Effects of Neonatal Handling on Playfulness by Means of Reversal of the Desire to Play in Rats (*Rattus norvegicus*)

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Abstract: In the present study the authors sought to establish whether the range of effects of neonatal handling stimulation (H), that is, brief daily periods of infant isolation, could be extended to the domain of social motivation. With this aim, the authors studied the innate motivation to engage in rough-and-tumble play (R&T) in adolescent rats (Rattus norvegicus) by means of a reversal design, in which half of the rats were first housed in isolation (Days 1–3), and then in company (Days 4 – 6), while the other half followed the reverse sequence of housing conditions. Results showed in a clear-cut manner that H fuelled playfulness, as measured by pin and dorsal contact episodes, with (relative) independence of trait-based differences in fearful behavior between handled and nonhandled rats. Given that the different levels of the rat's social brain are apparently sensitive to tactile stimulation in infancy, the authors propose that the vibrant R&T reported here could reflect an enduring alteration of genetically based, motivational systems underlying playfulness and, perhaps, positive social emotions like joy.

Introduction

More than 50 years ago, Levine reported that neonatal handling stimulation (H), that is, brief daily isolation of infant rats improved avoidance learning and attenuated physiological responses to stress. He even guessed right a few years later that these effects could be the result of changes in maternal behavior caused by dam-litter separations (Levine, 1957, 1960, 2005; Smotherman et al., 1977). Experiments from this time period also showed that H could increase resistance to stress associated with starvation, a crude and extreme protocol that impressively revealed that handled rats lived longer than controls (Denenberg & Karas, 1959). Then, an important protective neuronal effect was reported (Meaney et al., 1988): H prevented age-related neuronal atrophy within the hippocampus, as well as preserved spatial learning capacities, which require the integrity of this limbic structure. Liu et al. (1997) found converg- ing evidence supporting the hypothesis that changes in mother–infant interaction could mediate the effects of H. As compared with control dams of undisturbed litters, the dams of handled litters displayed more active maternal behavior, that is, higher frequency of licking, grooming and arched-back nursing. Interesting to note, Liu et al. (1997) also observed that offspring of dams with high scores in active care behavior were less susceptible to stress in adulthood than those bred by their low-scoring counterparts. Thus, the profile of fearfulness of handled rats corresponded with that of offspring of dams spontaneously displaying active maternal care.

Such a parallelism strongly suggested that the active principle of H could be one aspect of maternal care: The quantity of careful tactile stimulation provided by the dams. Since the pioneering work of Levine, Denenberg and others in the 1950s and 60s, the antianxiety effects of H have been exhaustively studied from peripheral physiology to the

brain through different indices of hypothalamic-pituitary-adrenal (HPA) activity and a host of neu- rotransmitter systems (e.g., Anisman et al., 2002b; Levine, 2005).

Basic social emotions in mammals are ingrained in ancient (subcortical) brain systems, which appear to be sensitive to the long-lasting effects of H. There is proof, for example, that the opioid neurons within the periaqueductal gray, hypothalamus, and amygdala are enduringly influenced by H, and that opioids modulate odor-touch associations underlying infant attachment, thus providing plausible neurobiological bases to expect direct effects of H on socially motivated behaviors (e.g., Gustafsson et al., 2008; Irazusta et al., 1999; Nelson & Panksepp, 1998; Panksepp, 1998; Ploj et al., 2001; 2003; Roth & Sullivan, 2006). Many of the neurons in the limbic system work with opioids as well as with oxytocin and vasopressin, substances that are all believed to be major contributors to social bond formation and/or maintenance (Nelson & Panksepp, 1998; Panksepp, 1998; Pank-sepp et al., 1980; Winslow & Insel, 2004; Young & Wang, 2004). The marked docility and social curiosity of handled rats, in conjunction with the impact of H on various levels of the limbic system, led us to consider the possibility that H could facilitate social behavior. However, per- haps because of research bias toward fear-based emotions in the field of early experiences (as in others; e.g., Burgdorf & Panksepp, 2006), the extent to which appetitive social emotions could be promoted by H remains practically unknown, for example, the proclivity of juveniles of many species to engage in episodic bursts of rough-and-tumble play (R&T; e.g., Burghardt, 2005; Panksepp, Siviy, & Normansell, 1984; Pellis & Pellis, 1998; Thor & Hollo- way, 1984). To our knowledge, the assessment of H effects upon playful behavior in rats was attempted for the first time, without success, by Arnold and Siviy (2002), and for the second time, now successfully, by Siviy and Harrison (2008). To

explain high play- fulness in handled rats they proposed two interpretations in terms of low fearfulness. The first one attributed the increased playfulness in handled rats to greater habituation to human contact, so that nonhandled rats were more fearful during testing because of contact with (or to the presence of) the "unfamiliar" experimenter; and the second (favored by the authors) attributed the increased playfulness to lower susceptibility to fear in threatening situations relative to nontreated controls. However, these authors did not consider the possibility of a genuine change induced by H in the motivational systems underlying the desire to play, with independence of other systems such as those for fear or incentive seeking (Gray, 1987; Panksepp, 1998). If play motivation were enhanced by H in such a specific way, it would suggest that there exists an evolutionarily ancient motivational system devoted to fuelling self-reinforcing enjoyable behaviors, sensitive at critical periods to enduring neurobiological changes, which could be the fundamental motivational source of pleasant emotions like joy and even flow experience in humans, but definitive evidence that a drive to play operates in this unitary fashion is lacking (Burghardt, 2005).

In the present work, we sought to establish whether H influences the desire to play in adolescent rats. In an attempt to rule out alternative explanations based on trait-based differences in fear- fulness (or novelty-induced activity), handled and nonhandled rats were tested in pairs during six consecutive days, following a reversal design to manipulate (between- and within-subjects) the motivation to play. Thus, half of the rats were first housed in isolation (Days 1-3), and then in company (Days 4-6), while the other half followed the reverse sequence of housing conditions, from company (Days 1-3) to isolation (Days 4-6). In doing so, we think that motivation to play is being manipulated in two ways: If the time of isolation determines in part the present level of play motivation, then rats

satiated in the first phase will exhibit the strongest motivation to play under social deprivation in the second phase, and vice versa (between- and within- subjects manipulations of the desire to play); and, if H induces enduring changes in brain systems relevant to social motivation, then the motivation to play will be chronically stronger in handled rats as compared with their nonhandled counterparts (between-subjects manipulation of the desire to play). Thus, our main hypothesis is twofold: First, isolation will increase motivation to play in handled and nonhandled groups as compared with their corresponding satiated controls; second, handled rats will exhibit stronger motivation to play when individually housed (in the two phases), relative to nonhandled (isolated) rats. As play levels could be minimal in satiated rats, the possibility of detecting H effects on some aspect of play behavior in this (presumably flat) motivational state will be open. Finally, once the rats were 3 months old, they were evaluated in the elevated plus maze to confirm that the H protocol used had the characteristic, long-term antianxiety effects.

Results

The number of pins is shown in Figure 1. For the first phase (Days 1–3), ANOVA yielded significant handling, $F(1, 35) = 11.18$, $p < .01$, $h2 = .24$; isolation, $F(1, 35) = 74.50$, $p < .001$, $h2 = .68$; and handling × isolation, $F(1, 35) = 10.48$, $p < .01$, $h2 = .23$, effects, as well as days, $F(2, 70) = 5.65$, $p < .01$, $h2 = .14$, and isolation × days, $F(2, 70) = 5.22$, $p < .01$, $h2 = .13$, effects, indicating (respectively) that both handling and isolation contributed to increase pinning behavior (main effects), although particularly in handled-isolated rats (interaction effect), and that the number of pins increased over days in isolated rats. Post hoc Duncan's test comparisons indicated that H-ISO > SAT rats pinned more than all groups, and that NH-

ISO > SAT pinned more than the satiated groups along the first three days ($p < .05$). For the second phase (Days 4 – 6), ANOVA yielded significant handling, $F(1, 35) = 10.30$, $p < .01$, $h2 = .23$; isolation, $F(1, 35) = 39.62$, $p < .001$, $h2 = .53$; and handling × isolation, $F(1, 35) = 7.28$, $p < .01$, $h2 = .17$, effects, as well as days, $F(2, 70) = 26.89$, $p < .001$, $h2 = .43$, and isolation × days, $F(2, 70) = 32.25$, $p < .001$, $h2 = .48$; effects, and a statistical trend for handling × isolation × days, $F(2, 70) = 2.75$, $p = .071$, $h2 = .073$, effects, indicating (respectively) that both handling and isolation contributed to increase pinning behavior (main effects), although particularly in handled-isolated rats (interaction effect), and that the number of pins increased over days in isolated rats, particularly in those treated with H. Post hoc Duncan's test comparisons indicated that H-SAT > ISO rats pinned more than all other groups along all days, and that NH-SAT > ISO rats pinned more than the satiated groups on Days 5 and 6 ($p < .05$). It should be noted that the H-SAT > ISO group was unique at showing a noticeable increment in pins the day after reversal (t test for related samples, $p < .05$).

The number of dorsal contacts is shown in Figure 2. For the first phase (Days 1–3), ANOVA yielded significant handling, $F (1, 35) = 6.40$, $p < .05$, $h2 = .15$, and isolation, $F (1, 35) = 122.78$, $p < .001$, $h2 = .78$, effects, as well as isolation × days, $F(2, 70) = 4.05$, $p < .05$, $h2 = .10$, effects, indicating (respectively) that both handling and isolation contributed to increase dorsal contacts (main effects), and that the number of dorsal contacts changed over days in isolated rats. Post hoc Duncan's test comparisons indicated that H-ISO > SAT and NH-ISO > SAT groups displayed more dorsal contacts than the two satiated groups along the three days ($p < .05$), while H-ISO > SAT was superior to NH-ISO > SAT on the third ($p < .05$). For the second phase (Days 4 – 6), ANOVA yielded significant handling, $F(1, 35) = 7.10$, $p < .05$, $h2 = .17$, and isolation, $F(1, 35) = 53.48$, $p < .001$, $h2 = .60$, effects, as well as days, $F(2, 70) = 17.35$, $p < .001$, $h2 = .33$, and isolation × days, $F(2, 70) = 12.11$, $p < .001$, $h2$

= .26, effects, indicating (respectively) that both handling and isolation contributed to increase dorsal contacts (main effects), and that the number of dorsal contacts changed over days, particularly as a function of isolation (interaction effect). Post hoc Duncan's test comparisons indicated that H-SAT > ISO rats displayed more dorsal contacts than all other groups on Day 4 and, in conjunction with NH- SAT > ISO rats, were superior to satiated groups on Days 5 and 6 ($p < .05$), while NH-SAT > ISO rats tended to be superior to its corresponding satiated group on Day 4 ($p = .056$).

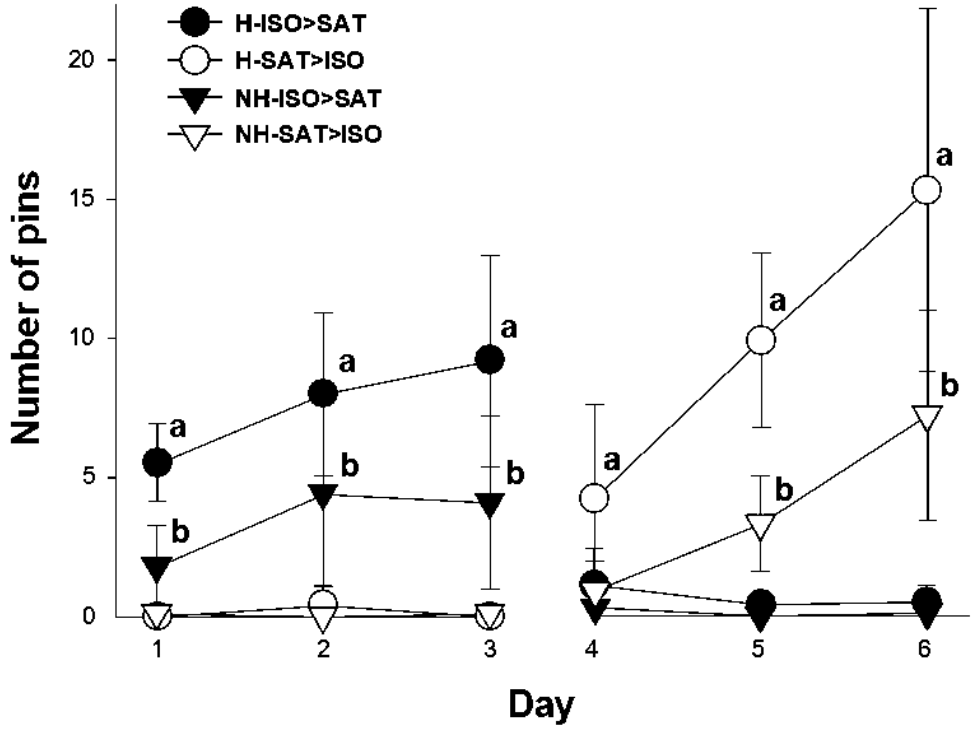

Figure 1. Mean (±95% confidence interval [CI]) of pins before (Days 1–3) and after (Days 4 – 6) reversal of housing conditions. Black circles are handled rats, which were first housed in isolation (Days 1–3) and then in company (Days 4 – 6 [H-ISO > SAT]), while white circles are handled rats, which were first housed in company (Days 1–3) and then in isolation (Days 4 – 6 [H-SAT > ISO]). Black triangles are nonhandled rats, which were first in isolation (Days 1–3) and then in company (Days 4 – 6 [NH-ISO > SAT]), while white triangles are nonhandled rats, which were first in company (Days 1–3) and then in isolation (Days 4 – 6 [NH-SAT > ISO]). H = handled; NH = nonhandled; ISO = housed in isolation; SAT = housed in pairs; > = reversal of prior housing conditions. a = versus all groups, $p < .05$; b = versus the two satiated groups, $p < .05$ (Duncan's test).

In addition, the number of pins was divided by the number of dorsal contacts displayed

in the last play session (i.e., the sixth, when play rates were highest), to obtain a measure of the proba- bility that a rat would exhibit pinning responding, relative to the frequency of dorsal contacts. ANOVA yielded handling, $F(1, 34) = 5.60$, $p < .05$, $h2 = .14$, and isolation, $F(1, 34) = 11.06$, $p < .001$, $h2 = .48$, effects, as well as a statistical trend for handling × isolation, $F(1, 34) = 3.63$, $p = .065$, $h2 = .096$, effects, indicating that both handling and isolation contributed to increase the probability of pins (main effects), particularly in handled-isolated rats (interaction effect). Post hoc Duncan's test comparisons indicated that the probability of pins was higher for H-SAT > ISO rats (mean ± 95% confidence interval [CI]: 0.462 ± .205) than in the other groups (NH-SAT > ISO: 0.221 ± .115; H-ISO > SAT: 0.040 ± .053; NH-ISO > SAT: 0.014 ± .032), as it was for NH-SAT > ISO rats relative to the two satiated groups ($p < .05$). Similarly, a measure of the probability to display defensive playful reactions only in response to playful solicitations/attacks (i.e., pins following dorsal contacts) was obtained by reanalyzing the videotapes of the last play session for H-SAT > ISO and NH-SAT > ISO groups (Reinhartet al., 2006). ANOVA yielded a main effect of handling, $F(1, 18) = 4.73$, $p < .05$, $h2 = .21$, the H-SAT > ISO rats being more prone to engage in defensive pins (0.185 ± .079) than their NH-SAT > ISO counterparts (0.092 ± .056). It must be noted that the correlation between the two obtained measures (number of pins divided by number of dorsal contacts vs. number of pins displayed only in response to dorsal contacts divided by number of dorsal contacts) was moderately high ($r = .73$, $p < .001$), suggesting that the two could measure the same thing, and so, that the simplest to obtain could be routinely used in future studies (if needed; i.e., number of pins divided by number of dorsal contacts).

With the aim of taking a global perspective on H and isolation effects, we calculated mean scores of pins and dorsal contacts across days, by grouping rat pairs sharing the same experimental condition; thus, we formed a general experimental condition of H-ISO rats (n =

20 pairs), grouping H-ISO > SAT rats from the first phase (10 pairs) with H-SAT > ISO rats from the second phase (10 pairs), as they had in common that they were tested when housed individually; the same was done for the rest of groups (H-SAT, n = 20, NH-ISO, n = 19 and NH-SAT, n = 19). The results for pins and dorsal contacts are shown in Figures 3 and 4, respectively. For pins, ANOVA yielded significant handling, $F(1, 74) = 20.14$, $p < .001$, $h2 = .21$; isolation, $F(1, 74) = 98.24$, $p < .001$, $h2 = .57$; and handling × isolation, $F(1, 74) = 15.94$, $p < .001$, $h2 = .18$, effects, indicating (respectively) that both handling and isolation contributed to increase pinning behavior (main effects), particularly in handled-isolated rats (interaction effect). For dorsal contacts, the same analysis showed significant handling, $F(1, 74) = 13.42$, $p < .001$, $h2 = .15$, and isolation, $F(1, 74) = 157.87$, $p < .001$, $h2 = .68$, effects, indicating that both handling and isolation contributed to increase dorsal contacts (main effects). Post hoc Duncan's test comparisons indicated, for both pin and dorsal contact episodes, that H-ISO rats were more playful than the rest of the groups ($p < .05$) and that NH-ISO rats were more playful than the two satiated groups ($p < .05$); in addition, H-SAT rats exhibited more dorsal contacts than the satiated nonhandled group ($p < .05$). Thus, these results confirm that handling in isolated rats fuelled playfulness to the maximum level relative to the rest of the groups.

Open arm behavior in the elevated plus maze is shown in Table 1. As two rats from each condition fell off the maze during the test, the final sample was of 14 handled rats and 15 nonhandled rats. ANOVA yielded handling effects for number of entries in the open, $F(1, 27) = 4.93$, $p < .05$, $h2 = .15$, and enclosed, $F(1, 27) = 13.64$, $p < .001$, $h2 = .34$, arms as well as for time spent in the open, $F(1, 27) = 5.87$, $p < .05$, $h2 = .18$, and enclosed, $F(1, 27) = 6.39$, $p < .05$, $h2 = .19$, arms: t test comparisons confirmed that all indices of fearfulness in this test converged to show that handled rats were less fearful than controls ($p < .05$). Tentatively, the

relationship between open arm behavior and play was explored (using a small sample from different experimental conditions, n = 27), to see whether individual differences in fearfulness in adult- hood could correlate with play rates in adolescence on the first day of testing, when fear to the situation was expected to be stronger.

Table 1
Mean (±95% Confidence Interval) of the Various Measures Taken from the Elevated Plus Maze

	Open arm entries	Time spent in the open arms	Enclosed arm entries	Time spent in the enclosed arms
Nonhandled	0.1 (0.1)	2.1 (4.4)	2.6 (1.1)	270.7 (22.2)
Handled	1.8 (1.7)	24.8 (20.4)	5.2 (1.1)	228.4 (28.7)

Note. Handled ($n = 14$) and nonhandled ($n = 15$) groups differed in all the measures, $p < .05$ (t test for independent samples).

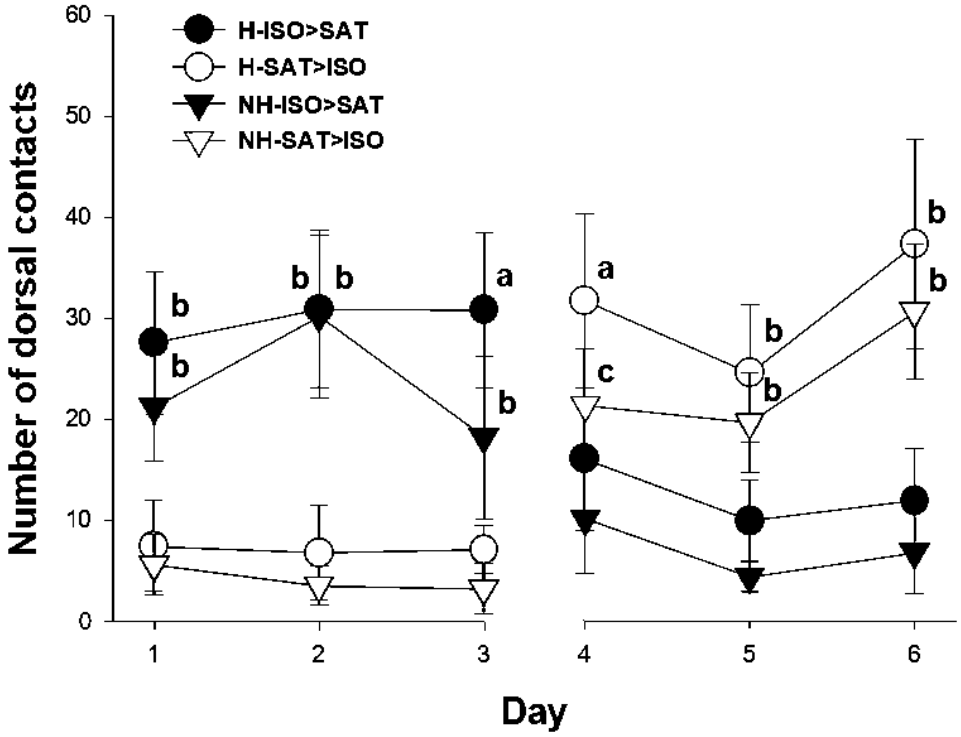

Figure 2. Mean (±95% confidence interval [CI]) of dorsal contacts before (Days 1–3) and after (Days 4 – 6) reversal of housing conditions. Black circles are handled rats, which were first housed in isolation (Days 1–3) and then in company (Days 4 – 6 [H-ISO > SAT]), while white circles are handled rats, which were first housed in company (Days 1–3) and then in isolation (Days 4 – 6 [H-SAT > ISO]). Black triangles are nonhandled rats, which were first in isolation (Days 1–3) and then in company (Days 4 – 6 [NH-ISO > SAT]), while white triangles are nonhandled rats, which were first in company (Days 1–3) and then in isolation (Days 4 – 6 [NH-SAT > ISO]). H = handled; NH = nonhandled; ISO = housed in isolation; SAT = housed in pairs; > = reversal of prior housing conditions. a = versus all groups, $p < .05$; b = versus the two satiated groups, $p < .05$; c = versus its corresponding satiated group, $p = .056$ (Duncan's test).

Correlational analyses showed that the number of open arm entries and time spent in them were independent of pins and dorsal contacts (from r = .06 to .13, p > .50). To explore correlations within separated groups, the same analyses were applied to the sample of isolated rats (n = 12) again yielding nonsignificant correlations (from r = .00 to -.20, p > .52), as well as to the sample of satiated rats (n = 15), giving rise to a significant positive correlation for the number of dorsal contacts with open arm entries (r = .53, p < .05; and r = .44, p = .101, with time spent in them); apparently, this moderate correlation indicates that the higher the number of dorsal contacts on the first day of play testing, the higher is the proclivity to visit the open

arms of the plus maze in adulthood, but only when rats were satiated, since the relationship dissipated under social deprivation; however, significant correlations did not emerge between pinning and open arm behaviors in these satiated rats (from r = -.10 to -.12, p > .67). Given the small sample of rats used, these analyses show (if anything) that behavior in the plus maze did not relate to pinning behavior, while dorsal contacts under satiation (i.e., flat motivational state to play) could be related to open arm activity in the plus maze, as the two types of behavior share a common locomotor activity component (play motivation being under satiation of no importance).

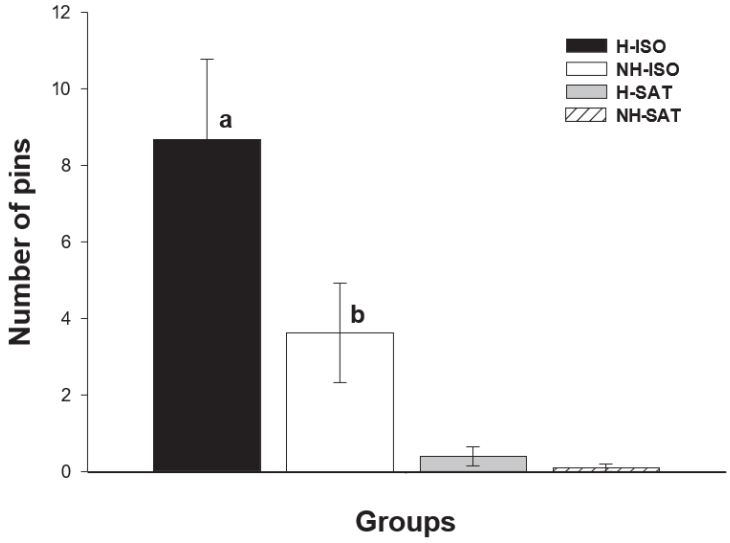

Figure 3. Mean (±95% confidence interval [CI]) of the sum of pins through all sessions, by grouping rat pairs sharing the same experimental condition on Days 1–3 and Days 4 – 6. The black bar (handled-isolated rats [H-ISO], n = 20) is the mean number of pins displayed in isolation for H-ISO > SAT (Days 1–3, n = 10) and H-SAT > ISO (Days 4 – 6, n = 10) rats, considered as a single group. The white bar (nonhandled-islolated rats [NH-ISO], n = 19) is the mean number of pins displayed in isolation for NH-ISO > SAT (Days 1–3, n = 10) and rats NH-SAT > ISO (Days 4 – 6, n = 9) rats, considered as a single group. The gray bar (handled satiated rats [H-SAT], n = 20) is the mean number of pins displayed in company for H-SAT > ISO (Days 1–3, n = 10) and H-ISO > SAT (Days 4 – 6, n = 10) rats, considered as a single group. The striped bar (nonhandled-satiated rats [NH-SAT], n = 19) is the mean number of pins displayed in company for NH-SAT > ISO (Days 1–3, n = 9) and NH-ISO > SAT (Days 4 – 6, n = 10) rats, considered as a single group. a = versus all groups, $p < .05$; b = versus the two satiated groups, $p < .05$ (Duncan's test).

The number of defecations (divided by two to compare with other tests) across days of testing did not vary between- or within- groups in the playfulness experiment (mean range = 0 - 0.4), suggesting that fear in this test was minimized. The level of fear that these rats could exhibit in different situations can be tentatively inferred from the following data on

defecations. The mean score (±SEM) for the total sample (n = 78) of the playfulness experiment was 0.08 ± 0.02; that of the rat subsample evaluated two months later in the plus maze (n = 29) was 1.3 ± 0.3; and that obtained from the open field arena (n = 75) was 3.4 ± 0.3. Because juveniles hardly defecated in the playfulness experiment, we evaluated them in the open field with the mere purpose of measuring defecations under a presumably more aversive situation. Coherently, rats defecated markedly more than in the play chamber

Discussion

Using a between-groups design with a within-subject reversal of housing conditions, we have shown here that a genetically based social behavior (R&T) was enhanced by H, a form of mild (early) stimulation, the main active principle of which probably is gentle physical contact, or touch, during infancy. Whereas H appeared to have a modest, though significant, effect on dorsal contacts across days, in both the pre- and post-reversal phases of the experiment, the evidence was clear-cut in the case of pinning behavior, in showing that handled-isolated rats systematically pinned more than all the groups across days and/or phases. These rats displayed an always-growing pattern of episodic bursts of vibrant and invig- orated R&T. In fact, the group of H-SAT > ISO rats was unique in showing a noticeable increment in pins the day after reversal and, as the global analyses revealed, H duplicated the proclivity to engage in R&T in isolated rats, even increasing playful solicitations in the satiated ones, what argues in favor of the robustness and magnitude of the effect. More direct measures of playful reactivity, such as the probability of responding to playful attacks (dorsal contacts) by displaying complete rotations to actively cope with the attacker (pins), consistently revealed clear differences between handled and nonhandled rats.

Anecdotal blind observations also suggested that certain aspects of the behavior of the handled isolated rats (i.e., handled rats strongly motivated to play) could be tentatively described as agitation, vibration, vigor, and perhaps, excitement or joy. Furthermore, it is worth noting that the range of episodes of pins and dorsal contacts recently reported by Siviy and Harrison (2008) was practically identical to that obtained in the present work, regardless of the fact that we used a different strain of rats (Wistar rats instead of Sprague-Dawleys) and a larger play chamber. Other procedural differences between the two studies were that H was done twice daily (Aguilar et al., 2002b), that the infants remained isolated during maternal separations (as originally done by Levine, 1960), and that the treatment lasted 18 days rather than 14 (originally 21). It is difficult to know whether these procedural differences could have had a differential impact on playfulness, although we suspect that our procedure exploits the tactile stimulation component better, and this component appears to be critical according to previous studies (Liu et al., 1997). We finished 3 days before weaning because we had observed in previous H experiments (this one included) that on this day a handful of very mischievous infants begin to climb onto the edge of our isolation cages (18 cm high). Interesting to note, this is the developmental point at which playful behavior appears to emerge, at least in nonhandled rats, although we suspect that the emergence of playfulness, like other genetically dictated maturing processes, is surely accelerated by H (a question still open to study). That our H treatment had the typical long-term antianxiety effects was shown by the increased number and duration of open arm visits of the elevated plus maze in handled rats, as compared with nontreated controls, when they were 3 months old.

Given the sensibility and fragility of playful behavior to various motivationally

aversive states (e.g., hunger or fear), some could reasonably argue that individual differences in fearfulness caused by H could account for the present results, as Siviy and Harrison (2008) were compelled to do in their own play experiment. Be- cause that experiment was part of a study exploring the usefulness of R&T as an index of fear of predators, we will briefly discuss exclusively the "play" part here, that is, prior to the phase when juveniles were exposed to cat odor. To explain enhanced playful- ness in handled rats they proposed two "hydraulic" interpretations, in terms of reduced fearfulness (i.e., when fear goes down play goes up). The first interpretation attributed the effects to the differential history of rat-experimenter contact, but it was partially excluded because "elevated levels of pinning in the handled rats stayed fairly constant throughout" (p. 5), a result incongruent with the hypothesis of gradual habituation to human contact, that would nonhandled rats and, thus, dissipation of group differences with repeated testing. The second, more general, explanation suggested that "handled rats may consistently perceive the testing environment as less threatening than nonhandled rats, perhaps making them more likely to engage in playful behaviors" (p. 5). Both explanations, therefore, make playfulness dependent on fearfulness. Though when we performed our experiment (November 2007) Siviy and Harrison's work had not been published yet, we attempted to circumvent exactly the kind of rival interpretations they had to deal with, and thus we applied a reversal design inspired by the study of Panksepp and Beatty (1980). With this design we attempted to prevent, at least in part, the influence of trait-based differences in fearfulness and novelty-induced activity, by manipulating the motivation to play between- and within- subjects.

For example, if fear of the experimenter and/or the situation had interfered with playful behavior, then NH-SAT > ISO rats would have exhibited more pins and dorsal

contacts in the second phase of the experiment than NH-ISO > SAT rats in the first phase, because in the second phase such fear was expected to diminish; additionally, if novelty-induced fear was involved, then group differences in defecations would be expected, particularly at the beginning of testing. Data did not fit these fear-based predictions. In this regard, it is important to note that, generally, H attenuates fear of actual and/or potential threat, but in the absence of threat, as when rats are in their home-cages (habituated to background stimulation), should we expect there to be any antianxiety H effects? Probably we should not. The same could be applied to the safe and fun play chamber located in the adjacent experimental room. Suppose that there was no threat in it: Would there be room for a significant antianxiety H effect? Again, we believe that there would not (i.e., floor effect for an anxiolytic-like action).

Recent preliminary results from our lab, evaluating nonhandled rats under a testing regime of seven consecutive days in isolation, indicate that the plateau level of pinning behavior is attained sooner than expected (in less than three days), suggesting that in a couple of days the influence of inadvertent fear-provoking stimuli in non- treated rats could be already minimized (unpublished data). And in the present study we have seen those relevant relationships between fearfulness in the plus maze and playfulness the first day of testing (when novelty/threat was highest) did not emerge. So, we believe that it is difficult to support with these findings that handled rats exhibited higher rates of R&T than nontreated controls because of lower fearfulness. One could still argue however that hypothetical differences between handled and nonhandled rats in their relative levels of “perceived” threat can always be present (as they are impossible to remove), affecting play via circuitous routes through fear-based mechanisms. Wouldn’t this be an example of a fear- biased argument

influenced by previous interpretations of H effects? The rival hypothesis would address the question in this alternative way: Could H affect the motivational systems for social behavior directly? Given that the skin surface represents the greatest (and probably, the most important) sensory organ of the rat neonate, in conjunction with the fact that it shares the embryologic origin of brain tissue, as well as that physical contact has well known comforting (the pleasure of touch) and calming effects after separation stress, it should not be surprising that long-term neuro- biological changes in the core of the rat's socio-affective brain could result as a consequence of tactile stimulation in infancy. Furthermore, one could argue that handled rats exhibited higher R&T because of differences in novelty-induced activity: that is, because of some form of unspecific (affectively appetitive) excitement, or "good arousal," induced by the fact of being transported 5 min daily to a fun situation (relative to normal housing), a wide and safe arena in darkness, suitable for chasing partners. If enhanced R&T in H-ISO > SAT rats during the first phase, and in H-SAT > ISO rats during the second phase, was solely a matter of "good arousal," then H-ISO > SAT rats are also expected to manifest (through increased R&T) an equivalent amount of excitement in the second phase, particularly on Day 4, as they would probably have learned well in the first phase that they were being transported to the play zone in the second phase. Data did not fit this possibility. These rats appeared to exhibit a specific, enhanced motivation for amicable contact and play when individually housed. If this "good arousal" is neither fear (fear interferes with R&T) nor unspecific excitement (if it depends on social deprivation, then it is specific), what is it? To us, the most parsimonious explanation is that such excitement must be an intense desire to play.

As Burghardt (2005) points out, one of the main issues in current research on play

motivation has to do with this question: “Is play controlled by a separable or unitary motivational (or behavioral) system for playful behavior that is separate from the internal states underlying serious performance?” (p. 136). Though previous research has provided interesting findings on the complex relationship between play and other motivational systems operating concurrently (e.g., hunger or aggression), “what we need are more detailed observations of play from the viewpoint of the various systems involved within what seems to be a single kind of play, such as play fighting” (p. 137). This approach has already given good dividends by permitting the distillation of the amicable character of R&T, from detailed analyses of the targets of bodily contacts or, more recently, of ultrasonic vocalizations, thus over- coming prior misconceptions based on superficial similarities with aggressive behaviors (Burghardt, 2005; Knutson et al., 2002; Pellis & Pellis, 1998).

Despite many years of research, however, definitive evidence that there exists a specific drive that operates like the primary motivational source of playful behavior is lacking. In this regard our findings can be theoretically important because they suggest the existence of such a unitary motivational system, from which the boisterous and invigorated R&T, as other variants of adult amicable behavior, would emerge. This system for the desire to play, apparently sensitive to physical contact at critical periods, would be devoted to fuelling self- reinforced enjoyable behaviors (i.e., those done for their own sake), and could be the motivational source of engrossing pleasant emotions like joy and even flow experience in humans (Burghardt, 2005; Csikszentmihalyi, 1999; Panksepp, 1998).

In fact, Panksepp et al. (1984) already proposed that mammals are born with this innate impulse to play. They observed in laboratory rats that R&T followed a well-defined developmental curve, beginning 18 days after birth, peaking between 30 and 45 days and

decaying at the end of adolescence. That this was not a learned behavior was demonstrated by impeding juveniles to play between Days 18 – 40, and when the restriction was abolished the characteristic pattern of invigorated R&T clearly emerged. That play acted as a natural (innate) reinforcer was also shown by the fact that juveniles learned to solve T-maze discriminations to have access to a playful partner. There is recent evidence indicating that R&T is accompanied by affective (50-kHz) vocalizations, and that this kind of rodent "laughter" can be psychogenetically selected by intercrossing rats with extreme scores in that trait, a putative psychogenetic rat model of human joy (Burgdorf et al., 2005; Knutson et al., 2002; Panksepp, 2005). Therefore, playfulness appears to be apt for psychogenetic experiments, as has been largely established for the trait of fearfulness (e.g., Escorihuela et al., 1999; Fernández-Teruel et al., 2002a; Gray, 1987). In this context, it is interesting to note that after receiving H, the fearful Roman low-avoidance rats, selected for poor 2-way shuttlebox avoidance performance, became behaviorally closer to (and in some cases, undistinguishable from) their fearless Roman high-avoidance counterparts, in a number of tests of fear and/or novelty-induced activity (e.g., Aguilar et al., 2002; Fernández-Teruel et al., 1997). Could the low-joy rats of Burgdorf et al. (2005) become closer to their high-joy counterparts by means of H? That is to say, could early experience reverse the unhappy profile of rats, so that they were more playful in adolescence and happier in adulthood? We have seen here that playful behavior effectively increases as a result of H in common rat strains (and we predict in passing that they will emit more laughing vocalizations in future studies), so that the first empirical step toward such theoretical possibilities has firm bases. Furthermore, it has been argued that sessions of R&T could facilitate maturation of the social brain, as well as attenuate some forms of excessive behavioral disinhibition in rats, reminiscent of some symptoms of children with ADAH (Panksepp, 1998, 2007). Because

we, like Siviy and Harrison (2008), have found that H increases R&T, and others that H strengthens latent inhibition (i.e., improves attention capacities; Feldon & Weiner, 1988; Peters, Gray, & Joseph, 1991), as well as reduces overall activity in some rat strains and tests (e.g., Colorado et al., 2006), perhaps nonhandled rats could serve as a rat analogue of child ADAH, when compared with their H-treated counterparts, which in this putative rodent model would act as a normal control group.

In summary, increased mother–infant interaction after separation stress could mediate the effects of H, as the dams of handled litters show more active maternal behavior, that is, high frequency of licking, grooming and arched-back nursing (Liu et al., 1997). The profile of fearfulness of handled rats is similar to that of the offspring of dams with high scores in active care behavior, suggesting that the active principle of H could be the amount of careful tactile stimulation provided by the dams. Negative correlations between the amount of care received and adult HPA- activity to stress rounded up the conclusion. The authors proposed congruently that through maternal care (early physical contact) dams were "behaviorally" programming the emotional brain of their progeny. That handled rats were less fearful and recovered faster than controls after stressor termination was explained in part by the fact that they presented more receptors of stress hormones in the hippocampus (the upper part of the HPA-axis), which could explain why they had a more efficient negative feedback system, and so were less susceptible to fear.

To date, a large body of evidence has been accrued demonstrating that some critical neuroendocrine systems underlying fearful behavior are permanently altered by H. In parallel with fear, we propose here that the same can be true for some components of the neuroendocrine systems mediating social behaviors such as R&T. However, more research is

needed to evaluate this possibility, provided that H-induced plasticity of some neurotransmitter systems relevant to social bonding has only recently begun to be studied (mainly opioids), whereas behaviors motivated by social emotions like R&T remain practically unexplored. In the same way that H has proved to be a useful tool for learning about the brain mechanisms of fear and stress (e.g., suggesting a key role for the hippocampus in emotion beyond cognition), profitable lines of research could be devoted to the study of the brain mechanisms underlying H-induced increments in R&T. As Panksepp states (1998):

> The fact that social deprivation increases the desire to play . . . suggests that it should be possible to produce such a state artificially. Only when someone has found a way to turn on play pharmacologically will we have achieved a profound neural understanding of playfulness . . . (p. 294).

Here we have found a way to turn on play through tactile stimulation in infancy, so that the next step should be to determine the neurobiological processes that fuel play motivation in these rats. Because characterization of the neural templates of playful- ness has shown to be rather elusive, more than for any other basic emotion, perhaps we could benefit by redirecting research efforts to the brain sites or systems affected by H in the search of new clues.

Materials and Methods

Subjects

The subjects were 78 male Wistar rats (Rattus norvegicus), coming from 16 litters

(10 to 13 male-female pups per litter), born and maintained at the Universidad de Málaga. The litters were housed with plentiful bedding material for nest building (sawdust blended with tiny bits of newspaper). After weaning (postnatal Day 22), the rats were group-housed (3 to 5 per cage: 42 × 26 × 18 cm) and maintained with food and water available ad lib, under conditions of controlled temperature (21°C to 22°C) and a light- dark schedule of 12 h each (lights on at 9 a.m.). When the experiment on playfulness began, half of the rats were housed in pairs and the other half in isolation. Thereafter, all rats remained in pairs.

Apparatus

To evaluate playful behavior in adolescent rats we used a square metal structure (80 × 80 × 30 cm) painted black, with the arena covered with abundant sawdust (3 cm deep). The unique source of illumination in the experimental room was a faint light from a 25 W red bulb mounted in a metallic frame, placed on the edge of (and outside) the walls of the apparatus, focusing obliquely on the center of the arena. Also, we used a wooden open field (90 × 90 × 37 cm) painted white and illuminated with a 200 W bulb to measure defecations in a more threatening environment. To evaluate fearful behavior in adult rats we used a wooden (elevated: 80 cm high) plus maze painted white, consisting of a cross (10 × 10 cm) with four arms (50 × 10 cm), as the sign plus, one pair of opposing arms with walls (40 cm high), and the other (unprotected) pair without walls. The experiments were recorded with a camera mounted above the apparatus.

Apparatus

On postnatal Day 1, the H treatment started. It consisted of removing first the mother and then the pups from the nest twice daily (the first time between 10:30 and 12:00, and the second time between 19:30 and 21:00). Sessions of H consisted of first separating each mother and then placing the pups individually in plastic cages (42 × 26 × 18 cm) lined with paper towel for a total period of 10 min, each pup being gently handled by the experimenter from the head to the tail with his forefinger for 5 s (one stroke per second). After 5 min in this situation, each pup was again handled for 5 s and returned to the same cage for the remaining 5 min. At the end of the 10-min period, each pup was gently handled for another 5 s and then

returned to its homecage. When all the pups from one litter were back in their homecage, the mother was returned to the homecage. The same procedure was repeated in the evening (second time). Eight litters were treated with H until postnatal Day 18 (3 days before weaning).

Behavioral assessment began when the youngest rat was 28 days old (the oldest one was at this time 31 days old). Rats were weighed just before they were housed according to their experi- mental condition, with 20 handled rats housed in pairs (H-SAT = 10 pairs of play-"satiated" handled rats; mean weight = 91 g) and 20 in isolation (H-ISO = 10 pairs of "isolated" handled rats: 88 g). Furthermore, 18 nonhandled rats were housed in pairs (NH-SAT 9 pairs: 88 g) and 20 in isolation (NH-ISO = 10 pairs: 84 g). Whichever the housing condition, rats were tested identically, in pairs formed by littermates (when possible) and in a counterbalanced manner. When the second rat of a pair was placed on the opposite corner of the arena, the 5-min period of play testing began. The number of pin episodes (when a rat lied down on its back with at least three paws elevated, with its partner's forepaws on its belly) and of dorsal contacts (when a rat contacted the skin surface on and within 2 cm of the partner's nape with its snout or forepaws) were scored as indices of playfulness. When the 5 min had elapsed, the rats were picked up from the arena and defecations measured and cleaned up. The rats were then returned to their homecages during an intertrial interval of 24 h. The same experimental routine was repeated the following 2 days. After testing on Day 3, the 39 rat pairs were assigned to the reverse housing conditions, that is to say, those that were in pairs in the first phase were housed individually in the second phase (i.e., from satiation to isolation: SAT > ISO), and those previously isolated were now housed together with their corresponding play partners (i.e., from isolation to satiation: ISO > SAT): thereby, Days 4, 5 and 6 constituted a within-subject reversal of prior housing conditions, and so, of play deprivation. Testing order for the four groups was again counterbalanced, so that rat pairs were evaluated at different hours in the two experimental phases. The six consecutive days of testing took place in the light phase of the 12 hours light– dark cycle, between 10:30 and 20:30. For convenience, the groups were named as H-SAT > ISO if they had received H and were in company (i.e., satiated) during the first phase (Days 1–3), and in isolation during the second phase (Days 4 – 6); or as H-ISO > SAT if they belonged to the complementary group. The same logic was followed to label nonhandled groups (NH-SAT > ISO and NH-ISO >

SAT). Two days after the end of the play experiment, open field defecations were registered with the purpose of taking a measure of fear from a presumably more threatening situation than the play chamber, so that comparisons of relative levels of fear could be made between tests. Two months later, a subsample of 33 rats was evaluated in the elevated plus maze, 16 treated with H (mean weight = 346 g) and 17 controls (350 g), from which the number of entries in each type of arm and the time spent in them during a 5-min period were measured. Testing began when the rats were placed in the center of the apparatus, facing an enclosed arm. The experimental room was illuminated by several fluorescent lights. The experiment was conducted during the illuminated phase of the light– dark cycle.

Interrater reliability correlations (between R.A. and J.M.C.) for pins, dorsal contacts, number of open and enclosed arm entries, time spent in the open and enclosed arms were (respectively) $r = .97, .87, .99, .98, .98$, and $.99$, all $p < .001$, $n = 100$ for observations of play (at least 15 for day of testing) and $n = 29$ for those of fear. Measurements were conducted in a blind manner.

Statistical analysis

A repeated-measures between-groups experimental design was used in the playfulness experiment. Separate three-way analyses of variance (ANOVAs; between-subjects factors: handling × isolation), with days as a within-subject factor, were applied to pins and dorsal contacts, the first for Days 1–3 and the second for Days 4 – 6. Duncan's multiple-range tests (for between-groups comparisons) and t test (for related samples) were applied after significant ANOVA results.

A between-groups experimental design was employed in the fearfulness experiment. The number of entries in each type of arm and the time spent in them were analyzed by one-way ANOVAs, and t tests (for independent samples) were applied for comparisons between groups after significant ANOVA results.

References.

Aguilar, R., Escorihuela, R. M., Gil, L., Tobeña, A., & Fernández-Teruel, A. (2002). Differences between two psychogenetically selected lines of rats in a swimming pool matching-to-place task: Long-term effects of infantile stimulation. Behavior Genetics, 32, 127–134.

Anisman, H., Zaharia, M. D., Meaney, M. J., & Merali, Z. (1998). Do early-life events permanently alter behavioral and hormonal responses to stressors? International Journal of Developmental Neuroscience, 16, 149 –164.

Arnold, J. L., & Siviy, S. M. (2002). Effects of neonatal handling and maternal separation on rough-and-tumble play in the rat. Developmental Psychobiology, 41, 205–215.

Burgdorf, J., & Panksepp, J. (2006). The neurobiology of positive emotions. Neuroscience and Biobehavioral Reviews, 30, 173–187.

Burgdorf, J., Panksepp, J., Brudzynski, S. M., Kroes, R., & Moskal, J. R. (2005). Breeding for 50-kHz positive affective vocalization in rats. Behavior Genetics, 35, 67–72.

Burghardt, G. M. (2005). The genesis of animal play: Testing the limits. Cambridge, MA: MIT Press.

Colorado, R. A., Shumake, J., Conejo, N. M., Gonzalez-Pardo, H., & Gonzalez-Lima, F. (2006). Effects of maternal separation, early handling, and standard facility rearing on orienting and impulsive behavior of adolescent rats. Behavioral Processes, 71, 51–58.

Csikszentmihalyi, M. (1999). If we are so rich, why aren't we happy? American Psychologist, 54, 821– 827.

Denenberg, V. H., & Karas, G. G. (1959). Effects of differential infantile handling upon weight gain and mortality in the rat and mouse. Science, 130, 629 – 630.

Escorihuela, R. M., Fernández-Teruel, A., Gil, L., Aguilar, R., Tobeña, A., & Driscoll, P. (1999). Inbred Roman high- and low-avoidance rats: Differences in anxiety, novelty-seeking, and shuttlebox behaviors. Physiology and Behavior, 67, 19 –26.

Feldon, J., & Weiner, I. (1988). Long-term attentional deficit in non- handled males: Possible involvement of the dopaminergic system. Psychopharmacology, 95, 231–236.

Fernández-Teruel, A., Escorihuela, R. M., Castellano, B., González, B., & Tobeña, A. (1997). Neonatal handling and environmental enrichment effects on emotionality, novelty/reward seeking, and age-related cognitive and hippocampal impairments: Focus on the Roman rat lines. Behavior Genetics, 27, 513–526.

Fernández-Teruel, A., Escorihuela, R. M., Gray, J. A., Aguilar, R., Gil, L., Giménez-Llort, L., et al. (2002a). A quantitative trait locus influencing anxiety in the laboratory rat. Genome Research, 12, 618 – 626.

Fernández-Teruel, A., Giménez-Llort, L., Escorihuela, R. M., Gil, L., Aguilar, R., Steimer, T., et al. (2002b). Early-life handling stimulation and environmental enrichment: Are some of their effects mediated by similar neural mechanisms? Pharmacology Biochemistry and Behavior, 73, 233–245.

Gray, J. A. (1987). The psychology of fear and stress. London: Cambridge University Press.

Gustafsson, L., Oreland, S., Hoffmann, P., & Nylander, I. (2008). The impact of postnatal environment on opioid peptides in young and adult male Wistar rats. Neuropeptides, 42, 177–191.

Irazusta, J., Tejedor-Real, P., Varona, A., Costela, C., Gilbert-Rahola, J., & Casis, L. (1999). Effect of neonatal handling on brain enkephalin- degrading peptidase activities. Neurochemistry International, 35, 357– 361.

Knutson, B., Burgdorf, J., & Panksepp, J. (2002). Ultrasonic vocalizations as indices of affective states in rats. Psychological Bulletin, 128, 961– 977.

Levine, S. (1957). Infantile experience and resistance to stress. Science, 126, 405.

Levine, S. (1960). Stimulation in infancy. Scientific American, 202, 880 – 886.

Levine, S. (2005). Developmental determinants of sensitivity and resistance to stress. Psychoneuroendocrinology, 30, 939 –946.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., et al. (1997). Maternal care,

hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science, 277, 1659 – 1662.
Meaney, M. J., Aitken, D. H., van Berkel, C., Bhatnagar, S., & Sapolsky, R. M. (1988). Effect of neonatal handling on age-related impairments associated with the hippocampus. Science, 239, 766 –768.
Nelson, E. E., & Panksepp, J. (1998). Brain substrates of infant–mother attachment: Contributions of opioids, oxytocin, and norepinephrine. Neuroscience and Biobehavioral Reviews, 22, 437– 452.
Panksepp, J. (1998). Affective neuroscience: The foundations of human and animal emotions. New York: Oxford University Press.
Panksepp, J. (2005). Beyond a joke: From animal laughter to human joy? Science, 308, 62– 63.
Panksepp, J. (2007). Can PLAY diminish ADHD and facilitate the con- struction of the social brain? Journal of the Canadian Academy of Child and Adolescent Psychiatry, 16, 57– 66.
Panksepp, J., & Beatty, W. W. (1980). Social deprivation and play in rats. Behavioral and Neural Biology, 30, 197–206.
Panksepp, J., Herman, B. H., Vilberg, T., Bishop, P., & DeEskinazi, F. G. (1980). Endogenous opioids and social behavior. Neuroscience and Biobehavioral Reviews, 4, 473– 487.
Panksepp, J., Siviy, S., & Normansell, L. (1984). The psychobiology of play: Theoretical and methodological perspectives. Neuroscience and Biobehavioral Reviews, 8, 465– 492.
Pellis, S. M., & Pellis, V. C. (1998). Play fighting of rats in comparative perspective: A schema for neurobehavioral analyses. Neuroscience and Biobehavioral Reviews, 23, 87–101.
Peters, S. L., Gray, J. A., & Joseph, M. H. (1991). Pre-weaning non- handling of rats disrupts latent inhibition in males, and results in persistent sex- and area-dependent increases in dopamine and serotonin turnover. Behavioral Pharmacology, 2, 215–223.
Ploj, K., Roman, E., Bergström, L., & Nylander, I. (2001). Effects of neonatal handling on nociceptin/orphanin FQ and opioid peptide levels in female rats. Pharmacology Biochemistry and Behavior, 69, 173–179. Ploj, K., Roman, E., & Nylander, I. (2003). Long-term effects of short and long periods of maternal separation on brain opioid peptide levels in male Wistar rats. Neuropeptides, 37, 149 –156.
Reinhart, C. J., McIntyre, D. C., Metz, G. A., & Pellis, S. M. (2006). Play fighting between kindling-prone (FAST) and kindling-resistant (SLOW) rats. Journal of Comparative Psychology, 120, 19 – 30.
Roth, T. L., & Sullivan, R. M. (2006). Examining the role of endogenous opioids in learned odor-stroke associations in infant rats. Developmental Psychobiology, 48, 71–78.
Siviy, S. M., & Harrison, K. A. (2008). Effects of neonatal handling on play behavior and fear towards a predator odor in juvenile rats (Rattus norvegicus). Journal of Comparative Psychology, 122, 1– 8.
Smotherman, W. P., Brown, C. P., & Levine, S. (1977). Maternal responsiveness following differential pup treatment and mother-pup interactions. Developmental Psychobiology, 10, 242–253.
Thor, D. H., & Holloway, W. R. (1984). Social play in juvenile rats: A decade of methodological and experimental research. Neuroscience and Biobehavioral Reviews, 8, 455– 464.
Winslow, J. T., & Insel, T. R. (2004). Neuroendocrine basis of social recognition. Current Opinion in Neurobiology, 14, 248 –253.
Young, L. J., & Wang, Z. (2004). The neurobiology of pair bonding. Nature Neuroscience, 7, 1048 – 1054