



Letter to Neuroscience

STRESS-INDUCED PREPROENKEPHALIN mRNA EXPRESSION IN THE AMYGDALA CHANGES DURING EARLY ONTOGENY IN THE RAT

C. P. WIEDENMAYER,^{a,b*} P. A. H. NOAILLES,^c J. A. ANGULO^c and G. A. BARR^{a,b,d}

^aDepartment of Psychiatry, Columbia University, 1051 Riverside Drive, Unit 40, New York, NY 10032, USA

^bDevelopmental Psychobiology, New York State Psychiatric Institute, New York, NY 10032, USA

^cDepartment of Biological Sciences, Hunter College, City University of New York, New York, NY 10021, USA

^dDepartment of Psychology, Hunter College, City University of New York, New York, NY 10021, USA

Key words: opioids, analgesia, periaqueductal gray.

Stress activates endogenous opioids that modulate nociceptive transmission. Exposure to a potentially infanticidal adult male rat suppresses pain-related behaviors in pre-weaning but not in older rats. This male-induced analgesia is mediated by μ opioid receptors in the periaqueductal gray, a midbrain structure that is innervated by amygdala projections. To determine whether enkephalin, a μ and δ opioid receptor agonist, is activated by male exposure, mRNA levels of its precursor, preproenkephalin, were measured in subdivisions of the amygdala and the periaqueductal gray. In 14-day-old but not in 21-day-old rats, 5 min of male exposure induced analgesia to heat and increased preproenkephalin mRNA levels in the central nucleus of the amygdala but not in the periaqueductal gray. The change in the activation of enkephalinergic neurons in the central amygdala may contribute to the change in stress-induced analgesia during early ontogeny. © 2002 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Aversive stimulation activates forebrain structures such as the amygdala (Gallagher and Chiba, 1996; Charney et al., 1998) and the midbrain periaqueductal gray (PAG) (Da Costa Gomez and Behbehani, 1995; Pavlovic et al., 1996; Helmstetter et al., 1998) and induces the release of opioids that inhibit afferent transmission of nociceptive signals (Fields and Basbaum, 1999). μ Opioid receptors, which are found in the amygdala and the PAG, play a major role in pain modulation; their antagonism blocks stress-induced analgesia (Pavlovic et al., 1996; Bellgowan

and Helmstetter, 1998; Tershner and Helmstetter, 2000). Enkephalin, a ligand to the μ and δ opioid receptor, is synthesized in neurons of the amygdala and the PAG (Harlan et al., 1987; Mansour et al., 1993; Smith et al., 1994; Veinante et al., 1997) and has been implicated in the modulation of nociception. Injection of an enkephalinase inhibitor or of the enkephalin gene into the amygdala or the PAG decreased nociceptive reactivity (Al-Rodhan et al., 1990; Kang et al., 1998), and paw inflammation induced enkephalin release in the PAG (Williams et al., 1995)

During development, as the animal matures, stimuli that are stressful and induce analgesia at a particular age may change their salience and no longer induce analgesia later in ontogeny. For example, young rats that are exposed to a potentially infanticidal adult male rat are analgesic only before weaning. Male-induced analgesia disappears by postnatal day 21 when the male no longer represents a deadly threat (Wiedenmayer and Barr, 1998, 2001). Male exposure seems to induce the release of opioids in the PAG because local injection of the selective μ opioid receptor antagonist CTOP into the ventrolateral part of the PAG suppressed male-induced analgesia (Wiedenmayer and Barr, 2000). The aim of the present study was to examine whether male exposure involves enkephalin activation in the amygdala and the PAG in young rats.

Fourteen-day-old rats exposed to an adult male differed in preproenkephalin (PPE) mRNA levels in selected brain areas (Fig. 1) from non-exposed rats (analysis of variance (ANOVA), $F(3,15) = 7.50$, $P < 0.01$). The male-exposed rats had significantly higher levels of PPE mRNA expression in the central amygdala compared to controls (Fig. 2, Newman–Keuls tests, $P < 0.001$). On day 21, PPE mRNA levels did not differ between male-exposed and control animals (Fig. 2). On day 14, male-exposed rats displayed significantly different paw withdrawal latencies from a heated surface compared

*Correspondence to: C.P. Wiedenmayer, Department of Psychiatry, Columbia University, 1051 Riverside Drive, Unit 40, New York, NY 10032, USA. Tel.: +1-212-543-5973; fax: +1-212-543-5467.

E-mail address: cpw14@columbia.edu (C. P. Wiedenmayer).

Abbreviations: ANOVA, analysis of variance; PAG, periaqueductal gray; PPE, preproenkephalin.

ERRATUM

ERRATUM TO “STRESS-INDUCED PREPROENKEPHALIN mRNA EXPRESSION IN THE AMYGDALA CHANGES DURING EARLY ONTOGENY IN THE RAT” [Neuroscience 114 (1) 7–11][☆]

C. P. WIEDENMAYER,^{a,b*} P. A. H. NOAILLES,^c J. A. ANGULO^c AND G. A. BARR^{a,b,d}

^aDepartment of Psychiatry, Columbia University, 1051 Riverside Drive, Unit 40, New York, NY 10032, USA

^bDevelopmental Psychobiology, New York State Psychiatric Institute, New York, NY 10032, USA

^cDepartment of Biological Sciences, Hunter College, City University of New York, New York, NY 10021, USA

^dDepartment of Psychology, Hunter College, City University of New York, New York, NY 10021, USA

The publisher regrets the greek letters in the abstract were incorrectly reproduced. The correct text is printed below.

Stress activates endogenous opioids that modulate nociceptive transmission. Exposure to a potentially infanticidal adult male rat suppresses pain-related behaviors in pre-weaning but not in older rats. This male-induced analgesia is mediated by μ opioid receptors in the periaqueductal gray, a midbrain structure that is innervated by amygdala projections. To determine whether enkephalin, a μ and δ opioid receptor agonist, is activated by male exposure, mRNA levels of its precursor, preproenkephalin, were measured in subdivisions of the amygdala and the periaqueductal gray. In 14-day-old but not in 21-day-old rats, 5 min of male exposure induced analgesia to heat and increased preproenkephalin mRNA levels in the central nucleus of the amygdala but not in the periaqueductal gray. The change in the activation of enkephalinergic neurons in the central amygdala may contribute to the change in stress-induced analgesia during early ontogeny.

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*Correspondence to: C. P. Wiedenmayer, Department of Psychiatry, Columbia University, 1051 Riverside Drive, Unit 40, New York, NY 10032, USA. Tel.: +1-212-543-5973; fax: +1-212-543-5467.

E-mail address: cpw14@columbia.edu (C. P. Wiedenmayer).

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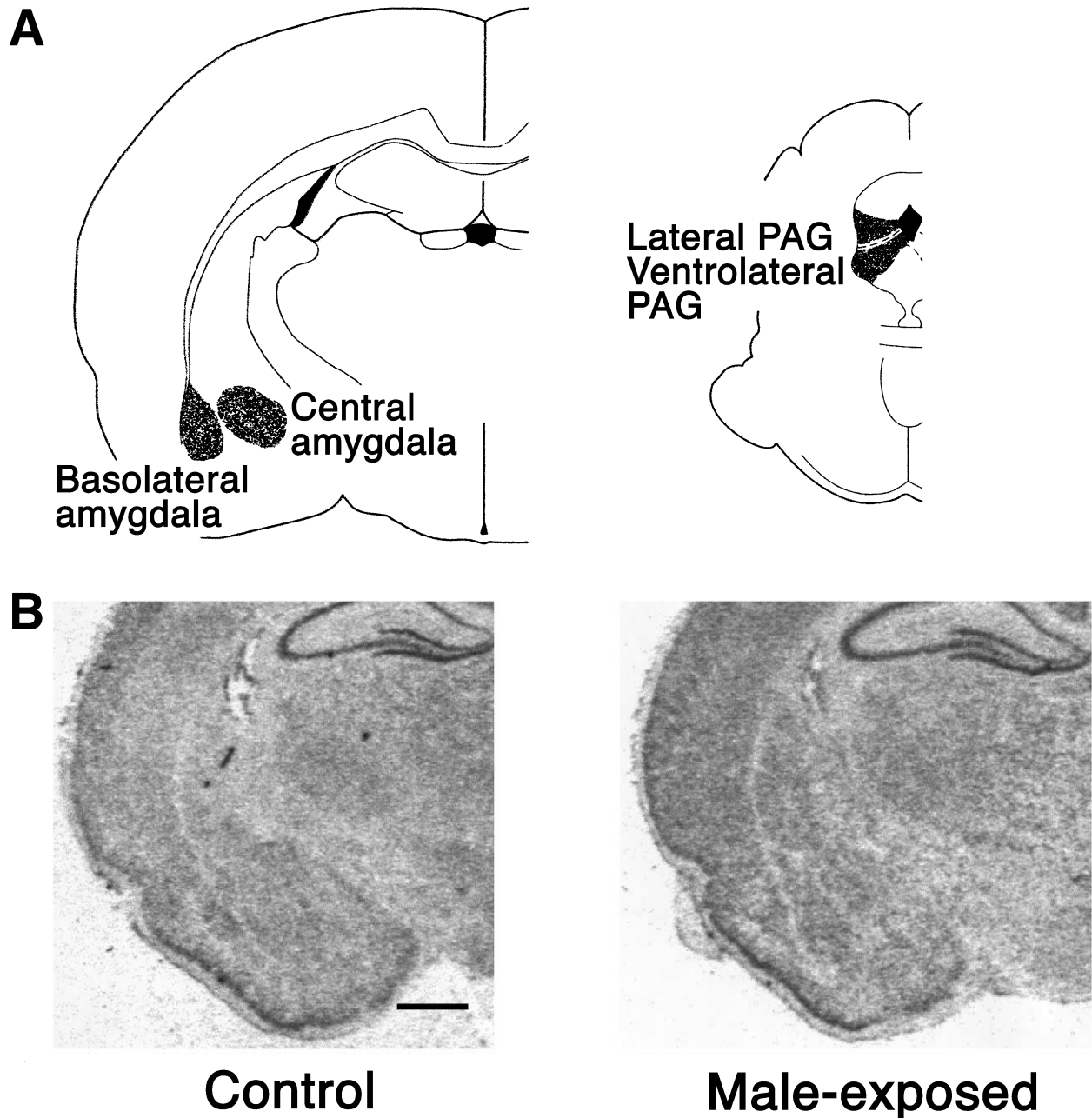


Fig. 1. (A) Schematic diagrams of the brain areas in which preproenkephalin mRNA levels were quantified. (B) Photomicrographs showing brain sections of a 14-day-old non-exposed control pup and a pup exposed to the adult male rat. Scale bar = 1 mm.

to controls (ANOVA, $F(3,15) = 3.89$, $P < 0.05$). Male-exposed rats took significantly longer to lift the paw compared to control rats (Fig. 3, Newman–Keuls tests, $P < 0.01$). On day 21, withdrawal latencies did not differ between conditions.

Several studies in adult rats have shown that stressful experience induces enkephalin gene expression in the hypothalamus (Iglesias et al., 1992; Helmreich et al., 1999; Dumont et al., 2000) and in the central amygdala (Petrovich et al., 2000). The results of the present study extend these findings and demonstrate that stimulus-induced enkephalin expression depends on the age of

the animal. Enkephalin biosynthesis in young rats after male exposure may replenish previously released enkephalin or result in accumulation, which is necessary to support a higher rate of utilization in a later encounter with stressors such as the male. However, it remains to be determined whether increased PPE mRNA levels indicate transcriptional activity. mRNA may not be translated into the enkephalin precursor PPE or not be processed further into enkephalin (Iglesias et al., 1992; Zagon et al., 1994).

Amygdala projections to the PAG seem to be involved in the inhibition of nociception. Lesion of the ventrolat-

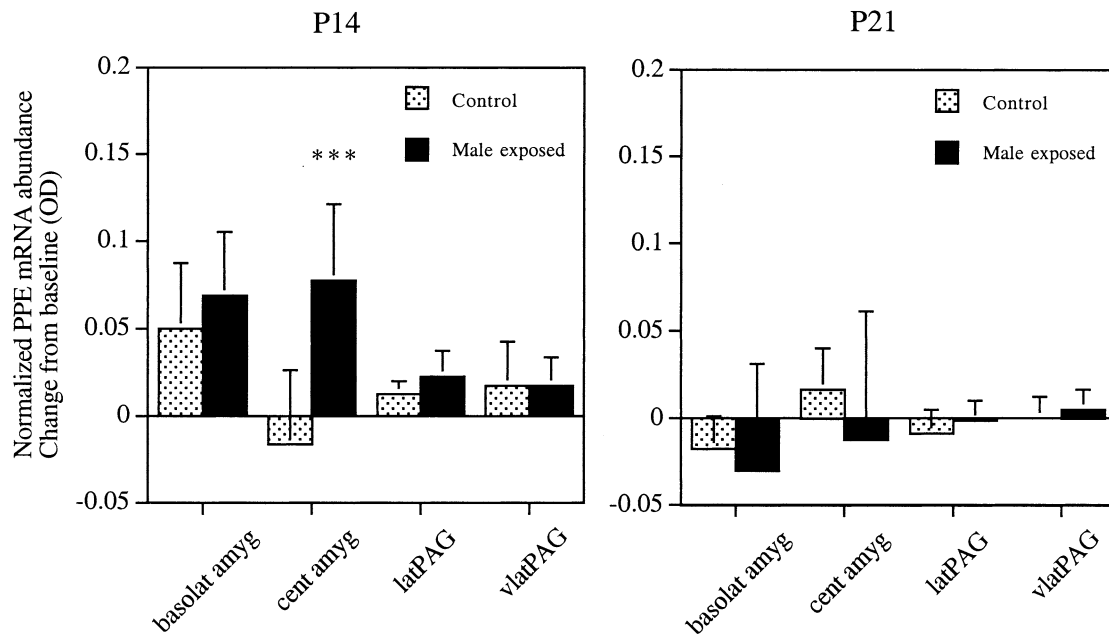


Fig. 2. PPE mRNA levels in the amygdala and periaqueductal gray of 14- and 21-day-old rats after exposure to an adult male rat. Control rats were not exposed to the male. The values represent change scores (means \pm S.E.M.) from baseline levels, *** P < 0.001. Basolat amyg, basolateral amygdala; cent amyg, central amygdala; latPAG, lateral periaqueductal gray; vlatPAG, ventrolateral periaqueductal gray.

eral PAG disrupted analgesia induced by stimulation of μ opioid receptors in the amygdala (Helmstetter et al., 1998). Injection of a μ opioid receptor antagonist into the ventrolateral PAG altered neuronal firing produced by stimulation of the central amygdala (Da Costa Gomez and Behbehani, 1995) and decreased analgesia produced by basolateral amygdala stimulation (Tershner

and Helmstetter, 2000). Enkephalin synthesis in the central amygdala may indicate enkephalin release in the PAG, which through μ opioid receptors mediates male-induced analgesia in 14-day-old rats (Wiedenmayer and Barr, 2000). Accordingly, differential activation of the amygdala may contribute to developmental changes in nociceptive reactivity.

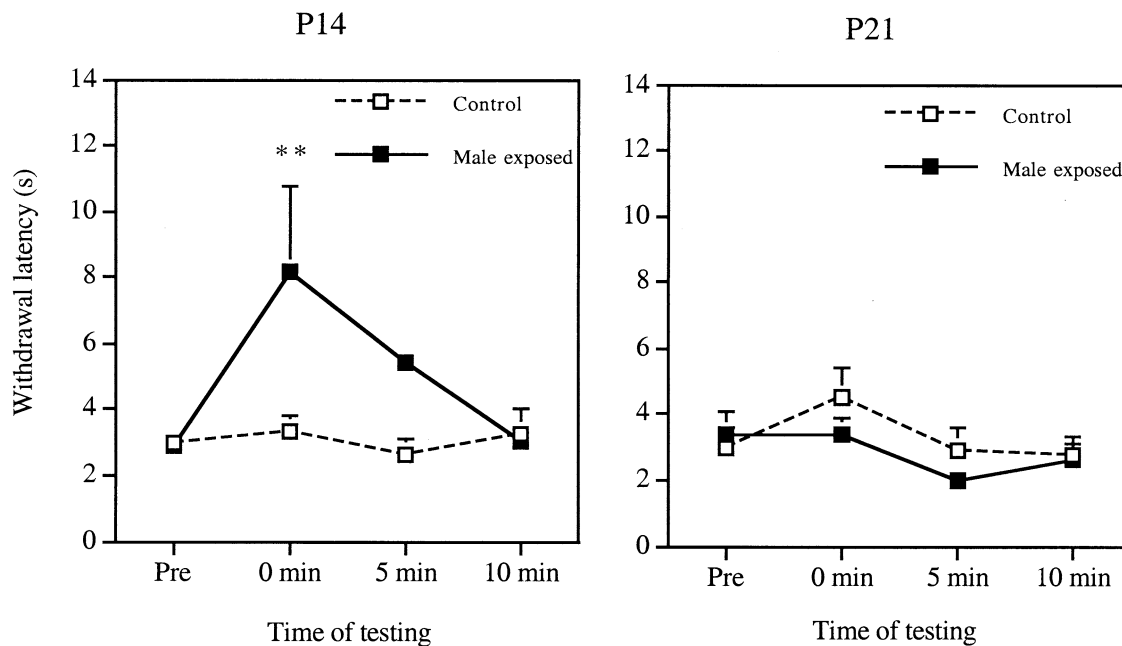


Fig. 3. Paw withdrawal latencies from a heated surface (39°C) of 14- and 21-day-old rats. Latencies were measured immediately before (Pre) and 0, 5 and 10 min after exposure to an adult male rat. Controls were not exposed to the male. Means \pm S.E.M., ** P < 0.01.

EXPERIMENTAL PROCEDURES

Animals

Long-Evans hooded rats were housed under standard laboratory conditions. Treatments were according to the guidelines of the Institutional Animal Care and Use Committee. The number of animal used is the minimum number required based on our prior experience to provide statistical significance.

Procedure and behavioral measures

Male rats were used throughout the experiments. Rats were tested in small huddles to decrease isolation-induced stress (Hofer and Shair, 1980). Of the three young rats, one rat was used to assess nociceptive reactivity, the other was used to determine enkephalin activity. This distinction was made because noxious thermal stimulation could have affected enkephalin activity. Testing procedures, as described previously (Wiedenmayer and Barr, 2001), consisted of exposing the young rats for 5 min to an adult male rat behind a wire-mesh screen. For nociceptive reactivity testing, the left forepaw of the first rat was put on an electric resistor heated at nominal temperatures of 39°C. Latency to withdraw the paw was assessed immediately before the male was placed in the testing cage (baseline), immediately after removal of the adult male, and again 5 and 10 min after exposure. Three rats from the same litter were used as control animals. They were submitted to the same procedure, but were tested without a male in the adjacent compartment. Three h after exposure, three rats per litter (see below) were removed from the home cage for the *in situ* hybridization procedure. Animals from six litters were tested on day 14 and another six litters on day 21.

Radiolabeling of the oligonucleotide probes

PPE mRNA was detected with antisense synthetic oligonucleotide probes (Oligos Etc., Wilsonville, OR, USA) that were labeled with ³⁵S-dATP. The probes correspond to amino acids 86–95 and 112–121, and their specificity was determined previ-

ously (Angulo et al., 1991). The probes were labeled at the 3' end by terminal transferase (Roche, Indianapolis, IN, USA).

In situ hybridization histochemistry

One rat from the male-exposed and one rat from the control group, which both had not been tested previously for nociceptive reactivity, were used for the assessment of PPE mRNA expression. In addition, baseline PPE mRNA levels were assessed in an unhandled littermate taken directly from the home cage. The rats were anesthetized, decapitated and their brains removed over dry ice. The frozen brains were sectioned coronally (20 µm). Three out of every 12 sections were collected per slide and fixed at 4°C in 2% paraformaldehyde. Sections were hybridized overnight at 37°C in a humidified environment (Angulo et al., 1991). The next day, hybridized sections were washed, dried and apposed to film (Hyperfilm MP, Amersham Pharmacia Biotech, Piscataway, NJ, USA) for 21 days.

Quantification of preproenkephalin mRNA levels

PPE mRNA levels were determined by quantifying gray levels on X-ray autoradiograms in regions corresponding to subdivisions of the amygdala and the PAG. The amygdala was subdivided into the basolateral complex (consisting of the lateral and basolateral nuclei; Maren, 1999) and central amygdala; the PAG was subdivided into lateral and ventrolateral PAG (Bandler and Shipley, 1994, Fig. 1). Gray levels were quantified with an image analysis system utilizing the NIH Image 1.49 software by a person without knowledge of treatment group. Film background values were subtracted from all sections. Sections of control and male-exposed animals were matched for corresponding neuroanatomical levels. To correct for variation between assays, values from unhandled animals were subtracted from values from control and male-exposed animals and change scores were used for the statistical analyses.

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