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SOCIAL BUFFERING IN RATS: PROLACTIN ATTENUATION OF ACTIVE INTERACTION , ^{1,2}

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Summary

Stress may result when the present environment is interpreted as threatening, and stress is known to increase the prolactin-secretory response. In the present study, rats ($N = 83$) were exposed to a conditioned-fear paradigm (environment paired with footshock), and on testing day, rats were exposed to the experimental chamber without shock while alone (Alone $n = 16$), with an object (Object $n = 17$), with a euthanized conspecific (Euthanized $n = 16$), or with a social partner (Social $n = 19$). The control group (Control $n = 15$) was exposed to the experimental chamber but was never shocked. The Control group had significantly lower levels of prolactin than the Alone, Object, and Euthanized groups; however, the Control group's levels of prolactin were not significantly different than that of the Social group, which was significantly lower than that for the Alone group. Social interaction decreased fear independent of the distraction provided by a stimulus in the chamber. Active touch appeared to be crucial for social buffering to occur.

Previous research has identified that social partners buffer stress (Kikusui, Winslow, & Mori, 2006). Several mechanisms of this social buffering effect are being identified (Kiyokawa, Kikusui, Takeuchi, & Mori, 2004; Wilson, 2007³). The purpose of the current study was to use a rat model to examine the role of touch by a social partner to decrease stress responses when exposed to a conditioned emotional response (CER) stressor.

In humans, stress occurs when an internal need or environmental demand is viewed as threatening or out of one's control (Clark, Rager, & Calpin, 1997). Stressors are often categorized as either physical or psychological. Physical stress is defined as a threat based on physical harm, pain, or the capacity to terminate life (Lovallo, 1997). This type of stress is experimentally induced in animal research by methods which include, but are not limited to, restraint (Kant, Eggleston, Landman-Roberts, Kenion, Driver, & Meyerhoff, 1985; Kant, Mougey, & Meyerhoff, 1986; Orr, Meyerhoff, Mougey, & Bunnell, 1990; Wilson, McKinley, & Young, 2000; Klenerova, Sida, Hynie, & Jurcovicova, 2001), forced swim (Greisen, Altar, Bolwig, Whitehead, & Wortwein, 2005; Ma, Shipston, Morilak, & Russell, 2005), and footshock (Huhman, Mougey, Moore, & Meyerhoff, 1995; Pitman, Natelson, Ottenweller, McCarty, Pritzel, & Tapp, 1995; Maslova, Bulygina, & Markel, 2003; Westenbroek, Ter Horst, Roos, Kuipers, Trentani, & den Boer, 2003; Penka, Bond, & Heinrichs, 2004).

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Psychological stressors are defined as situations perceived as threatening, and this interpretation leads to the engagement of a stress response regardless of whether the environment is actually harmful (Clark, *et al.*, 1997). Psychological stress is commonly induced in animal research by methods such as open-field exposure or a novel environment (Yelvington, Weiss, & Ratner, 1985; Muir & Pfister, 1986; Terranova, Cirulli, & Laviola, 1999; Wilson, 2000, 2001; Miserendino, Haile, & Kosten, 2003; Pijlman, Wolterink, & Van Ree, 2003), elevated plus maze (Belda, Marquez, & Armario, 2004; Mueller, Dolgas, & Herman, 2004; Marquez, Nadal, & Armario, 2005), and conditioned fear (Van de Kar, Lorens, McWilliams, Kunimoto, Urban, & Bethea, 1984; Przewlocka, Sumova, & Lason, 1990; Zhu & Onaka, 2003). Conditioned fear offers an animal model of stress based on prior experience. Female animals rarely have been examined in the CER stress literature; the conditioned-fear paradigm previously relied almost exclusively on male rats (Van de Kar, Lorens, Urbana, Richardson, Paris, & Bethea, 1985; Paris, Lorens, Van de Kar, Urban, Richardson-Morton, & Bethea, 1987; Lorens, Hata, Handa, Van de Kar, Guschwan, Goral, *et al.*, 1990). Therefore, one goal of the present study was to include female rats in the sample testing the animal model of learned stress based on prolactin responses to stress.

Previous research found prolactin secretion to increase in response to both physical and psychological stressors (Telner, Merali, & Singhal, 1982; Yelvington, *et al.*, 1985; Paris, *et al.*, 1987; Marti-Carbonell, Darbra, Garau, Sanz, & Balada, 1993). Prolactin increases as a direct function of stimulus intensities (Armario, Lopez-Calderon, Jolin, & Castellanos, 1986). Orr, *et al.* (1990) found tailshock to be more intense than immobilization, and immobilization more intense than exposure to a chamber without shock. Different intensities of the same stressor indicate gradations in the prolactin response. For example, Kant and colleagues found prolactin sensitive to increasing footshock intensities (Kant, Mougey, Pennington, & Meyerhoff, 1983). Based on these studies, rats were expected to display differences in prolactin across slight environmental variations even when exposed to the same stressor.

Responses to psychological stressors may be alleviated by social support, allowing the presence of another to act as a buffer against perceived threats (Bondi & Picardi, 1999). Among squirrel monkeys in a large social group, exposure to a caged live boa constrictor did not increase adrenocortical activities; however, the same stressor experienced in the absence of social partners induced elevated hormonal levels in squirrel monkeys (Coe, Franklin, Smith, & Levine, 1982). A similar effect was found in prolactin levels of rats exposed to an open-field stressor, with and without a social partner. Juvenile male and female rats exposed to a novel environment in the presence of a conspecific had significantly lower levels of prolactin than control rats individually exposed to the same stressor (Wilson, 2000). In this study, stress responses appeared to attenuate given the presence of a conspecific. However, the presence of a conspecific may have offered a distraction from the stressful environment. The present study included a condition with a novel object in the chamber to examine this possibility.

In addition to possible distraction, the conspecific offered active touch, which may be an important element in social buffering (Wilson, 2001). In a previous study, juvenile rats which were allowed to touch during an open-field stressor had lower levels of prolactin than rats not allowed to touch, even though all animals were able to see, hear, and smell the conspecific (Wilson, 2001). Although touch may indeed promote social buffering, one does not know if active touch is necessary. That is, the occurrence of social buffering may rely on reciprocated touch with an active animal, or it may rely simply on tactile stimulation. The exploration of comfort and tactile stimulation provided by living and nonliving stimuli dates back to Harlow's research (e.g., 1959). In the present study, the experimental animals were exposed to live and euthanized conspecifics to explore the function of touch in social

buffering. Live conspecifics reciprocated touch, and euthanized conspecifics functioned as tactile stimulation which received touch without response.

Increased touch may be confounded by increased activity, and activity has been associated with lower levels of prolactin (Marti-Carbonell, *et al.*, 1993; Wilson, 2000). Similarly, application of prolactin to the ventral hippocampus in female rats decreased ambulation (Alvarez & Banzan, 1994). In the present study, active touch associated with a live conspecific could be particularly problematic, as it would offer an alternative explanation for hypothesized reductions in prolactin. Therefore, to analyze general activity, freezing behaviors were quantified. When subjects are exposed to the conditioned emotional response (CER) paradigm, subsequent exposures evoke a freeze behavior in anticipation for the shock. Freezing does not allow the rat to engage in alternative active behaviors.

In the present study, rats were conditioned to fear a chamber by pairing the environment with footshock to produce a conditioned emotional response on the day of testing. Experimental conditions consisted of animals tested alone (Alone), animals paired with a novel object (Object), animals paired with a recently euthanized conspecific (Euthanized), and animals paired with a live social conspecific (Social). A fifth condition consisted of rats which were never conditioned to fear the chamber due to shock but were conditioned to the novel atmosphere of the chamber. These rats were tested alone on the day of experimentation and served as the control condition. The Alone, Object, and Euthanized experimental conditions were expected to show higher levels of prolactin than the Control condition. The Social condition (rats tested with an interactive littermate) was expected to exhibit prolactin levels similar to those of the Control condition and lower levels of prolactin than the Alone condition (animals exposed to the CER paradigm alone). Although activity was not expected to differ across treatment conditions, it was expected that touch would be enhanced in the Social condition.

Method

Subjects

Eighty-three (47 male and 36 female) juvenile Long-Evans Hooded rats were tested. Lighting was on a reversed 12:12 light:dark schedule, with the light phase beginning at 6:00 p.m. Rat chow was available *ad libitum*. Experimental rats were bred from animals obtained from Harlan SpragueDawley Laboratories. The day of birth was considered postnatal day (PD) 0. Litters were culled to 10 pups, and they remained with dams in Plexiglas cages (45.72 cm L × 22.86 cm W × 20.32 cm H) with pine bedding until weaning. On postnatal day 21, juveniles were weaned and group-housed with same-sex littermates in Plexiglas cages. On postnatal days 30 to 32, animals were transferred to smaller Plexiglas cages in groups of 2 or 3 same-sex littermates.

Testing Procedure

Rats were conditioned between postnatal days 30 to 32, and conditioning to the chamber occurred for three consecutive days. During conditioning, control animals were exposed to the chamber but never shocked. Rats in the remaining conditions were exposed to the chamber with scrambled footshock, achieved when the bar that ground out the shock is rapidly and constantly changed by the shock unit so animals could not fail to experience the shock by placing one foot on the grounding bar. Scrambled footshock was experienced for 10 sec. at the end of a 10-min. exposure session. The chamber was constructed of Plexiglas (30.48 cm L × 21.10 cm W × 29.20 cm H), with steel grid flooring through which 1 mA scrambled footshock was delivered (Van de Kar, *et al.*, 1985; Paris, *et al.*, 1987; Lorens, *et*

al., 1990). All training occurred between 12:00 p.m. and 2:00 p.m. under 120 W of red light and white noise.

After three days of conditioning to the chamber, on the following day animals were placed in the chamber for 10 min. without footshock. Animals were either in the control condition (Control), tested alone in the feared chamber (Alone), with a novel object (12-oz. soup can stripped of its label; Object), in the presence of a recently euthanized same-sex conspecific (Euthanized), or exposed to the chamber with an active same-sex littermate (Social). Other than the absence of footshock and the presence of appropriate stimuli for the specified conditions, the environment remained the same as on conditioning days.

Behavior

A video camera recorded behaviors of all animals during the 10-min. testing session. The number of touches, total duration of touch, the longest freezing bout, and latency to the bout during the session were scored using EthoLog (Ottoni, 2000), a computer program for event recording. Interrater reliability for behaviors was $r > .90$. Touch behaviors were excluded for one rat in the Object condition and one rat in the Social condition due to equipment malfunction.

Within 1 min. of testing, each rat was decapitated, and trunk blood was collected in a laboratory separate from the colony and experimental rooms. In the social condition, trunk blood was not collected from the stimulus animal; instead, that animal was used in the following condition which required a euthanized same-sex littermate. These stimulus animals were euthanized immediately prior to testing, and a different rat was used each time. Stimulus rats were euthanized via CO₂ in a sealed (45.72 cm L × 22.86 cm W × 20.32 cm H) Plexiglas chamber. Blood from the euthanized stimulus rat was not collected.

Hormonal Assays

All assays followed the methodology prepared by Cayman Chemical for their Enzyme Immunoassay kits (Ann Arbor, Michigan, USA). The limit of detection for this kit is 0.5 ng/ml, and the estimated interassay variability is 14%.

Results

Prolactin data were analyzed using a 2×5 (sex × testing condition) between-groups analysis of variance. The sex of rats was related to prolactin levels ($F_{1,73} = 10.76, p < .003, \eta^2 = .13$). Female animals had higher levels of prolactin than males ($M = 30.41, SEM = 4.44, n = 47$). Testing condition also affected prolactin levels ($F_{4,73} = 5.82, p < .001, \eta^2 = .24$). Fisher's protected t tests (Table 1) showed that the Alone, Object, and Euthanized conditions had significantly higher prolactin levels than the Control condition. Prolactin in the Social condition was significantly lower than that of the Alone condition; however, prolactin in the Social condition was not significantly different from the Control condition (see Table 1). Sex and testing condition did not interact ($F_{4,73} = .85, p = .50$); however, given the addition of females to this body of literature, means, standard deviations, and number of males and females are reported in Table 1.

A 2×3 (sex × testing condition) multivariate analysis of variance was used to analyze the total number of touches during a testing session and the total duration of touch in seconds. Touch was possible only in the Object, Euthanized, and Social conditions; therefore, the Control and Alone groups were omitted from this analysis. Testing condition affected number of touches ($F_{2,44} = 60.14, p < .001, \eta^2 = .73$), with more touches in the Social condition than the Object condition and Euthanized condition. The latter two conditions did not significantly differ from each other (see Table 2). The testing condition also affected

total duration of touch ($F_{2,44} = 7.33, p < .003, \eta^2 = .25$). As reported in Table 2, the Social group touched the stimulus rat more often than the Object and Euthanized groups ($p < .05$). The Euthanized group spent more total time touching the stimulus animal than the Object and Social groups ($p < .05$). Sex showed no main effect for either measure of touch ($p > .05$) and did not interact with testing condition ($p > .05$).

A 2×4 (sex $\times$ testing condition) multivariate analysis of variance was used to analyze latency to the longest bout of freezing and the duration of the freezing bout. Rats in the Control group would not have learned to fear the chamber and so do not exhibit the long freeze indicative of fear. Therefore, only the treatment conditions were compared in this analysis. No differences across sex or testing condition were found ($p > .05$), and the two variables did not interact to affect freezing ($p > .05$).

Pearson correlations were used to analyze the potential of relationships between prolactin and the two measures of touch, as well as freezing data. Correlations calculated separately by sex, with males and females showing relationships among behaviors. For males, prolactin was related to number of touches ($r_{29} = -.41, p < .05$). A significant relationship was also found among females; prolactin was significantly correlated with number of touches ($r_{21} = -.37, p = .05$; see Fig. 1); however, prolactin was not related to total duration of touch during the testing session for males ($r_{29} = .03, p > .05$) or for females ($r_{21} = -.03, p > .05$).

The behavior of freezing correlated with prolactin only on one measure, and the relationship was not statistically significant. Among males, prolactin tended to be related to the duration of the freeze exhibited during the second half of testing ($r_{38} = .27, p = .05$). However, this relationship was influenced by a single outlier with a high level of prolactin and long freeze duration. Removal of the outlier resulted in a nonsignificant correlation ($r_{37} = .21, p = .11$). Re-examining the touch data, removal of the same male rat did not abolish significance.

Discussion

In the present study, the experimental animals conditioned to the chamber by means of shock exposure displayed a stress response to the chamber on the day of testing, as demonstrated by elevated prolactin levels. Analysis indicated significant elevations in prolactin levels as a function of stress when comparing the prolactin from rats in the Control and Alone conditions. Prior research has focused on adult male rats in the CER paradigm, with higher prolactin associated with exposure to the chamber (Van de Kar, *et al.*, 1985; Paris, *et al.*, 1987; Lorens, *et al.*, 1990). The present study extends current knowledge to prolactin responses among female animals when exposed to a CER footshock paradigm. The use of juveniles allows for the analysis of females without the extraneous variable of estrus cycle that has been associated with prolactin fluctuations in rats (Gala, 1990).

The present study showed that prolactin levels were significantly higher in the Object condition than the Control condition, thus distraction may not explain the social buffering found in prior studies (Wilson, 2000, 2001). Similarly, prolactin levels were significantly higher in the Euthanized condition than the Control condition; therefore, it appears that social buffering did not rely on tactile stimulation. This result might be explained by the possibility that the experimental animals had knowledge that the stimulus rat was euthanized, even though sacrifice occurred immediately prior to testing. Researchers exploring tactile stimulation and social buffering may consider using a stimulus rat that is anesthetized; however, even anesthetizing the stimulus animal could create an artificial environment which could be interpreted as threatening.

It was hypothesized that some social buffering might result from tactile stimulation with the euthanized experimental rat. Although prolactin levels did not attenuate, touch behaviors did

change. Experimental animals spent significantly more time touching the euthanized rat than touching the object or the socially active stimulus rat. However, a long duration of touch may have indicated the animal's response to an unusual situation rather than touch associated with stress reduction. Importantly, touch duration and prolactin levels were not correlated.

The Social condition was the only experimental condition with prolactin levels that were not significantly higher than the Control condition. Furthermore, prolactin levels of socially active animals were significantly lower than that of animals tested alone in the feared environment. Based on prolactin levels in rats, the present study offers evidence that reciprocal touch may be an important element that influences social buffering.

The number of times rats touched the active stimulus animal was greater than the number of times rats touched either the object or the euthanized rat. Touch has been reported as a crucial component in stress reduction (Wilson, 2001), and the current study shows that reciprocal touch was effective in reducing prolactin, hence probably stress. Touching a passive companion does not appear to offer social buffering. This study shows a decrease in prolactin levels and an increase in touches across both male and female animals.

More touching in the Social condition was not indicative of more overall activity. Although rats in the Social condition touched more often than the other conditions, no significant differences were found for freezing, a measure of inactivity. Also, freezing behaviors did not correlate with prolactin levels. A single outlier explained a marginal correlation between freezing behaviors and prolactin among male rats. Thus, lower prolactin in the Social condition is not likely explained by an increase in activity.

The present study extends prior research by including female rats in an animal model of social buffering and stress as measured by prolactin. When ruling out exploration and tactile stimulation as catalysts for stress reduction, reciprocal touch appeared to be an important component for social buffering to occur. Social interaction, not social action, appeared to be important for social buffering to occur in this study.

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Fig. 1. Number of touches correlated with prolactin across male (a) and female (b) rats ($p \leq .05$). Removal of the apparent male outlier diet not affect significance.

TABLE 1

Number of Males and Females Across Treatment Groups, Prolactin Means, and Standard Errors

		Sex		
		Male	Female	Total
Control	<i>n</i>	9	6	15
	<i>M</i>	10.36	21.75	14.92
	<i>SE</i>	2.11	10.80	4.59
Alone	<i>n</i>	9	7	15
	<i>M</i>	38.07	73.77	53.69
	<i>SE</i>	4.69	20.61	10.10
Object	<i>n</i>	10	7	17
	<i>M</i>	34.50	56.63	43.61
	<i>SE</i>	7.49	7.69	5.93
Euthanized	<i>n</i>	8	8	16
	<i>M</i>	50.58	54.41	52.49
	<i>SE</i>	9.83	9.01	6.46
Social	<i>n</i>	11	8	19
	<i>M</i>	22.17	44.81	31.70
	<i>SE</i>	2.84	12.22	5.83

TABLE 2
Mean Number of Touches and Mean Duration of Touch (sec.) in Environmental Conditions

	Object			Euthanized			Social		
	<i>n</i>	<i>M</i>	<i>SE</i>	<i>n</i>	<i>M</i>	<i>SE</i>	<i>n</i>	<i>M</i>	<i>SE</i>
Touches	16	2.94	.74	16	2.31	.51	18	15.44	1.25
Duration	16	135.52	48.69	16	404.03	62.05	18	252.48	27.04