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Developmental Changes in the Behavioral and Autonomic Effects of Kappa Opioid Receptor Stimulation of the Midbrain Periaqueductal Gray

ABSTRACT: *Kappa opioid receptors stimulation with U50,488 is known to modulate behaviors during the early postnatal period, but the specific neuroanatomical locus of many of these effects is unexplored. In the present study, we infused U50,488 into the midbrain periaqueductal gray (PAG) and investigated the effects of this drug on behavior and heart rate of 1-, 2-, and 3-week-old rats. U50,488 increased activity most potently in 1- and 2-week-old subjects. Ultrasonic vocalization (USV) production was increased in 1-week-old subjects, but not in 2- or 3-week-old pups. Heart rate changes were similarly seen in younger aged subjects. At 1 week, U50,488 decreased heart rate, but at 2 weeks it increased heart rate. There was no effect of this drug on heart rate at 3 weeks. At 1 week, USVs were more potently elicited from dorsal than lateral PAG infusion sites. No other site-specific effects within the PAG were seen. The age-related decline in behavioral effects elicited by U50,488 is consistent with other published reports, and to the extent that kappa receptor activity mediates infant separation responses, implicates the PAG as a modulator of those responses. © 2004 Wiley Periodicals, Inc. Dev Psychobiol 46: 47–56, 2005.*

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INTRODUCTION

Rats given the kappa opioid receptor agonist U50,488 early in development exhibit behavioral responses that contrast with those evoked by this drug during adulthood. From the late embryonic period through the first 3 postnatal weeks, U50,488 increases general activity measures

(Jackson & Kitchen, 1989; Petrov et al., 1994). During the first 3 postnatal weeks, this drug also causes an increase in ultrasonic vocalization (USV) production (Carden, Bortot, & Hofer, 1993; Carden, Davachi, & Hofer, 1994; Kehoe & Boylan, 1994). In contrast, adult rats show no behavioral activation following subcutaneous (sc) administration of U50,488, and even show an inhibition of general activity following administration of other kappa opioid agonists (Jackson & Cooper, 1988). Not all effects of U50,488 are age dependent, however. The aversive properties of this drug are invariant across age, being observed in both preweanling and adult rats (Barr, Wang, & Carden, 1994; Mucha & Herz, 1985) as are its analgesic effects (Kehoe & Boylan, 1994; Vonvoigtlander, Lahti, & Ludens, 1983).

An interesting, yet unanswered, question regards the location of the kappa opioid receptors that mediate these

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effects of U50,488 in developing rats. For several reasons, we chose to examine the midbrain periaqueductal gray (PAG). First, the dorsal midbrain, including the PAG and midbrain tectum, has been shown to play a role in the organization of vocalization and analgesic responses to social isolation and social threats during the early postnatal period development (Wiedenmayer, Goodwin, & Barr, 2000). Second, kappa opioid receptors are expressed in the PAG as early as the first postnatal week (Kitchen, Kelly, & Viveros, 1990).

Currently, little is known about the functional development of the PAG. In adults, this midbrain structure has been shown to be the lowest level organizer of behavioral (e.g., vocalizations, escape and defensive behaviors) and autonomic (e.g., blood flow, blood pressure, and heart rate) responses to environmental threats (e.g., Bandler & Depaulis, 1988; Carrive, Dampney, & Bandler, 1987; Zhang, Davis, Bandler, & Carrive, 1994). Lesions of the PAG at postnatal Day 7 (P7) inhibit both the production of USVs and the analgesic response to social isolation at P10, demonstrating that it also is critical for the production of these defensive behaviors in infant rats (Wiedenmayer et al., 2000). In adult rats, kainate (a glutamate receptor subtype agonist) triggers a full range of behavioral and autonomic defensive responses (Bandler & Carrive, 1988). Microinjection of glutamate into this brain region at P10 triggers an analgesic response (Tive & Barr, 1992), but microinjection of kainate into this area at P7 or P14 does not increase USV production (Goodwin & Barr, 1998), suggesting this behavior is mediated by another type of receptor.

The present study was designed to extend our understanding of the function of the midbrain periaqueductal gray during early development by exploring the functional role of kappa opioid receptors within this structure in 1-, 2-, and 3-week-old rats. Activity and USV production were measured, and heart rate recordings were made to assess the potential for kappa opioid modulation of autonomic activity.

Infusion site also was examined, as studies of adult rats have suggested that the behavioral effects of PAG stimulation are dependent on the site of drug infusion. Specifically, flight or escapelike responses have been elicited primarily from the lateral region of the PAG (Depaulis, Keay, & Bandler, 1992); however, USV production in adult rats has been shown to be most potently elicited from the dorsal PAG (Yajima, Hayashi, & Yoshii, 1980). For these reasons, dorsal and lateral PAG cannula placements also were contrasted. Analgesic responses were not examined in the present study, as such testing involves the use of multiple stimulus modalities and intensities (for discussion, see Barr, 1992) and therefore would have greatly complicated the overall testing procedure.

MATERIALS AND METHODS

Subjects

Subjects were 129 Long-Evans hooded rats derived from 21 litters that were born in the AAALAC (Association for the Assessment and Accreditation of Laboratory Animal Care) accredited animal facility at the New York State Psychiatric Institute. All procedures used in this experiment were approved by the IACUC (Institutional Animal Care and Use Committee) at the New York State Psychiatric Institute. After mating, females were housed in plastic cages (40 × 20 × 24 cm) with pine shavings and were given ad-libitum access to Purina lab chow and water. Temperature was maintained at 22 ± 1°C with a 12:12 hr light:dark cycle (lights on at 0700 hr). Cages were checked daily for births at approximately 1000 and 1800 hr, and births noted at those times were termed 0 days of age. On postnatal Day 1 (P1), litters were culled to 10 pups without regard to the ratio of males to females. Pups were tested at P7 ($n = 44$), P14 ($n = 41$), or P21 ($n = 44$). Approximately equal numbers of males and females were used at each age, with male:female ratios of 25:19 at P7, 18:25 at P14, and 21:23 at P21. Analysis of sex means revealed no significant differences between males and females for any of the behavioral measures or for heart rate at any of the ages tested.

Four to 6 pups from each litter were used for testing at a given age. Most litters were tested at only one age (five litters at P7, six litters at P14, and seven litters at P21). Two litters contributed pups for testing at both P7 and P14, and one litter contributed pups for testing at P7 and P21. To minimize the potential for litter effects to confound dose effects, for each age of testing, no more than 2 pups from any given litter were tested at the same dose, and at least 1 pup from each litter was administered each dose of the drug. Because some subjects had to be excluded due to inaccurate cannula placements (discussed later), each litter did not necessarily contribute a data point at each dose. Overall litter differences were minimal, as one-factor ANOVAs did not reveal significant litter differences for any of the behavioral measures or for the overall heart rate at any age.

Procedure

Surgical Implantation of Electrodes and Intracranial Cannulas. On the day of testing, litters were removed from the dam, placed in a holding cage with clean pine shavings plus a handful of home cage shavings, and taken from the colony room to a holding area. The floor of the cage was maintained at 35 ± 2°C by a heating pad. Subjects were weighed and sexed. Prior to implantation of heart rate electrodes, pups were anesthetized with Metafane (Pittman-Moore). Initial anesthesia was obtained by placing the animal in a glass jar with Metafane-soaked cotton (~0.5 cc Metafane). The pup was removed from the glass jar after being sufficiently anesthetized, and anesthesia was maintained by placing a nose cone containing a Metafane soaked piece of cotton over the snout of the pup. Pups were implanted with sc silver-wire (30 ga) electrodes. The sc loops of wire traversed the front-right and left-rear thorax, and the ends exited near the spine where they were twisted together in a caudal direction. An amphenol male connector was crimped onto the

exposed wire. While still anesthetized, subjects were next implanted with a 26-ga intracranial guide cannula (Plastics One) directed at the PAG. The scalp of the subject was washed with Betadine (Purdue Fredrick), and then a 15-mm rostral-caudal incision was made along the midline of the head. The top of the skull was scraped to remove the periosteum and wiped with 70% EtOH. The subject was then placed in a Kopf stereotaxic apparatus. Although P14 and P21 subjects could be secured using standard earbars, P7 subjects required the use of a modified holder fitted to the stereotaxic apparatus (see Heller, Hutchens, Kirby, Karapas, & Fernandez, 1979). Coordinates were relative to lambda (P7: 0.8 mm posterior, 0.8 mm lateral, 3.5 mm ventral; P14: 1.0 mm posterior, 1.0 mm lateral, 4.0 mm ventral; P21: 1.0 mm posterior; 1.0 mm lateral, and 4.2 mm ventral). A hole was drilled through the skull, the guide cannula was lowered into position, and a thin layer of cyanoacrylate glue was spread on the surface of the skull. The cannula was then fixed in position with a layer of caulk grip cement (Dentsply) followed by a thicker layer of dental acrylic (Dentsply). After the surgery, the subject was returned to the holding cage with its littermates and allowed to recover for 5 hr before being tested. Subjects exhibited good recovery from this procedure by the time of testing. Longer recovery periods, though desirable, are difficult to accomplish in this age animal for two reasons. First when pups are returned to the home cage, the dam typically attempts to destroy the cannulae, making them unviable for testing within a few hours. Second, the rapid growth of the pups means that the cannula do not remain secured to the cranium for more than a day at best. Thus, although the short postsurgical recovery is a necessary compromise, it creates a potential confound in data interpretation—that drug effects interact with the effects of the surgery and/or anesthesia. Furthermore, if pups' recovery from the surgery is age dependent, this may further confound results. To help rule out such confounds, the consequences of central administration are compared with those of systemic administration in the discussion section.

Testing Procedure. Prior to testing, 1 nonimplanted littermate was anesthetized with urethane and placed in the testing chamber to help reduce some of the behavioral and heart rate responses normally induced by isolation (Hofer & Shair, 1978, 1987). The testing chamber consisted of a plastic tub (40 × 20 × 24 cm) with clean pine shavings on the floor. The floor of the cage was kept warm by a heating pad (35 ± 2°C). Axillary body temperatures were recorded using a BAT 12 Digital laboratory thermometer (Physitemp, Clifton, NJ); subjects then were placed in the testing chamber, and heart rate leads were attached. Following a 10-min acclimation period, an injection cannula (26 ga, Plastics One) was inserted into the guide cannula and either vehicle (distilled H₂O), 3.0-, 10.0-, or 30.0-nmol U50,488 in a volume of 0.8 µl was infused over a 60-s period. The injection of drug was verified by measuring the movement of a small air bubble in the tubing connecting the injection cannula to the syringe. The bubble also created an air gap between distilled water (used to initially fill the syringe, tubing, and cannula) and the vehicle. The experimenter was blind to the dose of drug administered. The injection cannula was left in place for an additional 30 s and then removed.

Following the drug injection, behavioral and cardiac responses were recorded for 10 min. The behavioral measures recorded at each age included the duration of several behavioral states and the number of USVs produced. Behavioral states and heart rate measures are discussed in detail later. USVs were transduced into an audible range for the experimenter using an AN-2 Bat detector (Ultrasound Advice) operated in a tuned mode set at 42 kHz. The number of vocalizations was counted manually using a hand counter. Following testing, subjects were removed from the testing cage, axillary body temperatures were recorded, and they were then returned to the holding cage.

Heart Rate Recordings. Heart rate (bpm) was determined from the output of a Grass Model 7 polygraph by a preprocessor and was passed via a parallel port to an IBM-compatible PC (for a detailed description, see Quigley, Shair, & Myers, 1996). The behavioral state (described later) of the subject was continuously monitored and recorded with key presses on the microcomputer so that they could be accurately correlated with the heart rate data. The heart rate and behavioral state data were stored on the hard drive of the PC. Following testing, data files for each individual subject were processed, and an average heart rate for the testing period was determined as well as the average heart rate recorded during each behavioral state exhibited during the testing period. By comparing overall heart rate and heart rate during different behavioral states, the potential for behavioral activity to affect heart rate could be examined. Heart rate recordings made during any behavioral state which lasted for less than 5 s were rejected because heart rate recorded during transient expressions of a behavioral state might not have reflected a true heart rate change resulting from the expression of that behavior.

Behavioral States. The operational definitions of all behavioral states and the ages at which they were observed are described in Table 1. Durations of four mutually exclusive behavioral states (quiet, general activity, wall climbing, and grooming) were recorded in the P7 subjects. In P14 and P21 subjects, the durations of five mutually exclusive behavioral states (quiet, immobile, general activity, groom, and wall climbing) were recorded. All subjects, regardless of age, were always in one of these states. The reason fewer behavioral states were recorded at P7 was the more limited behavioral repertoire of pups at that age. Immobility was not recorded in P7 rats because pups at this age normally rest their heads on the substrate, making it impossible to distinguish immobility from the quiet state as it was defined in this study. Note that in P7 pups, grooming consisted exclusively of face washing whereas in older pups it included limb and body grooming.

Histological Verification of Injection Sites. Following testing, subjects were euthanized by CO₂ inhalation, and 0.8 µl of India ink was infused into the PAG through the injection cannula to help visualize the location of the cannula track. The brains were removed by dissection and placed in a 10% buffered formalin, 30% sucrose solution until they sank. They were then removed and sectioned coronally (50 µm thickness) with a freezing microtome, mounted on glass microscope slides, stained with

Table 1. Summary of Behavioral States Recorded. Ages at Which the Behavioral State Could be Recorded are Denoted in Parentheses

Quiet	(P7, P14, & P21) The subject exhibits no movement except for twitches (as seen during active sleep) and respiration. P14 and P21 subjects in this state rest their head on the substrate and can be either awake or asleep.
Immobile	(P14 & P21) The subject exhibits no movement except for respiration and the head is held above the substrate and eyes are wide open. This behavioral state was not recorded in 7-day-old subjects.
General Activity	(P7, P14, & P21) The subject exhibits activities such as walking, sniffing, and rearing and was recorded whenever activity was observed that did not fall into any of the other categories.
Grooming	(P7, P14, & P21) The subject exhibits grooming of any part of its body and includes behaviors such as face washing and body grooming. Grooming does not include scratching with the hindlimb or allogrooming.
Wall Climbing	(P7, P14 & P21) The subject stands on its hindlegs while its forepaws are alternately moving back and forth as though the animal is trying to locomote up the wall.

cresyl violet, and cover slipped. Injection sites were determined with the aid of a light microscope. The section in which the ink was located most ventrally was used to mark the placement of the injection. If no ink was visible, that subject's data were excluded from the analysis. The placement could not be determined for 2 P14 and 2 P21 subjects. Examination of the ink spread indicated that the volume injected remained in the region surrounding the cannula tip. The experimenter was blind to the dose administered and to the response of the subject when determining the injection sites. A given placement was considered a "hit" if it was at least tangent to the boundary of the PAG. In the 7-day-old subjects, 40 of 44 subjects (90.9%) were hits. In the 14-day-old subjects, 34 of 41 subjects (82.9%) were hits, and in 21-day-old subjects, 31 of 44 (70.5%) were hits. Because there are no distinct histological markers that discriminate dorsal and lateral columns within the PAG, a placement was categorized as in either the dorsal or the lateral PAG if it fell above or below, respectively, a horizontal line drawn across the dorsal tip of the aqueduct in the plane of section used to determine placement. In P7, P14, and P21 subjects, 25.0, 52.9, and 83.9%, respectively, of all PAG placements were in the dorsal PAG. The cannula placements of all subjects are shown in Figure 1.

RESULTS

Statistical Analysis

Quantitative analysis was performed on data from subjects with placements that were inside or tangent to the PAG. Unless otherwise noted, the ANOVA model used to examine all behavioral and heart rate data was a two between-subject factor (Dose \times Placement) design. Body temperature was analyzed in 2 (Pretest vs. Posttest) \times 4 (Dose) within-between ANOVAs. Post hoc comparisons were made using Fisher's tests. When significant interactions between factors occurred in the analyses, main effects of those factors were ignored. Because heart rate specific to each behavioral state could only be recorded if the subject exhibited each behavior for at least 5 s, error degrees of freedom vary for the analysis of heart rate data. As described earlier, gender differences were not observed

for any of the dependent measures at any age tested, and thus data for males and females were combined for all analyses.

7-Day-Old Subjects

Behavior. There was a significant increase in general activity at the highest dose relative to the three lower doses, $F(3, 31) = 3.5, p < .05$ (Table 2). Conversely, time spent in a quiet state decreased significantly following administration of the highest dose relative to the three lower doses, $F(3, 31) = 3.2, p < .01$ (Table 2). The only effect of placement at this age was for USV rates. There was a significant Dose \times Placement interaction, $F(3, 32) = 11.4, p < .001$, on the rate of USV (Figure 2A). Animals with dorsal placements increased vocalization rates at the highest dose relative to all other doses. Animals with lateral placements did not increase their vocalization rates significantly at any dose. Neither wall climbing nor grooming was affected by any dose regardless of placement.

Heart Rate and Body Temperature. Rats at this age have limited thermogenic abilities (Alexander, 1975), and as expected, body temperature decreased significantly from the beginning to the end of the testing period in all groups (overall main effect: 34.6 vs. 33.9°C), $F(1, 30) = 8.7, p < .01$; this decline was not altered by any dose of U50,488. Overall heart rate was not affected by the dose of the drug, but some dose effects were evident for heart rate recorded during specific behavioral states (Table 3). Heart rate recorded when the subjects were quiet or engaged in general activity was decreased by dose, $F(3, 31) = 4.1, p < .05$, and $F(3, 28) = 3.3, p < .05$, respectively. In both cases, heart rate following administration of 10 nmol of U50,488 was reduced relative to vehicle and the 3.0 nmol dose. Heart rates when subjects were grooming or wall climbing could not be determined in enough subjects for analysis.

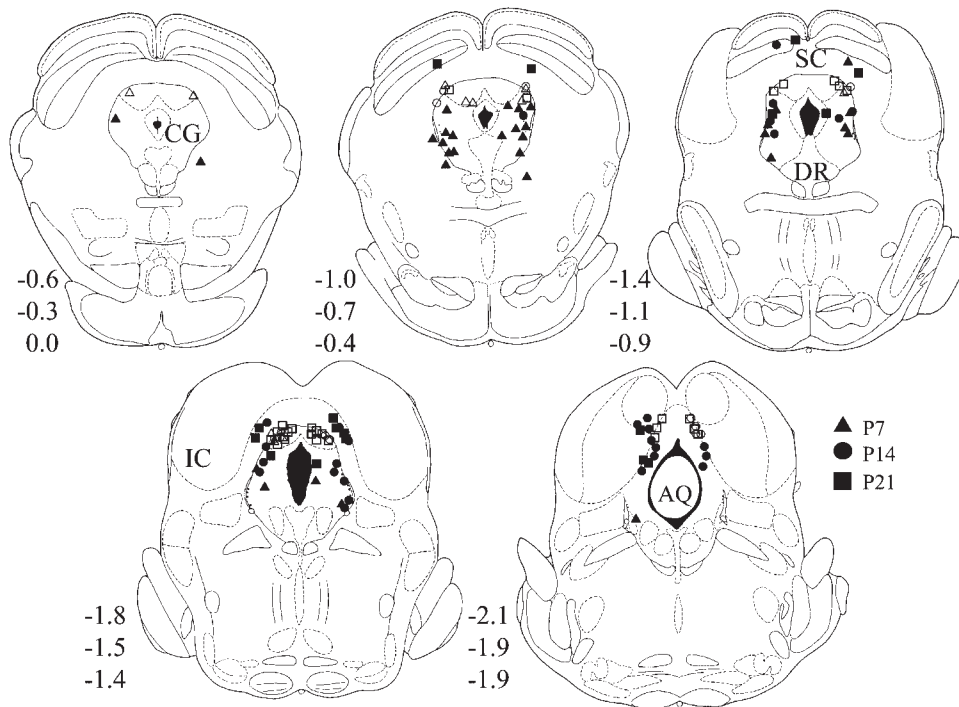


FIGURE 1 Injection sites of 7-, 14-, and 21-day-old subjects superimposed on coronal sections of the midbrain. The location of the planes of section in mm relative to the intraaural line is indicated to the left of each section. The top measure is for 7-day-olds, the middle measure is for 14-day-olds, and the bottom measure is for 21-day-olds. Open symbols indicate dorsal sites, and filled symbols indicate lateral sites. Sites outside the PAG (misses) are filled with horizontal lines. DR = dorsal raphe nucleus, CG = central grey, IC = inferior colliculus, SC = superior colliculus, AQ = aqueduct.

14-Day-Old Subjects

Behavior. Like the P7 subjects, the P14 subjects exhibited behavioral activation. Subjects spent less time quiet, $F(3, 26) = 21.3, p < .001$, and more time in general activity, $F(3, 26) = 17.0, p < .001$, following administration of all doses of U50,488 when compared to the vehicle (Table 2). These subjects also exhibited significantly more time wall climbing, $F(3, 26) = 8.7, p < .001$, at the highest dose of U50,488 relative to vehicle and the lowest two doses (Table 2). No significant main effects or interactions occurred in the analysis of immobility, grooming, or vocalization rate (Figure 2B).

Heart Rate and Body Temperature. There was a significant decline in body temperature from the pretest to the posttest measures (35.5 vs. 35.1°C), $F(1, 26) = 4.7, p < .05$, indicating that these animals were still not capable of maintaining a stable body temperature during the testing period. This decrease was not altered by any dose. Heart rate increased following administration of the highest two doses of U50,488, $F(3, 26) = 6.8, p < .01$, as did heart rate recorded during general activity, $F(3,$

25) = 5.8, $p < .01$ (Table 3). The only effect of placement at this age was seen in a Dose \times Placement interaction, $F(3, 19) = 4.4, p < .05$, of heart rate recorded when subjects were in the quiet state. Animals with lateral placements had a higher heart rate when given the highest dose than when they were given vehicle or the 3.0-nmol dose. In contrast, the dorsal placements had higher heart rates following the second-highest dose relative to all other doses and vehicle (Figure 3). There was insufficient data to analyze heart rate recorded when animals were immobile, grooming, or wall climbing.

21-Day-Old Subjects

Behavior. P21 subjects exhibited only a slight behavioral activation following administration of U50,488, which was reflected in a significant increase in the amount of time spent wall climbing following administration of the two highest doses of U50,488 relative to vehicle and the 3.0-nmol dose, $F(3, 24) = 4.1, p < .05$ (Table 2). There also was a significant suppression of the time spent grooming following injection of the 30.0-nmol dose relative to the 10.0-nmol dose, $F(3, 24) = 3.0, p = .05$. There were no

Table 2. Mean Time (s) and SEs of Behaviors by Age for all Subjects with Placements within the PAG

Behavioral State	Dose of U50,488 (nmol)			
	Vehicle	3.0	10.0	30.0
7-day-olds				
General activity	144.7 (23.0)	134.3 (33.9)	174.0 (64.8)	252.4 (15.4)**
Quiet	133.7 (21.8)	151.3 (37.1)	79.9 (57.8)	39.5 (48.1)**
Wall climbing	0.0	0.0	0.0	1.4 (1.4)
Groom	17.6 (9.07)	10.0 (6.22)	41.1 (13.45)	3.7 (2.92)
14-day-olds				
General activity	63.3 (22.3)	162.8 (22.3)*	221.4 (16.9)*	234.8 (13.2)*
Quiet	229.5 (22.1)	107.0 (29.2)*	55.4 (19.6)*	11.3 (6.7)**
Wall climbing	0.0 (0.0)	0.1 (0.1)	0.9 (0.8)	13.9 (4.1)*
Groom	0.4 (0.4)	22.9 (9.7)	16.4 (2.1)	11.3 (3.9)
Immobile	0.6 (0.6)	2.9 (2.8)	2.5 (2.5)	26.5 (13.6)
21-day-olds				
General activity	116.5 (32.1)	190.8 (10.3)	171.1 (16.3)	204.6 (21.5)
Quiet	61.3 (30.2)	40.2 (18.1)	7.8 (6.4)	52.7 (29.6)
Wall climbing	0.0 (0.0)	0.0 (0.0)	1.7 (0.8) [†]	2.0 (1.0) [†]
Grooming	60.4 (18.5)	63.7 (15.2)	96.3 (13.9)	15.2 (9.8) [‡]
Immobile	57.9 (36.2)	3.4 (3.2)	19.8 (12.7)	22.9 (9.1)

Note. *significantly different from vehicle. **significantly different from the lowest three doses. [†]significantly different from the lowest two doses. [‡]significantly different from 10.0-nmol dose. Placement did not interact with dose for any of the behaviors at any age.

statistical effects in the analysis of time spent quiet, immobile, in general activity, or vocalization rate (Figure 2C).

Heart Rate and Body Temperature. By 21 days of age, animals were able to maintain body temperature during the testing period. No main effects or interactions were observed for body temperature. There were no significant effects in the analysis of overall heart rate or heart rate recorded when the animals were quiet, immobile, engaged in general activity, or grooming. There were insufficient data to analyze heart rate recorded during wall climbing.

DISCUSSION

The results confirmed our primary hypothesis that the PAG is a site that mediates many of the behavioral effects of kappa receptor stimulation during early development. This does not rule out the possibility that kappa stimulation of other brain sites might produce similar effects. For example, the glutamate agonist kainate will evoke a similar repertoire of escapelike behaviors from multiple limbic-related brain sites in adult rats (Graeff, 1994). For most of the behavioral responses, the dorsal and lateral PAG were equally sensitive, with the exception that vocalizations in P7 subjects were more readily elicited by

dorsal sites. This is consistent with data from adult rats indicating that USVs are most readily elicited from the dorsal region of the PAG by electrical stimulation (Yajima et al., 1980). These two findings indicate that the dorsal PAG may be important for USV production throughout the life span, with the neurochemical mediator of vocalizations changing as rats approach weaning. Other studies have shown that in adult rats, microinjection of glutamate agonists into the PAG are very effective at inducing USV production (Bandler & Depaulis, 1988) whereas our own work indicates that stimulation of these receptors is ineffective in evoking USV production in preweaning pups (Goodwin & Barr, 1998).

Finally, heart rate was affected by kappa receptor stimulation during the first 2 postnatal weeks, albeit in opposite directions. The site-specific effects of U50,488 on heart rate in P14 subjects may reflect a greater sensitivity of dorsal sites to this drug's effects, as heart rate increases were induced at the 10-nmol dose for dorsal placement but at the 30-nmol dose for lateral placements. Although the dorsal placement did not evoke an increase in heart rate at the 30-nmol dose, this may reflect the inverted U-shaped dose–response relationship for U50,488 described by Carden et al. (1994) when this drug was administered peripherally. Though none reached significance, the duration of time grooming at all three ages and the duration of quiet and immobile states at P21

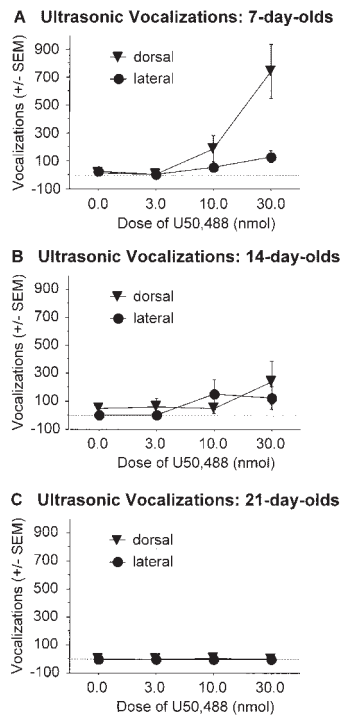


FIGURE 2 The total number of ultrasonic vocalizations produced in 7-day-old (A), 14-day-old (B), and 21-day-old (C) subjects averaged by dose and placement. Only 7-day-old subjects exhibited an increase in vocalization rate when the cannula was located in a dorsal site. Vocalization rates did not change significantly following administration of any dose of U50,488 in 14- or 21-day-old subjects regardless of cannula placement.

approximated inverted *U*-shaped dose–response relationships in the present study.

The increase in USV rates at 7 days of age are consistent with studies of the effects of U50,488 following systemic administration in 3- and 10-day-old pups (Carden et al., 1993; Kehoe & Boylan, 1994); however, the failure to find drug-induced increases in vocalization in 14- and 21-day-old subjects contrasts with a report of increased vocalization rates in 18-day-old pups following intraperitoneal administration of U50,488 (Carden et al., 1994). There are a couple of possibilities which might explain this discrepancy. One is that kappa opioid modulation of USV production by the PAG is limited to the first postnatal week, with kappa receptors in other brain areas playing a larger role in 2- and 3-week-old rats. Kappa opioid receptors are distributed in several limbic-related structures including the amygdala, hippocampus, and hypothalamus (Leslie & Loughlin, 1993), and these structures have been shown to be involved in the production of USVs in adult rats (Duncan, Knapp, & Breese, 1996; Fu & Brudzynski, 1994). Future studies could address this possibility by infusing U50,488 into multiple sites.

Another possibility is that a high-enough dose was not used in the present study to elicit vocalizations in the 2- and 3-week-old animals. In the report by Carden et al. (1994), 18-day-old subjects required a 10-fold higher dose than 10-day-olds to elevate vocalization rates. In addition, the peak vocalization rate of 18-day-olds was only about 20% of the peak rate of 10-day-olds. Although the highest dose in the present study was 10 times the lowest dose, it is possible that this high dose was still below a threshold necessary to elicit vocalizations in the oldest two ages tested. Nevertheless, it is important to note that both in the present study and in the Carden et al. (1994) report, an age-dependent decline in the ability of U50,488 to elicit vocalizations is observed between the end of the first to the end of the third postnatal week.

Animals at all ages exhibited some behavioral activation. General activity was increased by the drug only at 1 and 2 weeks of age; however, wall climbing was increased at 2 and 3 weeks of age. This behavioral arousal is more subdued than that seen following infusions of kainate, which triggers bouts of explosive running in 2- and 3-week-old subjects (Goodwin & Barr, 1998) as well as in adults (Carrive et al., 1987). Instead, U50,488 increased non-flight-like locomotion (as reflected in the general activity times) during the first 2 postnatal weeks, and then more specifically wall climbing during the second and third postnatal weeks. These findings are consistent with reports of an increase in activity scores following systemic administration of U50,488 in 10-day-old pups (Carden et al., 1993; Carden et al., 1994; Kehoe & Boylan, 1994). In the report by Carden et al. (1994), no changes in locomotor behavior were observed in 18-day-old pups, consistent with the present study which found no changes in general activity at P21.

Although U50,488 elicited increases in activity and vocalization rate in the present study as well as in previously published studies (Carden et al., 1994; Carden & Hofer, 1990; Kehoe & Boylan, 1994), increase in vocalization rate is probably not a by-product of this behavioral activation for a couple of reasons. First, the increases in activity of P14 and P21 subjects in the present study was not accompanied by increases in vocalization rate, and second, other studies have shown that these two measures are uncorrelated. For example, administration of benzodiazepines at doses that do not suppress locomotor activity are effective in suppressing USV production in P10 rats (Insel, Hill, & Mayor, 1986) whereas cocaine, a potent psychomotor stimulant in neonatal rats, suppresses vocalization rates (Kehoe & Boylan, 1992).

This is the first time the autonomic effects of infusing U50,488 into the PAG have been investigated in pre-weanling rats. Neurons in the PAG may alter heart rate by two possible means. One, known to operate in adult rats, is through projections to a region of the rostro-ventro

Table 3. Mean Heart Rate (bpm) and SEs by Behavioral State and Age for all Subjects with Cannula Placements within the PAG

Behavioral state	Dose of U50,488 (nmol)			
	Vehicle	3.0	10.0	30.0
7-day-olds				
Overall	365.7 (7.3)	357.9 (10.8)	331.6 (13.2)	341.4 (12.8)
General activity	370.2 (6.5)	369.9 (7.6)	334.5 (12.8) [†]	343.3 (12.2)
Quiet	357.8 (9.6)	353.1 (11.9)	316.4 (16.3) [†]	327.6 (12.5)
Groom	369.9 (7.8)	383.6 (6.8)	341.4 (14.5)	352.1 (48.3)
14-day-olds				
Overall	407.8 (12.6)	406.4 (10.1)	449.5 (14.1) [†]	469.5 (13.3) [†]
General activity	412.1 (12.4)	410.0 (10.2)	450.0 (13.7) [†]	469.6 (13.2) [†]
Quiet	406.2 (12.8)	392.5 (12.5)	448.6 (19.8)	437.1 (26.1)
21-day-olds				
Overall	451.4 (20.2)	440.2 (18.8)	451.2 (9.8)	467.9 (15.3)
General activity	458.2 (18.3)	437.2 (18.3)	438.9 (10.2)	469.4 (14.5)
Quiet	425.2 (23.5)	372.6 (20.2)	486.1 (0.0)	440.4 (25.8)
Groom	524.4 (14.5)	492.0 (6.71)	480.6 (11.4)	508.2 (10.2)
Immobile	439.1 (24.2)	441.0 (0.0)	412.8 (27.9)	485.5 (11.2)

Note. Heart rate specific to a behavioral state was recorded only if the behavior was exhibited for 5 consecutive s. Heart rate specific to wall climbing at P7, grooming, immobile, and wall climbing at P14, and wall climbing at P21 could not be calculated because there was insufficient data. [†]significantly different from vehicle and the 3.0-nmol dose. Placement interacted with dose for heart rates of P14 subjects recorded during a quiet state only.

medulla that regulates activity in both branches of the autonomic nervous system (Lovick, 1993). The other possibility is that heart rate changes are the indirect result of behavioral changes induced by drug stimulation. Several factors argue against this latter possibility. First, despite high levels of activity in P7 subjects, only decreases in heart rate were observed. Second, although the lowest dose given to P14 subjects significantly elevated general activity, it did not significantly elevate heart rate. Third, other reports have failed to show an activity-

induced increase in heart rate before 21 days of age (Hofer & Reiser, 1969). Finally, although a significant increase in wall climbing was observed in P21 subjects, no changes in heart rate were recorded. Although these results suggest it is unlikely that heart rate effects were secondary to behavioral changes, future studies will need to document these effects in nonbehaving animals. Incidentally, USV production is probably not secondary to alterations in autonomic activity induced by PAG stimulation, as pharmacological manipulations of autonomic activity are ineffective in changing USV production (Hofer & Shair, 1991).

The reasons for the opposite effect of U50,488 on heart rate in P7 and P14 subjects are not clear from the present study. Developmental changes either within the PAG or its efferent targets may be responsible. In general, excitatory amino acid or electrical stimulation of the dorsal and lateral PAG elicits increases in heart rate and blood pressure in adult rats (for review, see Carrive, 1993). These cardiovascular effects have been shown to rely on efferent projections to the ventral medulla, a region which modulates autonomic activity (Carrive, 1993; Lovick, 1993; van der Plas, Maes, & Bohus, 1995). The increase in heart rate seen at P14 is therefore consistent with the adult literature showing that stimulation of these regions of the PAG elicits increases in heart rate.

The reason for the decrease in heart rate at P7 is not clear; however, one possible explanation might stem from

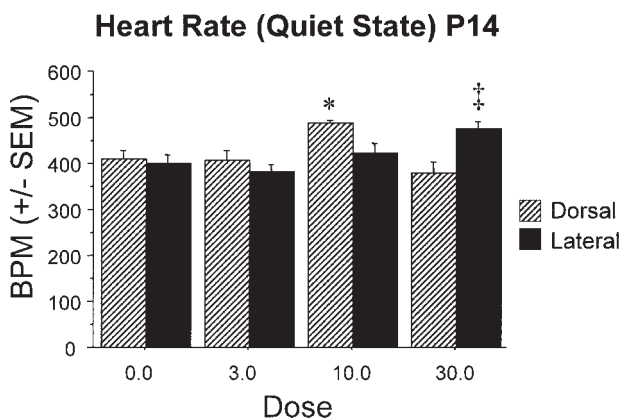


FIGURE 3 Average heart rate by dose and placement in 14-day-old subjects when they were in a quiet state (*significantly different from all other doses and vehicle; [†]significantly different from vehicle and the 3.0-nmol dose).

an earlier maturation of the PAG's control of blood pressure relative to heart rate. If true, then stimulation of the PAG at P7 might have resulted in an increase in blood pressure, but no corresponding direct effect on heart rate. The increase in blood pressure could have reduced heart rate indirectly via the baroreceptor reflex known to be functional by P14 (Hofer, 1985; Hofer & Reiser, 1969) (Younger ages have yet to be investigated.) This hypothesis is highly conjectural, but if true, then deafferentation of arterial baroreceptors should block the heart rate effects of intra-PAG infusions of U50,488 in P7 pups.

Whatever the cause of this developmental reversal, kappa receptor stimulation in the dorsal or lateral PAG no longer seems to affect heart rate by P21. Though no reports of the effects of dorsolateral infusions of U50,488 or any other kappa agonists exist in adult rats, there is one report of a suppression of heart rate and blood pressure resulting from kappa receptor stimulation in the ventro-lateral PAG (Keay et al., 1997), an effect also reported following peripheral administration of U50,488 (Sorrentino, Capasso, d'Emmanuele di Villa Bianca, & Pinto, 2001). One other article reports no effects on heart rate or blood pressure of U50,488 infusion into several brain regions including the dorsal hippocampus, paraventricular nucleus, or ventrolateral medulla (Sun, Liu, Li, & Ingenito, 1996). Thus, it would appear that the increase in heart rate observed in the present study in P14 subjects is a transient developmental phenomenon.

What may account for the developmental change in the behavioral and autonomic effects of kappa receptor stimulation in the PAG? One possibility that can be ruled out is that kappa receptor levels, or at least the $\kappa 1$ sites (i.e., the sites bound by U50,488) simply decline during the third postnatal week. Although whole brain $\kappa 1$ binding density decreases slightly from the second postnatal week through adulthood (Kitchen et al., 1990), autoradiographic studies demonstrate an increase in $\kappa 1$ binding in the PAG from the second postnatal week to adulthood (Kornblum, Hurlbut, & Leslie, 1987; Leslie & Loughlin, 1993). This suggests that something in the signal transduction pathway or in the output paths is changing during the preweanling period. At present, there are no studies available to suggest what the nature of these changes might be.

In conclusion, these results demonstrate that kappa receptors in the PAG are capable of producing unique behavioral and autonomic effects during the preweanling period. These behavioral responses (i.e., increased locomotion and USV production) closely resemble the same responses evoked by social isolation during the first postnatal week (Hofer, 1994; Noirot, 1968). Taken together with previous research showing that lesions of the PAG disrupt isolation-induced analgesia and vocali-

zations (Wiedenmayer et al., 2000), it is reasonable to hypothesize that an endogenous kappa opioid ligand released within the PAG stimulates some of these behavioral responses to social isolation in neonatal rats. Future research investigating the effect of infusing a kappa antagonist into the PAG will be necessary to prove such a hypothesis.

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