Repeated Exposure of Rat Pups to Isolation Attenuates Isolation-Induced Ultrasonic Vocalization Rates: Reversal with Naltrexone

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Young rat pups are dependent on the dam for their survival, thus isolation of the neonatal rat pup from the dam presents the young organism with a variety of stressors. The question examined in this study concerns the ability of the young rat pup to modify its response to isolation following repeated exposure to that isolation as well as the role played by endogenous opiates in this process. Following repeated isolations, pups were seen to decrease vocalization rates. Altering the context in an attempt to dishabituate animals failed to reverse the decreased vocalization rate. However, opiate receptor blockade attenuated this decrease when administered subsequent to the first isolation period but not prior to the last isolation period. These results suggest that the development of this attenuated response to isolation stress is opiate-mediated but that once established, its expression is not dependent on endogenous opiate release.

Introduction

In species which give birth to relatively altricial offspring, such as the rat, separation of neonatal offspring from the mother presents a variety of environmental stressors to the infants. The young rat pup depends on the dam to regulate its body temperature, assist in voiding, and provide nutrition and protection from predators. Neonatal rat pups appear to be sensitive to even short-term separation from the dam. For instance, 10-day-old (P10) rat pups subjected to 30 min of separation from the dam exhibit a

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rapid decline in growth hormone secretion (Kuhn, Butler, & Schanberg, 1978) as well as a decrease in ornithine decarboxylase (ODC) activity (which is thought to be an index of growth and differentiation) (Evoniuk, Kuhn, & Schanberg, 1979). This latter effect does not appear to be the consequence of a decrease in body temperature or nutritional deprivation, but seems to result from the absence of tactile stimulation (Pauk, Kuhn, Field, & Schanberg, 1986). Short-term isolation of this age rat for either 5 or 30 min also induces analgesia which appears to be mediated by an endogenous opiate release given that it is reversed by administration of opiate antagonists (Spear, Enters, Aswad, & Louzan, 1985; Kehoe & Blass, 1986). In contrast, corticosterone secretion, which is a common response to stress in adult animals, is only seen in the P10 pup in response to relatively longer periods of isolation from the dam (2 hr) (Kuhn, Pauk, & Schanberg, 1990).

Perhaps an even more sensitive response to isolation from the dam and litter are ultrasonic vocalizations which are seen almost immediately after the pup is removed from the litter and exposed to temperatures below nest temperature (about 35°C) (Noirot, 1968). These vocalizations appear to elicit retrieval behavior by the dam and to increase maternal behavior such as anogenital licking following retrieval (Smotherman, Bell, Starzec, Elias, & Zachman, 1974; Brouette-Lahlou, Vernet-Maury, & Vigouroux, 1992). In addition to these behavioral effects, these vocalizations appear to induce prolactin release in the dam (Terkel, Damassa, & Sawer, 1979; Voloschin & Tramezzani, 1984). These findings support the hypothesis that ultrasonic vocalizations represent a form of distress call produced by the pup, a view which is currently held by a number of researchers (e.g., Carden, Barr, & Hofer, 1991; Winslow & Insel, 1991a). In contrast, however, other investigators consider these vocalizations to be essentially a byproduct of a maneuver called laryngeal braking, a component of a thermoregulatory response, which may only serendipitously signal to the dam that the pup is in need of attention (Blumberg & Alberts, 1990).

An interesting question concerns the response of neonatal rats to repeated exposures of brief isolation stress. It is known that repeated exposure of adult rats to the same type of stressor often results in an attenuation of several of the behavioral and physiological responses produced by acute exposure to the aversive situation (Kennett, Dickinson, & Curzon, 1985; Ohi, Mikuini, Takahashi, 1989). This tolerance can be thought of as an adaptive response to stress which appears to be not only associated with alterations in monoamine activity (Kennett et al., 1985; Cancela, Volosin, & Molina, 1990; Cabib, Puglisi-Allegra, & Oliverio, 1984; Cancela & Molina, 1987; Stone, 1987), but is also dependent on release of endogenous opiates (Cancela et al., 1990; Cabib et al., 1984; Cancela, Rossi, & Molina, 1991). Thus, the combined administration of low doses of morphine with stressful experiences accelerates the onset of these adaptive changes and the administration of an antagonist of opiate sites prior to each daily stressor blocks the development of this adaptive change (Cancela et al., 1990).

Given that brief isolation of a neonatal rat pup from the dam and litter at room temperature is thought to elicit an endogenous opiate response (Kehoe & Blass, 1986; Spear et al., 1985), it was hypothesized that repeated isolation might result in an attenuation of some responses to this stressor, with this adaptation being mediated, as in the adult, by the release of endogenous opiates.

In adult animals, procedures typically used to administer chronic stress involve daily exposure to the stressor for 1 week or more; however, substantial developmental alterations occur across a similar amount of time in the neonate. For this reason, in the present study repeated isolations from the dam and littermates were given within

the same day. Because these isolations were given in close succession, it seemed possible that altered responses to this isolation might be explained in terms of nonassociative learning or habituation. To examine this possibility, one aspect of the isolation environment was changed for some pups after several exposures to the isolation stress in an attempt to dishabituate these animals.

Two experiments were performed in which pups were individually isolated five times, once every 30 min, while vocalizations were recorded. In the first experiment, the role endogenous opiates have in the expression of the response to repeated isolation was determined by administering the opiate antagonist naloxone prior to the last isolation. To examine the role endogenous opiates play in the development of this response, in the second experiment naloxone or the longer-acting opiate antagonist, naltrexone, was administered following the first isolation.

Methods

Subjects

Subjects were 172 postnatal Day 9–10 rat pups derived from Sprague-Dawley (Charles River) rats bred in our laboratory. Male and female adults were housed together in breeder cages in a temperature-controlled colony room with 14 : 10 hr light/dark cycle with lights on at 0700 hr. Breeding pairs were checked daily for births. The day of birth was considered postnatal Day 0. On P1 all litters were culled to between 8 and 10 pups. Eight to 11 pups were randomly assigned to each of the eight (2 isolation × 4 treatment) groups in each experiment, with no more than 2 pups per litter being randomly assigned to any given treatment group.

Procedure

In both Experiments 1 and 2, pups were tested on P9–10. On the day of testing, the home cage with the litter was removed from the colony room to a holding area outside the testing room. The parents were removed from the home cage and placed on clean shavings in a breeding cage adjacent to the home cage. The cage containing the pups was placed on a heating pad which maintained the surface of the shavings at 33 ± 2°C. The litter was randomly divided into two groups (4–5 pups each) which were separated by a Plexiglas partition so that repeated removal and replacement of pups in the chronically isolated group would not disturb animals in the other (acutely isolated) group. All pups were then weighed and sexed and allowed to sit undisturbed for 15 min.

At the onset of the session, pups in the chronic group were individually removed from their littermates in the home cage and isolated for 5-min period in a circular Plexiglas chamber (13-cm diameter) located in an incubator maintained at 25 ± 1°C. This isolation period was repeated for the pups in the chronic group every 30 min for a total of five isolations. During each isolation period, ultrasonic vocalizations were detected with a Mini-2 Bat detector (Ultra Sound Advice™) set at a frequency of 40 KHz that was interfaced with an Apple II+ computer which counted the number of vocalizations. Prior to placing each pup in the chamber, the floor was cleaned using a 50% ethanol solution and allowed to dry. Following each isolation, each pup's rectal temperature was recorded and the pup was returned to the huddle in the home cage prior to isolating the next pup.

Animals in the acute group were not isolated until the time of the fifth isolation of the chronic group. At this time, pups from the chronic and acute groups were alternately isolated, with ultrasounds being recorded during the 5-min isolation period for each group of pups. The inclusion of the acute group was necessary to compare the effects of repeated isolation, per se, to the effects associated with removal of various stimuli (tactile, olfactory, nutritional, etc.) originating from the dam.

In order to examine the possible consequences of endogenous opiate release on the expression and development of any consequences of repeated isolation on vocalization rates, opiate antagonists were administered either prior to the last isolation in the chronic group in Experiment 1 or 15 min following the first isolation in the chronic group in Experiment 2.

The basic design of Experiment 1 was 2 × 4 [Condition (acute vs. chronic) × Treatment (saline, 1.0, 2.0, mg/kg naloxone, dishabituation)] factorial. In this experiment, chronic animals were given four sessions of isolation before any ligands were administered. Then, 15 min prior to the final isolation for the chronic group (and only isolation for the acute group), animals in both acute and chronic groups were given a sc. injection of 0.9% saline, 1.0 mg/kg/3 cc or 2.0 mg/kg/3 cc naloxone HCl (DuPont). Both acute and chronic animals placed in the dishabituation condition did not receive any injection prior to this isolation test but were instead placed in a different context (a smaller circular Plexiglas chamber [17-cm diameter] with a wire mesh floor rather than a Plexiglas floor) during the final isolation period. The dishabituation group was examined in an attempt to determine whether an alteration in the isolation context would induce dishabituation in the chronically treated pups (as described in the Introduction). The order of testing of animals assigned to each treatment group was randomized for each litter.

The design for Experiment 2 was a 2 × 4 [Condition (acute vs. chronic) × Treatment (0, 1, or 2 mg/kg naloxone, 1 mg/kg naltrexone)] factorial. In this experiment, pups in the chronic group were given a sc. injection of 0.9% saline, 1.0 mg/kg/3 cc or 2.0 mg/kg/3 cc naloxone HCl (DuPont), or 1.0 mg/kg/3 cc naltrexone HCl (Research Biochemicals Inc.) 15 min following the first isolation. As in Experiment 1, doses were administered in a random order. Naltrexone was administered in addition to naloxone in this experiment because of the former's longer half life relative to naloxone. Animals in the acute group were given injections of the same drugs at the same chronological time as animals in the chronic group and were placed back in the huddle with other animals in the acute group. As in Experiment 1, animals in the acute group were not isolated until the time of the fifth isolation of pups in the chronic group at which time pups from the two groups were isolated/tested alternately.

Results

Experiment 1

The total number of ultrasounds recorded in the chronic group across the five isolation periods was analyzed using a 4 × 5 [Treatment (saline, 1.0, 2.0 mg/kg naloxone, dishabituation) × Isolation] analysis of variance (ANOVA). There was a significant effect of isolation, $F(4,136) = 24.978$, $p < .001$. Tukey's post-hoc analysis indicated that vocalization rates in the last four isolations were reduced when compared to the first. There was no significant effect of treatment nor was there a Treatment × Isolation interaction (Figure 1). The vocalization rates of the chronic animals recorded during

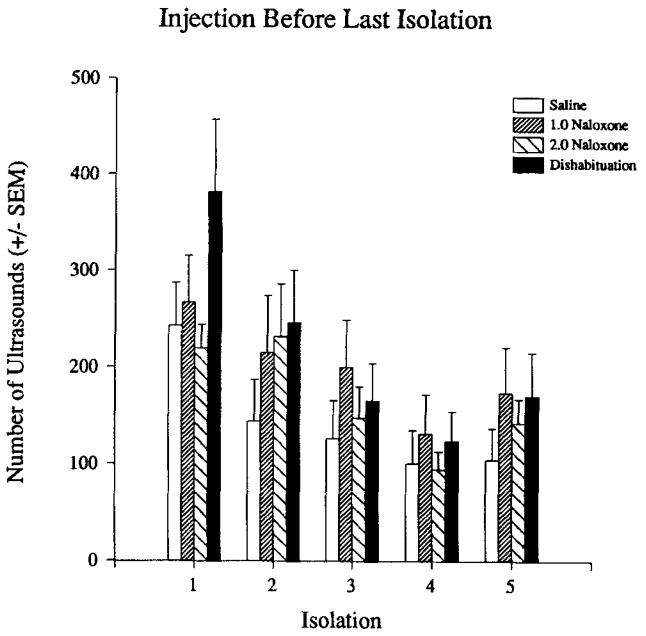

Fig. 1. Mean number of ultrasonic vocalizations recorded from chronic animals during each of the 5-min isolation periods in Experiment 1 of pups given 0.0 (0.9% Saline), 1.0, or 2.0 mg/kg naloxone or that were dishabituated as described in the methods. Vocalization rates of all pups were significantly reduced during the final four isolations.

the fifth isolation were compared to the vocalization rates of the acute animals in a 2 × 4 [Condition (chronic vs. acute) × Treatment] ANOVA. The ANOVA revealed a significant effect of condition, $F(1,71) = 17.265$, $p < .001$, with chronic animals vocalizing at a lower rate (147.74 ± 18.66) than acute animals (294.0 ± 30.29). Thus, the reduction seen across the five exposures to the testing incubator in the chronic group is not due only to reductions that might be induced by deprivation from the dam or from fluctuations that might occur as a result of the time of day at which the pups were tested.

Because the pharmacological and contextual manipulations were not administered until the final isolation, the repeated-measures ANOVA conducted across all isolation periods may not have been powerful enough to detect these treatment effects. For this reason, the vocalization rate of pups during the final isolation was expressed as a percentage of the mean vocalization rate of pups from the same treatment group during the first isolation. A 4 (Treatment) ANOVA on this data revealed no significant effect of treatment on vocalization rate (Figure 2a). A similar analysis conducted on vocalization rate of pups during the final isolation as a percentage of the mean vocalization rate of pups from the same treatment group during the isolation immediately prior to the drug treatment (i.e., the fourth isolation) likewise revealed no significant effects of treatment. Thus, in this experiment there was no evidence for a reversal of the decrease in vocalization rate observed in chronic animals by either administration of naloxone prior to the final isolation or by the introduction of a novel testing environment at that time.

The rectal temperatures taken from pups following each exposure to the testing incubator were analyzed in similar ANOVAs as were used to analyze the vocalization

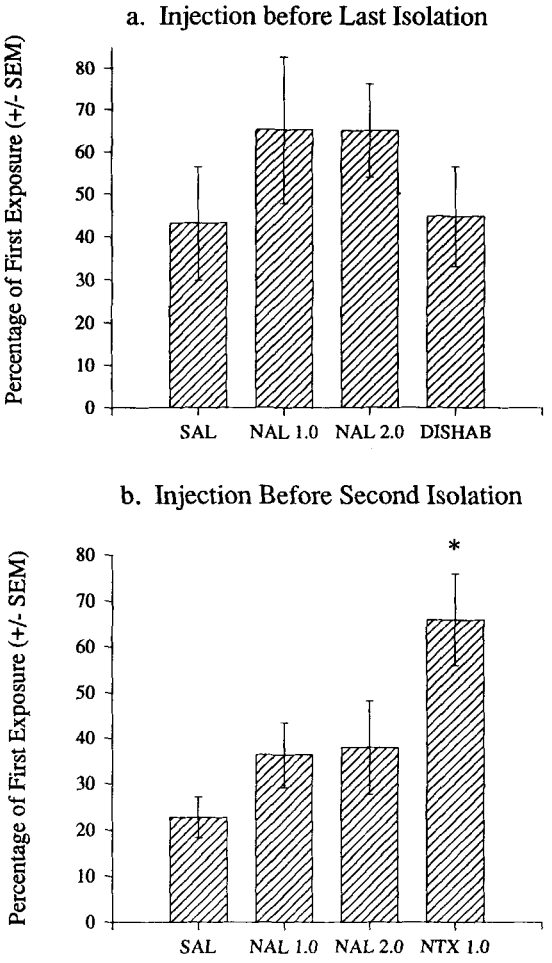

Fig. 2. Vocalization rates of pups during the fifth isolation expressed as a percentage of the mean number of vocalizations produced by each group during the first isolation in (a) Experiment 1 when pups were given 1.0 mg/kg naloxone (NAL 1.0), 2.0 mg/kg naloxone (NAL 2.0), or dishabituated (DISHAB) prior to the final isolation; and (B) in Experiment 2 when pups were given 1.0 mg/kg naloxone (NAL 1.0), 2.0 mg/kg naloxone (NAL 2.0), or 1.0 mg/kg naltrexone (NTX 1.0) prior to the second isolation, $*p < .05$.

data. A 4 × 5 (Treatment × Isolation) ANOVA of rectal temperatures revealed a significant main effect of isolation, $F(4,128) = 4.436, p < .05$. Tukey's post-hoc analysis indicated that rectal temperatures during the fourth and fifth isolations were increased when compared to the second isolation (Table I). A 2 × 4 (Condition × Treatment) ANOVA comparing the rectal temperatures of chronic animals following their fifth isolation and acute animals revealed no significant main effects or interactions. Thus, whereas chronic animals vocalized at a significantly lower rate than acute animals, this would not appear to be the result of differences in body temperature. The rectal temperatures of the chronic pups taken following the fifth isolation were also expressed as a percentage of the mean temperature taken from the same group of pups following the first isolation; A 4 (Treatment) ANOVA performed on this data revealed no effect of treatment.

Table 1
Mean Rectal Temperatures of Animals Recorded Following Each Exposure to the Testing Incubator

Experiment Condition	Isolation Mean/(*SEM*) 1	2	3	4	5
Experiment 1					
Chronic	31.48 (0.13)	31.15 (0.13)	31.34 (0.13)	31.51 (0.17)	31.62 (0.12)
Acute					31.41 (0.16)
Experiment 2					
Chronic	30.49 (0.16)	30.42 (0.16)	30.88 (0.10)	30.97 (0.11)	31.02 (0.13)
Acute					30.76 (0.11)

Condition	Treatment	Isolation Mean/(*SEM*) 1	2	3	4	5
Experiment 1						
Chronic	Saline	31.39 (0.33)	31.30 (0.37)	31.44 (0.40)	31.63 (0.35)	31.66 (0.30)
	1.0 Naloxone	31.35 (0.33)	31.13 (0.19)	31.19 (0.35)	31.60 (0.25)	31.43 (0.25)
	2.0 Naloxone	31.84 (0.21)	31.04 (0.25)	31.51 (0.22)	31.66 (0.26)	31.77 (0.22)
	Dishabituation	31.29 (0.19)	31.17 (0.23)	31.25 (0.19)	31.23 (0.41)	31.59 (0.24)
Acute	Saline					31.37 (0.36)
	1.0 Naloxone					31.57 (0.32)
	2.0 Naloxone					31.44 (0.31)
	Dishabituation					31.22 (0.35)
Experiment 2						
Chronic	Saline	30.51 (0.30)	30.59 (0.13)	30.87 (0.21)	30.83 (0.18)	30.80 (0.29)
	1.0 Naloxone	30.00 (0.37)	30.48 (0.25)	30.86 (0.29)	30.70 (0.18)	30.89 (0.09)
	2.0 Naloxone	30.58 (0.28)	30.54 (0.577)	30.93 (0.16)	31.00 (0.21)	31.15 (0.25)
	1.0 Naltrexone	30.78 (0.29)	30.09 (0.35)	30.89 (0.18)	31.33 (0.24)	31.27 (0.29)
Acute	Saline					31.08 (0.22)
	1.0 Naloxone					30.64 (0.29)
	2.0 Naloxone					30.62 (0.21)
	1.0 Naltrexone					30.69 (0.99)

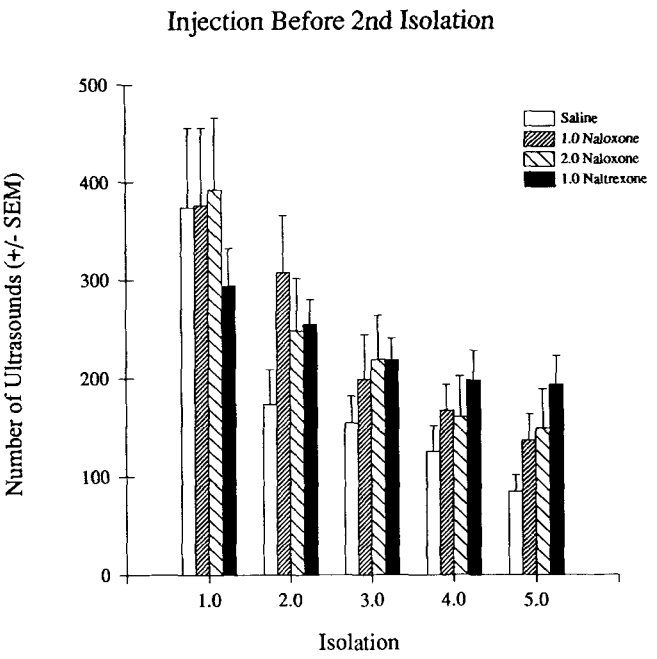

Fig. 3. Mean number of ultrasonic vocalizations recorded from chronic animals during each 5-min isolation period in Experiment 2 of pups given 1.0 mg/kg, or 2.0 mg/kg naloxone, or 1.0 mg/kg naltrexone. Vocalization rates of all pups were significantly reduced during the final four isolations.

Experiment 2

The total number of ultrasounds recorded from the chronic group during the five isolations was analyzed by a 4 × 5 [Treatment (saline, 1.0, 2.0 mg/kg naloxone, 1.0 mg/kg naltrexone) × Isolation] ANOVA. A significant effect of isolation, $F(4,144) = 31.958$, $p < .001$, was seen in the ANOVA. Tukey's post-hoc analysis indicated that vocalization rates in the last four isolations were significantly reduced compared to vocalization rates during the first isolation (Figure 3). There were no other main effects or interactions. The vocalization rate of chronic animals during the fifth isolation was compared to the vocalization rate of acute animals in a 2 × 4 (Condition × Treatment) ANOVA. As was the case in Experiment 1, this ANOVA revealed a significant effect of condition, $F(1,82) = 6.747$, $p < .05$, with chronic pups vocalizing less (137.98 ± 14.87) than acute pups (198.14 ± 17.36).

Analogous to the approach used for analysis of the data in Experiment 1, vocalizations measured during the fifth isolation of chronic animals were expressed as a percentage of the mean number of ultrasounds recorded from the same group of pups during the first isolation, with these data analyzed using 4 (Treatment) ANOVA. This ANOVA revealed a significant effect of dose, $F(3,36) = 5.527$, $p < .01$. Tukey's post-hoc analysis indicated that pups given naltrexone showed significantly less of a reduction in vocalization rate than pups given saline (Figure 2b). It should be noted that the baseline vocalization rate of pups given naltrexone was lower than that of pups given saline or either dose of naloxone. While this appears to be the result of sampling error, the lower baseline of naltrexone-treated pups in addition to their higher vocalization rate in the fifth isolation results in a lower percent reduction in these animals. Nevertheless, these are repeated measures and thus the reason for looking at the number of vocalizations

in the fifth isolation as a percentage of the baseline vocalization rates of the different groups is to correct for these differences in baseline vocalization rates.

Rectal temperatures measured following each isolation were analyzed in the same manner as vocalization rates. A 4 × 5 (Treatment × Isolation) ANOVA revealed a significant effect of isolation, $F(4,136) = 5.359$, $p < .001$. Tukey's post-hoc analysis indicated that rectal temperatures during the last two isolations were increased over rectal temperatures during the first two isolations (Table 1). A 2 × 4 (Condition × Treatment) ANOVA was used to compare rectal temperatures of chronic animals following the fifth isolation to temperatures of acute animals. This ANOVA revealed no significant main effects or interactions. No effect of treatment was observed in a 4 (Treatment) ANOVA on rectal temperatures of chronic pups from the fifth isolation expressed as a percentage of the mean temperature of pups from the same treatment group following the first isolation.

Discussion

In both experiments vocalization rates decreased significantly across the five isolation periods. Likewise, in both experiments, animals in the chronic group exhibited significantly lower vocalization rates than animals in the acute group. Therefore, the reduction seen in the chronic animals appears to be the consequence of repeatedly placing the pups in the testing incubator as opposed to some variable related to the prolonged absence of the parents or the time of day when testing occurred. The development of this attenuated response to isolation appears to be opiate-mediated, given that administration of naltrexone subsequent to the first isolation reduced this attenuation in ultrasound production.

It is possible only to speculate to what extent different variables associated with isolation are important in producing this attenuated response to isolation. When a pup is removed from the home cage and isolated, several components of the pup's environment change. For example, the pup is removed from a source of heat (the heating pad) and its ability to thermally insulate itself by huddling with its littermates is eliminated. In addition, the pup is removed from familiar tactile and olfactory nest stimuli and the chronic group is more frequently handled by the experimenters. In contrast to work such as that by Blumberg and Alberts (1990) and Hofer, Brunelli, and Shair (1993), the results of this experiment do not directly speak to the issue of which variables associated with the repeated isolation procedure are related to the reduction in ultrasound production.

The attempt in Experiment 1 to alter the context in which pups were tested in order to dishabituate animals did not significantly alter vocalization rates. If the alteration in context had restored vocalization rates, it would have been possible to conclude that nonassociative learning processes were involved in the decreased vocalization rates seen after repeated isolation. Because no changes were seen, it is unclear whether the manipulation failed to alter the testing context enough to dishabituate the animals or whether habituation is not involved in the attenuated vocalization response to repeated isolation.

It should be noted that significant changes in body temperature did occur across the repeated isolation periods in both experiments. It does not appear, however, that these account for the observed effects of chronic isolation on vocalization rate given that the body temperatures of chronic and acute animals did not differ when assessed immediately following the final isolation during which time vocalization rates differed

markedly. Thus, it appears that the repeated exposure of the 10-day-old rat pup to the stress of isolation from the litter and dam, in itself, results in an attenuation of the isolation-induced vocalization.

In Experiment 1, although there perhaps was a slight trend for naloxone to increase vocalization rates when administered prior to the fifth isolation, this was not statistically significant. This increase was not significant even when compared to the vocalization rates in the fourth isolation. In the second experiment, however, administration of the opiate antagonist naltrexone following the first isolation significantly attenuated the reduction in vocalization rate seen in chronic animals. While baseline calling rates of animals given naltrexone tended to be lower than calling rates of the other animals, this is thought to be a spurious effect given that animals were randomly assigned to each group before any vocalizations were recorded. There was a trend for both doses of naloxone to attenuate this reduction as well; however, as in Experiment 1, this was not a statistically reliable effect. One possible interpretation is that naltrexone, which is more potent than naloxone, may simply be more effective at blocking opiate receptors at the dose chosen than either dose of naloxone used. However, naltrexone also has a longer half-life than naloxone and so naltrexone's effects would diminish less rapidly than those of naloxone and would be more evident 2 hr postinjection. If this latter explanation is correct, then it would appear that it is easier to prevent the development of this attenuated response to isolation by blockade of opiate receptors than it is to reverse it once it is established.

These results may speak to the somewhat equivocal reports regarding the ability of opiate receptor blockade to increase vocalization rates of isolated rat pups. Early studies indicated that opiate receptor blockade was capable of increasing the vocalization rates of isolated rat pups (Kehoe & Blass, 1986; Kehoe & Sakurai, 1991; Carden & Hofer, 1990a), while other studies found no effect (Carden & Hofer, 1991; Winslow & Insel, 1991b). It is not clear whether what was reported as an increase in vocalization rate following naltrexone administration was actually an increase, per se, or whether it was the result of an attenuated reduction in vocalization rates that occurred within the test period. This is, in fact, pointed out by Kehoe and Blass (1986) as well as Carden and Hofer (1990a) who present minute-by-minute accounts of vocalization rates and show that animals given saline injections decrease vocalization rates across the isolation period while this decrease is not seen in animals given naltrexone. In the study by Winslow and Insel (1991b), no such decrease is seen in saline-treated animals, a discrepancy which is noted by these authors as a possible reason for their failure to replicate the findings of Kehoe and Blass (1986). In fact, the effects of naltrexone on ultrasonic vocalizations appear to be most robust in situations where stimuli are presented which decrease vocalization rates of isolated rat pups—for example, when an anesthetized littermate is present (Carden & Hofer, 1990b) or when intraoral infusions of milk or sucrose are given (Blass & Fitzgerald, 1988). Because these effects are naltrexone reversible, it is argued that the attenuation in vocalization rate is mediated by an endogenous opiate release. Thus, one possible reason for the conflicting findings reported above is that a single, brief period of isolation may not always be an adequate stimulus to induce a sufficient endogenous opiate release to reduce vocalization rates. The results of the experiments reported here indicate that repeatedly isolating young rats may be a means by which isolation, per se, can be made to reliably elicit an endogenous opiate release.

In relating the results of this experiment to those examining the response of adult rats to chronic stress, certain similarities can be seen. For example, repeated exposure of rat pups to isolation stress leads to an attenuation in a response to that stressor. In

adults, a comparable attenuation in stress responsiveness has been observed in animals previously exposed to the same type of stressor. For instance, behavioral suppression following acute stress is no longer evident in animals previously repeatedly exposed to the same aversive situation (Kennett et al., 1985; Cancela et al., 1991). Furthermore, as in adults, the attenuated response to isolation appears to be opiate-mediated, given that administration of an opiate antagonist prior to exposure to stress moderates the effect of chronic stress (Cancela et al., 1990, Cancela et al., 1991; Cabib et al., 1984). In the adult literature, these attenuated responses are thought to reflect an adaptation process (Kennett et al., 1985; Cancela et al., 1990; Cancela et al., 1991). It is not immediately clear whether the reduction in isolation-induced vocalization rates following repeated isolation is in some way reflective of an adaptation to isolation stress in the neonatal rat. However, given that chronic exposure of animals to stressors leads to behavioral abnormalities which have been associated with certain psychopathologies in humans (Willner, 1984), the ontogeny of the response of animals to chronic stressors is an important area for future study.

References

Blass, E. M., & Fitzgerald, E. (1988). Milk-induced analgesia and comforting in 10-day-old rats: Opioid mediation. *Pharmacology Biochemistry & Behavior, 29,* 9–13.

Blumberg, M. S., & Alberts, J. R. (1990). Ultrasonic vocalizations by rat pups in the cold: An acoustic by-product of laryngeal braking? *Behavioral Neuroscience, 104,* 808–817.

Brouette-Lahlou, E., Vernet-Maury, E., & Vigouroux, M. (1992). Role of pups' ultrasonic calls in a particular maternal behavior in Wistar rat: Pups' anogenital licking. *Behavioural Brain Research, 50,* 147–154.

Cabib, S., Puglisi-Allegra, S., & Oliverio, A. (1984). Chronic stress enhances apomorphine-induced stereotyped behavior in mice: Involvement of endogenous opioids. *Brain Research, 298,* 138–140.

Cancela, L., & Molina, V. A. (1987). Reduced apomorphine-induced sedation following chronic stress. *European Journal of Pharmacology, 134,* 117–119.

Cancela, L., Rossi, S., & Molina. V. A. (1991). Effect of different restraint schedules on the immobility in the forced swim test: Modulation by an opiate mechanism. *Brain Research Bulletin, 26,* 671–675.

Cancela, L., Volosin, M., & Molina, V. A. (1990). Opioid involvement in the adaptive change of 5-HT receptors induced by chronic restraint. *European Journal of Pharmacology, 176,* 313–319.

Carden, S. E., Barr, G. A., & Hofer, M. A. (1991). Differential effects of specific opioid receptor agonists on rat pup isolation calls. *Developmental Brain Research, 62,* 17–22.

Carden, S. E., & Hofer, M. A. (1990a). Independence of benzodiazepine and opiate action in the suppression of isolation distress in rat pups. *Behavioral Neuroscience, 104,* 160–166.

Carden, S. E., & Hofer, M. A. (1990b). Socially mediated reduction of isolation distress in rat pups is blocked by naltrexone but not by Ro 15-1788. *Behavioral Neuroscience, 104,* 457–463.

Carden, S. E., & Hofer, M. A. (1991). Isolation-induced vocalization in Wistar rat pups is not increased by naltrexone. *Physiology & Behavior, 49,* 1279–1282.

Evoniuk, G. E., Kuhn, C. M., & Schanberg, S. M. (1979). The effect of tactile stimulation on serum growth hormone and tissue ornithine decarboxylase activity during maternal deprivation in rat pups. *Communications in Psychopharmacology, 3,* 363–370.

Hofer, M. A., Brunelli, S. A., & Shair, H. N. (1993). Ultrasonic vocalization responses of rat pups to acute separation and contact comfort do not depend on maternal thermal cues. *Developmental Psychobiology, 26,* 81–95.

Kehoe, P., & Blass, E. M. (1986). Opioid mediation of separation distress in 10-day-old rats: Reversal of stress with maternal stimuli. *Developmental Psychobiology, 19,* 385–398.

Kehoe, P., & Sakurai, S. (1991). Preferred tastes and opioid-modulated behaviors in neonatal rats. *Developmental Psychobiology, 24,* 135–148.

Kennett, G. A., Dickinson, S. L., & Curzon, G. (1985). Enhancement of some 5-HT dependent behavioral responses following repeated immobilization in rats. *Brain Research, 330,* 253–263.

Kuhn, C. M., Butler, S. R., & Schanberg, S. M. (1978). Selective depression of serum growth hormone during maternal deprivation in rat pups. *Science, 201,* 1034–1036.

Kuhn, C. M., Pauk, J., & Schanberg, S. M. (1990). Endocrine response to mother–infant separation in developing rats. *Developmental Psychobiology, 23,* 395–410.

Noroit, E. (1968). Ultrasounds in young rodents. II. Changes with age in albino rats. *Animal Behaviour, 16,* 129–134.

Ohi, K., Mikuini, M., & Takahashi, K. (1989). Stress adaptation and hypersensitivity in 5-HT neuronal systems after repeated foot shock. *Pharmacology Biochemistry & Behavior, 34,* 603–608.

Pauk, J., Kuhn, C. M., Field, T. M., & Schanberg, S. M. (1986). Positive effects of tactile versus kinesthetic or vestibular stimulation on neuroendocrine and ODC activity in maternally deprived rat pups. *Life Sciences, 39,* 2081–2087.

Smotherman, W. P., Bell, R. W., Starzec, I., Elias, J., & Zachman, T. A. (1974). Maternal responses to infant vocalizations and olfactory cues in rats and mice. *Behavioral Biology, 12,* 55–66.

Spear, L. P., Enters, E. K., Aswad, M. A., & Louzan, M. (1985). Drug and environmentally induced manipulations of the opiate and serotonergic systems alter nociception in neonatal rat pups. *Behavioral and Neural Biology, 44,* 1–20.

Stone, E. (1987). Central cyclic-AMP-linked noradrenergic receptors: New findings on properties as related to the actions of stress. *Neuroscience and Biobehavioral Reviews, 11,* 391–398.

Terkel, J., Damassa, D., & Sawer, C. (1979). Ultrasonic cries from infant rats stimulate prolactin release in lactating mothers. *Hormones and Behavior, 12,* 95–102.

Voloschin, L., & Tramezzani, J. (1984). Relationship of prolactin release in lactating rats to milk ejection, sleep state, and ultrasonic vocalization by the pups. *Endocrinology, 114,* 618–623.

Willner, P. (1984). The validity of animal models of depression. *Psychopharmacology, 83,* 1–16.

Winslow, J. T., & Insel, T. R. (1991a). Infant rat separation is a sensitive test for novel anxiolytics. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 15,* 745–757.

Winslow, J. T., & Insel, T. R. (1991b). Endogenous opioids: Do they modulate the rat pups' response to social isolation? *Behavioral Neuroscience, 105,* 253–263.