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Social and environmental enrichment enhances sensitivity to the effects of kappa opioids: studies on antinociception, diuresis and conditioned place preference

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Abstract

Previous studies have reported that social and environmental enrichment can have a marked impact on the functional maturation of the central nervous system and may influence an organism's sensitivity to psychotropic drugs. The purpose of the present study was to examine the effects of social and environmental enrichment on sensitivity to drugs possessing activity at the kappa opioid receptor. Rats were obtained at weaning and randomly assigned to one of two housing conditions: isolated rats were housed individually with no visual or tactile contact with other rats; enriched rats were housed in groups of four in large cages and given various novel objects on a regular basis. After 7 weeks under these conditions, the effects of spiradoline, U69,593 and nalorphine were examined in the warm water, tail-withdrawal procedure. The effects of spiradoline were also examined on urine output and in the conditioned place preference procedure. Enriched rats were more sensitive to the antinociceptive effects of all the opioids examined in the tail-withdrawal procedure, and were more sensitive to the effects of spiradoline on urine output and in the conditioned place preference procedure. Following the conclusion of these tests, housing conditions were reassigned, such that isolated rats were transferred to enrichment cages, and enriched rats were transferred to isolation cages. After 7 weeks under these new conditions, the two groups were equally sensitive to the antinociceptive effects of spiradoline, indicating that the effects of the initial housing conditions were, in part, reversible. Collectively, these data suggest that enriched rats are more sensitive than isolated rats to the effects of kappa opioids, and that the kappa opioid receptor system is sensitive to social and environmental manipulations after weaning.

Keywords: Antinociception; Conditioned place preference; Diuresis; Enriched; Environment; Isolated; Kappa; Opioid; Rat

1. Introduction

Numerous studies indicate that social and environmental enrichment can have a marked influence on the functional maturation of the central nervous system. For example, animals reared under enriched conditions (i.e., conditions in which animals are housed together in large groups and given various novel objects on a regular basis) display increased cortical mass (Rosenzweig et al., 1962; Bennett et al., 1969) and neurotransmitter concentrations (O'Shea et al., 1983; Naka et al., 2002) and perform better on learning and memory tasks (Mohammed et al., 1990; Kobayashi et al., 2002) relative to animals reared in isolation. There is also a growing body of evidence that social and environmental enrichment may influence an organism's sensitivity to psychotropic drugs. In studies conducted with psychomotor stimulants, for example, it was reported that enriched rats are more sensitive than isolated rats to the locomotor and rewarding effects of amphetamine (Bowling et al., 1993; Bowling and Bardo, 1994; Bardo et al., 1999), and are more sensitive to amphetamine-induced dopamine release and metabolism in the nucleus accumbens (Bowling et al., 1993; Bardo et al., 1999).

A limited number of studies have examined the effects of social and environmental manipulations on sensitivity to

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other psychotropic drugs. Studies conducted with mu opioids, for instance, indicate that group-housed rats are more sensitive than isolated rats to the antinociceptive (i.e., analgesic) effects of morphine in the tail-shock and tail-compression tests (Czlonkowski and Kostowski, 1977; Panksepp, 1980), and are more sensitive to the rewarding effects of morphine and heroin in the conditioned place preference procedure (Schenk et al., 1983; Wongwitdecha and Marsden, 1996; Coudereau et al., 1997). Although the mechanisms responsible for these differences are not known, they may involve differences in sensitivity of the opioid receptor system between the two subject populations. A previous study reported that opioid receptor density (i.e., B_{max}) was increased in group-housed rats relative to isolated controls (Schenk et al., 1982), which may explain the increased sensitivity to mu opioid agonists in group-housed animals. Unfortunately, the effects of social and environmental manipulations on sensitivity to drugs acting at other opioid receptor subtypes are not known.

The purpose of the present study was to examine the effects of social and environmental enrichment on sensitivity to drugs possessing activity at the kappa opioid receptor. To this end, rats were obtained at weaning (21 days) and randomly assigned to one of two housing conditions. Isolated rats ($n = 8$) were housed individually in opaque laboratory cages with no visual or tactile contact with other rats. Enriched rats ($n = 8$) were housed in groups of four in large cages with various enrichment stimuli (e.g., tubes, ladders, toys) that were added to their cages daily. After approximately 7 weeks under these conditions, the antinociceptive effects of two kappa opioids (spiradoline, U69,593) and one mixed-action opioid possessing activity at the kappa receptor (nalorphine) were examined in the warm water, tail-withdrawal procedure. The effects of spiradoline were further examined on urine output (i.e., diuresis) and in the conditioned place preference procedure. Following these tests, housing conditions were reassigned, such that isolated rats were transferred to enrichment cages, and enriched rats were transferred to isolation cages. The antinociceptive effects of spiradoline were then reexamined under these new conditions 7 weeks later.

2. Method

2.1. Animals

Sixteen male, Fisher 344 rats were obtained at weaning (21 days) from Charles River Laboratories (Raleigh, NC) and randomly assigned to one of two conditions. Isolated rats ($n = 8$) were housed individually in opaque laboratory cages (interior dimensions: 43 × 21 × 20 cm) and had no visual or tactile contact with other rats. Enriched rats ($n = 8$) were housed in groups of four in large cages (interior dimensions: 92 × 38 × 40 cm) that permitted extensive social interactions between cagemates. Rats in this group received supplemental enrichment from a variety of objects (e.g., cardboard and PVC tubes, ladders, Ping-Pong balls, animal toys) that were changed daily. The two cage sizes, as well as the number of rats per cage, were selected as to ensure that the floor area per rat was held constant across the two groups (~ 875–900 cm^2 per rat). All rats were housed under these conditions for 7 weeks prior to behavioral testing. Rats in both groups were kept in a large colony room maintained on a 12-h light/dark cycle with food and drinking water available ad libitum in the home cages. Body weight, food intake and general health were similar between the two groups upon arrival, and remained very similar throughout all phases of the study. All subjects were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of Davidson College and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996).

2.2. Antinociception

During antinociceptive testing, subjects were restrained in plastic restraint tubes (Fischer Scientific, Pittsburgh, PA, USA) and tail-withdrawal latencies were measured with a hand-operated stopwatch with a time resolution of 0.01 s. Water was maintained at 50 and 55 °C via thermostat-controlled water baths (Fisher Scientific).

Antinociceptive testing was conducted according to procedures described previously (Smith and Gray, 2001; Smith and French, 2002). Briefly, rats were habituated to both the injection procedure and restraint tube confinement during two 30-min habituation sessions conducted on consecutive days prior to the first test session. Unpublished data from our laboratory, as well as published data from other laboratories (e.g., Calcagnetti and Holtzman, 1990) suggest that such habituation sessions are sufficient to minimize the influence of injection- and restraint-related stress on antinociceptive testing. During antinociceptive tests, rats were removed from their home cages and placed into restraint tubes with their tails hanging freely off the edge of a table. The distal 10 cm of the tail was then immersed into an insulated mug containing either 50 or 55 °C water, with the order of stimulus presentation counterbalanced across rats. Using a hand-operated stopwatch, the latency for each rat to withdraw its tail from the water was recorded. A cutoff latency of 15 s was employed in all tests to prevent tissue damage. During these tests, and during all subsequent tests conducted during the session, approximately 3 min separated the two stimulus presentations.

All drugs were administered using a cumulative dosing procedure. In this procedure, each rat was removed from its restraint tube, injected intraperitoneally with the lowest dose of the test drug, and then immediately returned to the tube. After a 15-min interval, the latency for each rat to withdraw its tail from the 50 and 55 °C water was determined. Immediately following testing at both temperatures, each rat was administered the next dose of the test drug, such that

the dose increased the cumulative amount of drug received in that session by 0.5 or 1.0 log unit. For example, the administration of 1.0, 2.0, 7.0 and 20.0 mg/kg over the course of a testing session yielded cumulative doses of 1.0, 3.0, 10.0 and 30.0 mg/kg. A total of 3–4 doses were tested during each test session. For individual rats, when a maximal response was observed at one water temperature, no further tests were conducted at that temperature.

2.3. Diuresis

One day prior to diuresis testing, each rat was habituated to a sound-attenuating operant-conditioning chamber (Med Associates, St. Albans, VT) for 2 h. Saline (control) and drug sessions were conducted over the next two consecutive days, with the order of saline and drug administration counterbalanced across rats. During these sessions, each rat was administered either saline or 10 mg/kg spiradoline, and urine was collected over 2 h in stainless steel pans located beneath the grid floor of the chamber. All rats were normally hydrated at the beginning of the session, but no food or drinking water was available during the session.

2.4. Conditioned place preference

Conditioning took place in a three-chamber place preference chamber (Med Associates, St. Albans, VT, USA). The chamber consisted of two choice compartments (interior dimensions: 25 × 20 × 20 cm) separated by a smaller center compartment (interior dimensions: 13 × 20 × 20 cm). One choice compartment was painted black and had a floor that consisted of stainless steel rods covering corncob bedding. The other choice compartment was painted white and had a mesh floor covering pine bedding. The center compartment was painted a neutral gray and had a solid PVC floor. Each choice compartment was separated from the center compartment by a manually operated guillotine door. During habituation and test sessions (see below), behavioral activity was recorded by a video camera mounted 1.5 m above the chamber.

One day prior to the first conditioning trial, each rat was given 15 min to habituate to the conditioning chamber. During this habituation session, rats were placed in the center (neutral) compartment and given free access to the entire chamber by opening the guillotine doors separating the two choice compartments from the center compartment. Entrance to a compartment was noted when the head and both forepaws passed completely through a doorway. The amount of time spent in each of the three compartments was recorded over the entire 15-min session.

Over the next eight consecutive days, rats received daily conditioning trials in which they were injected with either 10 mg/kg spiradoline or saline and placed into one of the two choice compartments for 30 min. For half of the rats in each group, the white compartment served as the drug-paired compartment; for the other half, the black compartment served as the drug-paired compartment. Both guillotine doors were closed during these conditioning trials, and rats were confined to the appropriate compartment for the duration of the trial. Drug and saline administration alternated daily such that each rat received four conditioning trials with both the drug-paired and saline-paired compartments.

On the day immediately following the last conditioning trial, place preference was assessed in each rat. During this test session, rats were placed in the center compartment and both guillotine doors were opened. Rats were given free access to the entire chamber for 15 min, and the amount of time spent in each of the three compartments was recorded.

2.5. Schedule of testing

Rats were maintained under isolated or enriched conditions for 7 weeks prior to behavioral testing. The effects of spiradoline and U69,593 in the tail-withdrawal procedure were tested during the 8th and 9th week of the study, respectively. The diuretic effects of spiradoline were tested during the 10th week of the study, and the effects of spiradoline in the conditioned place preference procedure were tested during the 12th and 13th week of the study. The effects of nalorphine in the tail-withdrawal procedure were tested during the 14th week of the study. Following antinociceptive testing with nalorphine, housing conditions were reversed such that isolated rats were transferred to enrichment cages and enriched rats to isolation cages. The effects of spiradoline in the tail-withdrawal procedure were then reexamined under these new conditions, 7 weeks later.

2.6. Drugs

Spiradoline mesylate was obtained from Sigma (St. Louis, MO, USA). U69,593 and nalorphine hydrochloride were generously supplied by the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, NC, USA). All compounds were dissolved in distilled water and injected intraperitoneally in a volume of 1.0–2.0 ml/kg of body weight.

2.7. Data analysis

Tail-withdrawal latencies were converted to percent antinociceptive effect using the following equation: % antinociceptive effect=[(observed − baseline)/(15 sec − baseline)] ×100. Area under the curve (AUC) estimates were then made for each rat using the trapezoidal rule (Procedure 25, Tallarida and Murray, 1987). In this analysis, the function of the dose–effect curve is divided into segments, and the area of each individual segment is calculated. When using dose–effect data, the area of each segment is determined by its width (measured in log units of the dose) and height (measured in percent maximal

effect). The areas of each segment are then added together to get a total AUC. This analysis was selected because it takes into account the response produced by each individual dose of the drug, which is desirable under conditions in which a drug fails to produce the maximal possible effect across the dose range tested (Morgan and Picker, 1996; Morgan et al., 1999; Smith and French, 2002). Using AUC estimates from each rat, differences across conditions were analyzed via repeated-measures analysis of variance (ANOVA), with group serving as the between-subjects variable and temperature serving as the within-subjects variable.

In the diuresis test, between-group differences in the amount of urine collected over the 2-h session after spiradoline administration was compared via an independent-sample *t* test.

In the conditioned place preference procedure, difference scores were obtained for each rat by subtracting the amount of time spent in each compartment before conditioning (i.e., during the free-access habituation session) from the amount

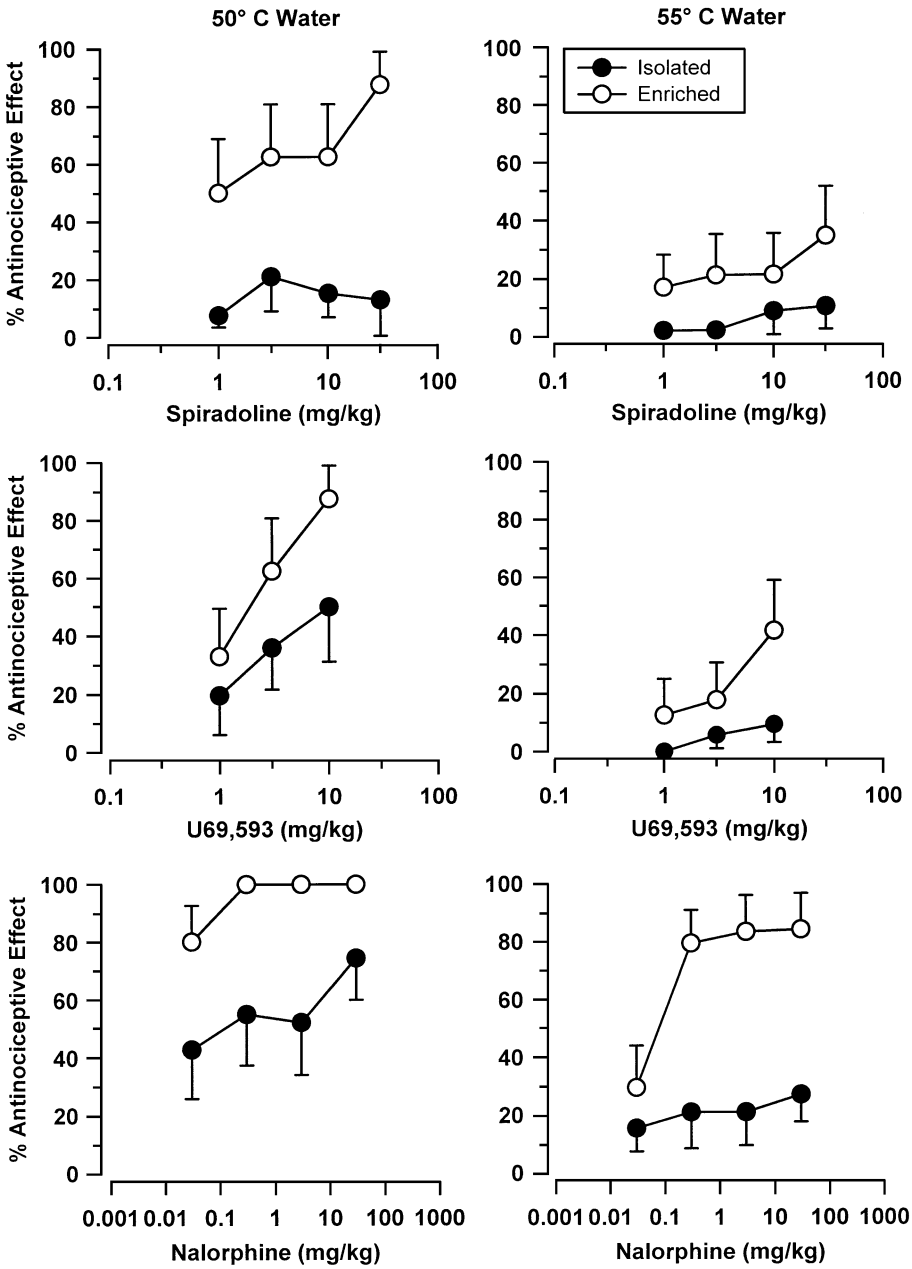

Fig. 1. Effects of cumulative doses of spiradoline (upper panels), U69,593 (middle panels) and nalorphine (lower panels) in the warm water, tail-withdrawal procedure. Left and right panels depict data collected in 50 °C and 55 °C water, respectively. Vertical axes reflect tail-withdrawal latencies and are expressed as a percentage of the maximal possible effect. Horizontal axes reflect doses in milligram per kilogram of body weight. Vertical lines on data points represent the S.E.M.; where not indicated, the S.E.M. fell within the data point.

of time spent in each compartment after conditioning (i.e., during the free-access place preference test). Using these difference scores, group effects were determined via an independent-sample t test.

The alpha level was set at .05 for all statistical tests.

3. Results

3.1. Antinociception

Baseline tail-withdrawal latencies (i.e., tail-withdrawal latencies in the absence of drug administration) were greater at the low temperature than at the high temperature, but were similar across the two groups of subjects. Mean (S.E.M.) tail-withdrawal latencies were 11.22 (0.27) and 8.34 (0.36) s in the isolated group at the low and high temperatures, respectively. In the enriched group, these values were 11.28 (0.17) and 8.69 (0.18) s at the low and high temperatures, respectively.

As shown in Fig. 1, 30 mg/kg spiradoline produced maximal levels of antinociception ($\geq$ 80% antinociceptive effect) in enriched rats at the low temperature. In contrast, this dose of spiradoline produced only a 21% antinociceptive response in isolated rats. Differences in sensitivity were also observed at the high temperature, where 30 mg/kg spiradoline produced responses of 35% and 10% in the enriched and isolated groups, respectively. A repeated-measures ANOVA revealed significant main effects for group [$F(1,14) = 6.76$, $P < .05$] and temperature [$F(1,14) = 8.41$, $P < .05$].

Similar to that seen with spiradoline, U69,593 produced maximal levels of antinociception in enriched rats at the low temperature (Fig. 1). U69,593 also increased tail-withdrawal latencies in isolated rats, but failed to produce a maximal response up to a dose of 10 mg/kg. Differences in sensitivity were also observed at the high temperature, where 10 mg/kg produced responses of 42% and 9% in the enriched and isolated groups, respectively. Consistent with these observations, significant main effects were observed for group [$F(1,14) = 5.33$, $P < .05$] and temperature [$F(1,14) = 6.92$, $P < .05$].

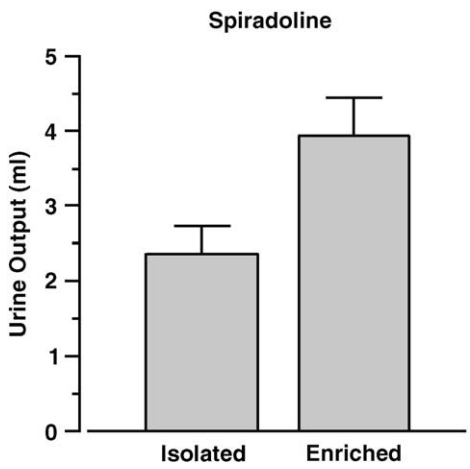

Fig. 2. Effects of 10 mg/kg spiradoline on urine output. Data reflect volume (ml) of urine collected over a 2-h session. Vertical lines represent the S.E.M.

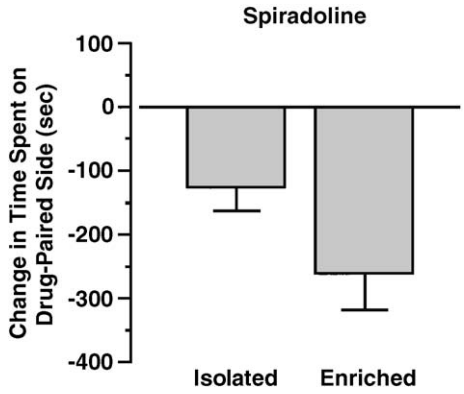

Fig. 3. Effects of 10 mg/kg spiradoline in the conditioned place preference procedure. Data reflect time (s) spent in the spiradoline-paired compartment after conditioning minus time (s) spent in the spiradoline-paired compartment before conditioning. Vertical lines represent the S.E.M.

At the low temperature, nalorphine produced maximal levels of antinociception in enriched rats at all doses tested (Fig. 1). In isolated rats, nalorphine increased tail-withdrawal latencies, but failed to produce a maximal response up to a dose of 30 mg/kg. At the high temperature, this dose of nalorphine produced an antinociceptive response of 83% in enriched rats, but failed to produce greater than a 27% response in isolated rats. A repeated-measures ANOVA revealed significant main effects for group [$F(1,14) = 12.91$ $P = < .05$] and temperature [$F(1,14) = 15.68$, $P < .05$].

3.2. Diuresis

Urine output was negligible under baseline conditions: a mean (S.E.M.) of 0.05 (0.01) and 0.10 (0.03) ml of urine were collected after saline administration in the isolated and enriched groups, respectively. As shown in Fig. 2, administration of 10 mg/kg spiradoline produced large increases in urine output in both groups of rats, with this effect being significantly greater in the enriched group [$t(14) = 2.55$, $P < .05$].

3.3. Conditioned place preference

Prior to conditioning, isolated and enriched rats spent a mean (S.E.M.) of 318 (42) and 405 (51) s in the drug-designated compartment, respectively. After conditioning, these values decreased to 191 (19) and 142 (23) s, respectively. As shown in Fig. 3, both groups spent less time in the spiradoline-paired compartment after conditioning, but this effect was significantly greater in the enriched group [$t(14) = 2.67$, $P < .05$].

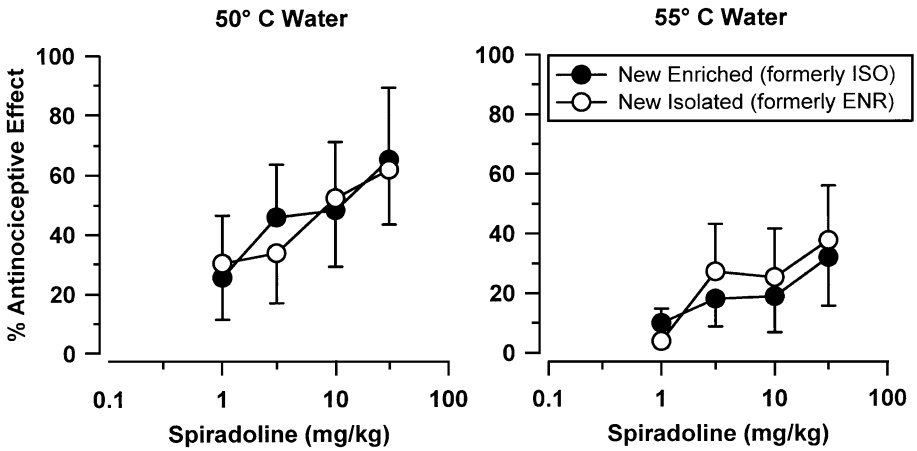

Fig. 4. Effects of cumulative doses of spiradoline in the warm water, tail-withdrawal procedure 7 weeks after housing reassignment. Left and right panels depict data collected in 50 °C and 55 °C water, respectively. Other details are as described in Fig. 1.

3.4. Housing reversal

Housing conditions were reversed during the 14th week of the study, such that isolated rats were transferred to enrichment cages, and enriched rats to isolation cages. The majority of rats in both groups failed to exhibit a nociceptive response under baseline (i.e., nondrug) conditions in antinociceptive tests conducted 2 and 8 days after housing reversal (data not shown). In these tests, baseline tail-withdrawal latencies reached the cutoff latency (i.e., 15 s) in the majority of rats tested, which precluded further testing with spiradoline as originally planned. Baseline measures of nociception returned to normal 7 weeks after reversal, and testing was conducted with spiradoline as scheduled. As shown in Fig. 4, the two groups did not differ in their sensitivity to spiradoline at this time point. At the low temperature, 30 mg/kg spiradoline produced responses of 65% and 63% in the former isolated and enriched rats, respectively. At the high temperature, this dose of spiradoline produced responses of 32% and 37% in these groups, respectively. A repeated-measures ANOVA revealed a main effect for temperature [$F(1,14)=7.84$, $P<.05$], but the effect of group was not significant. Compared to that observed prior to housing reassignment, sensitivity to the effects of spiradoline decreased at the low temperature in enriched rats transferred to isolation cages, whereas sensitivity to the effects of spiradoline increased at both temperatures in isolated rats transferred to enrichment cages (compare Figs. 1 and 4).

4. Discussion

The purpose of this study was to examine the effects of social and environmental enrichment on sensitivity to opioids possessing activity at the kappa receptor. Previous studies (e.g., Adler et al., 1975; Czlonkowski and Kostowski, 1977; Panksepp, 1980; Bardo et al., 1997) examining the effects of social and environmental manipulations on opioid sensitivity have used isolation (animals housed individually), group (animals housed together in large groups) and/or enriched housing (animals housed together in large groups and given various novel objects on a regular basis). In the present study, we chose to compare animals reared in isolation vs. animals reared under enriched conditions, as previous studies suggest that these two subject populations exhibit the greatest between-group differences in sensitivity to psychotropic drugs (Bowling and Bardo, 1994; Bardo et al., 2001).

Enriched rats were significantly more sensitive to the antinociceptive effects of spiradoline, U69,593 and nalorphine in the tail-withdrawal procedure, and these effects were apparent at both the low and high nociceptive intensities. Spiradoline and U69,593 are highly selective agonists for the kappa receptor (France et al., 1994; Butelman et al., 1998), and to our knowledge, this is the first demonstration that social and environmental enrichment enhances sensitivity to their antinociceptive effects. It should be noted that these data are similar to those obtained in previous studies with mu opioids, which indicate that group-housed rats are also more sensitive to the antinociceptive effects of morphine (Czlonkowski and Kostowski, 1977; Panksepp, 1980). This is also the first demonstration that enrichment enhances sensitivity to the antinociceptive effects of nalorphine, a mixed-action opioid possessing weak agonist activity at mu and kappa receptors (Emmerson et al., 1996; Zhu et al., 1997; Selley et al., 1998; Remmers et al., 1999). We previously reported that social and environmental enrichment enhances sensitivity to the antinociceptive effects of the mixed-action opioids butorphanol and nalbuphine (Smith et al., 2000); however, it is not clear whether these findings reflect an enhanced sensitivity to their mu component of action, their kappa component of action, or a combination of both.

Despite large differences in sensitivity to the antinociceptive effects of spiradoline, U69,593 and nalorphine, baseline measures of pain sensitivity did not differ between

the two groups of subjects. Previous studies examining the effects of social and environmental manipulations on pain sensitivity have reported that group housing increases (Panksepp, 1980), decreases (Czlonkowski and Kostowski, 1977), and does not change (Adler et al., 1975) sensitivity to nociceptive stimuli. A number of different assays were used in these studies (tail shock vs. tail compression vs. hot plate), making it possible that the effects of social and environmental enrichment on pain sensitivity depend upon the type of nociceptive stimulus employed (electrical vs. mechanical vs. thermal, respectively). Although this explanation would account for the findings described above, the authors do not know of any previous studies that have specifically addressed this issue.

Spiradoline produced robust increases in urine output in both groups of rats, which is in agreement with numerous studies reporting that kappa opioids produce pronounced diuretic effects in this species (Leander et al., 1987; Cook et al., 2000; Craft et al., 2000). Enriched rats were significantly more sensitive than isolated rats to the diuretic effects of spiradoline, despite only minimal differences in urine output under baseline conditions. These findings are consistent with those obtained in the tail-withdrawal procedure (see above), and suggest that the effects of social and environmental enrichment on sensitivity to kappa opioids extend across behavioral measures.

In the conditioned place preference procedure, rats in both groups spent less time in the compartment paired with spiradoline. This finding is in agreement with previous studies reporting that kappa opioids produce aversive stimulus effects in rats (Shippenberg and Herz, 1987; Bals-Kubik et al., 1993; del Rosario Capriles and Cancela, 2002) and dysphoric subjective effects in humans (Pfeiffer et al., 1986; Reece et al., 1994; Rimoy et al., 1994). To our knowledge, this is the first study to examine spiradoline in the conditioned place preference procedure, and it is appropriate to comment on the parameters used to establish conditioning. The dose employed (10 mg/kg) was selected because it was high enough to produce robust effects in the diuresis procedure, but not so high as to produce ataxia, which is a common problem encountered with high doses of spiradoline (Coltro Campi and Clarke, 1995). Although only one dose was examined, it is unlikely that the effects observed at this dose were atypical, as previous studies report that doses of kappa agonists generally produce linear effects in this procedure (Shippenberg and Herz, 1988; Suzuki et al., 1992; Funada et al., 1993). The eight conditioning trials (four drug; four saline) and the three-chamber place preference apparatus are both typical of place-conditioning studies (e.g., Morutto and Phillips, 1997; Subhan et al., 2000; Ren et al., 2002). Thus, it is not surprising that the magnitude of effect seen in the present investigation (125–250 s decrease in time spent in the drug-paired compartment) is very similar to that reported in previous studies that have employed kappa agonists in this procedure (e.g., Bals-Kubik et al., 1988, 1993; Shippenberg et al., 1988; Funada et al., 1993).

Similar to that seen in the antinociceptive and diuresis tests, enriched rats were significantly more sensitive than isolated rats to the effects of spiradoline in the conditioned place preference procedure. This observation is consistent with the general finding that group-housed rats are more sensitive than isolated rats to the effects of psychotropic drugs in this procedure, regardless of the drug employed (Schenk et al., 1983; Bowling and Bardo, 1994; Wongwitdecha and Marsden, 1996; Bardo et al., 1997; Coudereau et al., 1997). It has been suggested that these findings may reflect differences in the ability of isolated and group-housed rats to learn drug-context associations, but differences in this procedure have been observed in the absence of differences in global learning abilities. For instance, Coudereau et al. (1997) reported that isolated mice failed to exhibit a conditioned place preference for morphine under conditions in which a robust place preference was observed in group-housed mice. These effects were observed even though the two groups did not differ in spatial learning ability in the Morris water maze, or in their ability to acquire an avoidance response in a passive avoidance task. Such findings suggest that isolated and enriched rats do not differ in their ability to learn drug-context associations, but rather, in their sensitivity to the rewarding/aversive properties of psychotropic drugs (Coudereau et al., 1997).

The effects of isolation and enrichment were not permanent. Seven weeks after housing conditions were reassigned (i.e., when isolated rats were transferred to enrichment cages and enriched rats to isolation cages), the two groups did not differ in their sensitivity to the antinociceptive effects of spiradoline. These findings are markedly different from those obtained prior to housing reassignment, and suggest that the effects of the initial housing conditions were, in part, reversible. Although we had originally planned to test the effects of spiradoline at earlier time points, rats in both groups failed to show baseline nociceptive responses 2 and 8 days after housing reassignment. It is not known why baseline tail-withdrawal latencies were elevated at these time points, but it may be related to the stress of housing reassignment. Indeed, there is an extensive literature showing that social and environmental stress produces short-term antinociceptive effects, and elevates nociceptive thresholds in a variety of behavioral assays (Miczek et al., 1985; Kehoe and Blass, 1986; Vivian and Miczek, 1998; Wiedenmayer and Barr, 2000).

The mechanisms responsible for these differences in sensitivity between isolated and enriched subjects are not known, but some possibilities deserve mention. It is possible that these differences are due, in part, to underlying pharmacokinetic differences between the two conditions. Indeed, previous studies have reported that enriched animals have lower body weights (Bardo and Hammer, 1991), smaller livers (Black et al., 1989) and larger cerebral capillaries (Sirevaag and Greenough, 1988), all of which may contribute to greater drug bioavailability in this subject population. A second explanation for these

findings involves potential pharmacodynamic differences between the two groups. Schenk et al. (1982) reported that binding sites labeled by [^{3}H] naloxone were increased in group-housed rats relative to isolated controls, suggesting an increase in opioid receptor density in these subjects. In contrast, Bardo et al. (1997) reported that binding sites labeled by [^{3}H] DAMGO did not differ between isolated and enriched rats, suggesting that the density of mu opioid receptors are not affected by social and environmental manipulations. It is possible that kappa receptors, which are labeled by [^{3}H] naloxone but not by [^{3}H] DAMGO, are functionally up-regulated in enriched animals. This explanation is consistent with the data described in the present study, and would account for the apparent discrepancy between the studies described above.

It is important to emphasize that two variables were manipulated in the present study: number of rats per cage and whether novel objects were added each day. The relative contribution of these variables to the observed findings is not known, but data from previous studies suggest that both play contributing roles. For example, enriched rats are more sensitive than both isolated and group-housed housed rats to the effects of amphetamine on locomotor activity (Bowling and Bardo, 1994), indicating that the presence of novel objects (i.e., environmental enrichment) is the critical variable mediating sensitivity to amphetamine's locomotor effects. However, isolated rats respond more for intravenous amphetamine infusions than both enriched and group-housed rats (Bardo et al., 2001), indicating that the number of rats per cage (i.e., social enrichment) is the critical factor mediating amphetamine's positive reinforcing effects. Evidently, both social and environmental factors contribute independently to differences in sensitivity to amphetamine, and it is likely that both make independent contributions to differences in sensitivity to other drugs as well. The degree to which these variables individually contribute to differences in kappa sensitivity awaits further study.

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