. The middle compartment was painted gray and communicated with each lateral compartment through 10 x 10 cm doors. The front wall of the middle compartment was made of fine wire mesh, which allowed observation of the animals inside the apparatus. When a rat was in the place preference apparatus a piece of Plexiglas was place over the top to prevent the rat from (attempting to) escaping. The place preference apparatus was located in a quiet room (in suite 311) illuminated with a dim white light.

Drugs

Estradiol Benzoate (30 µg/0.2 ml Canola oil; Sigma Chemical Corporation, St. Louis) was administered (s.c.) 18-24 hours prior to testing or conditioning days.

Experimental Design

Three test groups were run: an intact group (n=10), a group with bilateral ovariectomies (n=17), and a group with bilateral ovariectomies with estrogen replacement via sc injections (n=10).

During place preference conditioning training, subjects are given 4 conditioning trials during which the experimental treatment under evaluation is paired with the cues associated with one of the lateral compartments of the PPC apparatus. On alternate trials, a control or non-treatment condition is paired with the other lateral compartment of the PPC apparatus. Following training, an increased tendency to approach or spent time in the experimental location is considered to demonstrate that the treatment produced reinforcing effects. Conversely, an increased tendency to avoid the conditioned location indicated the development of a conditioned place aversion (CPA), and is taken as evidence that the treatment produces aversive effects.

Procedure

Subjects were first given a ten-minute baseline session during which they were allowed free access to all parts of the device. The amount of time spent in both side chambers was recorded to the nearest on-tenth of a second, using stopwatches. A subject will be considered to have entered the chamber, when both front paws break the plane of the door. The side in which the animal spends most time was designated as the initially or originally preferred side. The other side was designated as the side for place conditioning, and the initial preferred side was the unconditioned location. This biased procedure is considered to be a more stringent test than the unbiased method, in which the animal is randomly assigned to one side or the other, for conditioning purposes.

Following the baseline session, conditioning trials, one per day, began. Four conditioning trials was alternated with four non-conditioning trials. During a conditioning trial, subjects will first be placed for fifteen minutes in a holding chamber, along with a female non-specific target. A novel target animal will be used for each trial. Following this, subjects will then be confined in the appropriate side of the PPC apparatus for thirty minutes. They will then be returned to their home cage. On the non-conditioning trials, subjects will be place in the holding chamber alone for fifteen minutes, then confined for thirty minutes I the other chamber of the PPC apparatus. After training is completed, subjects will be given another ten minute free choice trial, and the amounts of time spent in each chamber again is recorded. A place preference will be considered to have been developed, if the animals shows a reversals in initial preference, or reduce the time in the originally preferred side, and increase time spent in the initially non-preferred side.

Statistical analysis

For evaluating conditioning effects of social interaction on the place preference, the difference between time spent in the preferred cage and time spent in the non-preferred cage before conditioning was compared with the difference between time spent in the cages after conditioning. A two tail t-test (StatMost™, DataMost Corporation) was used to determine if the change from pretest to posttest was significant.

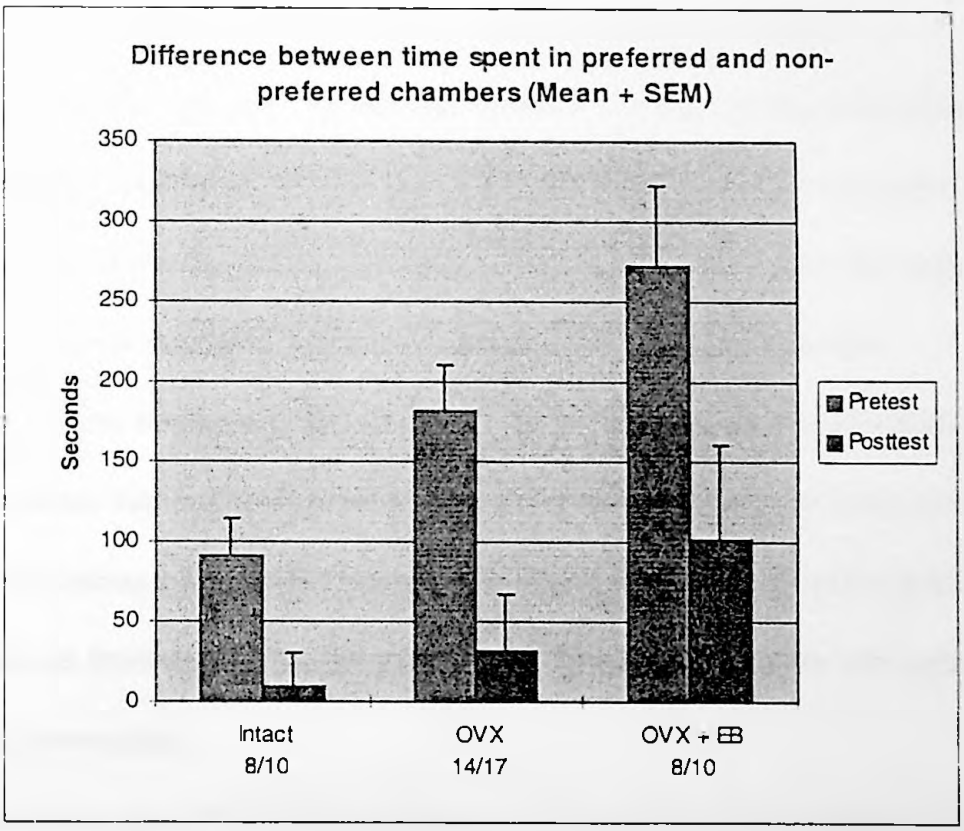
Results

As seen in Figure 1, social interaction in the female rat was able to cause a significant place preference conditioning in all three test groups (intact, OVX, and OVX + EB). In the intact group of rats, 8 of the 10 rats showed a decrease in the difference of time spent in the preferred and non-preferred chambers between the pretest and the posttest. The decrease in the difference of time spent in the preferred and non-preferred chambers represents a place preference to the non-preferred chamber. In the OVX and OVX + EB replacement groups, 14 of the 17 and 8 of the 10 rats showed a place preference, respectively.

When the magnitude of the place preference produced by the three test groups was compared, there was a tendency for the OVX group to have a slightly stronger conditioning effect, although the difference was not significantly different.

Figure 1

Place preference in female rats exposed to social interaction via interaction with another rat. Female rats were either paired with a female rat for positive reinforcement or left alone for negative reinforcement to cause a place preference. Values are M ± SE. The numbers below the bar indicate the proportion of animals changing preference between the pretest and the test. The light colored bars represent the pretest and the dark colored bars represent the posttest.


Difference between time spent in preferred and non-preferred chambers (Mean + SEM)
Seconds
350
300
250
200
150
100
50
0
Intact
8/10
OVX
14/17
OVX + EB
8/10
Pretest
Posttest

Discussion

These results show that social interaction in the female rat can produce a place preference. This suggests that social interaction induces a reward state to allow association between the state and the environmental cues of the conditioning side of the place preference conditioning apparatus. The fact that the animals were subjected to social interaction in a separate chamber from the conditioning chamber excludes the possibility that the reward is limited to the time of social interaction. Suggesting that social interaction causes a reward that continues to act as a positive reinforcer after the social interaction is ended.

The present data does allow for any conclusion as to what role the opiate system may have in the reward caused by social interaction. The link Normansell and Panksepp (1990) made between morphine, naloxone, and play behavior in juvenile rats may give some merit to the suggestion that opioids may have a role in rewards caused by social interaction.

In their natural habitat, rats have a defined social structure. Dominant males establish individual territories that may include several burrows. Each burrow may house several females. The females of a colony collectively nurtured their young, and when reproductively active, they excluded other rats from the burrow (Nowak, 1991). This may be a reason why social interaction in female rats is rewarding.

Meisel and Joppa (1994) found aggressive encounters to be rewarding only to the dominant rat. Floody and Pfaff (1977) noted that group housed rats have already established an order of dominance within their group and when paired with an individually housed rat the group housed rat will keep the same position, whether dominant or subordinate. In our experiments, no effort was made to determine dominance of rats during their social interaction. Rats used in our experiments were paired with group housed rats. Although weighing took place before every

conditioning, rats were paired randomly with no discrimination to larger or smaller rats for social interaction partners.

The tendency for the OVX group to have a stronger place preference than the intact and OVX + EB replacement groups may suggest the ovarian hormones may have a role in social interaction conditioning. The lack of a significant change between the groups suggests that the role of the ovarian hormones as tested seem to be minor. Although, Edwards and Pfeifle (1983) found estrus to decrease aggression and increase social behavior.

The estrous cycle in the intact group was not monitored so it is unclear as to what effect the cycle had on conditioning. In the estrogen replacement group, the doses used (0.2ml) were considerably higher than those used by Zelman and Adler (1977) to induce estrous.

To summarize, the present study shows that social interaction is rewarding in female rats and that the ovarian hormones may have a role in determining if social interaction is rewarding.

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Appendix A

INTACT				
ID #	Pre_CS-	Pre_CS+	Post_CS-	Post_CS+
2	177	6	172	106
3	211	123	122	219
4	255	75	133	136
5	230	146	189	130
9	222	148	168	134
10	168	147	199	69
11	288	61	87	138
14	187	176	136	139
16	163	141	151	124
18	205	175	137	211

OVX				
ID #	Pre_CS-	Pre_CS+	Post_CS-	Post_CS+
66	274	13	94	190
73	30	27	19	260
67	450	2	106	47
72	230	96	158	63
65	207	2	168	41
60	285	5	283	0
10	195	70	185	140
8	310	14	220	173
12	262	40	287	83
22	261	33	192	132
14	164	164	200	117
17	78	72	193	70
11	319	26	158	177
28	199	158	120	260
25	283	78	59	299
7	230	117	115	171
13	226	7	215	11

OVX + EB				
ID #	Pre_CS-	Pre_CS+	Post_CS-	Post_CS+
180	470	23	94	364
900	280	17	208	209
160	187	113	139	163
190	383	0	219	179
210	251	77	290	159
270	201	53	255	73
150	581	0	421	4
600	253	61	230	125
200	340	44	272	107
240	290	124	395	125

Appendix A

INTACT					OVX					OVX + EB				
ID #	Pre_CS-	Pre_CS+	Post_CS-	Post_CS+	ID #	Pre_CS-	Pre_CS+	Post_CS-	Post_CS+	ID #	Pre_CS-	Pre_CS+	Post_CS-	Post_CS+
2	177	6	172	106	66	274	13	94	190	180	470	23	94	364
3	211	123	122	219	73	30	27	19	260	900	280	17	208	209
4	255	75	133	136	67	450	2	106	47	160	187	113	139	163
5	230	146	189	130	72	230	96	158	63	190	383	0	219	179
9	222	148	168	134	65	207	2	168	41	210	251	77	290	159
10	168	147	199	69	60	285	5	283	0	270	201	53	255	73
11	288	61	87	138	10	195	70	185	140	150	581	0	421	4
14	187	176	136	139	8	310	14	220	173	600	253	61	230	125
16	163	141	151	124	12	262	40	287	83	200	340	44	272	107
18	205	175	137	211	22	261	33	192	132	240	290	124	395	125
					14	164	164	200	117					
					17	78	72	193	70					
					11	319	26	158	177					
					28	199	158	120	260					
					25	283	78	59	299					
					7	230	117	115	171					
					13	226	7	215	11					

Appendix B

Intact			OVX			OVX + EB		
Pre	Post	Diff	Pre	Post	Diff	Pre	Post	Diff
171	66	105	261	-96	357	447	-270	717
88	-97	185	3	-241	244	263	-1	264
180	-3	183	448	59	389	74	-24	98
84	59	25	134	95	39	383	40	343
74	34	40	205	127	78	174	131	43
21	130	-109	280	283	-3	148	182	-34
227	-51	278	125	45	80	581	417	164
11	-3	14	296	47	249	192	105	87
22	27	-5	222	204	18	296	165	131
30	-74	104	228	60	168	166	270	-104
		82	0	83	-83			
			6	123	-117			
			293	-19	312			
			41	-140	181			
			205	-240	445			
			113	-56	169			
			219	204	15			