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Significance of Neuronal Cytochrome P450 Activity in Opioid-Mediated Stress-Induced Analgesia

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Abstract

Stressful environmental changes can suppress nociceptive transmission, a phenomenon known as "stress-induced analgesia". Depending on the stressor and the subject, opioid or non-opioid mechanisms are activated. Brain μ opioid receptors mediate analgesia evoked either by exogenous agents (e.g. morphine), or by the release of endogenous opioids following stressful procedures. Recent work with morphine and neuronal cytochrome P450 (P450)-deficient mice proposed a signal transduction role for P450 enzymes in μ analgesia. Since μ opioid receptors also mediate some forms of stress-induced analgesia, the present studies assessed the significance of brain P450 activity in opioid-mediated stress-induced analgesia. Two widely-used models of opioid stress-induced analgesia (restraint and warm water swim) were studied in both sexes of wild-type control and P450-deficient (*Null*) mice. In control mice, both stressors evoked moderate analgesic responses which were blocked by pretreatment with the opioid antagonist naltrexone, confirming the opioid nature of these responses. Consistent with literature, sex differences (control female > control male) were seen in swim-induced, but not restraint-induced, analgesia. *Null* mice showed differential responses to the two stress paradigms. As compared with control subjects, *Null* mice showed highly attenuated restraint-induced analgesia, showing a critical role for neuronal P450s in this response. However, warm water swim-induced analgesia was unchanged in *Null* vs. control mice. Additional control experiments confirmed the absence of morphine analgesia in *Null* mice. These results are the first to show that some forms of opioid-mediated stress-induced analgesia require brain neuronal P450 activity.

Keywords

stress; analgesia; cytochrome P450; brain; opioid; μ opioid receptors

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1. Introduction

Environmental changes which threaten an organism's survival can evoke a plethora of defensive/survival responses. Among these, the suppression of pain transmission by the CNS following exposure to stressful stimuli is well documented. Laboratory studies have demonstrated the existence of multiple forms of such responses, collectively referred to as “stress-induced analgesia” (Kelly, 1986;Bodnar, et al., 1980). The pharmacological characteristics of a particular stress-induced response are known to depend upon the intensity, duration and nature of the stressor; the characteristics of the subject (gender, physiological and psychological state) are also important. Stress-induced analgesic responses have been classified as either “opioid” or “non-opioid”, and as either “neuronal” (i.e. mediated exclusively by the nervous system) or “hormonal” (mediated by a combination of neuronal and humoral factors) (Terman, et al., 1984; Watkins and Mayer, Kelly, 1986;Bodnar, 1990). Brain μ opioid receptors are important for both neuronal-opioid and hormonal-opioid forms of stress-induced analgesia, but δ opioid and κ opioid receptors may also participate (Contet, et al., 2006;Labuda, et al., 2000).

Brain μ opioid receptors respond to both endogenous (endorphins, endomorphins, enkephalins) and exogenous (e.g. morphine) μ agonists, and are therefore crucial for many physiological, psychological and pharmacological processes. Morphine acts on μ opioid receptors in the periaquectuctal grey (PAG), the rostral ventromedial medulla, and spinal dorsal horn to attenuate pain transmission. In the PAG, μ activation inhibits pre-synaptic GABAergic activity, leading to stimulation of descending, pain-relieving circuits (Heinricher and Ingram, 2008). However, the cellular mechanisms by which μ agonists produce this effect remain uncertain. While exploring the opioid analgesic mechanisms in the PAG, we recently discovered that knockout mice with deficiencies in brain neuronal cytochrome P450 monooxygenase (P450) lacked normal analgesic responses to morphine (Conroy, et al., 2010). Although P450 enzymes are best known for performing drug metabolism, they also catalyze many endogenous lipid metabolic reactions (Spector, 2009). Based on the defective responses in P450-deficient mice and earlier results (Vaughan, et al., 1997), Conroy et al. (2010) proposed a new analgesic mechanism whereby μ opioids inhibit PAG GABAergic activity by stimulating the release and P450-mediated epoxidation of arachidonic acid. The epoxide products are reported to have analgesic or anti-allodynic activity (Terashvili, et al., 2008;Wagner, et al., 2013).

As mentioned, μ opioid receptors mediate both physiological (stress-induced) as well as pharmacological (morphine-induced) analgesia. Since brain P450 activity was shown to be important for analgesia produced by μ opioids like morphine (Conroy, et al., 2010), it seemed likely that similar mechanisms might be significant for opioid forms of stress-induced analgesia. This hypothesis was tested presently by studying two forms of opioid-mediated stress-induced analgesia in brain P450-deficient (*Null*) and wild-type control mice.

2. Results

2.1. Restraint-induced analgesia

Restraint increased nociceptive latencies in both male and female saline-treated, control mice, effects which subsided ten min later (Fig. 1A). This analgesia was prevented by naltrexone pre-treatment (Figs. 1A, 1B). ANOVA (wild-type latencies in Fig. 1 A, between groups [factor #1]: gender; [factor #2]: drug; within groups [factor #3, repeated measures]: time) found significant main effects of drug ($F_{1,14}$=8.3, P<0.02), time ($F_{2,28}$=13.0, P<0.001), with a significant drug by time interaction term ($F_{2,28}$= 5.0, P < 0.02). No gender differences were detected in main effects or in gender-related interaction terms. Additional ANOVAs of 30 min latencies (sexes analyzed separately) and 30 min % MPE data (pooled sexes) documented the naltrexone effects. Post-hoc testing confirmed the inhibition by naltrexone at the 30 min time point in both sexes (Fig. 1A, 1B).

Among saline-treated animals, restraint-induced analgesia was highly attenuated in male and female *Null* mice as compared with respective wild-type controls (Figs. 1C, 1D). ANOVA (latencies in Fig. 1C, between groups [factor #1]: gender; [factor #2]: genotype; within groups [factor #3, repeated measures]: time) found a significant main effect of time ($F_{2,30}$=17.5, P<0.001), with a significant genotype by time interaction term ($F_{2,30}$= 3.7, P < 0.05). No gender differences were detected in the main effects or gender-related interaction terms. Additional ANOVAs of the 30 min latencies (sexes analyzed separately) and % MPE data (pooled sexes) documented significant main effect differences between genotypes at this time. Post-hoc testing confirmed the reduction in restraint-induced analgesia in *Null* mice (vs. wild-type) at the 30 min time period in both sexes (Fig. 1C, 1D).

2.2. Swim-induced analgesia

Warm water swimming increased nociceptive latencies in both sexes of saline-treated control mice 5 min after the end of swimming; the effect was considerably larger in females vs. males (Fig. 2A, 2B). Both effects vanished 5 min later (i.e. at the 10 min point, Fig. 2A). Naltrexone pretreatment attenuated swim-induced analgesia in both sexes (Fig. 2A, 2B). ANOVA (wild-type latencies Fig. 2A, between groups [factor #1]: gender; [factor #2]: drug; within groups [factor #3, repeated measures]: time) found significant main effects of gender ($F_{1,42}$=12.2, P<0.01) and time ($F_{2,84}$=24.3, P<0.001), with a significant drug by time interaction term ($F_{2,84}$= 5.4, P < 0.01). The absence of significant gender-drug interactions confirmed that the inhibition by naltrexone occurred in both genders. A separate ANOVA of 5 min latencies (between groups [factor #1]: gender; [factor #2]: drug) found significant main effects of gender ($F_{1,42}$=5.4, P < 0.05) and drug ($F_{1,42}$=11.2, P < 0.01), with no interactions. Nearly identical results were found from ANOVA of 5 min % MPE scores (Fig. 2B), further supporting a sex difference in swim analgesia among WT mice, and the antagonism of both responses by naltrexone. Post-hoc testing confirmed these differences (Fig. 2A, 2B). Although female WT mice showed nearly twice the magnitude of swim-induced analgesia as compared with males, the naltrexone-sensitive component of each was approximately the same (ca. 15% MPE). Thus, essentially all of the male analgesia, but only about one-half of the female analgesia was inhibited by naltrexone (Fig. 2B).

Unlike the case of restraint-induced analgesia, no genotype differences were detected in swim stress-induced analgesia (Fig. 2C, 2D). ANOVA (latencies in Fig. 2C, between groups [factor #1]: gender; [factor #2]: genotype; within groups [factor #3, repeated measures]: time) found significant main effects of gender ($F_{1,44}$=14.1, P<0.01) and time ($F_{2,88}$=44.3, *P*<0.001), with a significant gender by time interaction term ($F_{2,88}$= 4.7, P < 0.02). No significant genotype differences were detected in either main effects, or in genotype-related interaction terms. The same results emerged from additional ANOVAs of 5 min latencies and 5 min % MPE scores. The gender difference in swim-induced analgesia (females > males) was seen in both *Null* and control mice, (post-hoc testing in Figs.2C, 2D).

2.3. Morphine analgesia

Previous studies documenting the importance of brain P450 activity in morphine analgesia (Conroy, et al., 2010) used nociceptive test methods which differed from those used in the present stress experiments. To confirm that the method used presently to measure stress-induced analgesia (the hot plate test) would also detect the previously-reported genotype differences with morphine, additional experiments (Fig. 3) were performed with morphine analgesia in wild-type and *Null* mice. A low dose of morphine (5.6 mg/kg, s.c.) produced antinociception in control mice comparable to that seen after restraint or swim; this effect was completely absent in *Null* mice (Fig. 3A, B). ANOVA of the latencies in Fig. 3A (between groups [factor #1]: gender; [factor #2]: genotype; [factor #3]: drug; within groups [factor #4, repeated measures]: time) found significant main effects of drug ($F_{1,24}$ = 18.8, P<0.001) and genotype ($F_{1,24}$ = 15.3, P<0.001) with significant time by genotype ($F_{1,24}$ = 7.2, P<0.05), time by drug ($F_{1,24}$ = 20.2, P<0.001), and time by genotype by drug ($F_{1,24}$ = 11.6, P<0.01) interaction terms. Post-hoc analyses of the 30 min latencies or %MPE values confirmed the attenuated analgesic responses in *Null* mice (Fig. 3A, 3B).

3. Discussion

Morphine-like opioids relieve pain by activation of G protein-linked μ opioid receptors in the brain and spinal cord, but the cellular mechanisms responsible for μ analgesia have remained elusive. Transduction mechanisms for these receptors include inhibition of adenylate cyclase, inhibition of voltage-sensitive calcium channels, activation of voltage-gated potassium channels, and opening of G protein-gated inwardly rectifying channels (Williams, et al., 2013;Law, 2011). In the PAG, opening of voltage-gated potassium channels in pre-synaptic GABAergic terminals (thereby reducing GABA release) best accounts for the activation of descending, pain-relieving circuits (Vaughan, et al., 1997). Activation of this conductance is mimicked by arachidonic acid (AA) or an AA metabolite (Vaughan, et al., 1997). AA metabolism is highly complex, and can occur through lipoxygenase, cyclooxygenase, epoxygenase, and other mechanisms (Morisseau and Hammock, 2013). Conroy et al. (2010) reported inhibition of morphine analgesia by several types of P450 epoxygenase inhibitors, and showed defective analgesia in *Null* mice lacking neuronal cytochrome P450 activity. Brain levels of morphine were unchanged in the mutant (vs. control mice), indicating no role for morphine metabolism in this differential response to morphine. The density and affinity of brain μ opioid receptors were also unaffected by the P450 mutation. These and other findings led to an epoxygenase hypothesis for μ opioid

analgesia (Conroy, et al., 2010), which proposed that activation of μ opioid receptors leads to stimulation of calcium-dependent phospholipase A_2, AA release, and P450-catalyzed epoxidation of AA, which produces pain-relieving epoxyeicosatrienoic acids (Wagner, et al., 2013). More recent in vivo (Conroy, et al., 2013) and in vitro (Zhang and Pan, 2012) findings support this mechanism of μ action in the brain stem.

Deficits in morphine analgesia were previously documented in *Null* mice (Conroy, et al., 2010) by use of the tail immersion test (a spinally-mediated response to thermal nociception) and the tail pinch test (a supraspinally-organized response to mechanical nociception). However, based on a considerable amount of earlier work (Marek, et al., 1992;Kavaliers and Innes, 1992;Kavaliers and Innes, 1987;Mogil, et al., 1996), the present stress-induced analgesia experiments used the hot plate test (a supraspinally-mediated response to thermal stimuli). To unify the drug and stress studies related to brain P450 activity, it was important to ensure that *Null* mice would show a deficit in both morphine analgesia and stress-induced analgesia on the same nociceptive test. The complete absence of morphine analgesia in *Null* mice (Fig. 3) confirms the phenotype of these mice when tested with the hot plate. A low dose of morphine was used (5.6 mg/kg) in order to simulate the magnitude of the stress effects in control mice (compare size of control responses in Fig. 3 with those in Figs. 1 and 2).

Mu opioid receptors are not only activated by exogenous drugs, but also by endogenous opioid hormones and transmitters. Critical evidence for the importance of endogenous opioids and opioid receptors came from the characterization of stress-induced analgesia. Important early studies in rodents (Lewis, et al., 1980;Watkins and Mayer, 1982) classified these responses as either “opioid” or “non-opioid”. “Opioid” responses showed inhibition by opioid antagonists, developed tolerance with repeated daily exposure, and demonstrated cross-tolerance following repeated morphine dosing (Kelly, 1986). “Non-opioid” forms showed none of these characteristics, although some stimuli can evoke mixtures of these responses (Marek, et al., 1992;Mogil, et al., 1996;Kavaliers and Choleris, 1997). Although three opioid receptors in the CNS can reduce pain transmission under specific circumstances (μ, δ, and κ), many “opioid” forms of stress-induced analgesia are thought to be mediated at least in part by μ opioid receptors (Contet, et al., 2006;Labuda, et al., 2000). Since brain neuronal P450s were recently shown to participate in *morphine*-activated μ analgesia (Conroy, et al., 2010), the present studies evaluated the significance of neuronal P450 activity in *stress*-activated μ analgesia.

The nature of the analgesic responses following exposure to stressful events depends on the properties of stress paradigm and the subject. In addition to the opioid/non-opioid classification, responses have been further divided into “hormonal” and “non-hormonal” (i.e. neuronal) forms, based on hypophysectomy and adrenalectomy experiments (Watkins and Mayer, Kelly, 1986; Terman and Liebeskind, Kelly, 1986;Bodnar, 1990). In general, brief exposures to stressors evoke neuronal responses, whereas more prolonged or intermittently-applied stressors produce hormonal-type analgesia. Among the former, the body region affected or the intensity of the stressor can produce opioid or non-opioid responses. For forced swimming or foot shock, moderate water temperatures or low current intensities evoke opioid responses, whereas colder water or higher currents elicit non-opioid

responses (Mogil, et al., 1996; Watkins and Mayer, Kelly, 1986). Presently, we used two well-characterized models of opioid-mediated, stress-induced analgesia: 1) restraint stress-induced analgesia (Amir and Amit, 1979;Porro and Carli, 1988;Kavaliers and Innes, 1987;Seo, et al., 2011), and 2) warm water swim stress-induced analgesia (Christie, et al., 1982;Kavaliers and Galea, 1995;Contet, et al., 2006;Mogil, et al., 1996;Rubinstein, et al., 1996).

The present findings, demonstrating that 30 min of restraint produces robust, short-lived analgesia in both genders of control mice (Fig. 1A, B), resemble earlier results in rats and mice (Porro and Carli, 1988;Amir and Amit, 1979;Lipa and Kavaliers, 1990;Kavaliers and Innes, 1992). Complete antagonism of these responses by naltrexone (Fig. 1A, 1B) confirms the “opioid” classification. Kavaliers et al. (1987; 1992) measured restraint-induced analgesia in several varieties of deer mice and reported greater responses in males vs. females. In contrast, the present findings found no sex differences in restraint-induced analgesia in control mice (Fig. 1A, B). Genetic backgrounds or species differences could account for the discrepancy.

The nearly-complete absence of restraint-induced analgesia in P450-deficient *Null* mice (Fig. 1C, 1D) is compelling evidence that neuronal P450 enzymes are essential for some of the brain's adaptive responses to stress. Since *Null* mice have defective analgesic responses to morphine (Fig. 3), the attenuated *Null* responses to stress are most likely due to defective μ opioid receptor signaling. However, the significance of neuronal P450s in δ opioid or κ opioid analgesia has not yet been evaluated.

The precise mechanism by which restraint evokes antinociception in rodents is not known. In rats, restraint-induced analgesia requires an intact pituitary gland (Amir and Amit, 1979;Porro and Carli, 1988) and utilizes CNS opioid receptors (Amir and Amit, 1979;Kavaliers and Innes, 1987). The existence of beta endorphin in the pituitary, the elevations in brain and blood endorphin levels following restraint stress, and the ability of blood-borne endorphin to produce μ analgesia all support a stress-induced release of hypophyseal beta endorphin (Akil, et al., 1986). According to this idea, beta endorphin in plasma, like morphine, would relieve pain by the same P450-dependent μ analgesic signaling pathway in the brain . This hypothesis would account for the absence of restraint-induced analgesia in *Null* mice (Fig. 1C, 1D), but a role for pituitary beta endorphin has not confirmed for restraint-induced analgesia. In mice, the requirement for an intact pituitary gland for restraint-induced analgesia has not been found in the literature, although the opioid nature of this response has been widely confirmed (Kavaliers and Innes, 1987;Lipa and Kavaliers, 1990;Kavaliers and Innes, 1992).

Two other hypophyseal hormones (vasopressin and oxytocin) are also liberated by stress, and have been implicated in stress-analgesic mechanisms (Bodnar, 1986;Mogil, et al., 2011;Robinson, et al., 2002). Robinson et al. (2002) used several physiological and molecular genetic tools to conclude that neuronal oxytocin mediates restraint-induced analgesia. The authors proposed hypothalamo-spinal oxytocinergic fibers to be the critical pain-relieving elements (not the pituitary). The experiments in this important study (Robinson, et al., 2002) did not include either naltrexone nor hypophysectomy. The oxytocin

hypothesis is consistent with earlier work showing blockade of oxytocin analgesia by opioid antagonists (Ge, et al., 2002). Notwithstanding a role for either humoral or neuronal vasopressin, the present results show that restraint stress evokes an opioid-dependent analgesic response which utilizes neuronal P450 enzymes.

The present results from swim stress experiments (Fig. 2) closely conform to earlier findings with swim stress-induced analgesia in control mice (Kavaliers and Galea, 1995;Contet, et al., 2006;Mogil, et al., 1996;Rubinstein, et al., 1996). Sex differences in the magnitude of control responses (females > males) and the antagonism of these responses by naltrexone in both genders (Contet, et al., 2006) were also observed presently (Fig. 2). Neuronal beta endorphin has been suggested to mediate warm water swim-induced analgesia (Rubinstein, et al., 1996). Unlike the case for restraint-induced analgesia, however, the normal swim-induced responses in *Null* mice implies no P450 requirement for this opioid response. In theory, the discrepant results could be due to either differences in analgesic receptors mediating these two stress responses, or due to differential localizations of the same receptors. Since μ opioid receptors are thought to function in both of these responses, the second explanation is more likely.

The nature of the P450 deficit in the *Null* mouse brain may also help to explain the discrepant analgesia results between the two present stress experiments. The P450 reductase gene (*Cpr*) is not deleted in all neurons of the *Null* mouse brain. In *Null* mice, the *loxP*-flanked *Cpr* gene is only deleted in Cre-expressing CNS neurons via *Camk2a-cre*, a transgene under control of the *Camk2a* promoter (Conroy, et al., 2010). *Camk2a* is predominantly expressed in forebrain (implying that *Cpr* deletion might be limited to the forebrain in these mice (Dragatsis and Zeitlin, 2000)), but a population of ventrolateral PAG neurons was also shown to lack the P450 reductase in the *Null* mice (Conroy, et al., 2010). Since the *Camk2a* promoter is not active in all neurons, only a subset of *Null* neurons possess a P450 deficit. Because of these circumstances, the detection of a phenotype in these mice (e.g. deficits in morphine- and restraint-induced analgesia) is powerful evidence for the importance of brain neuronal P450. However, when *Null* mice do not show a deficit in a response (as in the presently-observed swim-induced analgesia), a relevant P450 could simply be in a non-*Camk2a*-expressing portion of the brain. Taken together, the discrepant results from the two stress-induced analgesia experiments are most likely explained by differential locations of the relevant μ opioid receptors. Although additional studies are needed to better understand brain P450-associated mechanisms, the present results are the first to demonstrate a physiological, stress-related role for these enzymes in brain function.

4. Experimental Procedure

4.1. Drugs and solutions

Naltrexone hydrochloride (Sigma-Aldrich, St. Louis, MO) and morphine sulfate (Mallinckrodt, MO) were dissolved in saline.

4.2. Animals

The mouse genome contains over 100 functional P450 genes (Nelson, et al., 2004), but microsomal P450 activity requires the enzyme cytochrome P450 reductase, encoded by a

single gene (*Cpr*, also known as *Por*). Brain neuron-specific P450-deficient mice (designated here as *Null*) were produced by targeted deletion of the *loxP*-flanked *Cpr* gene in Cre-expressing brain neurons. This Cre expression (via *Camk2a-cre*) was under control of the *Camk2a* promoter, as described (Conroy, et al., 2010). *Null (Cre* $^{+/-}$ $Cpr^{lox/lox}$) and wild-type control (*Cre* $^{-/-}$*Cpr*$^{lox/lox}$) adults (greater than 10 weeks of age, 19 – 44 g) of either sex were used for all studies. Animals were maintained on a 12-h light/ dark cycle (lights on from 0700 to 1900), with food and water freely available. All animal experiments were approved by the Institutional Animal Care and Use Committee of Albany Medical College.

4.3. Nociceptive testing

Baseline, post-stress, and post-drug nociceptive scores were measured with the hot plate nociceptive test (Mogil, et al., 1996). Mice were placed in a Plexiglass cylinder (20 cm diameter, 29 cm high) resting on a 56°C metal surface, and the latency to vigorous hind-paw flutter, hind paw lift and lick, or jump was recorded. Subjects that did not respond after 45 sec (cutoff latency) were removed from the apparatus to minimize tissue damage.

4.4. Experimental design

Two forms of stress-induced analgesia (restraint and warm water swim) were separately studied in three groups of subjects: wild-type mice (saline pre-treatment), wild-type mice (naltrexone pre-treatment), and *Null* mice (saline pre-treatment). For each stress paradigm, two hypotheses were tested: 1) the opioid nature of the stress analgesia was confirmed by comparing the effects of naltrexone vs. saline in wild-type mice, and 2) the significance of brain P450 activity in stress analgesia was determined by comparing *Null* vs. wild-type responses after saline pre-treatment. Thus, saline/wild-type subjects served as the control group for both of the other two groups. All experiments were performed in both sexes, since gender differences have been documented for some forms of stress-induced analgesia. All subjects were only used once.

4.4.1. Restraint stress-induced analgesia—Restraint stress was induced by methods similar to published studies (Kavaliers and Innes, 1992;Seo, et al., 2011). Subjects were tested for baseline nociceptive responses, injected with either naltrexone hydrochloride (10 mg/kg, i.p.) or saline, and placed in a sealed translucent 50 ml Falcon polypropylene centrifuge tube with air vents (3×11.5 cm) for 30 min. Mice were prevented from backing out of the tube by inserting a ‘notched cork’ into the tube. The notch permitted free movement of the tail. Nociceptive responses were re-assessed immediately after removal from the tube and again 10 min later. Non-stressed control mice were baseline tested, returned to their home cage, then re-tested as described. Re-test latencies for unstressed mice were less than 10 sec (data not shown).

4.4.2. Swim stress-induced analgesia—A warm water swim stress procedure was used (32 °C water for 3 min), which has been widely reported to produce “opioid-mediated” analgesia (Marek, et al., 1992; Mogil et al., 1996). Mice received i.p. injections of either saline or naltrexone hydrochloride (10 mg/kg). Thirty min later, they were tested for baseline nociceptive responses and were gently placed in a rectangular water bath (14 × 15 cm, water depth 13 cm) maintained at 32°C (Precision Scientific, 280 Series, Winchester,

VA) for 3 min. Subjects were then removed, briefly hand-dried under a lab bench pad, and placed in a cage lined with paper towels to further air dry for a total of 2 min. Nociceptive latencies were re-assessed 5 and 10 min after the initiation of the forced swim.

4.4.3. Morphine analgesia—Mice were baseline tested, received saline or morphine sulfate (5.6 mg/kg, s.c.) and were re-tested 30 min later.

4.5. Data Analysis

Results are given in both latencies (sec, mean ± SEM) and analgesia scores. The latter are given as % maximum possible effect (MPE), calculated using the following formula:

$$\%MPE = \frac{(post\ latency - baseline\ latency)}{(45 - baseline\ latency)} \times 100$$

All data were analyzed by analysis of variance (ANOVA), followed by Neuman-Kuels post-hoc testing where permitted (Statistica, StatSoft, Tulsa, OK). Data are graphed with Prism 5.04 (Graphpad, San Diego, CA).

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Abbreviations

AA	arachidonic acid
PAG	periaqueductal grey
P450	cytochrome P450 monooxygenase

- Pain relief produced by opioid drugs require neuronal cytochrome P450 activity.
- Stress also produces opioid-mediated analgesia.
- Restraint - induced analgesia is highly attenuated in neuronal P450 deficient mice.
- Opioid analgesia produced by some stressors require brain neuronal P450 activity.

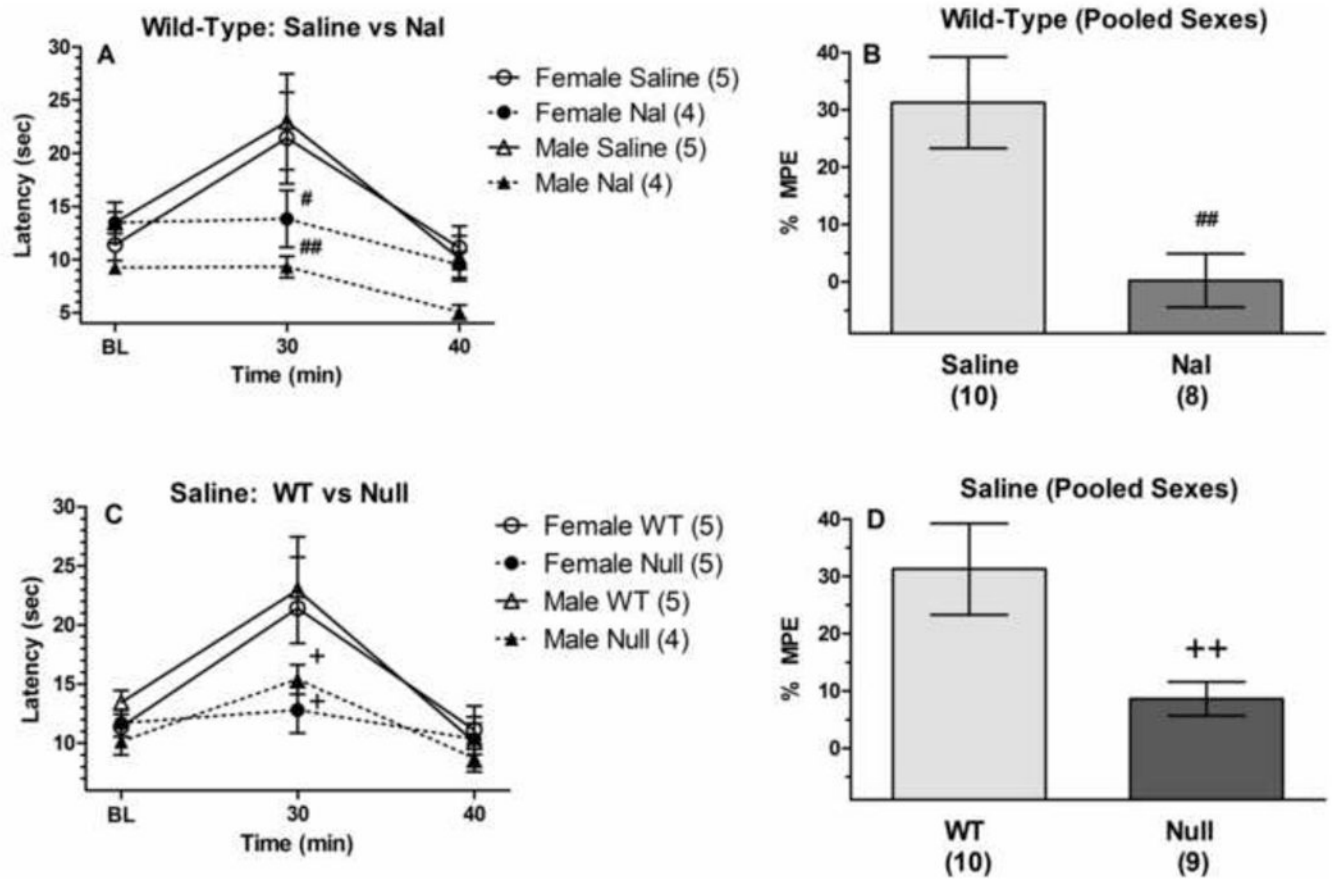

Figure 1.

Restraint- induced analgesia in brain P450-deficient (*Null*) and control (WT) mice. Subjects of either gender were tested for baseline (BL) nociceptive latencies, received either saline or naltrexone (Nal), were restrained for 30 min, then re-tested immediately (30 min) and ten min later (40 min). In panels A and C, ordinates show latencies (sec, mean ± SEM) for the number of subjects in parentheses at the three test times (abscissa). Latency data (30 min) in A and C were pooled for both sexes and are shown as analgesic scores (% MPE, mean ± SEM) in B and D, respectively. A, B: Effects of naltrexone in male and female WT mice. C, D: Latencies and analgesic scores are shown for saline-treated, male and female *Null* and WT mice. $^{\#,\#\#}$

P<0.05, 0.01, respectively for naltrexone vs. saline in the same gender/time group. +,++ P<0.05, 0.01, respectively for *Null* vs. WT in same gender/time group.

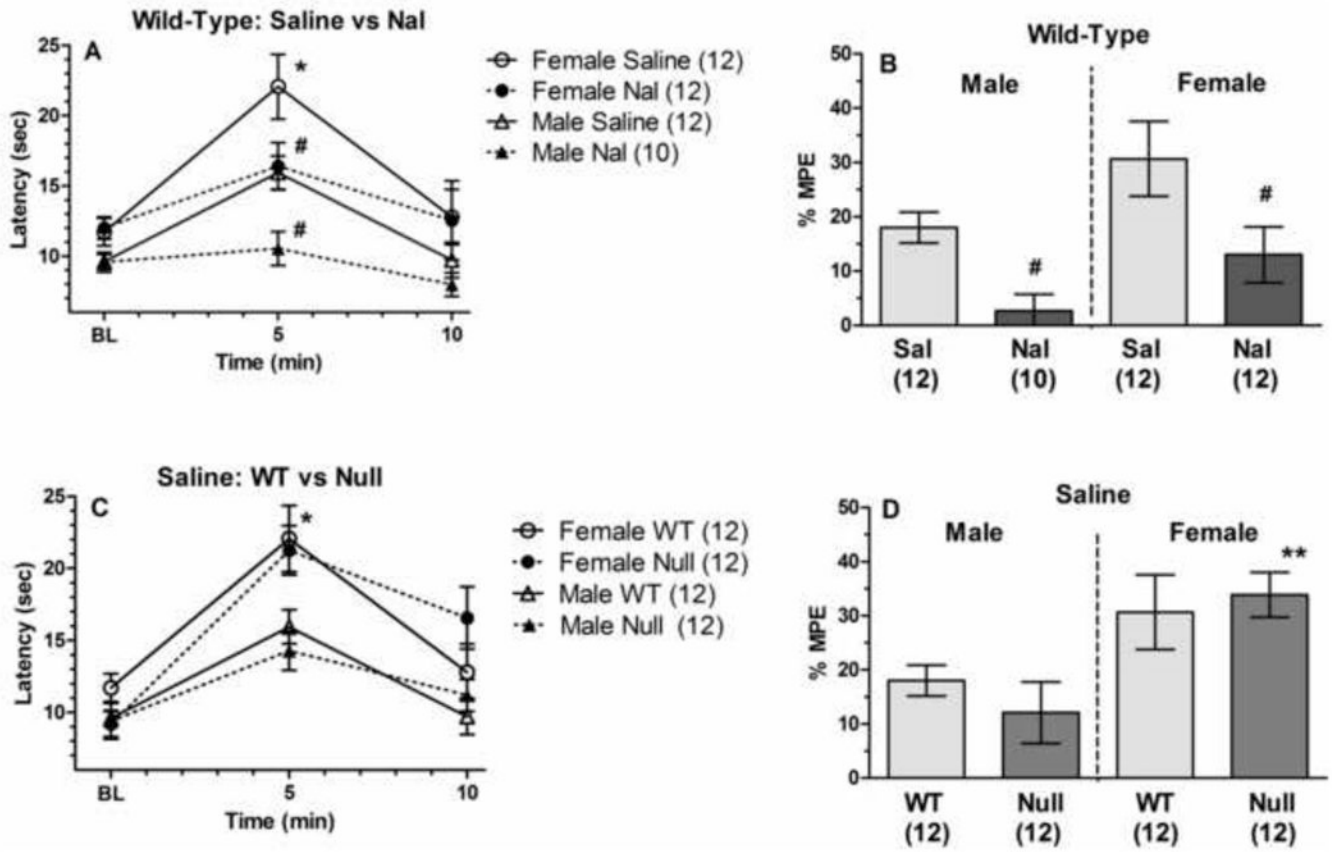

Figure 2.

Swim stress-induced analgesia in brain P450-deficient (*Null*) and control (WT) mice. Subjects were pre-treated (30 min) with either saline (Sal) or naltrexone (Nal), followed by baseline (BL) nociceptive testing. They were then placed in 32° water for 3 min, allowed to dry for 2 min, re-tested immediately (5 min) and again 5 min later (10 min). In panels A and C, ordinates show latencies (sec, mean ± SEM) for the number of subjects in parentheses at the three test times (abscissa). Latency data (5 min) in A and C were calculated as analgesic scores (% MPE, mean ± SEM) in B and D, respectively. A, B: Effects of Nal in male and female WT mice. C, D: Latencies and analgesic scores are shown in saline-treated, male and female Null and WT mice. $^{\#}P<0.05$ for naltrexone vs. saline in the same group. *, **$P<0.05$, 0.01, respectively for gender differences in saline-treated subjects of the same genotype.

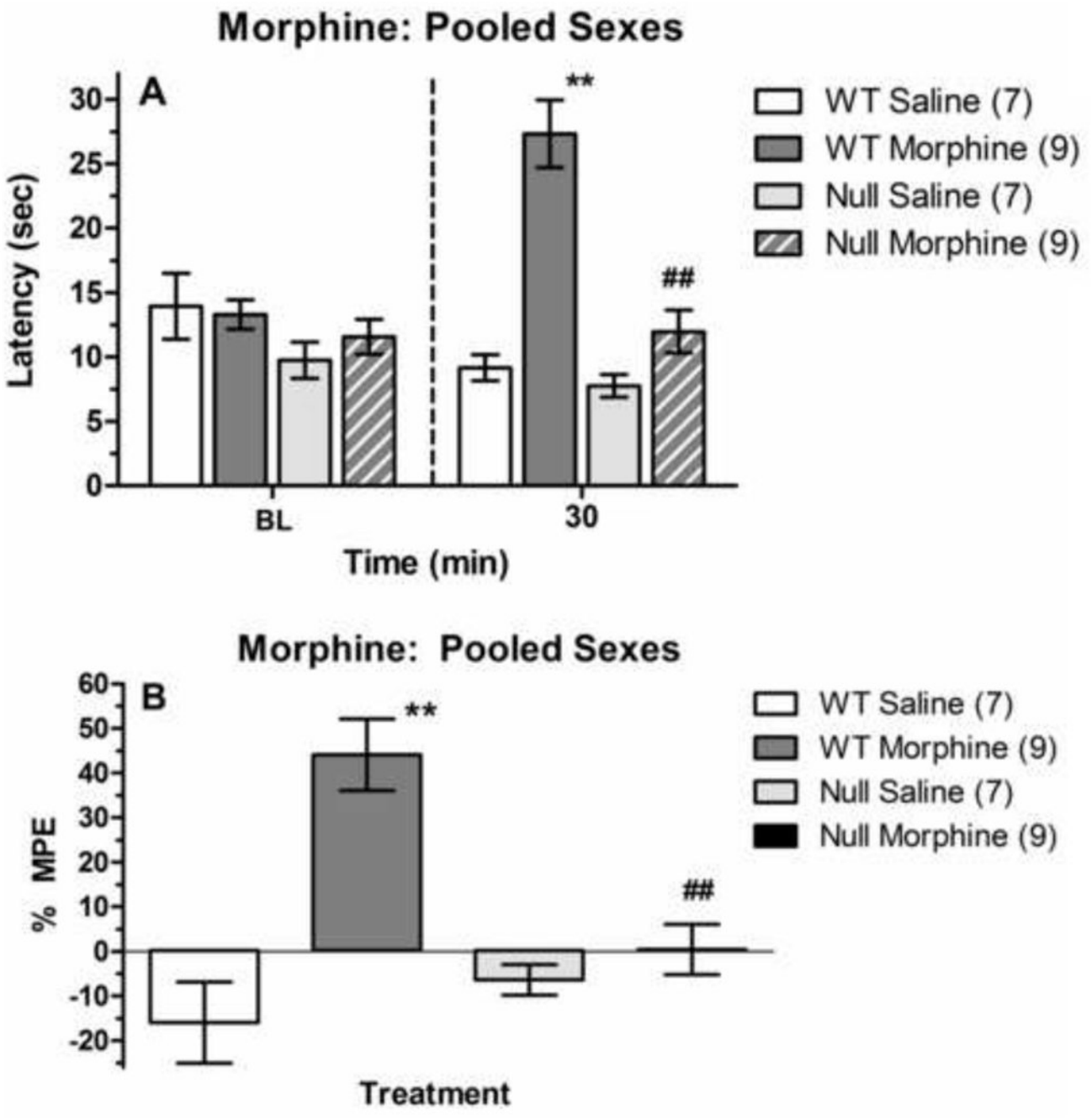

Figure 3.

Morphine analgesia in brain P450-deficient (*Null*) and control (WT) mice. Subjects were tested for baseline (BL) nociceptive latencies, and received morphine (5.6 mg/kg, s.c.) or saline. Thirty min later, they were re-tested. A) Ordinate shows latencies (sec, mean ± SEM) for the number of subjects in parentheses at the two test times (abscissa). B) Ordinate shows thirty min analgesic scores (% MPE, mean ± SEM). **P<0.01 vs. WT saline at the same time. ##P<0.01 *for Null* vs. WT after morphine.