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SEPARATION, MOTIVATION, AND DEPRESSION: NEONATAL ISOLATION REDUCES FOOD-REWARDED OPERANT RESPONDING IN RATS

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One symptom of depression is loss of motivation, which can be defined as responsiveness to response-eliciting stimuli and quantified as reward-related behavioral output. Long-term changes in reward-related behavior have been shown to follow early life stress. Most rodent studies investigating the effects of postnatal separation, an early stress, on reward-related behavior have used drug rewards and few have used natural rewards. Given that separation has been implicated in depression in humans, who may experience impaired motivation without drug experience, it is important to understand how separation affects motivation for natural reward. We hypothesized that neonatal isolation would slow the acquisition of and reduce levels of food-rewarded operant responding, a measure of motivation, in rats. Eight male Long-Evans rats were individually isolated from dams and littermates for 1 hr on postnatal days 2 through 9 while the dam stayed with remaining pups. Seven male siblings were handled to the same extent but without the isolation. When tested as adults on a lever pressing task under fixed and progressive ratio schedules of food reward, isolated rats acquired the operant response significantly more slowly than handled siblings and showed significantly lower levels of responding under all schedules. These results indicate that early separation causes a reduction in motivation, which may be one mechanism of human depression.

A major symptom of depression is reduced motivation. Additionally, early separation stress has been associated with depression. Experiences of loss, such as death or separation, are correlated both with the early life histories of patients with depression (Gilmer & McKinney, 2003; Heim & Nemeroff, 2001; Luecken, 2000), and with the onset of primary depressive episodes (Kendler, Hettema, Butera, Gardner, & Prescott,

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2003). Furthermore, disruptions in parent-child attachment are thought to lead to a variety of psychopathologies and vulnerabilities that include depression (Beebe & Lachmann, 2002; Fonagy, 2001; Schore, 1996). Given that both early stress and reduced motivation are associated with depression, we sought to investigate whether or not there exists a direct relation between early separation stress and impaired motivation.

The subjugation of rodents to early separation is considered to be a model of depression and has generated findings on the long-term effects of early stress on adult neurophysiology and behavior (Nestler et al., 2002). Manipulations include maternal separation (MS), in which the litter is separated from the dam for an extended period such as 24 hours; repeated maternal separation (RMS), in which a litter is separated from the dam for periods ranging from 1 to several hours over a span of days within the first several weeks of birth; and neonatal isolation (NI) or early deprivation (ED), in which individual pups are isolated from both the dam and littermates for a period of 1 to 6 hr over a span of days within the first several weeks of birth (see Pryce & Feldon, 2003, for review). Using these manipulations, studies have revealed a number of common features between the neurophysiology of these animals and human subjects with depression. For example, it is now well documented that both humans with depression and rodents affected by early separation show dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (for human reviews, see Erickson, Drevets, & Schulkin, 2003; Gutman & Nemeroff, 2003; Heim & Nemeroff, 2001; for rodent reviews, see Cirulli, Berry, & Alleva, 2003; Levine, 2001; Pryce & Feldon, 2003).

Early life stress has also been correlated with changes in dopamine (DA) neuronal systems, key substrates for reward and motivation. For example, early separation experience leads to increased striatal levels of DA and decreased prefrontal turnover of DA (Matthews, Dalley, Matthews, Tsai, & Robbins, 2001), reduced tyrosine hydroxylase-immunoreactive fibers in some regions of frontal cortex (Braun, Lange, Metzger, & Poeggel, 2000) and increased tyrosine hydroxylase-immunoreactive fibers in others (Poeggel, Nowicki, & Braun, 2003). Given these changes, it might be expected that early separation would lead to changes in reward-related behavior.

Indeed, early separation paradigms have been used to investigate the effects on reward-related behavior. Most of these studies have incorporated drug rewards, pharmacological manipulations, or both (e.g., Kehoe, Shoemaker, Triano, Callahan, & Rappolt, 1998; Kosten, Miserendino, & Kehoe, 2000; Matthews, Hall, Wilkinson, & Robbins, 1996; Matthews, Robbins, Everitt, & Caine, 1999; Zhang, Sanchez, Kehoe, & Kosten, 2005). However, given that many humans develop depression without any experience with exogenous chemicals, it is important to understand the effects of early separation on motivation in relation to natural rewards. Only a few studies to date have done so.

No differences have been found in basic consummatory behavior between early separated animals and comparison groups (Iwasaki,

Inoue, Kiriike, & Hikiji, 2000; Matthews, Hall, et al., 1996). However, differences have been found in conditioned responding for natural reward in maternally separated rats. RMS resulted in reduced anticipatory locomotion in response to food (Matthews, Hall, et al., 1996; Matthews, Wilkinson, & Robbins, 1996) and reduced adjustment of lever pressing rates in response to both negatively and positively contrasted reward (Matthews, Hall, et al., 1996). The only study to test operant responding in RMS rats found no differences in sucrose-maintained lever pressing under fixed and progressive ratio schedules between RMS rats and both handled and nonhandled rats (Shalev & Kafkafi, 2002).

To our knowledge only two published studies examined the effects of neonatal isolation on instrumental responding maintained by natural rewards at the time the current study was conducted. In the first of these two studies, isolated male rats tested as adults showed no difference in the number of days to acquire food-maintained lever pressing on a fixed-ratio 1 (FR1) schedule of reinforcement in 30-min sessions, with a maximum of 50 pellets to be earned (Kosten et al., 2000). The second study, from the same lab, found enhanced acquisition in the same protocol for female adult rats (Kosten, Sanchez, Zhang, & Kehoe, 2004). These studies addressed whether neonatal isolation affects acquisition of an operant response but did not address whether it affects motivation to respond after acquisition has occurred.

Because of the strong connections between motivation and depression, and between early stress and depression, we were interested in investigating whether or not neonatal isolation leads to changes in motivation to respond for natural reward, once the operant response is acquired. In the present study, therefore, we used an operant conditioning procedure designed to assess motivation for food. We hypothesized that rats that experienced neonatal isolation would acquire the lever press response more slowly and would respond at lower rates for food under both fixed or progressive ratio schedules of reinforcement than rats not exposed to neonatal isolation.

Since this study was conducted, two additional studies have been published which examined operant responding for natural reward after neonatal isolation. In one study, isolated male rats separated during the dark phase showed reduced responding for sucrose on a progressive ratio schedule when compared to nonhandled controls, although rats isolated during the light phase showed normal responding (Ruedi-Bettschen, Pedersen, Feldon, & Pryce, 2005). In the second, isolated males showed significantly lower responding for food reward under a fixed-ratio schedule in comparison to nonhandled controls, although no differences were found under progressive ratio schedules (Zhang et al., 2005).

Method

Subjects

The experimental protocol described here was approved by the Queens College Institutional Animal Care and Use Committee and is

in accordance with the Animal Welfare Act. Subjects were male Long-Evans rats, born to four dams who were shipped timed-pregnant (Charles River Laboratories, Raleigh, NC) at 13 days of gestation. Dams were individually housed in 25- x 46- x 20-cm opaque plastic cages and were kept on a 12:12 hr light:dark cycle (lights on at 0700 hr) in a temperature-controlled environment (20 °C). All dams had water and food (Purina Rat Chow, LabDiet 5001) available ad libitum. Litters born before 5 p.m. were considered as postnatal day (PND) 0. On PND 1, litters were culled to 12 pups and sex balanced where possible.

Isolation Procedure

On PNDs 2 through 9, 3 male pups from each litter to be designated as "isolated" were placed for 1 hr in individual compartments measuring 5 x 8.5 x 5 cm each in a polypropylene storage container with a lid. Each compartment contained approximately 5 ml of bedding from the home cage to retain odors of the nest, so pups would mainly be affected by the absence of the dam rather than exposure to a novel environment. The isolation container was placed on a heating pad (TheraTherm, Chattanooga Group, Hixton, TN) set to 32 °C. The pups could not touch neighboring pups. Upon retrieval, the pups were marked dorsally with permanent marker and placed back with their littermates, at which time the remaining males of each litter were placed in the same container for 10 s, marked, and returned to the nest. Because these pups received the same amount of handling as isolated pups, minus the isolation period, they were considered "handled." Female pups were also marked to ensure that all pups would have the same novel scent. To limit stimulation, the pups were not weighed, as this paradigm has been shown to produce no differences in weight gains between groups (Kehoe et al., 1998).

Immediately prior to removal or return of pups to the nest, dams were removed from the nest and placed in a clean bin. The dams stayed in this bin during the time that pups were removed or returned to the nest, after which the dams were returned to the nest. Dams therefore were with the remaining nonisolated pups during the entire isolation period, minus the brief periods of transition at the beginning and end of the hour.

Pups were weaned at PND 25 and group-housed by sex. Between 75 and 80 days of age, rats were transferred to a reversed-light room (lights off at 0600 hr). After a week of adjustment to the new light phase, rats were weighed and then placed on a restricted feeding diet that reduced and maintained their weights to 85% of their free-feeding values.

Apparatus

Experimental sessions were conducted in operant conditioning chambers measuring 30 x 21 x 18 cm (l x w x h). Each chamber consisted of clear plastic sides and top, except for an aluminum wall equipped with two levers, two stimulus lights, and a food trough. Each lever was positioned 2.5 cm away from the edge of the wall and extended 2 cm from the wall. A white stimulus light was positioned 3 cm above each lever. The food trough measured 5 x 5 cm and was centered between the two levers

at a height of 3 cm from the floor. The floor of each chamber consisted of aluminum rods, and each chamber was housed in a ventilated, sound-attenuating box.

Operant Conditioning Testing

On PND 98, 8 isolated and 7 handled rats began testing. The isolated group was composed of 2 rats per litter, and the handled group contained 2 rats each from two litters and 3 rats from a third litter. Rats were placed in operant chambers every day for 10-min sessions. Presses on the active lever resulted in the delivery of one food pellet (45 mg, BioServ) and the illumination of the stimulus light above that lever for 1 s. Presses on the inactive lever resulted in no consequences. Initially, the rats were rewarded with a food pellet on a fixed-ratio 1 (FR1) schedule of reinforcement until they demonstrated stable lever pressing, at which time the schedule of reinforcement was changed. Stable responding was operationally defined as three consecutive sessions in which total responses per session fell within ±10% of the mean for the three sessions and did not show upward or downward trends. When responding by a rat stabilized, the schedule of reinforcement was changed to FR5 and was maintained at this setting until stable responding (as defined above) was demonstrated. The schedule was changed in this manner again to FR10 and then to a progressive ratio (PR) schedule of reinforcement, at which time sessions were increased to 1 hr.

Under the PR schedule, the response requirement for the first reward was set to 1 and increased exponentially for each subsequent reward according to the formula: $5 \times e^{(\text{reward \#} \times 0.22)} - 5$ (with the progression being 1, 3, 5, 7, 10, 14, 18, 24, 31, 40, 51, 65, 82, 104, 131, 164, etc.). Eventually, the ratio requirement becomes so high that rats cease to respond. The point at which rats stop responding is referred to as the break point (BP). BPs were operationally defined as the final number of ratios completed (which resulted in the delivery of a food reward) within 30 min of the previous one. After BPs stabilized on the PR schedule, the number of food pellets per reward was changed from one to two to assess for any differences in responding as reward magnitude increased, and kept there until stable BPs developed and then returned to one pellet, to see if responding changed as reward magnitude decreased, until stable BPs developed again. Stable BPs were operationally defined as three consecutive sessions in which the number of rewards per session fell within ±10% of the mean for the three sessions and did not show upward or downward trends.

Data Analysis

Performance on the first and last days of responding under the FR1 schedule was compared using a 2 x 2 ANOVA (day x group) with repeated measures on day. Performance on the first 5 days of responding under the FR1 schedule, beginning with the first day in which a lever press was recorded, was analyzed using a 2 x 5 ANOVA (day x group) with

repeated measures on day. Because sphericity was not assumed in these data, a Greenhouse-Geisser degrees of freedom correction was used. A significant day by group interaction was followed by tests of simple main effect of day at each level of the group factor. BPs on the PR schedule were compared using two 2 x 2 ANOVAs (pellets x group) with number of pellets as a repeated measures factor; the first compared performance between one and two pellets, and the second compared performance on two pellets and the second phase of testing with one pellet.

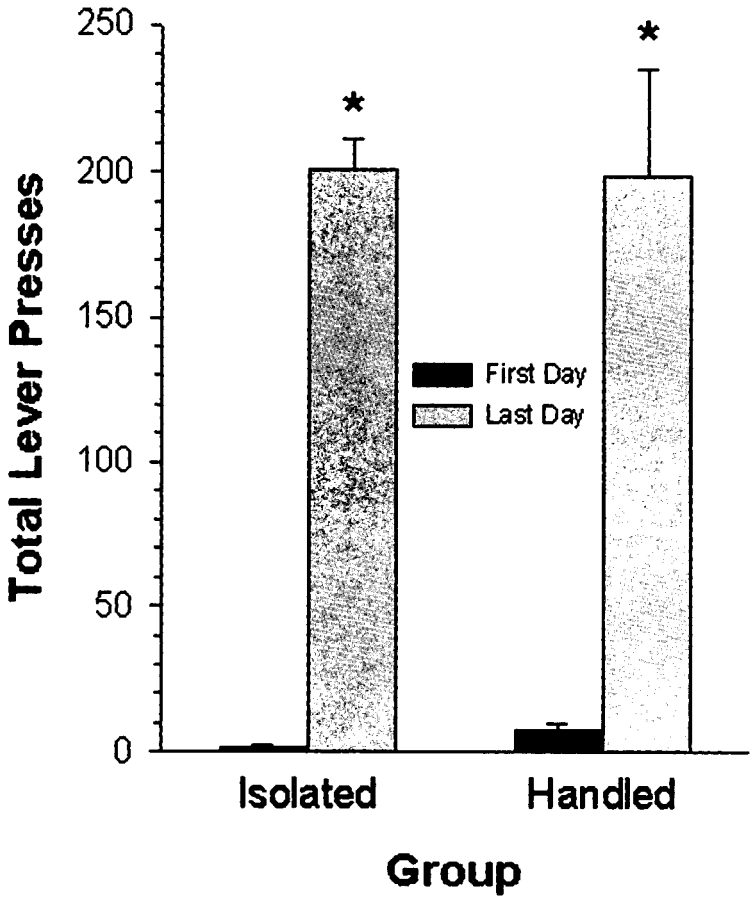

Figure 1: Total number of lever presses for isolated and handled rats on the first day and the last day of responding under a fixed-ratio 1 schedule of food reinforcement.
* indicates a significant difference between responding on the first day and the last day. Vertical lines represent the standard error of the mean.

Results

Weight

There was no significant difference in the average free-feeding weights between isolated rats (495 ±31 g) and their handled siblings (484 ±49 g).

Acquisition of Response

Both isolated and handled rats demonstrated significantly greater lever pressing on their last day of responding under the FR1 schedule than on their first day of responding under this schedule (see Fig. 1; a two-way ANOVA with group and day [repeated measures] as factors revealed a significant day effect, $F(1,13) = 114.219$, $p < .0005$). However, isolated rats acquired the lever press response significantly more slowly than did

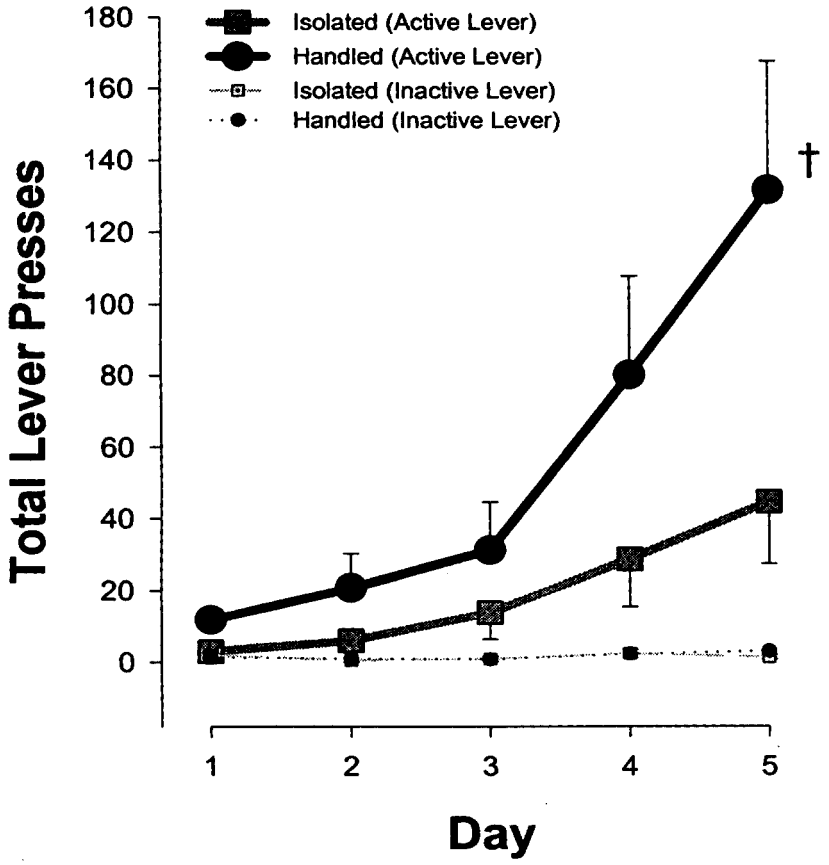

Figure 2. Total number of lever presses per session for the first five sessions under the FR1 schedule of reinforcement, beginning with the first session in which at least one lever press was recorded. † indicates a significant difference between responding on the 1st day and the 5th day. Vertical lines represent the standard error of the mean.

their handled siblings through the first 5 days of acquisition (see Fig. 2; a two-way ANOVA revealed a significant day by group interaction, $F(4, 52) = 4.456$, $p < .05$ with Greenhouse-Geisser correction; tests of simple main effect of day at each level of group revealed a significant effect in the handled group, $F(1,52) = 19.260$, $p < .001$). In contrast to the increased pressing on the active lever, presses on the inactive lever did not change for either group over the first 5 days of acquisition (see Fig. 2; a two-way ANOVA revealed no significant group differences or significant group by day interactions).

Fixed and Progressive Ratio Responding

Isolated rats pressed the lever significantly less than did handled siblings under all fixed ratio schedules of reinforcement (see Fig. 3; a two-way

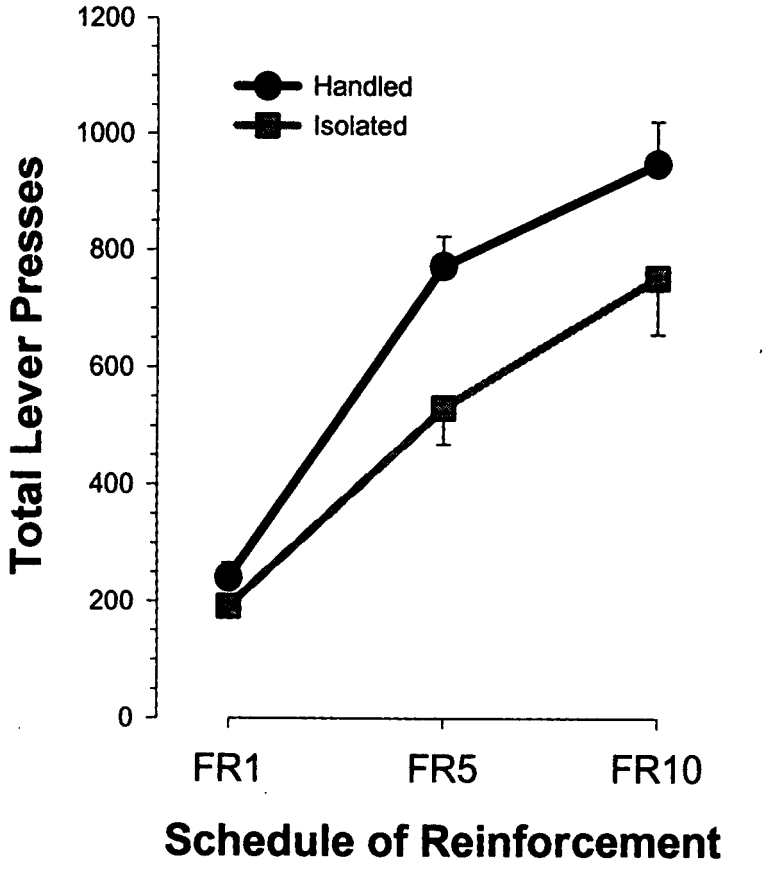

Figure 3. Mean total number of lever presses during the last 3 days of responding under FR1, FR5, and FR10 schedules of reinforcement. Vertical lines represent the standard error of the mean.

ANOVA on group and schedule [repeated measures] revealed no significant interaction, but a significant group effect, $F(1,13) = 6.317$, $p < .05$).

When comparing BPs on progressive ratio responding for one pellet compared to two, isolated rats showed significantly lower BPs when responding under a PR schedule for either one or two food pellets, and neither group showed increased pressing for two pellets (see Fig. 4; a two-way ANOVA on group and number of pellets [repeated measures] revealed a significant group effect, $F(1,13) = 5.592$, $p < .05$, but no

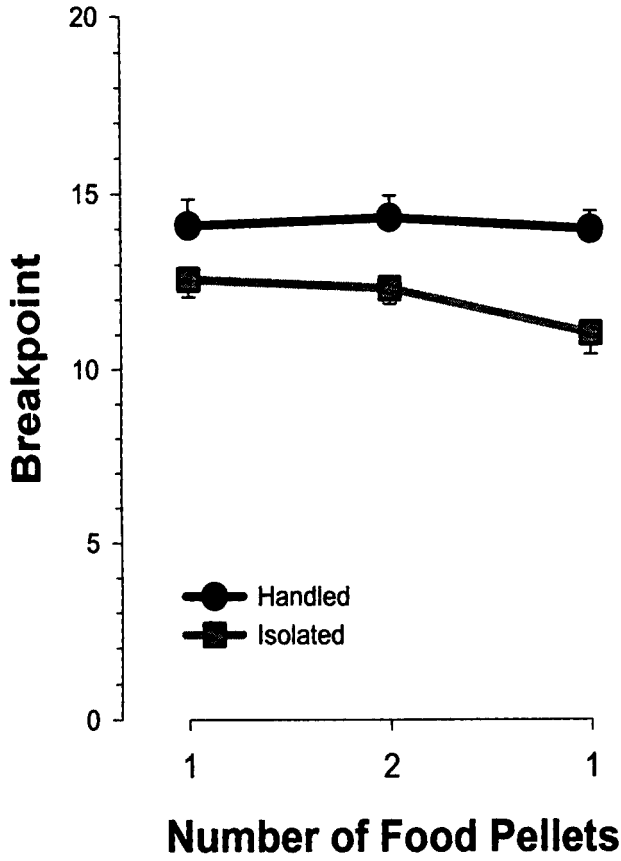

Figure 4. Break points in responding under a PR schedule of reinforcement with one and two food pellets as rewards. Break points were operationally defined as the last reward earned within a 30-min period following the previous one. The abscissa depicts the levels of food reward magnitude and the chronological order in which they were experienced by all rats; the first level (labeled "1") represents the first determination of stable break points for one food pellet; the second level (labeled "2") represents the determination of stable break points for two food pellets and in all cases this value was determined after the determination of stable break points for one pellet; the third level (labeled "1") represents the second determination of stable break points for one food pellet and in all cases this value was determined after the determination of stable break points for two food pellets. Vertical lines represent the standard error of the mean.

significant effect of pellet and no significant interaction). When the reward was switched from two pellets back to one pellet, isolated rats continued to show lower responding at both levels, and both groups showed a decrease in pressing when comparing one pellet to two, with isolated rats seeming to show a greater decline, although the interaction was not significant (see Fig. 4; a two-way ANOVA on group and number of pellets [repeated measures] again revealed a significant group effect, $F(1,13) = 12.687$, $p < .005$ as well as a significant effect of pellet, $F(1,13) = 4.683$, $p = .05$, but no significant interaction).

Discussion

In this study, we found that male Long-Evans rats that were isolated for 1 hr each on PNDs 2-9 showed lower levels of food-maintained operant responding as adults than did their handled siblings. Total lever presses were lower under three fixed-ratio schedules, and BPs were lower under a PR schedule at each of two levels of food reward. Isolated rats also demonstrated a delayed acquisition of the operant response. These data suggest that early life isolation under certain conditions leads to reduced motivation for natural rewards.

Because there was no difference in free-feeding weights between the groups, it is unlikely that the reduced performance of the isolated rats was caused by a basic difference in consummatory behavior. Progressive ratio break points indicate the amount of work that an organism expends to obtain a reward—by definition an animal's motivation for the reward. Thus, the difference in break points between the groups is best attributed to differences in motivation. It is conceivable that cognitive impairment, rather than altered motivational processes, could have contributed to the group differences. If that were the case, we might have expected to see a lack of discrimination between levers as the lever press response was being acquired. However, the data indicate that the isolated rats discriminated between levers to the same extent as the handled rats, as both groups showed equally low levels of pressing on the inactive lever across the first five sessions. We therefore conclude that the difference between groups was motivational.

As mentioned earlier, little data exists on the relationship between early separation and operant responding maintained by natural reward. To this date, only five studies have examined this relationship, and an important caveat must be mentioned before the results of these studies are compared: Differences in procedure (both between maternal separation and isolation, and different protocols of isolation) may account for different outcomes, and we should be mindful that research in this area is just at its beginning. Shalev and Kafkafi (2002) used a maternal separation procedure; Kosten and colleagues (Kosten et al., 2000, 2004; Zhang et al., 2005) used a 1-hr isolation procedure with no bedding in which all pups were removed from the dam during the isolation period; and Ruedi-Bettschen and colleagues (2005) used a 4-hr isolation on

sawdust in which all pups were removed from the dam. In contrast, our method involved isolating only 3 pups, so the dam herself is not completely alone during the isolation period, as she is in the other protocols. In addition, we used bedding from the home cage to retain smells from the mother, as we are interested in investigating not the stress reaction to novelty per se, but to being unattended to and alone in a familiar environment; in other words, a closer approximation of the stress of loss and separation in humans. Other important differences may affect outcomes: For example, dams were shipped timed-pregnant in the Shalev and Kafkafi study and in ours, whereas dams were bred on-site in the Ruedi-Bettschen and Kosten studies (Kosten et al. do not specify origin of the dams so we assume they were bred on-site). Lehmann and Feldon (2000) note that the various types of maternal separation manipulations produce conflicting results and the specific types of manipulations must be researched systematically. Indeed, the various factors of prenatal stress, isolation in the presence or absence of other pups, temperature level, and the presence or absence of familiar smells may interact in complex ways in their long-term effects on motivated behavior, and future research will need to flesh out those interactions.

The variety of parameters which are involved in early separation manipulations therefore make it impossible to say at this point which factors in the isolation experience account for long-term differences. A variety of "regulators" mediate the relationship between pup and dam (Hofer, 1994), including smell, sound, temperature, physical stimulation, and nutritive factors, all of which are potentially altered by separation and may have differing effects on pups and dams. Certainly the pup may be affected by isolation, as this procedure has been found to raise corticosterone levels (McCormick, Kehoe, & Kovacs, 1998). However it is very possible that a greater factor in long-term outcomes is the behavior of the dam, either during the isolation period in relation to the remaining pups, or towards the isolated pups upon reunion (which could be greater or lesser degrees of maternal attention), or both. Maternal behavior, in particular ano-genital licking, has been shown to be a critical determinant in long-term HPA regulation (Francis, Diorio, Liu, & Meaney, 1999) and is affected by handling of pups (Denenberg, 1999). However, maternal behavior was not observed in this study so it is not known whether pups received greater, lesser, or equal amounts of maternal attention following reunion. This study did not aim to investigate the components of the isolation experience, and future research should illuminate these factors further.

Given the differences in procedures used in the studies completed to date, it is not surprising that the findings are mixed. Shalev and Kafkafi (2002) found that maternally separated (RMS) rats showed no difference in fixed or progressive ratio responding maintained by sucrose in comparison to both early handled and nonhandled controls. These findings clearly contrast with those of the present study. However, their rats were not food deprived, and it is possible that the effects of food deprivation account for the discrepancy. Thus, it is possible that we observe motivational deficits where they did not because these deficits

are revealed only when animals are motivated to expend more effort to obtain rewards due to food deprivation, or because underlying deficits emerge only in the context of the stress of food deprivation.

Our findings are partially in line with the three other studies to date on operant responding in rats following early separation, all of which used the neonatal isolation procedure. First, we found no differences in the ultimate acquisition of the operant response task between isolated and handled male rats, just as two previous studies found no differences between isolated and nonhandled male rats (Kosten et al., 2000; Zhang et al., 2005). Using the criteria of acquiring 50 pellets in a 5-min period, all animals in those studies acquired the operant response regardless of treatment. (A third study by Kosten et al., 2004, from the same lab found enhanced acquisition in the same protocol in female rats. As the authors note, gender differences affect long-term responses to early stress and should be investigated further, so it is not useful to compare those findings to those of the current study.)

Furthermore, however, we also measured behavior across days so that each subject's responding could be compared to its initial performance. In this analysis, our data demonstrate that neonatal isolation significantly slows the *rate at which the acquisition of operant responding occurs* in male rats. This is interpreted as a reduction in the ability to adapt to a changing environment, or, effectively, a reduction in the ability to learn. In this regard our findings are similar to those of Matthews, Hall, and colleagues (1996) who found delayed acquisition of conditioned locomotor activity in response to food in RMS rats, suggesting that their early separation procedure caused delayed *classical* learning. Our data, showing that neonatal isolation slowed the rate of acquiring a lever press task, suggest that early life separation can also impair the acquisition of *operant* learning. It is possible that the slower rate of learning in isolated rats results from the reduced reinforcing effectiveness of the rewarding stimulus, which may indicate a dysregulation in the neural circuits underlying motivated behavior.

In addition, our findings on fixed-ratio responding are in line with those of Kosten and colleagues (2004) who found significantly reduced responding on a FR15 schedule for food reward in comparison to nonhandled rats. However, our findings on progressive ratio responding fall between those of the two other studies testing this schedule. Kosten et al. (2004) found no differences on two PR schedules, whereas Ruedi-Bettschen et al. (2005) found that 4-hr isolation led to reduced responding for sucrose on a progressive ratio schedule compared to nonhandled controls, when the animals had been separated during the dark phase (under both cold and warm conditions). Animals experiencing isolation during the light phase showed no reductions in breakpoints compared to controls. As our rats were separated during the light phase and did show reductions in PR responding, further research is needed to explore this discrepancy.

Several differences between these studies and ours may account for the discrepant findings. First, differences in isolation protocols may have

distinct long-term effects: in the other two protocols, all pups are isolated at once, so the dam has a different experience than our dams do, who are left with remaining pups during the isolation period. Second, the other protocols compare isolated rats to nonhandled rats from different litters; ours compares isolated rats to handled siblings, which may accentuate certain differences and obscure others. These two considerations apply to the differences between our study and the other two studies.

Furthermore, a specific difference between the PR schedules used may account for the fact that we found reduced responding on a PR schedule and Kosten et al. (2004) did not, as the slope of our schedule was steeper, perhaps giving the rats a better chance to demonstrate differences in motivation. Further research using varied PR schedules may clarify this question.

A likely explanation for the difference between ours and the Ruedi-Bettschen study is that those rats were not food deprived during testing and ours were, and, as mentioned above, increased motivation for food during food deprivation may reveal differences between groups that are revealed during our protocol but masked in the other protocol, which tests rats under conditions of satiety. Alternatively, these differences may emerge only with, or be accentuated by, the catalyst of the stress of food deprivation. Given the strong evidence from that study that isolation affects rats differently depending on the light/dark phase in which they are isolated, it may be that the effects of isolation on motivation are milder when rats are separated during the light phase, but nevertheless existent and observable when motivation is enhanced by food deprivation, as in this study.

Surprisingly, neither our isolated nor handled groups showed a significant increase in responding under the PR schedule of reinforcement when the reward was changed from one to two pellets. In fact, BPs in both groups declined through the three phases of testing, with isolated rats showing a greater decline, although not significantly, when reward was changed from two pellets back to one. In relation to the finding of Matthews, Wilkinson, et al. (1996) that RMS rats show impaired sensitivity to both positive and negative contrasts, one might expect to observe in our isolated rats a failure to increase responding for a greater reward, but this does not explain why our handled rats also fail to increase responding for a greater reward. It appears that under the conditions of this protocol rats do not experience a positive contrast between one and two 45-mg food pellets, food magnitudes that were chosen arbitrarily as a starting point in this initial study. The establishment of positive contrasts between two magnitudes of food reward under the PR schedule may require larger differences between the food levels, such as 1 versus 5 pellets, which we chose not to use in case of satiety effects. However, the greater decrease in isolated rats' responding as a function of changing reward from two pellets to one pellet, although not significant, suggests that neonatal isolation may enhance the effects of negative contrasts on reward-related responding. Further research is required to better address this possibility.

In summary, we found that neonatal isolation experience in rats resulted in a slowed acquisition of a food-rewarded operant response as well as reduced levels of operant responding under both FR and PR schedules of reinforcement. Although there are some differences between our findings and others which have also examined operant responding, a general picture is emerging that neonatal isolation under certain conditions leads to reductions in motivated behavior in relation to natural reward. This finding may have important implications for a link between separation experiences in human children and the development of depression in adulthood. It is possible that early separation experiences lead to a basic deficit in motivation or a vulnerability to impaired motivation in response to stress, or a combination of the two, which may account for some of the behavioral and subjective aspects of depression.

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