Predictors for response to anti-inflammatory analgesics

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PREDICTORS FOR RESPONSE TO ANTIINFLAMMATORY ANALGESICS

by

Belinda Elizabeth Giles

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge it contains no material previously published or written by another person no material which to a substantial extent has been accepted for the award of any other degree or diploma at UNSW or any other educational institution, except where due acknowledgment is made in the thesis. Any contribution made to the research by others, with whom I have worked at UNSW or elsewhere, is explicitly acknowledged in the thesis.

I also declare that the intellectual content of this thesis is the production of my own work, except to the extent that assistance from others in the project's design and conception, or in style, presentation and linguistic expression is acknowledged.

Belinda Elizabeth Giles

ABSTRACT

PREDICTORS FOR RESPONSE TO
ANTIINFLAMMATORY ANALGESICS

by Belinda Elizabeth Giles

"The true use of chemistry is not to make gold but to prepare medicines"[1]

To most, the very idea of pain is abhorrent. Therefore, the relief of pain and the restoration of function are of paramount importance, and has indeed been the clinician's goal since antiquity. However, pharmacological intervention is hampered by interindividual variability in response, which has not been adequately explained. The aim of this thesis was to determine some predictors for response to antiinflammatory analgesics – with particular focus on sex, sex hormone status and genetics, using three major approaches. *Firstly*, in humans, the effect of sex and sex hormone status on basal nociception and response to ibuprofen using an electrical model was explored. *Secondly* an animal model of pain (tail pinch method) was used to explore the effect of the female sex hormones oestrogen and progesterone on basal nociception and ibuprofen analgesia in ovariectomized rats. *Finally*, the effect of genetic strain on basal nociception and paracetamol analgesia was determined using a murine model.

The results have shown that sex hormone status is a good predictor for basal nociceptive sensitivity, and also for analgesic response. For example, those subjects with a high level of the sex hormone oestrogen exhibit no ibuprofen analgesia, while those subjects with lower levels of oestrogen do. In addition, male subjects' responses to ibuprofen were dependent upon their expectancy – that is, when they believed an analgesic was to be given, analgesia resulted. In the rodent model, while the sex hormones had no influence on basal nociceptive sensitivity, ibuprofen only produced its analgesic effects when oestrogen was present. In the murine model, strain was a good predictor for both basal nociceptive sensitivity, and paracetamol response (DBA/2j mice had reduced pain sensitivity and reduced paracetamol analgesia, compared to C57BL/6j mice).

While much further work needs to be done, especially with regard to the mechanisms responsible for eliciting the effects outlined in this thesis, clinicians now need to consider the patient's sex and sex hormone status when prescribing medications for pain relief. The results of this thesis should convince clinicians and experimenters alike that sex, sex hormone status and genes play an important role in predicting basal nociceptive sensitivity and analgesic response.

[1] Philippus Aureolus Theophrastus Bonbastus von Hohenheim (1493-1541), In: Mackay A.L. A Dictionary of Scientific Quotations (1991), IOL Publishing Ltd., Bristol, UK. Pp 190

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To GlaxoSmithKline for their generous monetary assistance, and the provision of ibuprofen and placebo for the study outlined in Chapter 3 – and especially to Ms Maryanne Murray, Ms Fiona Dunagan and Mr Ian Adams from the Consumer Healthcare division in Sydney for their interest in the project. Thanks must go to the South Eastern Area Laboratory Service, and their personnel for generously performing the haematological and biochemical analyses in Chapters 2 and 3. In addition, I'd like to thank Prof Jeff Mogil, Dr William Lariviere and Ms Sonya Wilson for allowing the use of their pharmacodynamic data in chapter 6. Dr Andrew McLachlan from the University of Sydney for his assistance with the population pharmacokinetic analysis in Chapter 6.

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Belinda Elizabeth Giles

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ABBREVIATIONS

CCK	cholecystokinin
CGRP	calcitonin gene-related peptide
CL	apparent clearance
C_{max}	Maximal plasma or serum concentration
CNS	central nervous system
COX	cyclooxygenase
E	oestrogen
EDTA	ethylene-diamine-tetraacetic acid
ERT	oestrogen replacement therapy
FSH	follicle stimulating hormone
GABA	γ-amino-butyric acid
GnRH	gonadotropin releasing hormone
HPLC	high performance liquid chromatography
HRT	hormone replacement therapy (*i.e.* oestrogen + progesterone)
LH	luteinizing hormone
NMDA	*N*-methyl-D-aspartate
NSAID	nonsteroidal antiinflammatory drug
OFEXH	older female taking exogenous hormones (postmenopausal)
OFNIL	older female not taking exogenous hormones (postmenopausal)
OM	older male
P	progesterone
QTL	qualitative trait locus
SIA	stress induced analgesia
$t_{1/2}$	terminal half life
V	volt
V_D	apparent volume of distribution
VAS	visual analogue scale
YF	younger female
YM	younger male

LIST OF PUBLICATIONS AND ABSTRACTS

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Giles BE & Walker JS (1999) Gender differences in pain. *Current Opinion in Anaesthesiology* **12**: 591-595.

Giles BE & Walker JS (2000) Sex differences in pain and analgesia. *Pain Reviews* **7**: 181-193.

ABSTRACTS

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Carmody JJ, Giles BE, Ting ML & Walker JS (2001) Sex hormones and NSAID analgesic response in rats. British Pharmacological Society, Dublin, Ireland

Giles BE, Ting ML, Carmody JJ & Walker JS (2001) Nociception and NSAID analgesia in female rats: importance of sex hormones. IUPS Satellite Symposium on Pain Mechanisms, Sydney, NSW, 20-22 August, 2001

Giles BE, Knowles AE, Walker JS & Carmody JJ (2002) Influence of sex hormones on analgesic response to experimental pain in healthy human volunteers. Australian Pain Society National Meeting, Sydney, NSW April 2002.

Giles BE, Knowles AE, Walker JS & Carmody JJ (2002) Influence of sex hormones on placebo response to experimental pain in healthy human volunteers. Australian Pain Society National Meeting, Sydney, NSW April 2002.

Chapter 1

INTRODUCTION

1.1 IMPLICATIONS

To most, the very idea of pain is abhorrent. It is enveloped in mystery and mythology – even today pain is associated with sin. For example, Christian teachings suggest that "The wicked man trevaileth with pain all his days, and the number of years is hidden to the oppressor"[1] and "Why do you cry out over your wound, your pain that has no cure? Because of your great guilt and many sins I have done these things to you"[2]. Buddhist teachings state, "If a man speaks or acts with an evil thought, pain follows him, as the wheel follows the foot of the ox that draws the carriage. All that that we are is the result of what we have thought: it is founded on our thoughts, it is made up of our thoughts. If a man speaks or acts with a pure thought, happiness follows him, like a shadow that never leaves him."[3] Indeed, the word pain itself is derived from the Latin *poena*, meaning penalty. A recent Australian study found that 17.1% of males and 20.0% of females were in chronic pain, with 11.0% of males and 13.5% of females reporting interference with daily activities because of their pain (Blyth *et al.*, 2001). Therefore, the relief of pain and restoration of function is of paramount importance, and has indeed been the clinicians' goal since antiquity. However, too often pharmacological

[1] Job 15:20, King James Version Bible
[2] Jeremiah 30:15, New International Version Bible
[3] attributed to the teachings of the Dalai Lama

intervention is a "hit and miss" affair, with adequate pain relief not achieved rapidly and with minimal side effects. For example nonsteroidal anti-inflammatory drugs (NSAIDs) are amongst the most widely used therapeutic agents, both as prescription drugs for arthritis and as over-the-counter medications for a wide variety of minor ailments including musculoskeletal pain, headache and primary dysmenorrhoea. In Australia, over 6.9 million prescriptions were written in 1998 for NSAIDs (Australian Bureau of Statistics, 1999), and many more were sold as over-the-counter preparations. Although they are widely used, there is a large degree of inter-individual variability associated with NSAIDs that is yet to be adequately explained (Walker *et al.*, 1997; Palmer & de Lapp, 2000). Often this variability is so great in patients that NSAIDs have to be stopped, changed and restarted until an effective therapy is found. This causes unnecessary delays in pain relief, which could be prevented if we understood the responsible mechanisms. The aim of this thesis is to explore some possible "predictors for response to anti-inflammatory analgesics" with a focus on the NSAIDs ibuprofen and paracetamol, in particular.

1.2 PAIN

The relief of pain has been the goal of physicians since antiquity, and while new pharmacological agents target this goal, our understanding of the pathophysiology of pain is far from complete. In spite of this, our knowledge of pain mechanisms has advanced enormously over the last 35 years.

1.2.1 Anatomy of the Pain System

Noxious stimulation (such as that produced by chemical, mechanical, thermal or electrical stimulation) is detected by the nociceptors, which are the free nerve endings of fast conducting Aδ-myelinated afferent fibres and slowly conducting C-unmyelinated afferent fibres (Tracey, 1992). Several classes of nociceptors have been identified, for example, some which are polymodal (responsive to chemical, thermal and mechanical stimuli; Besson, 1999), and some which are only be activated under the pathological condition of inflammation, called *sleeping nociceptors* (Schaible & Schmidt, 1988). The nociceptors can be *sensitised* by repeated activation of second messenger systems activated by the release of inflammatory mediators such as bradykinin, prostaglandins, serotonin and histamine (for review see Besson, 1999).

The Aδ and C fibres then enter the spinal cord in the dorsal roots, and ascend through the central nervous system via multiple pathways. These include the spinothalamic tract, the spinoreticular tract, the spinomesencephalic tract (Tracey, 1992; Willis & Westlund, 1997). These inputs are projected to both the primary and secondary somatosensory cortex, and the anterior cingulate cortex. A brief overview of the neural circuitry involved in the ascending pain pathways is shown in Figures 1.1.

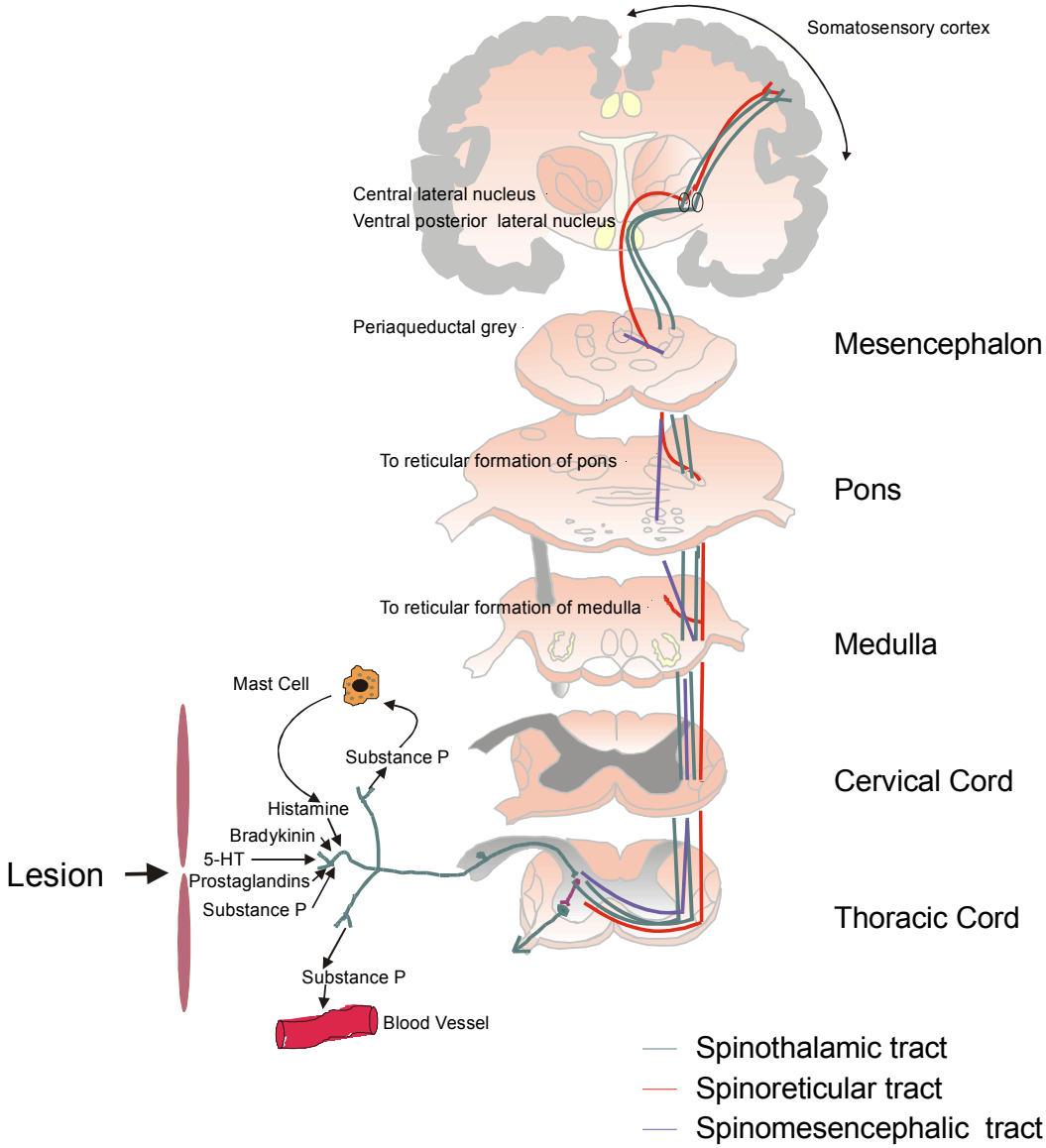


Figure 1.1: Schematic diagram of the anatomy of the ascending pain pathways. Adapted from Jessell & Kelly, 1991 pg 391.

One of the more remarkable aspects of pain is that the intensity of sensation often does not correlate well with the intensity of the stimulus. This is a direct result of the *descending inhibition of pain*. For example stimulation of the periaqueductal grey matter (Hosobuchi, 1988), or the region of the medulla containing the nucleus raphe magnus produces analgesia. The descending inhibitory process is important as it allows the modulation of pain from *within* the subject/patient. Indeed it is only when the net effect of the ascending input is greater than the descending output

that pain is perceived. Several pathways, and structures are involved, including the periaqueductal gray, locus ceruleus, subceruleus, Kölliker-Fuse nuclei and the nucleus raphe magnus (for review see Willis & Westlund, 1997). In addition, structures at higher levels of the central nervous system, such as the hypothalamus, and the cerebral cortex, also contribute to the analgesia pathways (Willis & Westlund, 1997). Several different neurotransmitters are involved, for example, opioids, 5-hydroxytryptamine, and/or catecholamines (Willis & Westlund, 1997).

The descending inhibition of pain process is one area where sex differences may be apparent. This is discussed further in Section 1.5.4.

1.2.2 Classes of Pain

Pain is often divided into two classes: *nociceptive pain* or pain which results from noxious stimulation in otherwise intact tissue and reflects normal functioning of the pain system (for example acute, subacute and inflammatory pain) and *pathophysiological pain* or pain which arises following injury to neural tissue, and reflects abnormal functioning of the pain system (for example neuropathic, radiculopathic, deafferentation and central pain; Devor, 1999). Pain has also been classified into acute, subacute and chronic. Acute pain is that felt at the moment of injury and results from the activation of nociceptive sensory endings in the affected tissue. Subacute pain is pain that may last for hours, days or weeks, for example pain following a muscle sprain. Chronic pain is pain that is much longer lasting (Devor, 1999).

1.2.3 Sensitisation

There are two types of sensitisation: *peripheral* and *central*. *Peripheral sensitisation* was discussed in part in Section 1.2.1, and involves inflammatory mediators sensitising the nociceptors to generate sensory impulses in response to stimuli that would normally be too weak, resulting in allodynia and hyperalgesia (Niv & Devor, 1999). Nonsteroidal anti-inflammatory drugs are particularly useful in reducing pain arising from peripheral sensitisation. *Central sensitisation* is an abnormal degree of amplification of the incoming sensory input at the central nervous system level, particularly at the spinal cord. Central sensitisation is caused by an increase in the synaptic strength of pre-existing, but previously subliminal spinal synaptic terminals, giving rise to amplification of the post-synaptic spinal neurones. The glutamate receptor *N*-methyl-D-aspartate (NMDA) is particularly involved in the central sensitisation process, and therefore, the NMDA receptor is a potential target for the development of analgesic drugs to combat central sensitisation (Niv & Devor, 1999).

1.3 Nonsteroidal Anti-Inflammatory Drugs

The NSAIDs are often prescribed in both acute and chronic pain states. All NSAIDs have three effects: anti-inflammatory, analgesic, and anti-pyretic. Most can also inhibit platelet function, in particular platelet aggregation (Day, 1988). The traditional mechanism for these functions has been explained on the basis of their peripheral action on prostaglandin synthesis by the inhibition of cyclooxygenase 2 (see Figure 1.2 and Vane, 1971; Day, 1988; Cashman, 1996), however, a comprehensive survey of the clinical analgesic performance of NSAIDs found no relationship between ability to inhibit prostaglandin synthesis *in vitro* and analgesic

activity (McCormack & Brune, 1991). For example, while diflusinal 1000mg, naproxen 550mg and tolmetin 400mg demonstrate analgesic efficacy greater than aspirin 650mg, they are all weak inhibitors of prostaglandin synthesis. Conversely, diclofenac 100mg and etodolac 200mg whilst being potent inhibitors of prostaglandin synthesis, showed no better analgesic efficacy than aspirin 650mg (McCormack & Brune, 1991).

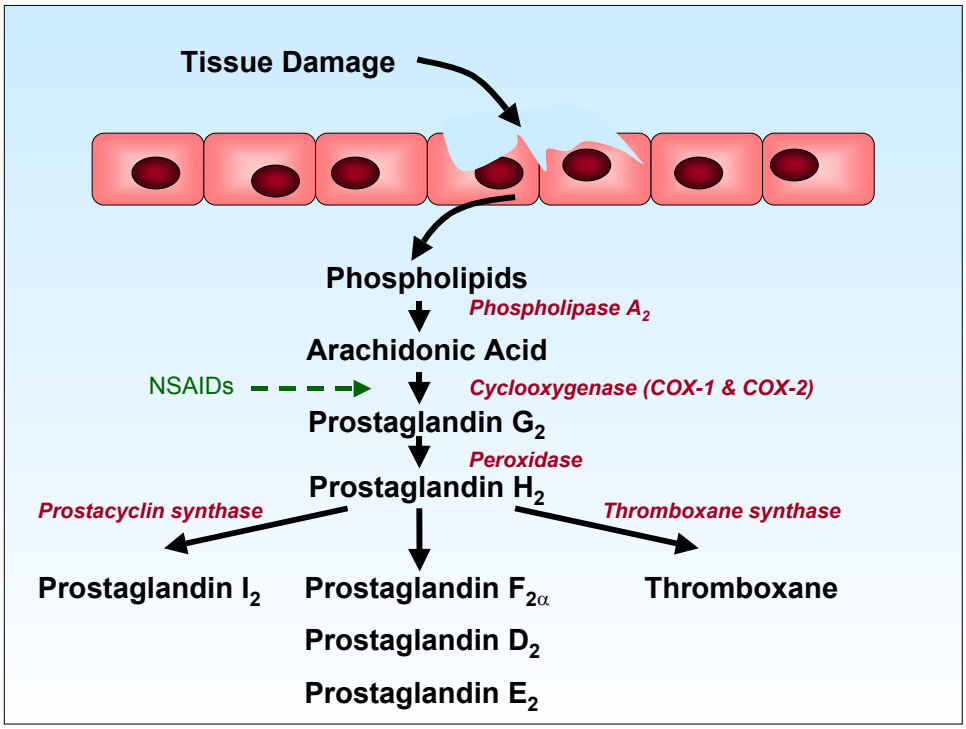


Figure 1.2: The inhibition of prostaglandin synthesis by NSAIDs occurs at the level of cyclooxygenase enzymes (COX-1 & COX-2) in the periphery. The dashed arrow represents inhibition. Enzymes are italicised and printed in red.

Ample evidence demonstrates that NSAIDs also exert effects at central sites, for example, the spinal cord and brainstem (Willer *et al.*, 1989; Jurna & Brune, 1990; Fabbri *et al.*, 1992; Vaughan *et al.*, 1997; Vaughan & Christie, 1997). Several mechanisms have been postulated to account for the central action of NSAIDs:

- *The central antinociceptive effect may be the result of interference with the formation of prostaglandins in the CNS (Cashman, 1996).*

- *NSAIDs exert an effect on the transmitters or modulators of the nociceptive system, for example, the central action may be mediated, in part, by endogenous opioids (such as β-endorphin; Martini et al., 1984), by blocking serotonin (Björkman, 1995), or by inhibiting the excitatory amino acids (including glutamate and aspartate) or N-methyl-D-aspartate (NMDA) receptors (Malmberg & Yaksh, 1992a; Malmberg & Yaksh, 1992b).*

The NSAIDs, like ibuprofen, are useful for pain arising from both peripheral and central sensitisation (for review see McCormack, 1994). Their effectiveness on central sensitisation is evidenced by the fact that they can modulate glutamate and NMDA activity centrally (Niv & Devor, 1999). Therefore, NSAIDs are useful for pain of musculoskeletal origin that involves both peripheral and central sensitisation.

Paracetamol (acetaminophen) is another widely used NSAID. Historically, paracetamol was not classed as a NSAID because of its very weak inhibition of COX-1 and COX-2 enzymes. However, recent evidence suggests that paracetamol has a selective action on a third cyclooxygenase form: COX-3 (Simmons *et al.*, 2002), and that it potently inhibits prostaglandin production in intact cells in a COX-2 dependent system (Graham *et al.*, 2001).

1.3.1 Ibuprofen

Ibuprofen was first introduced as an anti-inflammatory drug for human use in the late 1960s. It has weak anti-inflammatory effects, its other properties are similar to aspirin, but with considerably fewer side effects (Kantor, 1979). The clinical uses of ibuprofen include arthritis, dental pain, headache, menstrual pain, soft-tissue injury and other musculoskeletal pain.

Ibuprofen (like other 2-arylpropionic acids) exists as R- and S-enantiomers (Figure 1.3), but is marketed as a racemate (a mixture of R- and S-). While the S-

enantiomer is considered to be responsible for the anti-inflammatory and anti-platelet effects of ibuprofen (via the inhibition of prostaglandin synthesis, see Figure 1.2; Williams & Day, 1985; Williams *et al.*, 1994), both enantiomers are equally responsible for the analgesic effect (Brune *et al.*, 1991), possibly mediated centrally (see above).

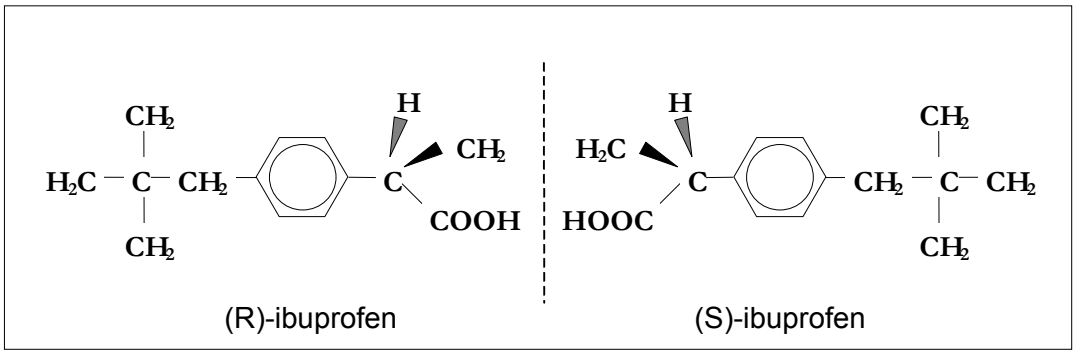


Figure 1.3: The structure of the enantiomers of ibuprofen.

Metabolism & Pharmacokinetics

Following administration of single doses, ibuprofen is rapidly absorbed, with peak plasma concentrations observed within 3 hours post-treatment (Davies, 1998). Ibuprofen is 99% protein bound to a single primary site on human serum albumin (Mills *et al.*, 1973), and this binding is freely reversible. It is metabolised by liver oxidation (by P450 CYP2C9; Hamman *et al.*, 1997; Klose *et al.*, 1998) to form two inactive metabolites, (+)2-[4-(2-hydroxy-2-methylpropyl]phenyl) propionic acid and (+)2-[4-(2-carboxypropyl]phenyl) propionic acid. About 50-60% of an oral dose is excreted in the urine as these metabolites or as their glucuronide conjugates within 24 hours of administration (Mills *et al.*, 1973), less than 1% is excreted in the urine unchanged (Lee *et al.*, 1985), and the remainder is eliminated in the faeces both as metabolites and as unabsorbed drug (Mills *et al.*, 1973). Excretion in humans is virtually complete within 24 hours of oral administration.

The published pharmacokinetic parameters of ibuprofen in healthy individuals are shown in Table 1.1:

Table 1.1: Published pharmacokinetic parameters of ibuprofen in humans following 800mg oral dose (Lee et al., 1985; Davies, 1998).

Parameter	Value
Oral availability (%)	>80
Urinary excretion (as unhanged drug; %)	<1
Bound in plasma (%)	>99
Clearance ($mL.min^{-1}.kg^{-1}$)	0.75 ± 0.20
Volume of distribution ($L.kg^{-1}$)	0.15 ± 0.02
Half-life (h)	2.0 ± 0.5
Therapeutic concentration window ($mg.L^{-1}$)	10-50

Side Effects

All NSAIDs are associated with a high incidence of side effects when administered chronically, however, there are substantial differences in overall toxicity. For example, NSAIDs have been reported to produce hives, pruritus, rash, oedema, mucosal ulcers, blurred vision, vertigo, headache, tinnitus, heartburn, nausea, vomiting, upper and lower abdominal pain and diarrhoea (Fries *et al.*, 1991). Ibuprofen has low incidences of major side effects compared with naproxen, indomethacin and tolmetin (Fries *et al.*, 1991). In their examination of the relative toxicity of NSAIDs, each NSAID was given a "Toxicity Index", which represents a summary index of all the side effects of a particular medication, with higher scores given to more toxic drugs. According to their study, ibuprofen was statistically less toxic than meclofenamate, tolmetin and indomethacin (Fries *et al.*, 1991). A review of clinical trial data on the adverse reactions from ibuprofen at over-the-

counter dosage (*i.e.* 400 mg as a single dose or 1.2 g per day in adults) showed that (Rainsford, 1998):

- No serious adverse events were reported that required medical attention (for example gastrointestinal bleeding).
- Most adverse reactions were minor symptomatic (non-pathologic) reactions in the GI tract and CNS.
- No adverse reaction was permanent.

Common side effects of ibuprofen therapy include nausea, dyspepsia, diarrhoea, constipation, headaches and vertigo (Polisson, 1996). More rarely are peptic ulceration, oedema, tinnitus, urticaria, rashes, blurred vision, drowsiness and shortness of breath (Polisson, 1996). Severe but rare side effects include vomiting blood, increased faecal blood wasting, and drug induced asthma (Polisson, 1996).

1.3.2 Paracetamol

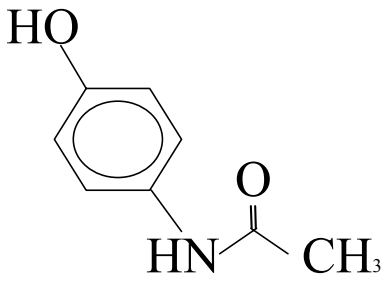


Figure 1.4: Structure of paracetamol

Paracetamol was synthesised by Morse in 1878, and was introduced into clinical practice in 1893 by von Mering. In 1894 it was shown to have equal antipyretic properties to antipyrine and phenacetin (Prescott, 2000). However, it was not until 1943, when paracetamol was recognised as the main metabolite and active analgesic form of phenacetin, that it began to be extensively used (Haas, 1983). Paracetamol was first marketed in Australia in 1956, as the nephrotoxicity of phenacetin began to be recognised (Prescott, 2000).

Mechanism of Action

Despite its wide usage, the mechanism of action of paracetamol is poorly understood. Traditionally it was thought to only weakly inhibit COX-1 and COX-2; although recent evidence shows it can strongly inhibit COX-2 in intact cells (Graham *et al.*, 2001). Others have demonstrated suppression of prostaglandin E_2 in the dorsal horn of rat with concomitant analgesic effect (Muth-Selbach *et al.*, 1999). Finally, paracetamol appears to have selective effects on a new variant of COX, namely COX-3 (Simmons *et al.*, 2002).

Metabolism and Pharmacokinetics

Paracetamol is rapidly and almost completely absorbed from the gastrointestinal tract, with peak plasma concentrations of approximately 15mg/L occurring between 30 and 60 mins following therapeutic doses (*i.e.* 1g as a single dose, or 4g daily in adults); its plasma half-life is approximately 2 hours. After therapeutic doses, paracetamol is widely distributed with 90-100% of the drug able to be recovered in the urine as conjugates of glucuronic acid (~60%), sulfuric acid (~35%), or cysteine (~3%) – see Figure 1.5. (Limbird *et al.*, 1996)

Side Effects

Paracetamol is generally well tolerated at therapeutic doses, although skin rashes (erythematous or urticarial) and other allergic reactions occur occasionally. Very rarely, paracetamol usage is associated with neutropenia, thrombocytopenia and pancytopenia. The most serious side effects are associated with acute over dosage: potentially fatal hepatic necrosis, renal tubular necrosis and hypoglycaemic coma (Insel, 1996).

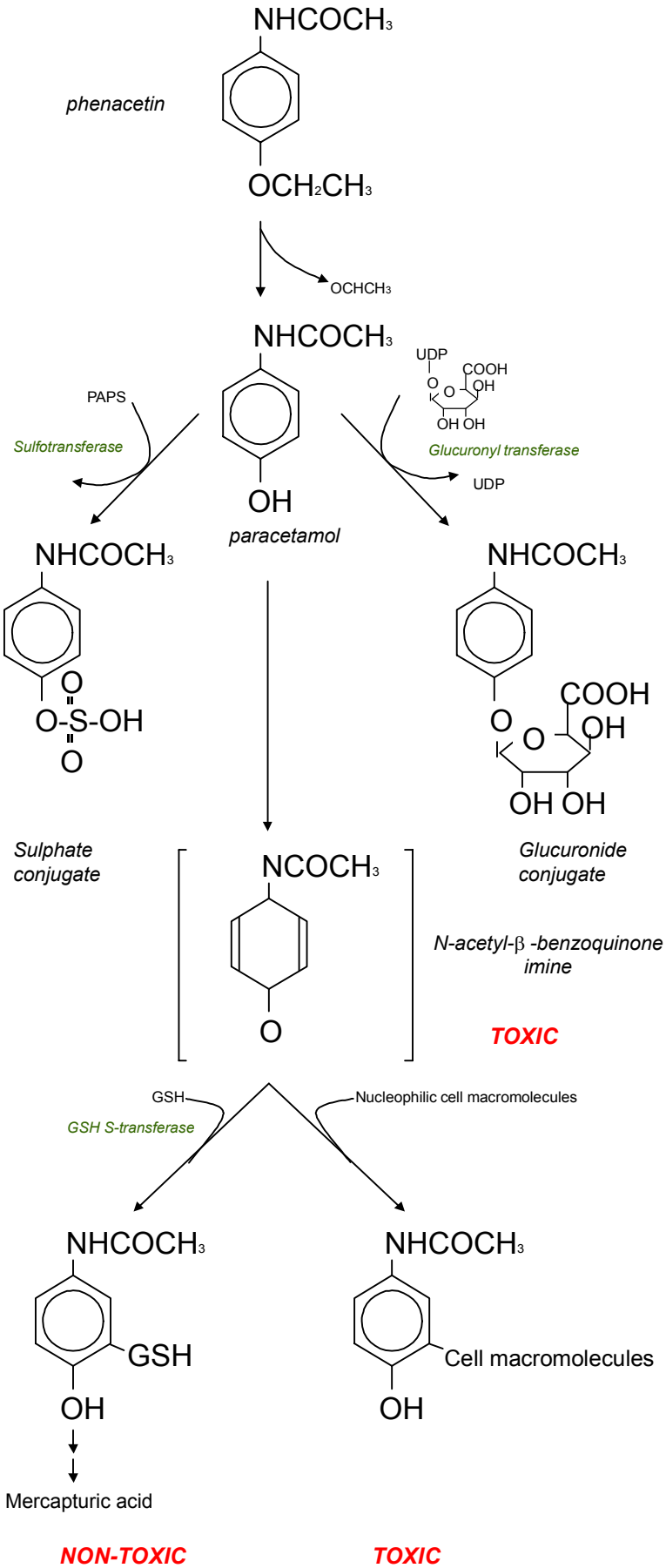


Figure 1.5: Metabolism of paracetamol

1.4 VARIABILITY IN RESPONSE TO NSAIDS

Variable response to NSAIDs in the patient population is very common. Significant variability in osteoarthritis and rheumatoid arthritis patients as well as in subjects with experimental inflammation or pain has allowed classification of subjects as *Responders* or *Non-Responders* to a range of NSAIDs (Walker *et al.*, 1994; Walker *et al.*, 1997; Walker & Carmody, 1998). The scientific basis for inter-individual variability remains unknown, although several hypotheses have been put forward (Baber *et al.*, 1979; Cush *et al.*, 1990; for review see Walker, 1995). These include: pharmacokinetic differences; biochemical mechanisms; tolerance; age and sex (see below).

1.4.1 Pharmacokinetic Differences

When patients respond differently to analgesic drugs, the first line of investigation is usually to determine whether there are inter-patient differences in the pharmacokinetic handling of the drug. This is primarily because pharmacokinetic analyses are easy to perform.

Most NSAIDs (including ibuprofen) exist as enantiomers, and the analgesic activity was generally attributed to the S-isomer (Williams & Day, 1985). However, recent evidence has shown that both enantiomers are equally responsible for the analgesic effect (Brune *et al.*, 1991). Thus the arguments that when an NSAID is marketed as a racemate, patients who ineffectively convert the R-enantiomer to the S-enantiomer might be expected to have reduced analgesia, is probably incorrect.

There are theoretical possibilities that there are differences in absorption, distribution and metabolism may contribute to response variability to drugs (for

review see Beierle *et al.*, 1999). As yet, differences in pharmacokinetics have been unable to explain pharmacodynamic differences in NSAID analgesia (Walker & Carmody, 1998).

1.4.2 Biochemical Mechanisms

Differences in arachidonic acid metabolism have been reported to influence NSAID response. For example, only responders to indomethacin have a positive correlation between the plasma indomethacin concentration and the percentage inhibition of platelet malonyldialdehyde production (a product of prostaglandin synthetase activity; Baber *et al.*, 1979; Orme *et al.*, 1981). Baber and colleagues concluded that there were different effects of the various prostaglandin synthetase enzymes in responders compared to non-responders, and that the platelet would be a good model for further study of this difference. However, as we now know that NSAID analgesia is induced via a prostaglandin-inhibition-independent mechanism, the relevance of this biochemical difference to analgesia is queried.

Others have reported differences between *responders* and *non-responders* in the anti-inflammatory, but not analgesic effects of NSAIDs (MacGregor, 1976; Cush *et al.*, 1990; Høyeraal *et al.*, 1993; Walker *et al.*, 1997).

1.4.3 Tolerance

Tolerance is the term usually used to define the phenomenon where a subject is less susceptible to the effect of a drug as a result of prior administrations, and is traditionally associated with the opioid drug class. Tolerance also has been shown to occur with NSAIDs (Walker *et al.*, 1996). Many theories have been proposed to explain this phenomenon that is classically associated with opiates, for example,

pharmacological tolerance (this is observed when a subject takes repeated high doses of a drug over a short period of time) is due to changes at the cellular or receptor level (Walker *et al.*, 1996). These may occur at the G-protein level, for example through interference with leukotriene synthesis, inhibition of membrane transport, or suppression of neutrophil aggregation and degranulation, or via various other endogenous systems (see Walker *et al.*, 1996). If NSAIDs produce analgesia via endogenous opioid peptides (Martini *et al.*, 1984), then tolerance may occur via the "traditional" mechanisms reported to be important in opioid tolerance. It is exciting therefore that a functional synergism between NSAIDs and opioids has been reported by one group (Vaughan *et al.*, 1997; Vaughan & Christie, 1997).

1.4.4 Age

A great proportion of NSAID prescriptions are for the older population: e.g. for rheumatoid arthritis and osteoarthritis sufferers. Despite this, little has been done to examine the effect of age on NSAID response. In rodents, there are age-dependent changes in pharmacokinetics of ibuprofen (Satterwhite & Boudinot, 1991), although the relevance of this to the pharmacodynamic properties of ibuprofen are yet to be established.

1.4.5 Sex

Sex differences in response to NSAIDs have been reported in the literature (Walker & Carmody, 1998), and these will be closely examined in Section 1.6.2.

The rational prescription of NSAIDs to patients would be easier if it were possible to determine reliable predictors of response to these drugs. Sex as a basis for the

inter-individual variability in analgesic response to NSAIDs is an emerging concept, and is discussed at length in Section 1.6.2. If sex were a predictor of analgesic drug response, it would be relatively simple to rationalize the prescription of these drugs on this basis.

1.5 SEX DIFFERENCES IN PAIN

The interest in sex differences in pain sensitivity has substantially stemmed from the epidemiological observations that many chronic painful conditions are more prevalent amongst females *e.g.* migraine and non migraine headaches, facial, oral, back and musculoskeletal pain, and temporomandibular disorders (for reviews see Berkley, 1996; Unruh, 1996; Riley *et al.*, 1998; Sun, 1998; Lamberg, 1999). Initially it was hypothesised that there was a sociocultural explanation for these differences, however, as they are reported in both animals and humans, this factor alone cannot account for them (Sternberg, 1998). Many clinical and experimental studies have examined sex differences in pain sensitivity to determine the mechanisms responsible for the effect. Whilst these largely remain unresolved, several factors have been suggested to be important in determining them (Berkley, 1997):

- Stimulus specific factors: type, timing and spatial aspects of the stimulus
- Sex-role expectations: including the influence of the sex of experimenter, subject's willingness to report pain, interest in symptoms etc.
- Psychological factors
- Neural differences: differences in receptor populations or pain neuromodulators
- Sex-determined influences on function: for example via the sex hormones.

1.5.1 Stimulus Specific Factors

In humans a number of studies have reported sex differences in nociception, usually based upon observations made in the experimental setting (See Table 1.2). However, not all studies of sex influences have found differences, and the inconsistencies may be due to the type of noxious stimulus used to elicit the pain (Lautenbacher & Rollman, 1993; Riley *et al.*, 1998). The three most common experimental methods used to evoke pain sensations in humans are: pressure, thermal and electrical. Pressure and electrical methods appear to be the most consistent in producing sex differences in pain threshold (the least stimulus intensity at which a subject perceives pain (Merskey, 1979) and pain tolerance (the greatest stimulus intensity causing pain that a subject is prepared to tolerate; Merskey, 1979) measures, with men having higher threshold and tolerance levels than women (pressure (Otto & Dougher, 1985; Ellermeier & Westphal, 1995) electrical (Lautenbacher & Rollman, 1993; Walker & Carmody, 1998); see Table 1.2). By contrast, with thermal stimulation sex differences have been reported only in pain tolerance measures (Feine *et al.*, 1991; Lautenbacher & Rollman, 1993). Despite the discrepancies, when differences are found they are in the same direction (*i.e.* males exhibiting greater pain thresholds and tolerances, and females showing greater pain ratings and sensitivities, Table 1.2).

The reason for the discrepancies are probably due to the different methodologies used *within* each stimulus modality. In both of the *pressure* stimulations (Table 1.2), a dull weighted edge was placed on the subjects finger – this stimulation produces a aching pain which gradually increased in intensity (Otto & Dougher, 1985; Ellermeier & Westphal, 1995). In the first study, males had a higher pain

tolerance than females (Otto & Dougher, 1985), in the other, females rated their pain intensity higher than males (Ellermeier & Westphal, 1995).

The *electrical* models were either constant current (Walker & Carmody, 1998) or constant voltage (Lautenbacher & Rollman, 1993). Both are thought to elicit Aδ and C fibres sequentially (Walker *et al.*, 1993), and both exhibited sex differences, with males having higher pain thresholds and tolerances than females (Lautenbacher & Rollman, 1993; Walker & Carmody, 1998).

Thermal heat stimuli showed the greatest variability in terms of methodologies, and in terms of support for the hypothesis that males have greater pain thresholds and tolerances than females. There are two main methodologies: the method of levels (where pre-set temperatures are presented to the subject and they are asked to rate the temperatures as painful or not painful) and the method of limits (where the temperature rises until the subject perceives it as painful). The *method of limits* can be affected by reaction times, with faster rates of rise more substantially influenced by differences in reaction time, while slower rates of rise have an increased temporal component, which may have important implications for sex differences. In addition, different rates of rise have been used. Some studies used a slow rate of rise (*e.g.* 0.7°C/s, Lautenbacher & Rollman, 1993; or 0.5°C/s, Fillingim *et al.*, 1999), which are thought to activate C fibres preferentially, and showed *inconsistent* sex differences, for example one found no difference (Lautenbacher & Rollman, 1993), while the other found males have higher pain thresholds (Fillingim *et al.*, 1999); others used faster rates of rise (*e.g.* 6°C/s, Feine *et al.*, 1991; or 10°C/s, Fillingim *et al.*, 1998), which activate Aδ fibres preferentially, and do show sex differences (males have higher pain thresholds and tolerances). The most

consistent sex differences are found using the *method of levels* (Fillingim *et al.*, 1999).

Table 1.2: *Reports of sex differences in pain threshold, tolerance and sensitivity for experimental pain procedures. Data expressed as mean ± SEM.*

Species	Stimuli	Difference Present?	Magnitude Male *cf* Female	Direction of Difference	Reference
Human	Pressure (Mechanical)		Latency to Pain Threshold		Otto & Dougher (1985)
	(0.7mm x 640g stimulus)	Yes	64.1 ± 8.5 *cf* 32.5 ± 3.3 s	M > F	
			Latency to Pain Tolerance		
		Yes	172.6 ± 16.5 *cf* 98.9 ± 16.2 s	M > F	
Human	Pressure (Mechanical)		Verbal-Numerical Categorization Score	Pain Rating	Ellermeier & Westphal (1995)
			(From 0-50) at Pain Tolerance Level		
	750 kPa	No	16.9 ± 1.9 *cf* 16.9 ± 1.9		
	940 kPa	No	24.6 ± 1.2 *cf* 27.3 ± 1.5		
	1190 kPa	Yes	32.7 ± 2.2 *cf* 36.9 ± 1.5	F > M	
	1500 kPa	Yes	38.5 ± 0.8 *cf* 46.2 ± 1.2	F > M	
Human	Thermal		Temperature at Pain Threshold		Fillingim *et al.* (1999)
			Ascending method of limits		
	38-50 °C (1 °C/s) session 1	No	46.3 ± 0.9 *cf* 45.4 ± 0.8 °C		
	38-50 °C (1 °C/s) session 2	No	47.3 ± 0.9 *cf* 45.4 ± 0.7 °C		
			Method of levels		
	38-50 °C (0.5 °C/s) session 1	Yes	46.6 ± 0.6 *cf* 42.9 ± 0.8 °C	M > F	
	38-50 °C (0.5 °C/s) session 2	Yes	47.3 ± 0.9 *cf* 43.9 ± 0.9 °C	M > F	
	38-50 °C (4 °C/s) session 1	Yes	47.1 ± 0.8 *cf* 45.2 ± 0.9 °C	M > F	
	38-50 °C (4 °C/s) session 2	Yes	48.6 ± 0.6 *cf* 46.3 ± 0.7 °C	M > F	
Human	Thermal (rate = 6°C/s)		Mean VAS Score for low and high	Pain Rating	Feine *et al.* (1991)
	Method of limits		temperature ranges		
	45-49 °C (low)	Yes	36.9 *cf* 50.8 (low; upper lip)	F > M	
	46-50 °C (high)	Yes	44.8 *cf* 60.4 (high; upper lip)	F > M	
Human	Thermal (rate = 10°C/s)		Temperature at Pain Threshold		Fillingim *et al.* (1998)
	Method of limits	Yes	46.7 ± 0.2 *cf* 45.3 ± 0.5 °C	M > F	
	41.5-50 °C	No	46.3 ± 0.3 *cf* 45.3 ± 0.5 °C (left face)		
		No	45.5 ± 0.2 *cf* 44.8 ± 0.4 °C (left arm)		
			Temperature at Pain Tolerance		
		Yes	47.8 ± 0.1 *cf* 46.7 ± 0.3 °C (right face)	M > F	
		Yes	47.7 ± 0.2 *cf* 46.3 ± 0.3 °C (left face)	M > F	
		Yes	47.2 ± 0.2 *cf* 46.2 ± 0.3 °C (left arm)	M > F	
Human	Thermal (rate = 0.7°C/s)		Temperature at Pain Threshold		Lautenbacher & Rollman (1993)
	40-52 °C	No	43.3 °C *cf* 43.5 °C (right foot)		
		No	44.0 °C *cf* 43.5 °C (right hand)		
	Electrical		Current at Pain Threshold		
	(Constant Voltage)	Yes	3.5 mA *cf* 1.5 mA (hand)	M > F	
			Current at Pain Tolerance		
		Yes	7.5 mA *cf* 3.5 mA (hand)	M > F	
Human	Electrical		Voltage at Pain Threshold		Walker & Carmody (1998)
	(Constant current)	Yes	18 ± 0.3 V *cf* 15 ± 0.3 V (earlobe)	M > F	
			Voltage at Pain Tolerance		
		Yes	24 ± 0.4 V *cf* 21 ± 0.4 V (earlobe	M > F	
Rat	Pressure (Mechanical)	Yes	Tail Pressure	M > F	Kayser *et al.* (1996)
	Randall and Selitto Test	No	Hind-paw Pressure		
Rat	Chemical (Formalin)	Yes	0.1% Formalin		Aloisi *et al.* (1995)
		No	10% Formalin	Pain Behaviours F > M	
Mouse	Chemical (Formalin)	Yes	5% Formalin	Pain Behaviours F > M	Kim *et al.* (1999)

1.5.2 Sex-Role Expectations

While the emphasis of this thesis is that there are sex differences in biological mechanisms involved in pain perception, and analgesic drug handling, other researchers suggest that the differences are as a result of differences in learning history, socialization, behaviour and risk factors. However, it is far more likely that there is a contribution from both influences that combine to produce the sex differences seen experimentally and clinically.

Sex-role expectations in pain begin from an early age when children *learn* from their parents and carers to label physiological stimuli such as pain (Kupers, 1997; Munafo, 1997). Whilst boys are often expected to be heroic and uncomplaining, girls are encouraged to show their emotions (including expressions of pain). According to Kupers, this may cause "boys and girls to get dissimilar clues from their environment about how to label their physiological arousal" (Kupers, 1997). As adults this may produce differences in willingness to report pain, resulting in higher pain report in women compared to men (Berkley, 1997). This hypothesis may influence the epidemiological observations that a greater percentage of women (compared to men) suffer from severe chronic pain syndromes (*e.g.* rheumatoid arthritis and temporomandibular disorder; for review see Berkley, 1997), and suffer greater pain with the same objective pathology (Puntillo & Weiss, 1994). In the acute pain setting (such as pain resulting from tooth extraction), women also report greater pain than men (Averbuch & Katzper, 2001). From these observations, Unruh suggests that women actually experience more frequent and more severe pain than men (Unruh, 1996), independently of their willingness to report pain. In contrast to this hypothesis, recent evidence indicates that gender-

role expectations (*i.e.* the socially acquired aspects of being male or female) are influential in the areas of pain report, pain sensitivity and willingness to endure pain, with both men and women rating men as less likely to report pain and to be less pain sensitive (Robinson *et al.*, 2001). In further studies, an individual's gender-role expectation of pain was able to predict pain tolerance in a thermal experimental pain paradigm (Wise *et al.*, 2002).

Despite the evidence that psychosocial factors play a role in determining sex differences in the experimental pain setting, they cannot be the only causal factor. In an experimental paradigm using an autonomic indicator (pupil dilation following noxious pressure stimulation of the middle finger), Ellermeier and Westphal (1995) demonstrated sex differences in pain ratings (females having higher pain ratings than males). Since differences were found using this autonomic indicator suggests that sex differences must have sensory or affective components of pain rather than purely attitudinal or response-bias (sex role expectations) aspects. Thus both psychosocial and biological sex differences are likely to both contribute to produce the observed sex differences in nociception.

Effect of Experimenter Sex

It has been hypothesised that the sex of the experimental operator is an important factor in the severity of reported pain, however, this experiment heightened sex-role expectations by the use of an excessively attractively dressed experimenter (Levine & de Simone, 1991). Other researchers found pain report to be independent of experimenter sex (Otto & Dougher, 1985; Feine *et al.*, 1991). While this is hypothesis is interesting, the fact that sex differences in nociception are found

across a wide range of experimental set-ups (with varying experimenter sex) it is unlikely that this factor is solely responsible for the differences observed.

1.5.3 Psychological Factors

Women utilize health care services for pain more often than men, which may reflect the fact that women attend to and manage pain more readily than men (Unruh, 1997). Alternatively it may reflect differences in willingness to report pain, as outlined above. The National Health Survey, Use of Medications, Australia (1999) found that 27% of women reported using a pain relieving medication recently compared to only 20% of men (Australian Bureau of Statistics, 1999), whilst this difference is relatively small, it does highlight differences in willingness to access and utilise analgesic drugs.

Psychological conditions such as depression and anxiety are often accompanied by chronic pain conditions. Furthermore, anxiety and depression may cause an increase in pain severity, especially in women (Haley *et al.*, 1985). In addition, women suffer more often than men from unipolar depression, anxiety disorders and somatization disorder (Benedetti, 1997), with the possibility of accompanying painful somatic symptoms. Affective disorders such as depression and anxiety are thought to be caused by a functional deficit in 5-HT that may be compounded in women by fluctuations in the levels of sex hormones (which have been shown to alter expression of 5-HT receptors in rat brain (Fink *et al.*, 1998), peak levels of oestrogen and progesterone decrease 5-HT release from the hypothalamus (Gundlah *et al.*, 1998). The anti-depressant drugs (especially the 5-HT uptake inhibitors) have been used successfully as adjuncts to analgesic therapy in painful conditions for many years (McQuay *et al.*, 1996).

1.5.4 Neural Differences: The Role of Sex Hormones

Although psychological differences between men and women may account for some of the sex differences in pain perception, the fact that women possess greater pain discrimination than men (Feine *et al.*, 1991) implies that differences in pain perception are also due to physiological differences (Vallerand, 1993). Furthermore, the finding that women exhibit greater pain sensitivity for some and not other experimental pain measures (Lautenbacher & Rollman, 1993; Fillingim & Maixner, 1996) also points to a neural disparity accounting for the differences.

Descending Inhibition of Pain

Like many other of the body's systems, nociceptive excitatory input is often balanced by inhibitory output (see Section 1.2.1, and Lautenbacher, 1997). When there is not an input-output balance, pathological pain conditions may arise. Since women suffer more chronic painful conditions, perhaps there is a difference in the excitatory-inhibitory balance, possibly due to differences in the descending (modulatory) component of the pain pathway (Sternberg, 1998).

This issue has been examined using the stress-induced analgesia (SIA) animal model. According to this model, exposure to a stress prior to noxious stimulation, would be expected to result in a reduction of pain sensitivity (Aloisi *et al.*, 1998). Reflecting the experimental findings in pain sensitivity, female rodents display lower levels of SIA than males (when differences are present; Romero & Bodnar, 1986; Kavaliers & Innes, 1987a; Lipa & Kavaliers, 1990). Not only does the expression of SIA differ between the sexes, but the neurochemical quality of the analgesia also differs: *i.e.* in opioid mediated SIA paradigms, males exhibit greater levels of analgesia than females (Romero & Bodnar, 1986). Non-opioid mediated

paradigms in male mice may be mediated by NMDA receptors (Mogil *et al.*, 1993), while female mice exhibit naloxone (Sternberg *et al.*, 1995) and NMDA-antagonist insensitive SIA (Lipa & Kavaliers, 1990; Sternberg *et al.*, 1995).

Sex hormones influence the expression of SIA. Female mice exposed to testosterone during the neonatal period exhibit NMDA-mediated SIA (even in the presence of oestrogen; Sternberg *et al.*, 1995), whilst those not exposed to testosterone exhibit oestrogen-dependent swim SIA. Since changing the sex hormonal profile can alter the neurochemical basis for SIA, there must be organisational (developmental) effects of sex steroids on endogenous analgesia (Kavaliers & Galea, 1995).

Sex Hormones

Sex hormones produce a wide range of effects that can be considered non-reproductive, for example cognitive function, motor activity, seizure susceptibility as well as pain sensitivity (McEwen *et al.*, 1998). As early as the 1940s, sex hormones were reported to influence pain responses (Selye, 1941). During a course of experiments initially designed to determine steroid toxicity in rats, Selye found that injections of sex hormones (eg progesterone), resulted in deep anaesthesia (Selye, 1941). More recent research has examined the analgesic properties of the pregnane derivatives alphadalone and alphaxalone (Goodchild *et al.*, 2000; Nadeson & Goodchild, 2000; Nadeson & Goodchild, 2001; Goodchild *et al.*, 2001).

Differences in pain sensitivity across the oestrus cycle have been demonstrated in animal models, although the results are conflicting, probably due to different

stimulation methods (Frye *et al.*, 1993; Kayser *et al.*, 1996; Giamberardino *et al.*, 1997; for review see Fillingim & Ness, 2000)

Whilst animal findings suggest that pain sensitivity would also vary across the human menstrual cycle, the nature of its effect on pain responses are not so clear. Some pain syndromes are aggravated by exogenous hormones (for example, temporomandibular disorder; LeResche *et al.*, 1997, for review see Warren & Fried, 2001), and fluctuations in endogenous sex hormones can also alter some pain syndromes (for example, fibromyalgia (Østensen *et al.*, 1997), rheumatoid arthritis (Cardoe *et al.*, 1977), and irritable bowel syndrome (Mathias *et al.*, 1998). These further suggest a role for the sex hormones in clinical pain perception.

Experimental pain data also indicate that sex hormones play a role in pain perception, although these results are not as clear as the animal findings. This may be due to methodological variability, cyclical variability, lack of standardised definitions and methods for identifying menstrual cycle phase or variability in the way we determine pain threshold and tolerances (Riley *et al.*, 1999). Riley and colleagues reviewed sixteen published studies that examined the relationship between the pain perception of experimentally induced pain and menstrual cycle phase in healthy females, by performing meta-analysis to quantitatively analyse the data. They found, for the most part, that during the follicular phase females exhibited the greatest pain threshold and tolerances (with the exception of electrically induced pain which produced the greatest pain threshold during the luteal phase; Riley *et al.*, 1999).

Sex steroids may be influential in the development (organization) or the regulation (activation) of neural systems involved in pain response. The effect of sex steroids

on both early stages of development and on the adult brain have been reported in the literature (Aloisi, 1997). Research by a group in Japan has given insight into a possible biochemical cause for sex differences in pain (Yuri & Katawa, 1994). This group demonstrated a relationship between calcitonin gene related peptide (CGRP) and sex steroids centrally in the preoptic hypothalamus, the intensity and number of CGRP-immunoreactive neurones were up regulated in female rats by oestrogen by way of the oestrogen receptor (Yuri & Katawa, 1994). In male rats, CGRP-immunoreactive neurones were greater in castrated animals compared with intact males, with this effect reversed by testosterone treatment (Popper & Micevych, 1989; Popper & Micevych, 1990). In contrast, in the *peripheral* nervous system (specifically in the dorsal root ganglion) the percentage of CGRP immunoreactive neurones was significantly lower in the female than the male rat (Yang *et al.*, 1998). This may reflect differences in central versus peripheral pain processing.

Another possible mechanism is through sex hormonal regulation of β-endorphin (Veith *et al.*, 1984; Forman *et al.*, 1985; Nakano *et al.*, 1991; Tomimatsu *et al.*, 1993; Desjardins *et al.*, 1993; Aloisi *et al.*, 1995). Oestradiol has been shown to be neurotoxic to hypothalamic β-endorphin neurones in the arcuate nucleus (Desjardins *et al.*, 1993), and, perhaps in consequence decreases levels of β-endorphin in both hypothalamus and plasma (Forman *et al.*, 1985). This would be expected to increase nociception especially given the fact that noxious stimulation increases β-endorphin release from the arcuate (Zangen *et al.*, 1998). Furthermore, this effect on nociception might be reinforced by the fact that oestrogen treatment reduces opioid binding sites in the anterior hypothalamus (Wilkinson *et al.*, 1985). Despite the contradictions apparent in the literature, it is

clear that there is a sex hormonal effect on human pain perception that warrants further investigation.

1.6 SEX DIFFERENCES IN ANALGESIA

The issue of sex differences in analgesic response has been very difficult to resolve. This is primarily due to a lack of data examining the analgesic responses of men *and* women. Up until the early 1990's women were routinely excluded from clinical trials of analgesic drugs because they were thought to "confound" the results due to menstrual cycle variability, and secondly due to the obvious ethical issue of studying women of childbearing potential. However in the early nineties the governments of several countries (including Australia) changed the guidelines for drug research: stating both men and women *must* be utilised in trials of new drugs, except where the inclusion or exclusion is essential for the purpose of the research (National Health and Medical Research Council, 1999). This has allowed researchers to examine several sex-related issues, including the effects of menstrual cycle, menopausal status and oral contraceptives on the pharmacokinetics and/or pharmacodynamics of the drug in question.

1.6.1 Opioids

Sex differences in opioid effects are documented in the literature in both animal and human models of pain. For example, male rats display greater analgesia following systemic (Kavaliers & Innes, 1987b; Baamonde *et al.*, 1989; Candido *et al.*, 1992; Cicero *et al.*, 1996; Cicero *et al.*, 1997; Binder *et al.*, 2000) and central (*i.e.* intracerebroventricular) administration of the μ-opioid agonist morphine (Kepler *et al.*, 1989; Kepler *et al.*, 1991) as well as greater

antinociception following systemic administration of alfentanil (Cicero *et al.*, 1997). The sex differences (when found) could not be explained in terms of pharmacokinetics, since the blood and brain concentrations of morphine were the same (Cicero *et al.*, 1997). Bartok and Craft, however, found female rats showed greater antinociception following the μ-agonist buprenorphine (Bartok & Craft, 1997), and Sarton and co-workers found greater morphine potency, but slower onset and offset in women (Sarton *et al.*, 2000). This might suggest that sex differences are *drug specific* rather than *class specific*. However, buprenorphine acts as a partial agonist, and although very potent, can also act as an antagonist to other opioids, furthermore it has significant κ-opioid receptor activity (McDonald, 2001) and κ-opioid agonists have been shown to be more potent in women than in men (see below). Thus specificity for the μ-receptor may also play a role in determining sex differences in analgesia. In Sarton and colleagues' study, the electrical stimulation of the skin overlying the tibia may have caused inherent sex differences due to differences in skin thickness in this region (which alters conductivity), elicitation of other neural fibres unrelated to pain responses, or involvement of twitching of the underlying muscle.

Sex differences in analgesic response to the κ-opioid drugs pentazocine, nalbuphine and butorphanol in a human clinical pain model (molar extraction) have also been reported, with men less sensitive to the analgesic effects (Gear *et al.*, 1996a; Gear *et al.*, 1996b). However, perhaps more intriguing was the fact that women exhibited a *bell-shaped* dose response curve in response to nalbuphine, that is, the maximal effect was achieved following a 10 mg and not a 20 mg dose (Gear *et al.*, 1999). That the analgesic effect of nalbuphine can be so

markedly different in males and females points to a pharmacodynamics influence. Their finding was strengthened by that of Bartok and Craft who found female rats to be more sensitive to the effects of U-69,593 than male rats (Bartok & Craft, 1997). These findings were re-examined by our own laboratory where responses to thermal and mechanical analgesimetry following asimadoline (a peripherally selective κ-agonist) or PNU-50,488H (a centrally acting κ-agonist) were measured in an animal model of chronic pain (Binder *et al.*, 2000). Whereas asimadoline indicated no gender differences with the mechanical test, PNU-50,488H produced greater analgesia in female rats. In contrast, using a thermal analgesia test both asimadoline and PNU-50,488H produced greater anti-nociception in female rats (Binder *et al.*, 2000), consistent with others findings (Gear *et al.*, 1996a; Gear *et al.*, 1996b; Bartok & Craft, 1997; Gear *et al.*, 1999). This may point to a difference in *peripheral* versus *central* handling of κ-opioid drugs; perhaps the mechanical and thermal testing procedures differentially activate *peripheral* or *central* pain pathways; *or* there are different receptor densities in these two regions with respect to sex.

1.6.2 NSAIDs

Our laboratory has reported a sex difference in analgesic efficacy following ibuprofen administration: women were refractory to the effects of 800mg of ibuprofen in an experimental pain model (Figure 1.6; Walker & Carmody, 1998). This result is the basis of the experiments outlined in this thesis.

Figure 1.6: Analgesic effect of placebo (white bars) and ibuprofen 800 mg (black bars) in healthy young men and women (Walker & Carmody, 1998).

1.6.3 Mechanisms

Several mechanisms have been reported to be important in determining sex differences in analgesic response. These include differences in pharmacokinetics or pharmacodynamics, differences in tolerance or differences in central nervous system (CNS) mechanisms of action of the drugs. Sex hormones may modulate analgesic response by acting on one or more of these systems.

Pharmacokinetics

Differences in pharmacokinetics were the initial target of investigation, possibly because they are simple to observe. Many authors have reported gender differences in pharmacokinetics (for review see Beierle *et al.*, 1999), although few focus on analgesic drugs. There are four areas linked to pharmacokinetics that exhibit gender differences: absorption/bioavailability; distribution/protein binding; metabolism; and excretion.

Firstly, *absorption and bioavailability*. There are sex differences in gastric acid secretion and gastric emptying: women secrete less acid (Fletcher *et al.*, 1994) and

have slower gastric emptying than men (Gryback *et al.*, 1996; Degen & Phillips, 1996; Hermansson & Sivertsson, 1996). This results in differences in bioavailability of some drugs and differences in absorption time respectively.

Secondly, *distribution and protein binding*. The distribution of a drug is affected by its physiochemical properties, the vascular and tissue volume of distribution, and the ratio of lean body mass to adipose tissue mass (Beierle *et al.*, 1999). Considering that the ratio of muscular to adipose tissue of men and women differ, it is not surprising that there are sex differences in distribution. Furthermore, physiological changes in the menstrual cycle, such as fluctuations in water and electrolyte balance also influence drug distribution. However, as there is no sex difference in albumin levels in men and women (Beierle *et al.*, 1999), drugs that primarily bind to albumin do not usually show sex differences. Furthermore, as sex differences in volume of distribution seem to be mainly related to weight and fat proportion, this element may be of very little relevance to clinical practice (Beierle *et al.*, 1999).

Metabolism also exhibits sex differences, primarily due to a difference in the activity of the metabolic enzyme status, however, clinical significance is only reached in isolated cases (Beierle *et al.*, 1999).

Excretion is the major route of elimination for many drugs, and there is a sex difference in glomerular filtration rate, however, sex differences in renal excretion have only been identified for a few drugs, and again either do not reach clinical significance, or need to be further evaluated (Beierle *et al.*, 1999).

Despite sex differences in pharmacokinetics, the question as to whether pharmacokinetic differences can account for sex differences in analgesic effect is

contentious. While some evidence suggests that the efficacy of morphine is greater in males than in females (see above), none has indicated a pharmacokinetic explanation. Firstly, reports show no difference in blood or brain (Cicero *et al.*, 1997) levels of morphine in mice. Further, the demonstration of sex differences following intracerebroventricular injections of morphine argues against a pharmacokinetic explanation (Kest *et al.*, 1999). Lastly studies of ibuprofen in humans have also shown sex differences in analgesia in the absence of differences in pharmacokinetics (Walker & Carmody, 1998).

Pharmacodynamics

Whilst very limited research has occurred in this area several researchers have demonstrated sex differences in pharmacodynamics. Firstly, our own laboratory has examined sex differences in the analgesic effects of ibuprofen (Walker & Carmody, 1998), and found that despite no differences in pharmacokinetics, there were significant sex differences in analgesic effect of ibuprofen (to the extent that females were refractory to the effects of 800mg ibuprofen; Walker & Carmody, 1998). More recently, Gear and co-workers have reported sex differences in the efficacy of nalbuphine, a κ-opioid, in a clinical pain model, and despite similar responses to placebo, women experienced significantly greater analgesic response than men for all doses of nalbuphine (Gear *et al.*, 1999). Others have reported no sex differences in the pharmacokinetics of nalbuphine (Wilson *et al.*, 1986; Jaillon *et al.*, 1989)

Tolerance

Drug tolerance is traditionally associated with the opioid drug class (*e.g.* morphine (Craft *et al.*, 1999) and cocaine (van Harren & Meyer, 1991)), however, it also

occurs with NSAIDs (*e.g.* ibuprofen; Walker *et al.*, 1996). The issue of sex differences in tolerance has potential clinical significance in chronically dosed patients. Further research needs to be conducted to assess the effect of sex on drug tolerance.

Central Nervous System Mechanisms

Another mechanism of sex differences in analgesia may involve differences in the central nervous system processing of pain. There is a sexual dimorphism in NMDA involvement in the expression of non-opioid in SIA (see Section 1.5.4), furthermore, the NMDA antagonist (NPC 12626) has been shown to attenuate analgesic effects of both non-opioid- and opioid-induced analgesia in male mice, whilst having no significant effect in female mice (Kavaliers & Choleris, 1997). Others have reported striking hormonal influences on the expression of NMDA (glutamate) receptors in the central nervous system (Weiland, 1992).

Genetics

Morphine's potency has been shown to vary between subpopulations of a single species (for review see Mogil *et al.*, 1996), as well as varying by sex. Mogil's laboratory has been particularly interested in genetics as a factor in nociceptive and analgesic variability. The technique of molecular gene mapping can be used to find the qualitative trait locus (QTL) for morphine analgesia (Mogil *et al.*, 1996), stress induced analgesia (Mogil *et al.*, 1997) etc. There are *sex specific* QTLs, in particular, Mogil's group has demonstrated a large QTL associated with variability in swim stress-induced analgesia in female but not male mice (Mogil *et al.*, 1997). This finding provides additional evidence that there are female specific mechanisms of nociceptive modulation in the rodent at least. Should such sexually dimorphic

pain-modulatory systems also be found in humans, it would revolutionise the way that we treat patients with painful conditions.

"The experience of being female or male depends on complex interactions among multiple endogenous and exogenous variables. These include obvious anatomical differences such as body size, genital organs, and muscle mass; differing levels and temporal patterns of gonadal hormones; psychosocial factors such as emotional experience and sex role expectancies; and multiple environmental and cultural influences" (Fillingim, 2000). Clearly merely being male or female involves complex interactions, which must have some inherent variability. Thus, researchers face the task of delineating what is intra-sex variability and what is inter-sex variability. The fact that researchers have reported sex differences in analgesia, despite the difficulties of this research, makes their findings even more powerful.

There is scope for much more work. The interaction of other classes of analgesic drugs with gender needs to be examined, as well as an evaluation of the mechanisms involved. Clearly both researchers and clinicians alike must consider sex as a significant variable that affects pain and analgesic response.

1.7 THE SEX HORMONES

The major focus of this thesis is the influence of sex hormones on analgesic response. Therefore this introduction would not be complete without a brief overview of the human sex hormones.

1.7.1 The Hypothalamic-Anterior Pituitary-Gonadal Axis

In both sexes, the gonads are responsible for the secretion of sex hormones under the control of the hypothalamic-pituitary-gonadal axis (Males see Figure 1.7; Females see Figure 1.8).

The androgens (*e.g.* testosterone) are the steroid sex hormones that are responsible for masculinizing, whilst the oestrogens are feminising. Both are present in men and women, but their levels are dependent upon sex. The testes secrete large amounts of androgens (primarily testosterone), as well as small amounts of oestrogen, whilst the ovaries secrete large amounts of oestrogen and progesterone, with small amounts of androgens. Both sexes produce androgens from the adrenal cortex.

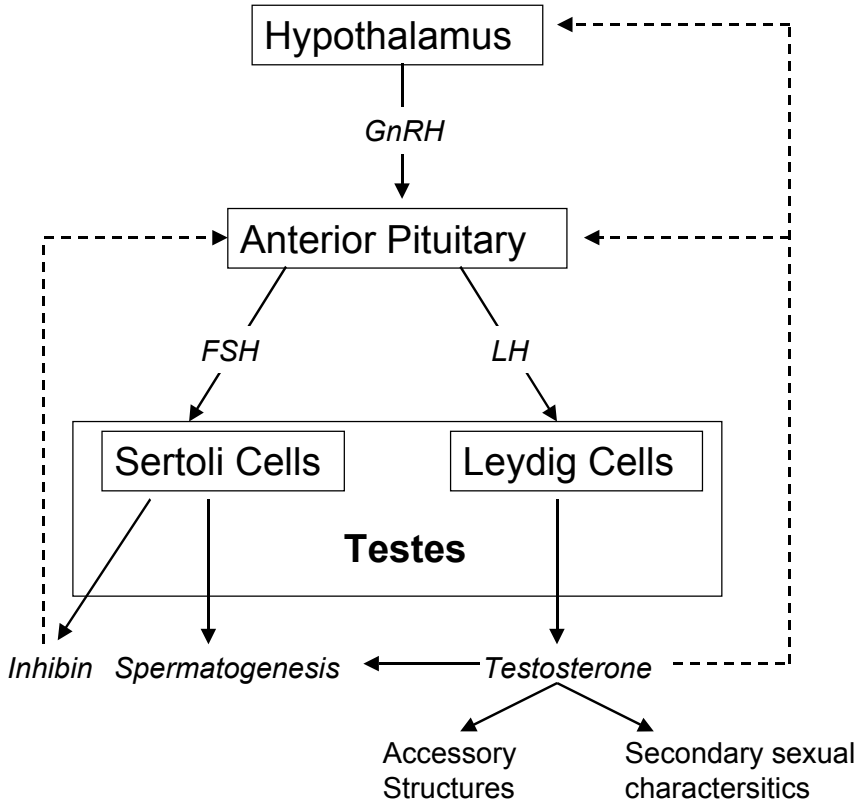


Figure 1.7: Hypothalamic-pituitary-gonadal control of sex hormone secretion in males. Adapted from Moffett et al. (1993) pg 702.

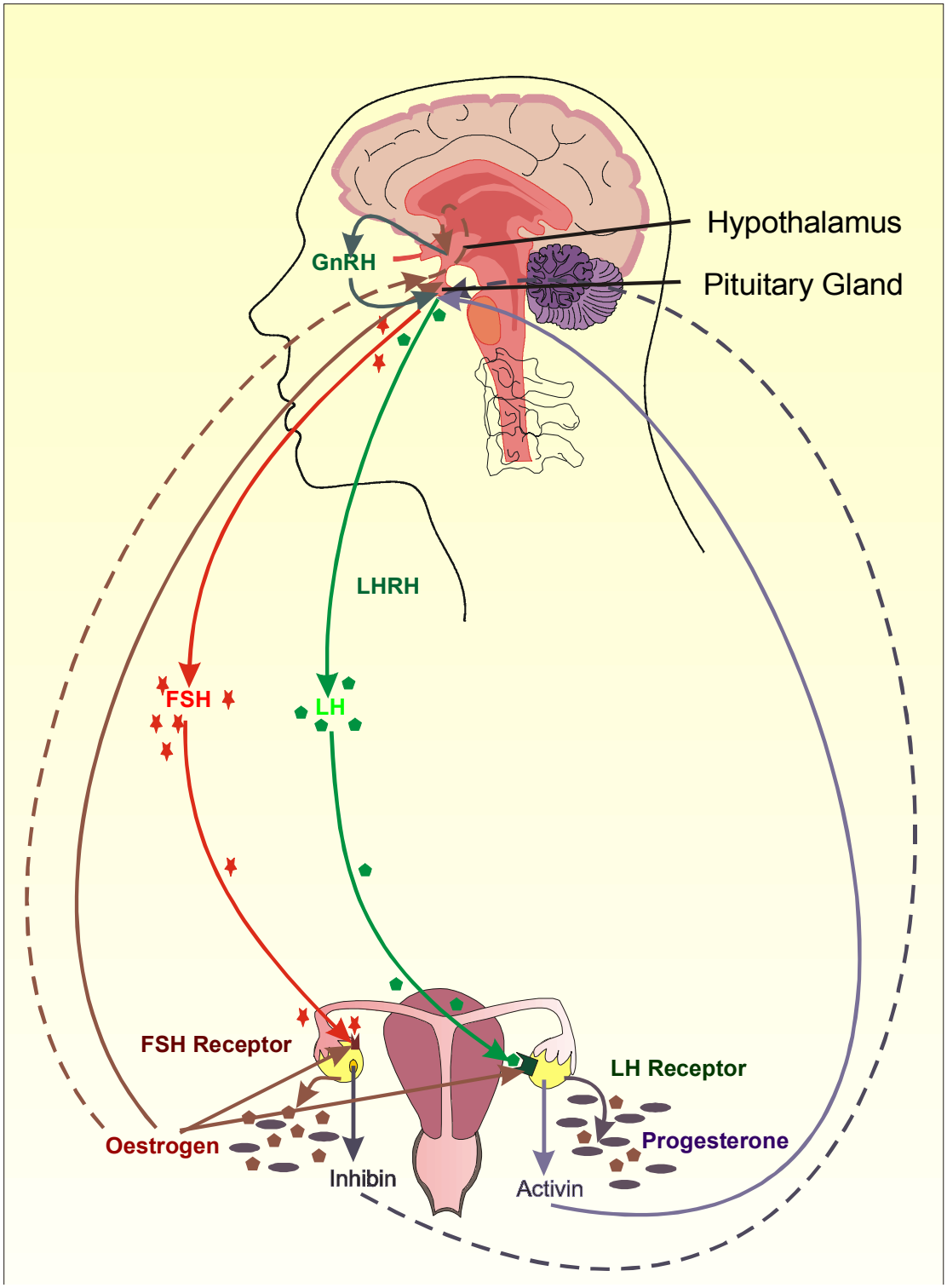


Figure 1.8: The control of sex hormone secretion in post-pubescent, pre-menopausal women. Dashed lines represent inhibition.
Note that oestrogen exerts positive feedback on the pituitary, FSH and LH receptors during the follicular phase, whiles it exerts negative feedback on the pituitary and hypothalamus during the luteal phase.

1.7.2 The Menstrual Cycle

The human female menstrual cycle is characterised by cyclical changes in sex steroid levels over a period of approximately 28 days (see Figure 1.9). There are three phases: follicular, luteal and menses.

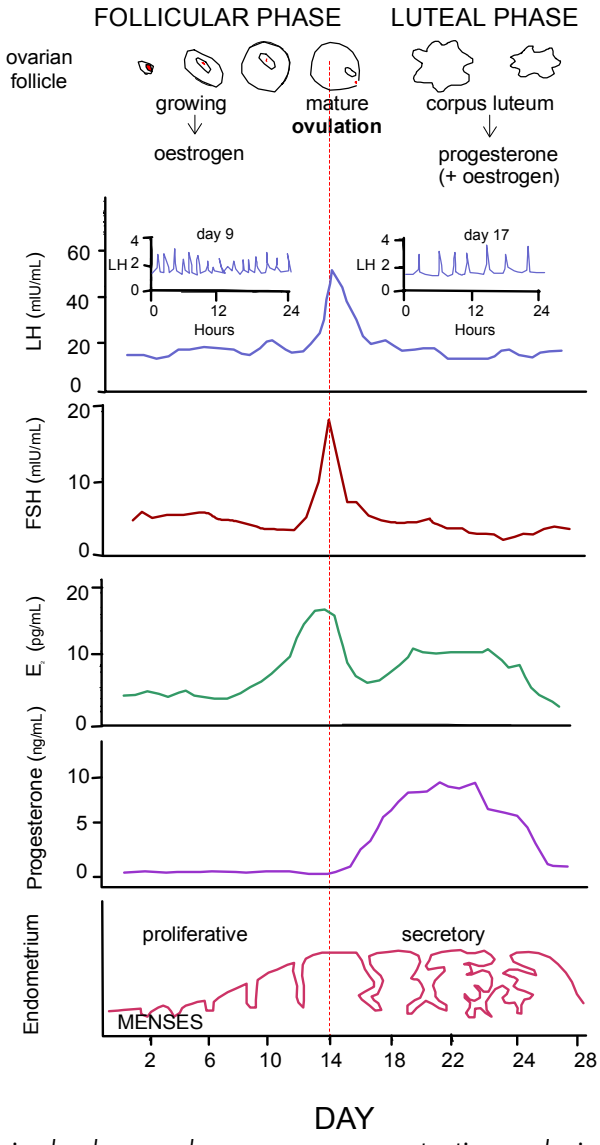


Figure 1.9: Typical plasma hormone concentrations during a normal 28 day human menstrual cycle. LH Luteinizing Hormone; FSH Follicle Stimulating Hormone, E_2 Oestradiol. Adapted from Williams & Stancel (1996) pg 1416.

Menstruation

The levels of oestrogen and progesterone are at a minimum, and the endometrium breaks down and is expelled (bleeding). Negative feedback on the hypothalamic-pituitary axis is at a minimum, and the levels of Follicle Stimulating Hormone (FSH) increase the growth of the ovarian follicles.

The Follicular Phase

The Follicular Phase is characterised by increasing levels of oestrogen (peaking 24 hours prior to ovulation) and low levels of progesterone. FSH levels are greater than Luteinizing Hormone (LH), and the ovarian follicle and endometrium are growing. At the end of this phase, the oestrogen levels peak stimulating positive feedback on the anterior pituitary and the hypothalamus, instigating the FSH and LH surges, resulting in ovulation 24 hours later.

The Luteal Phase

The Luteal Phase is characterised by the formation of the corpus luteum, increasing levels of oestrogen, and a peak in progesterone levels. The endometrium transforms from being proliferative to becoming secretory. Towards the end of this phase, there are declining levels of oestrogen and progesterone.

1.7.3 Control of Fertility – The Oral Contraceptives

The oral contraceptive pill is based on the negative-feedback effect of oestrogen and progesterone on the hypothalamic-pituitary axis. The pill (usually containing low levels of synthetic oestrogen and a higher level of synthetic progesterone) is taken daily for 21 days beginning on the 5th day of menstruation. The hormones mimic the effects of endogenous oestrogen and progesterone, however, as their levels are artificially raised from the first day, the hypothalamus ceases to release

Gonadotropin Releasing Hormone (GnRH), resulting in a lack of FSH and LH secretion. In their absence, follicular development, ovum maturation and ovulation cannot occur. The synthetic hormones promote endothelial proliferation and secretion, and once the hormone support is lost (at day 22), menstruation occurs.

1.7.4 Menopause

The World Health Organisation defines menopause as "the permanent cessation of menstruation resulting from the loss of ovarian follicular activity. Natural menopause is recognized to have occurred after 12 consecutive months of amenorrhea, for which there is no other obvious pathological or physiological cause. Menopause occurs with the final menstrual period that is known with certainty only in retrospect a year or more after the event. An adequate biological marker for the event does not exist". Menopause is caused by a change in ovarian function rather than a change in the hormonal control system (Figure 1.8, above), which is evidenced by a rise in gonadotropin levels after menopause (see Figure 1.10). This is due to the ovaries becoming unresponsive to GnRH, probably because of the decline in number of primordial follicles (Shoupe *et al.*, 1997). The ovaries then largely cease production of progesterone and 17β-oestradiol which leads to a reduction of negative feedback on the hypothalamus and anterior pituitary, ultimately leading to increased levels of GnRH, FSH, and LH (Shoupe *et al.*, 1997).

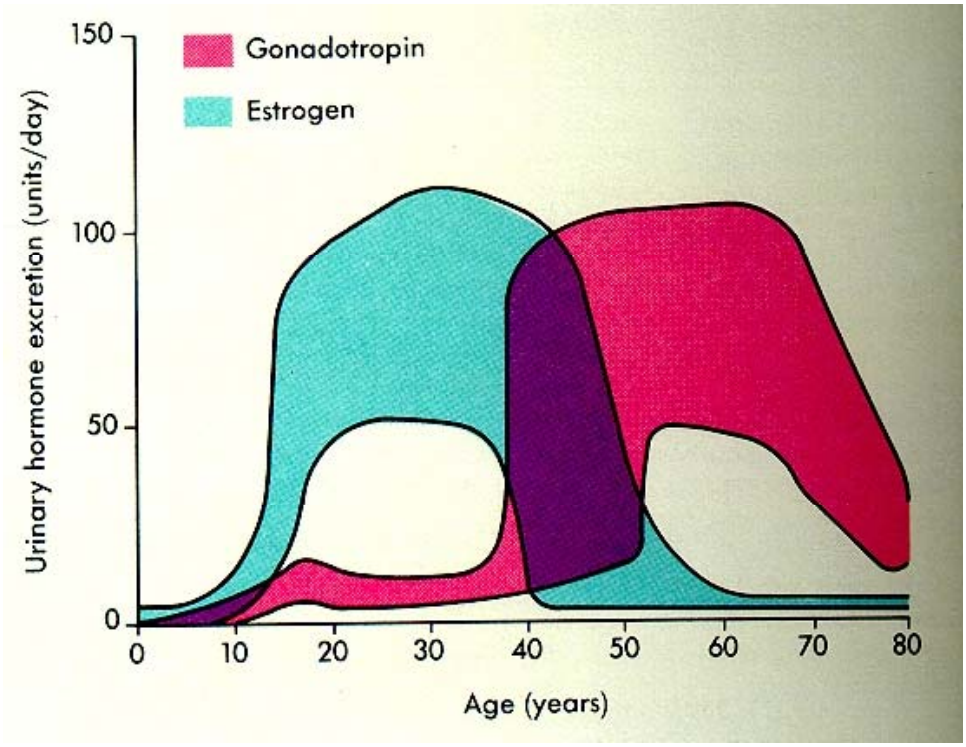


Figure 1.10: Normal rates of urinary excretion of gonadotropin and oestrogen in a population of women of various ages. These rates are rough indicators of the plasma levels of the corresponding hormone. During the reproductive years, much of the variation in the range of normal values is due to changes over the menstrual cycle. From Moffett et al. (1993) pg 710.

There are several symptoms common after ovarian function has ceased. These include: uterine and vaginal atrophy, hot flushes, inappropriate sweating, decreased vaginal lubrication, palpitations, and paraesthesias (Moffett *et al.*, 1993).

Hormone Replacement Therapy

Hormone replacement is used in postmenopausal women to reduce: symptoms associated with a decline in oestrogen production (see above); the incidence of coronary heart disease; and the risk of osteoporosis (Shoupe *et al.*, 1997). As oestrogen replacement therapy can increase the risk of cancer (both endometrial and breast), progesterone is commonly co-prescribed (Shoupe *et al.*, 1997).

1.8 THESIS OUTLINE

To date, predictors for response to painful stimuli and analgesic efficacy have not been adequately explored or addressed, thus an examination of these issues is paramount. There are several aims of the present work:

- To confirm that there are sex differences in basal nociception, and in response to ibuprofen in a human experimental model of pain (Chapters 2, 3 and 4);
- To investigate whether these differences are due to differences in sex hormone status rather than sex per se in the same experimental model (Chapter 2);
- To assess the role of sex (and sex hormone status) on placebo and drug expectancy in a human experimental model of pain (Chapter 3);
- To determine which sex hormone(s) are responsible for this effect (if any) in a rodent model of pain (Chapter 5);
- To find out whether acetaminophen analgesia is dependent upon genotype in a murine model (Chapter 6).

Chapter 2

INFLUENCE OF SEX HORMONE STATUS ON ANALGESIC RESPONSE TO EXPERIMENTAL PAIN IN HEALTHY VOLUNTEERS

2.1 INTRODUCTION

> *"We used to think that if we knew one, we knew two, because one and one are two. We are finding that we must learn a great deal more about 'and'."*[1]

Nonsteroidal anti-inflammatory drugs (NSAIDs) are usually the first line of treatment in rheumatoid arthritis, osteoarthritis, back pain and other musculoskeletal conditions. Although they are widely used, they show a large degree of interindividual variability that is yet to be adequately explained (Walker *et al.*, 1997; Palmer & de Lapp, 2000). Failure to obtain clinical relief with one drug usually leads to a rotation through a series of NSAIDs until either a satisfactory response is obtained or an unsatisfactory outcome is acknowledged and further therapeutic options are contemplated. Clearly, understanding the mechanisms responsible for variability is important, and would have significant implications for the rational prescribing of these therapeutic agents to patients with musculoskeletal pain.

[1] Sir Arthur Stanley Eddington (1882-1944) In Mackay A.L., A Dictionary of Scientific Quotations (1991) IOP Publishing Ltd, Bristol UK. Pg 79.

Hoping to find a predictor for this clinically troublesome variability, Walker and Carmody (1998) performed a study in healthy young men and women to determine whether sex played an important role in the inter-subject variability seen in the analgesic response to the NSAID ibuprofen. Using an electrical pain model, these studies showed for the first time that men and women responded differently to ibuprofen therapy, and that women were refractory to the analgesic effects of an 800mg dose of ibuprofen (see also Figure 1.7; Walker & Carmody, 1998).

The aim of the present study was, therefore, to further examine this intriguing result by determining whether sex hormone status rather than sex *per se* were the determinant of analgesic response following ibuprofen administration. Two hypotheses were postulated:

1. That males would exhibit greater baseline pain responses (pain threshold and tolerances) than women.
2. That ibuprofen would produce greater analgesia in males.
3. That response to treatment would be dependant upon sex hormonal status.

2.2 METHODS

2.2.1 Volunteers

Subjects were recruited from within The University of New South Wales in Sydney and its surrounds using posters, flyers, broadcast email and newspaper advertisements. The subjects were healthy, pain-free volunteers, aged 18 to 65, who had provided written, informed and free consent, in accordance with institutional guidelines (UNSW HREC Approval No. 97058). They were given modest payment for their participation and were free to withdraw at any time.

A total of 256 people responded to the advertisement, potentially unsuitable subjects were dissuaded from participating by an initial discussion with Belinda Giles (see inclusion and exclusion criteria in Appendix A). 71 subjects completed the trial (41 female and 30 male) of which 68 were included in the analyses (3 were excluded entirely owing to protocol violations or technical difficulties). Subjects were divided into one of 5 groups according to their sex and sex hormone status:

1. young females, post-pubertal, pre-menopausal (YF, n=19);
2. young post-pubertal males (YM, n=19);
3. older postmenopausal females taking exogenous hormones (OFEXH, n=10);
4. older postmenopausal females, without exogenous hormones (OFNIL, n=10);
5. older males (aged-matched to subjects in groups 3 and 4; OM, n=10).

Before entry into the trial, the subjects underwent a medical examination (undertaken by Dr John Carmody, UNSW), which included a full medical history, a relevant clinical examination, a blood sample for biochemical and haematological screening (tests included: full blood count, liver function tests, urea, creatinine and

electrolyte levels, and oestrogen, progesterone and testosterone levels; all were kindly performed by South Eastern Laboratory Services, Prince of Wales Hospital, Sydney, NSW) and routine urinalysis. The full range of tests is described in Appendix B. Significant abnormalities led to exclusion of the subject from the study (only 1 subject was rejected). Other exclusion criteria included pregnancy; regular use of medication, especially analgesics; NSAID-sensitive asthma; alcohol abuse; participation in a trial of an investigational drug in the preceding month or during the study; surgery within the previous three months; or symptoms of a clinically important illness within four months of the study (no subjects were in this category as they were dissuaded from participating following an initial telephone screening by Belinda Giles). All the young females were cycling normally, and if oral contraceptives were taken this information was recorded upon entry to the study. The stage of the young female's menstrual cycle was recorded on each testing day, and, in the older females, the type of hormone therapy taken was recorded upon entry into the study (*i.e.* oestrogen therapy (ERT) or oestrogen and progesterone therapy (HRT)).

2.2.2 Experimental Procedure

One week before entry into the study each subject was familiarized with the analgesic testing apparatus and procedures for 20 minutes. Consistency of reporting is crucial for the reliability of this method, *i.e.* every time a subject terminates the noxious stimulus pain perception should be the same. This was optimised by giving standard instructions to each participant, which were repeated on each experimental day, at each time-point.

Subjects were required to refrain from alcohol, caffeine and analgesic drugs for 24 hours prior to the experimental day, and to have at least 8 hours' sleep the night before. They were also requested to have a light breakfast 1 hour before the beginning of the observation. Testing was conducted in an air-conditioned room between approximately 8 am and 2 pm, with subjects seated upright in a comfortable chair. The same trained observer (either Ms Belinda Giles [1998 (B.Sc (Hons) studies), 2000 and 2001 (PhD studies), 45 subjects] or Ms Amy Knowles [1999 (B.Sc. (Hons) studies) 27 subjects]) supervised all pain measurements during the day. A light snack was made available 1 hour after consumption of the treatment capsules (fruit juice and biscuits). The insistence on breakfast, and the provision of a standardised diet during the experiments were intended to minimise any gastrointestinal disturbances or possible influences of plasma glucose concentration on nociception (Morley *et al.*, 1984).

Subjects were told that ibuprofen was to be administered but to prevent possible bias were not informed that a placebo would be given (Walker *et al.*, 1994; Wall, 1994; Walker & Carmody, 1998). They were also told that this study was to elucidate why responses to analgesic drugs differed amongst individuals.

A schedule for a typical experimental day is shown below in Table 2.1.

Table 2.1: Sequence of events during an experimental day

8.30 am	Adaptation to surroundings Blood sample 1 Ear thickness measurement Electrode placement	9.40 am	Drug/Placebo administration
9.00 am	Stimulus trial *Pre-drug 1*	10.40 am	Stimulus trial *Post-drug 1* Blood sample 2 Snack and drink
9.15 am	Stimulus trial *Pre-drug 2*	11.40 am	Stimulus trial *Post-drug 2* Blood sample 3
9.30 am	Stimulus trial *Pre-drug 3*	12.40 pm	Stimulus trial *Post-drug 3*
		1.40 pm	Stimulus trial *Post-drug 4* Blood sample 4

2.2.3 Electrical Stimulation

The chosen noxious stimulation method was constant-current electrical stimulation of the earlobe. It is a development of the work of Hallin and Torebjörk, which distinguished the early (*pain threshold*) and late (*pain tolerance*) components of electrically induced pain which correspond to Aδ- and C-fibre activation respectively (Hallin & Torebjörk, 1973). The present technique was developed in order to establish a simple and reliable experimental pain model that could distinguish between the analgesic effects of NSAIDs and placebo in human volunteers (Walker *et al.*, 1993). It is a useful model, as it satisfies the 10 requirements for ideal pain stimuli originally proposed by Beecher (1959), and is also easy to apply, inexpensive, and reliable (Walker *et al.*, 1993). It has been used successfully previously to discriminate between dosages of 800mg of ibuprofen and placebo at the pain tolerance level (Walker *et al.*, 1993; Walker & Carmody, 1998). Previous reports have suggest that "threshold measures", which only produce first pain sensations (in the present study this correlates with the 2nd

button press) by activating Aδ fibres, are unreliable in detecting analgesic effects (Walker *et al.*, 1993).

Surface electrodes [pre-gelled, paediatric, disposable silver/silver chloride electrocardiogram electrodes, 34mm diameter, Medicotest, Olstykke, Denmark] were affixed to each surface of the alcohol-cleaned, earlobe on the subject's non dominant side (to avoid dextrality effects; Haslam, 1970) and connected via fine wires to the computer-controlled, battery-operated stimulator [maximal output 60V, designed by Edward Crawford, Electrical Engineer, Department of Physiology & Pharmacology, UNSW, Sydney, Australia, compliant to AS3221]. Test stimuli (one **stimulus train**) were 1ms square wave pulses delivered continuously at 20Hz, with the amplitude increased by 1V every 2 seconds (from an initial 1V). During exposure to the stimulus train, each subject was asked to indicate (by pressing a button), three grades of perception: *firstly,* when the stimulus was first felt (tingling, tickling); *secondly,* when the stimulus first was perceived as sharp and painful (which was called *Pain Threshold*) and *lastly* when the subjects perceived the stimulus as deep and burning (which was called *Pain Tolerance*). It may be argued that this is not a true measure of pain tolerance (the International Association for the Study of Pain's definition of pain tolerance is *the greatest stimulus intensity causing pain that a subject is prepared to tolerate;* Merskey, 1979) – however subjective behaviour (wincing, pulling away from stimulator, sighing etc) suggested that this closely approached the traditional definition of pain tolerance.

The computer captured the data from each button press and the stimulation was terminated at the third button press. After the third press, the subject was asked to rate "how bad the pain was" at the pain tolerance level using a computerised

visual analogue score (VAS) on a scale of 0 ("not bad at all") to 100 ("the most intense bad feeling possible").

A **stimulus block** comprised of four stimulus trains (described above) and were carried out over a period of approximately 5 minutes. Starting at 8.30am, painful stimulus blocks were administered 3 times in the hour prior to drug/placebo treatment (*baseline*), and then hourly for four hours thereafter (see Table 2.1). The training of subjects on this apparatus was very important, and was standardised across all subjects to ensure as much consistency of reporting as possible. Furthermore, the experimenters (BG or AK) were very careful not to cue responses in subjects following treatment administration – although neither subject nor experimenter knew whether a drug or placebo was involved as the treatments had been blinded by Dr Carmody. In addition, the experimenter was careful not to cue males and females differently, in light of the results of (Walker & Carmody, 1998), who found that 800mg dosages of ibuprofen were ineffective in women.

2.2.4 Drugs and Dosing

Subjects were randomly assigned, in a double-blind manner, to receive ibuprofen 800mg (four capsules 200mg ACT3, Wyeth, Sydney, Australia), or ibuprofen 400mg (two capsules 200mg ACT3 and two placebo capsules of identical appearance) or placebo (4 capsules placebo), once each on one of their (3) weekly visits. These doses were chosen partly because 800mg of ibuprofen is effectively analgesic with our stimulation technique (Walker & Carmody, 1998), without inducing any discernable sense of drug action or producing side effects (Walker *et al.*, 1996), and partly because the lower dose of 400mg (2 capsules) is

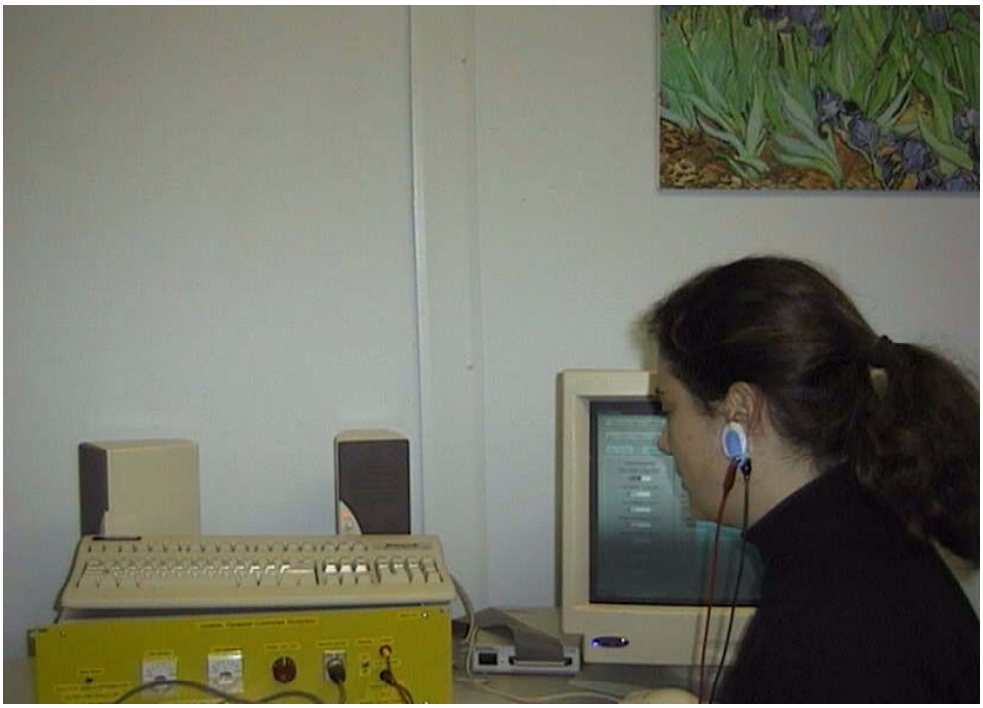
the commonly recommended dosage when patients buy this drug in Australian pharmacies.

2.2.5 Blood Collection and Ibuprofen Assay

For the determination of plasma concentrations of ibuprofen, blood was collected from an antecubital fossa vein (by Dr John Carmody or Ms Belinda Giles), pre-treatment (time 0) and then 1, 2 and 4 hours following treatment administration into Vacuette® Tubes containing 1.8mg/mL ethylenedaminetetraacetic acid (EDTA) powder [Greiner Labortechnik, Stonehouse, UK]. Technical difficulties precluded the successful collection of some samples. The collected samples were centrifuged (4,800rpm, 5min), the plasma transferred to microcentrifuge tubes, and stored at –70°C until analysis. Plasma concentrations of ibuprofen were then determined by a methodology that was a refinement of published HPLC procedures. The ibuprofen extraction procedure was taken from Pargal *et al.* (1996), whilst chromatography parameters (column type, wave-length, phosphate buffer concentration etc) were adapted from Walker *et al.* (1993). The internal standard and its concentration, as well as the mobile phase constitution, were chosen on the basis of preliminary HPLC analysis. An aliquot internal standard (50μL 0.5mM naproxen) was added to plasma (500μL), and the mixture acidified with 0.1M hydrochloric acid (500μL) then extracted with dichloromethane (5mL). The solvent layer was evaporated until dry [SpeedVac® Plus SC210A, Thermo Savant, Holbrook NY, USA], and the residue redissolved in sodium dihydrogen orthophosphate buffer (200μL, 0.025M NaH_2PO_4 with orthophosphoric acid to pH 3.4).

Samples (50 μL) were injected onto a non-chiral HPLC system, which consisted of a C8 HPLC column [Platinum EPS C8 100Å, 5μm 150 x 4.6mm; Alltech Associates (Aust.) Pty Ltd., Sydney, NSW, Australia], and eluted with a mixture of 0.025M NaH_2PO_4 buffer (pH 3.4) and methanol (50:50) at a constant flow of 1mL/min. The effluent from the column was detected by UV (220nm) using a UV-Vis detector [SPD-10Avp, Shimadzu Corporation, Kyoto, Japan]). Retention times for the internal standard (naproxen) and ibuprofen were 11.5 and 20.8 minutes, respectively. The limit of determination of ibuprofen was 5μg/mL, and the coefficients of variation were 2.7% and 5.3% for ibuprofen standard concentrations of 12.5μg/mL and 125μg/mL respectively (the reason for the smaller coefficient of variation at lower sample concentration is unknown).

A



B

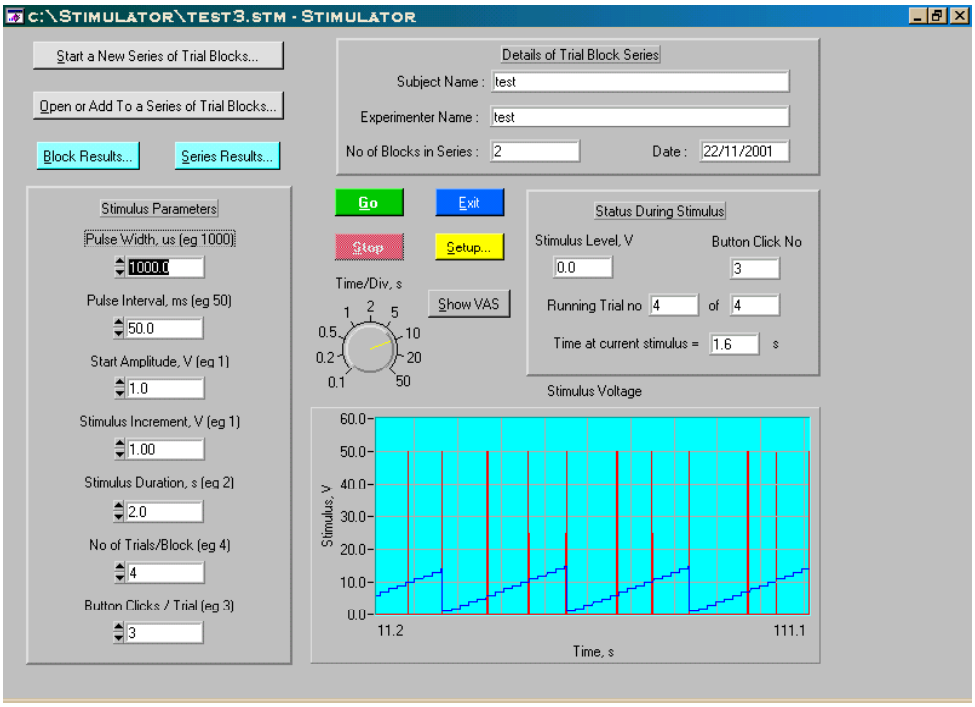


Figure 2.1: Experimental set-up: A Stimulator (left), computer (middle) and subject (right) set up B Stimulator computer output screen. The left hand panel allows the user to define the stimulus parameters, while the lower right hand side shows the voltage output from the stimulator (blue steps), and the time (and voltage level) at which the buttons were pressed (red lines).

2.2.6 Statistical Considerations

Belinda Giles performed all statistical analyses.

Pharmacodynamic Analysis

All data were tested for normality and equal variances. Data are expressed as mean ± SEM for normally distributed data and median [range] for data that did not fit the normal distribution. Analyses were performed using the statistical program SPSS version 11 [SPSS Inc., Chicago, Illinois, USA]; with values of $P < 0.05$ considered statistically significant.

Because of the volume of data generated in this experiment, the data will be divided into three areas: sex differences; group differences; and sex hormonal status differences.

1. Sex differences

Sex differences in demographic variables were explored using unpaired *t*-tests.

Pain threshold, pain tolerance and VAS scores for each time-point were calculated as the average of the last 3 stimulus trials. This was performed as previous experience with this technique has shown that the voltage measurements in the first train of each stimulus block are always lower than subsequent readings as the subject becomes familiar with the procedure (Walker *et al.*, 1993). Therefore in all measurements the results from the first train were rejected. Differences in baseline nociception (pain threshold, pain tolerance and VAS scores) which might follow from differences in sex, were explored using unpaired *t*-tests for normally distributed data or Wilcoxon Rank-Sum tests for data which proved to differ from the normal distribution.

Analgesia was defined simply as *a statistically significant increase in the voltage at the pain tolerance level post-treatment compared with baseline*. Pain tolerance data were normalised to baseline by subtracting baseline pain tolerance (as calculated above) from every pain tolerance voltage. Thus all baseline voltages became zero, and the subsequent pain tolerance voltages became the change in voltage from baseline. The effects of treatment were analysed with single factor repeated-measures ANOVA with drug treatments as a covariate. *Post hoc* analyses were performed on the pre-planned comparisons using the Bonferroni correction (Wallenstein *et al.,* 1980).

2. Group differences

Group differences in demographic variables were explored using one-way ANOVA with Bonferroni correction, as multiple comparisons were involved (Wallenstein *et al.*, 1980).

Pain threshold, pain tolerance and VAS scores were calculated as above, and differences in them which might follow from a difference in group, were examined using one-way ANOVA with *post hoc t*-tests and Bonferroni correction for normally distributed data or Kruskal-Wallis z-test for data that deviated from normal.

Effects of treatment and any group differences in analgesia were determined as above.

3. Sex Hormone Status Differences

For the purposes of examining sex hormone status differences, the subjects were grouped according to their presumed levels of female sex hormones, thus OM, YM and OFNIL were grouped together and called '-SH' (meaning female sex

hormones minimal), and YF and OFEXH were grouped together and called '+SH" (meaning a greater level of female sex hormones present). Sex hormone status differences in demographic variables were explored using unpaired *t*-tests.

Pain threshold, pain tolerance and VAS scores for each time-point were calculated as above, and differences which might follow from differences in sex hormone-status were explored using unpaired *t*-tests for normally distributed data or Wilcoxon Rank-Sum tests for data which proved to differ from the normal distribution.

4. Endogenous and Exogenous Hormone Differences

For the purposes of examining exogenous and endogenous hormone influences on pain and analgesia, the young females were divided by the stage of their menstrual cycle (menses, follicular and luteal; if cycling physiologically), or by the activity of their oral contraceptive preparation (menses and active; in those women taking oral contraceptives). In the young women cycling physiologically, menstrual cycle phase was determined retrospectively: menses (all days of bleeding), follicular (from the end of bleeding to about day 14) and luteal (the 14 days prior to bleeding). For subjects with cycles longer than 28 days, the extra days were assigned to their follicular phase, following customary practice (Hapidou & Rollman, 1998). The results from women taking oral contraceptives and those who were not were combined, so that comparisons between menses (pooled result), follicular, luteal and active phases could be made.

Pain threshold, pain tolerance and VAS scores for each time-point were calculated as above, and differences which might follow from differences in menstrual cycle

phase were explored using one-way ANOVA for normally distributed data or the Kruskal-Wallis z-test for data which proved to differ from the normal distribution.

Effects of treatment were determined as for the group analysis (see point 2 above).

The analysis of exogenous hormone use (ERT *vs.* HRT) in older women proceeded as for the +SH and –SH (point 3), above, except that comparisons were made between exogenous hormone type.

Pharmacokinetic Analysis

Sex, sex hormone status and group differences in plasma ibuprofen concentrations were explored using repeated-measures ANOVA with *post hoc t*-tests and Bonferroni correction. Blood concentration-time data for each subject were used to derive pharmacokinetic parameters using non-compartmental methods. The single compartment method assumes that the rates of absorption, metabolism and excretion are directly proportional to the concentration of the drug in the compartment from which the drug is transferring. This is the simplest method which assumes that the body is a single compartment through which the drug will distribute evenly, and has been used previously to describe the pharmacokinetic behaviour of ibuprofen (Walker *et al.*, 1993; Walker & Carmody, 1998). The initial plasma concentration, C(0), is determined by the dose (D), the bioavailability of the drug (F), and the volume of the compartment (Vd), such that C(0)=F*D/Vd. The drug concentration in plasma at a defined time after Cp(0), depends on the rate of elimination of the drug, K_e. Therefore drug concentration at any given time can be described by the equation: $Cp(t) = Cp(0)e^{(-K_e xt)}$

The volume of distribution was calculated as the half-life multiplied by the clearance and all divided by the natural log of 2 (for equations see Appendix C).

The apparent volume of distribution at steady state and the terminal half-life of ibuprofen were calculated by moment analysis and linear regression of the terminal plasma concentration data, respectively.

2.3 RESULTS

2.3.1 Subject Recruitment and Demographics

Subject recruitment formed a major and ongoing part of this trial. Several methods of recruitment were used: newspaper advertising, posters around the university, brochures at pharmacies, posters and brochures at Prince of Wales Hospital and broadcast email advertisements. Of all these, the email messages were the most successful in recruiting subjects. A total of 257 people responded to the advertisements, of which 71 people were enrolled (28%).

Of 71 subjects who were enrolled in the trial, 68 are included in the analyses. These subjects had *at least two testing sessions (out of three) that could be included in the analysis*. Two subjects tested by BG were excluded completely, the first (YM20) owing to protocol violations (the subject did not understand electrical endpoints), and the second (YF12), because two testing days were excluded on account of technical difficulties (subjects earlobe was small, and the electrodes repeatedly slipped). The other subject that was not included in the analysis, prematurely discontinued from the study due to other commitments (OFEXH8).

Eight subjects tested by AK, and three subjects tested by BG had one arm of the study excluded because of technical difficulties (e.g. electrodes slipping, and therefore uncertainty regarding the consistency of the stimulus) or because of protocol violations (e.g. caffeine intake). The subjects (and dose) were YF3 (0), YF9 (0), YM3 (0), YM11 (0), YM18 (0), OFEXH1 (0), YF13 (400), YF17 (400), YM9 (400), YM10 (800), OFNIL5 (400), and OM7 (800). The majority of the data of this thesis, therefore, came from the experiments performed by Ms Belinda

Giles. The demographics of all subjects included in the analyses are summarised in Table 2.2.

Table 2.2: Demographics of subjects included in subsequent analyses by group.

Group	Age (years)	Weight (kg)	Ear Thickness (cm)
Young Females (n=19)	26.3 ± 1.9	61.9 ± 2.5	0.52 ± 0.02
Young Males (n=19)	29.1 ± 2.0	74.7 ± 3.8	0.46 ± 0.03
Older Females EXH (n=10)	57.6 ± 1.7	65.0 ± 2.8	0.42 ± 0.04
Older Females NIL (n=10)	56.6 ± 1.2	68.8 ± 2.7	0.52 ± 0.03
Older Males (n=10)	57.5 ± 1.8	82.6 ± 4.3	0.53 ± 0.04

Of the 19 young females included in the analyses, 6 were taking oral contraceptives (YF4, 6, 9, 13, 14 and 19). There were two types of exogenous hormones taken by subjects in the OFEXH group – 4 of 10 were taking ERT (oestrogen replacement therapy), whilst 6 of 10 subjects were taking HRT (oestrogen and progesterone replacement therapy; see also section 2.2.1). The type of exogenous hormones taken by women included in the analyses in the OFEXH group, is shown in Table 2.3:

Table 2.3: Types of exogenous hormones taken by women in the OFEXH group.

Subject Number	Oestrogen Replacement	Progesterone Replacement
OFEXH1	Premia 5 continuous (625 μg conjugated oestrogens)	Premia 5 continuous (5mg medroxyprogesterone)
OFEXH2	Ogen 0.625 (730 μg piperazine oestrone sulphate, equivalent to 625 μg sodium oestrone sulphate)	
OFEXH3	Premarin 0.625 (625 μg conjugated oestrogens)	
OFEXH4	Sandrena gel (~1 mg/24 hours oestradiol)	Duphaston (10 mg dyrogesterone)
OFEXH5	Premarin 0.300 (300 μg conjugated oestrogens)	Provera (14/28 days) (medroxyprogesterone acetate)
OFEXH6	Premarin 0.300 (300 μg conjugated oestrogens)	Provera (medroxyprogesterone acetate)
OFEXH7	Premia continuous (conjugated oestrogens)	Premia continuous (medroxyprogesterone)
OFEXH8	Sandrena Gel (1mg/g sachet oestradiol)	
OFEXH9	Premarin 0.625 (625 μg conjugated oestrogens)	Progesterone 10 mg
OFEXH10	Estracombi 50 (4 mg patch) (~50 μg/24 hours oestradiol)	
OFEXH11	Menorest 75 (6.57 mg patch) (~75 μg/24 hours oestradiol)	

2.3.2 Sex Differences

Summary

- *No sex difference in baseline pain variables (pain threshold, pain tolerance or VAS scores)*
- *Analgesia in females only occurred following administration of placebo at 2 hour time point; ibuprofen 400mg at 4 hour time point; and ibuprofen 800mg at 1 hour time point.*
- *Analgesia in males occurred over the entire time-course of the experiment following all treatments (placebo, ibuprofen 400mg and ibuprofen 800mg).*
- *No sex differences in pharmacokinetic profiles.*

Baseline Pain

To test the primary hypothesis (that males would exhibit greater baseline pain responses), baseline pain levels and visual analogue scores were first partitioned by sex (Table 2.4).

Table 2.4: Baseline pain threshold and tolerance levels in Volts, and baseline Visual Analogue Scale score (0-100) by sex. Pain threshold and tolerance values are mean ± SEM, VAS scores are median [range].

Subject Group	Pain Threshold (Volts)	Pain Tolerance (Volts)	Visual Analogue Scale Score
Females (n=39)	13.1 ± 0.6	17.9 ± 0.6	40.7 [3.7 - 77.8]
Males (n=29)	13.0 ± 0.8	18.6 ± 1.1	47.7 [5.0 - 82.9]

There were no statistically significant differences in pain threshold, pain tolerance or Visual Analogue Scale scores as a function of sex ($P \approx$ 0.94, 0.56 and 0.08 respectively, repeated measures ANOVA). It is interesting to note though, that the difference in VAS scores between men and women approached significance – however the magnitude of this difference is unlikely to be clinically significant.

Time-course of Pain Tolerance

No subject reported any noticeable effects following the administration of ibuprofen or placebo. The time-courses of pain tolerances following ibuprofen and placebo

in all women and all men are depicted in Figure 2.2. Because of the difficulty of comparing the analgesic effects of ibuprofen and placebo due to differing baselines, the data were normalised to baseline. The time-courses of baseline corrected pain tolerances following ibuprofen and placebo in all women and all men are depicted in Figure 2.3.

In *females*, statistically significant analgesia was produced by placebo only at 2 hours post-treatment, by a dosage of 400mg of ibuprofen only at 4 hours post treatment, and by a dosage of 800mg of ibuprofen 2 hours post-treatment ($P < 0.05$, repeated measures ANOVA with *post hoc t*-tests and Bonferroni correction). However, there were no overall statistically significant differences in the time-courses of baseline corrected pain tolerance between placebo, ibuprofen 400mg and ibuprofen 800mg ($P \approx 0.95$, repeated measures ANOVA).

In *males*, statistically significant analgesia was produced at a greater number of time-points following all treatments; following placebo, analgesia was present at all post-treatment time points (hours 1 to 4); following ibuprofen 400mg, analgesia was present between the 2nd and 4th hours post-treatment; and following ibuprofen 800mg, analgesia was present at all post-treatment time-points, except at the 3rd hour ($P < 0.05$, repeated measures ANOVA with *post hoc t*-tests and Bonferroni correction). Thus, in all cases, analgesia was prolonged in males, while it was not in the females. In addition, in males, as in females, there were no statistically significant differences in the time-courses of baseline corrected pain tolerances between treatment groups ($P \approx 0.54$, repeated measures ANOVA).

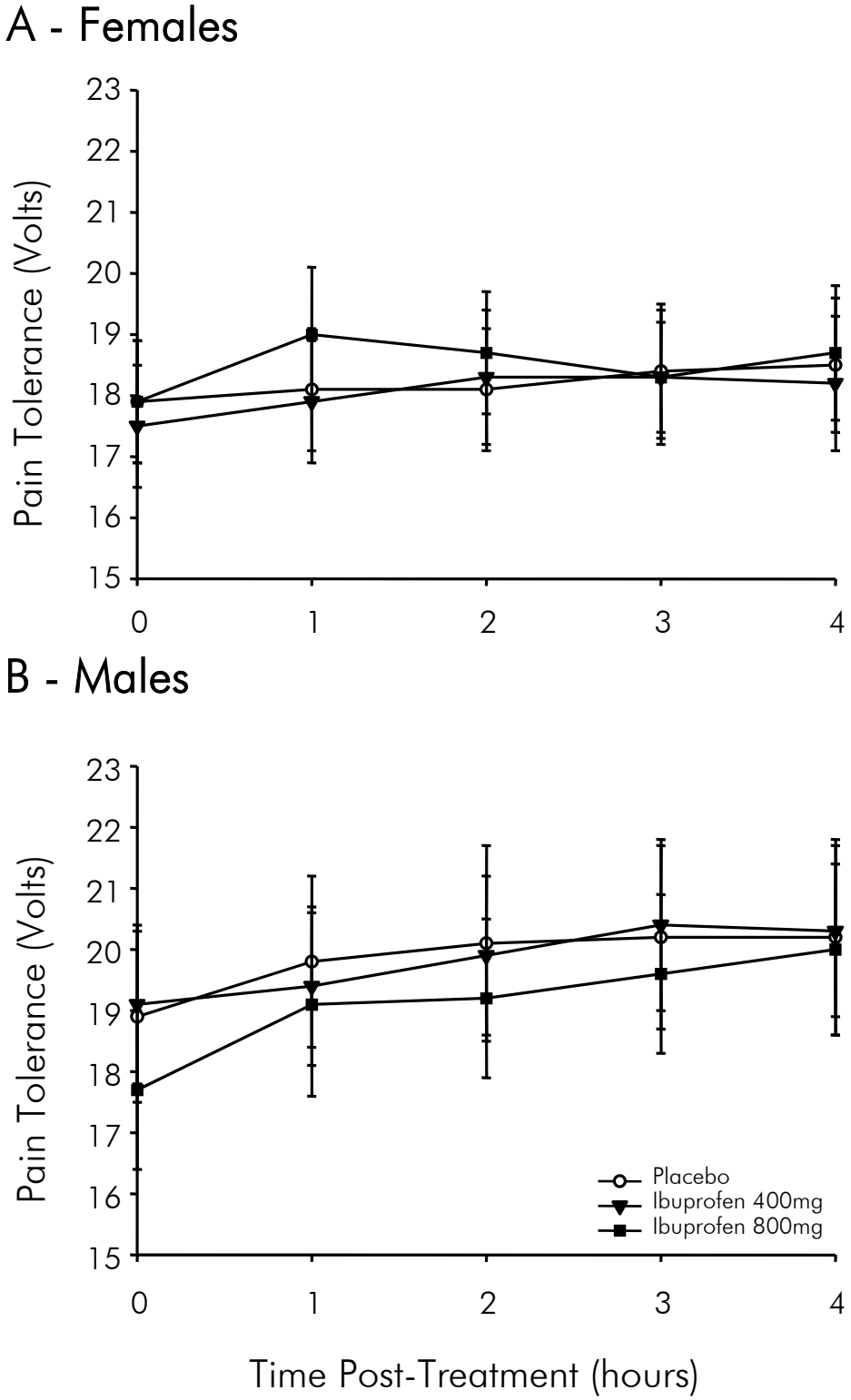


Figure 2.2: Time-course of pain tolerances in A women and B men following placebo (open circles), ibuprofen 400mg (closed triangles) and ibuprofen 800mg (closed squares) p.o. *as a single dose.*

The standard error of a single observation as calculated from the ANOVA matrix was 0.3V for females and 0.4V for males.

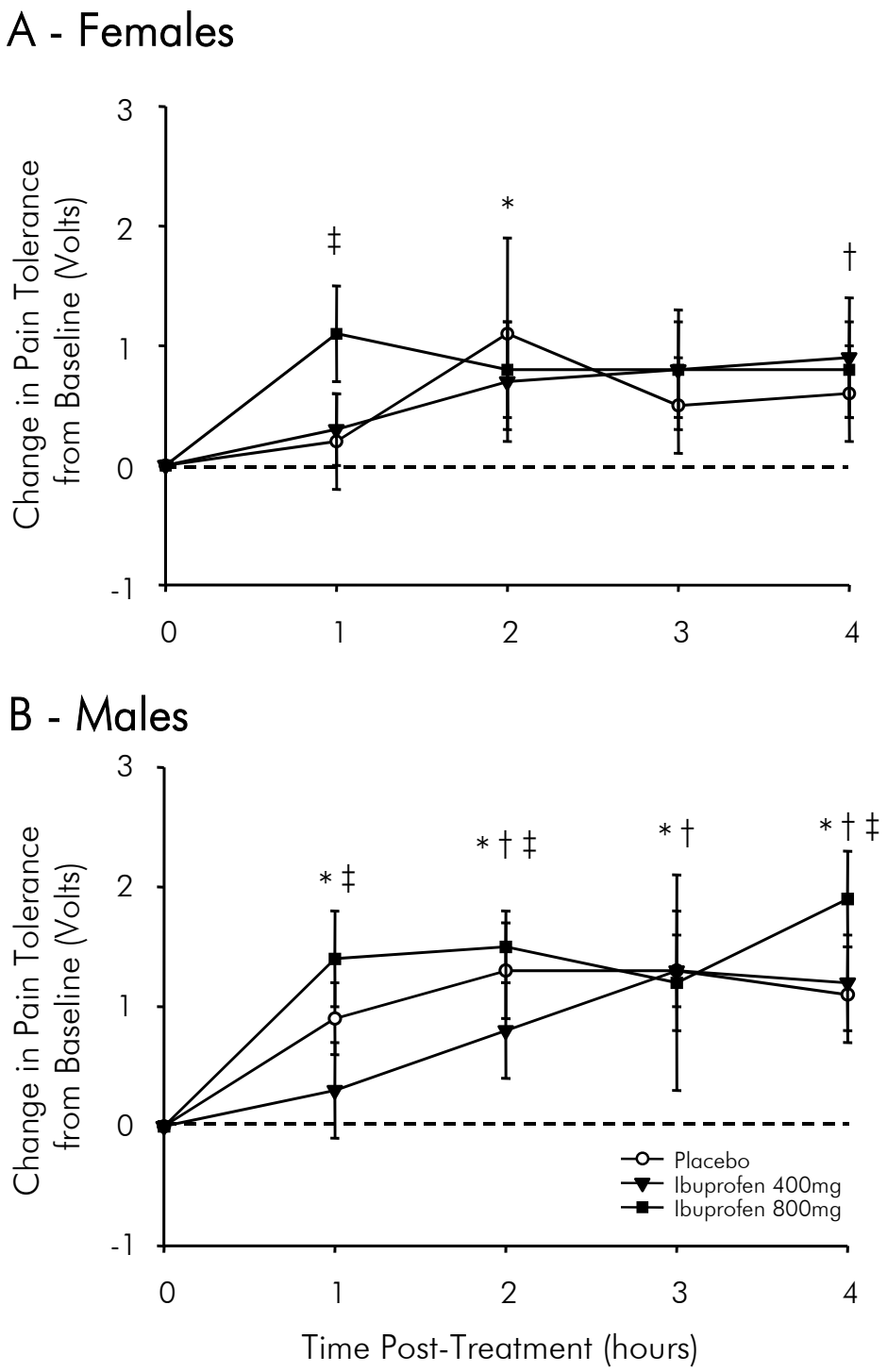


Figure 2.3: Time-course of baseline corrected pain tolerances in A women and B men following placebo (open circles), ibuprofen 400mg (closed triangles) and ibuprofen 800mg (closed squares) p.o. *as a single dose.*

*The standard error of a single observation as calculated from the ANOVA matrix was 0.1V for both females and males. * denotes significant difference from placebo baseline (P < 0.05, repeated measures ANOVA); † denotes significant difference from ibuprofen 400mg baseline (P < 0.05, repeated measures ANOVA); and ‡ denotes significant difference from ibuprofen 800mg baseline (P < 0.05, repeated measures ANOVA).*

Pharmacokinetics

The time-course of plasma ibuprofen concentrations in all men and all women following administration of ibuprofen 400mg and 800mg is shown in Figure 2.4, and the calculated pharmacokinetic parameters in Table 2.5. Note that difficulties in obtaining a complete set of blood samples for each subject over the time-course of the experiment precluded some subjects from the pharmacokinetic analyses. Subject numbers are shown in the table.

Note that the peak plasma concentration in Table 2.5 was calculated as the mean peak plasma concentration from each subject, regardless of whether that occurred at 1 hour post-treatment, or at the 2nd hour post-treatment. Therefore, peak plasma concentrations in the Table will not necessarily have the same value as the mean 1 hour post-treatment plasma concentration shown in Figure 2.4.

Table 2.5: Mean pharmacokinetic parameters of ibuprofen according to sex and dose. C_{max} = maximal plasma concentration, AUC = area under the plasma concentration-time curve.

	MALE		FEMALE	
Parameter	400mg ibuprofen (n=18)	800mg ibuprofen (n=18)	400mg ibuprofen (n=17)	800mg ibuprofen (n=25)
C_{max} ($\mu g.mL^{-1}$)	21.4 ± 1.9 (n=22)	36.0 ± 3.1 (n=21)	26.0 ± 2.1 (n=21)	41.6 ± 2.4 (n=30)
AUC ($\mu g.mL^{-1}.h^{-1}$)	85.4 ± 8.1	130.3 ± 10.3	94.8 ± 8.6	154.3 ± 11.4
Half-Life (h)	1.82 ± 0.19	1.53 ± 0.12	1.74 ± 0.13	1.75 ± 0.18
Clearance ($L.h^{-1}$)	5.89 ± 0.81	6.70 ± 0.45	5.14 ± 0.70	5.91 ± 0.43
Clearance ($mL.min^{-1}.kg^{-1}$)	1.23 ± 0.18	1.43 ± 0.10	1.28 ± 0.15	1.58 ± 0.11
Volume of Distribution (L)	17.2 ± 2.6	18.2 ± 1.4	14.5 ± 1.6	16.5 ± 1.5
Volume of Distribution ($L.kg^{-1}$)	0.21 ± 0.03	0.23 ± 0.02	0.22 ± 0.02	0.27 ± 0.02

There was no difference in peak plasma concentrations between men and women following 400mg or 800mg doses of ibuprofen ($P \approx 0.11$ and 0.07 respectively, unpaired *t*-tests); nor was there any sex difference in plasma concentration-time profiles ($P > 0.05$, ANOVA) or pharmacokinetic parameters ($P > 0.05$, unpaired *t*-tests).

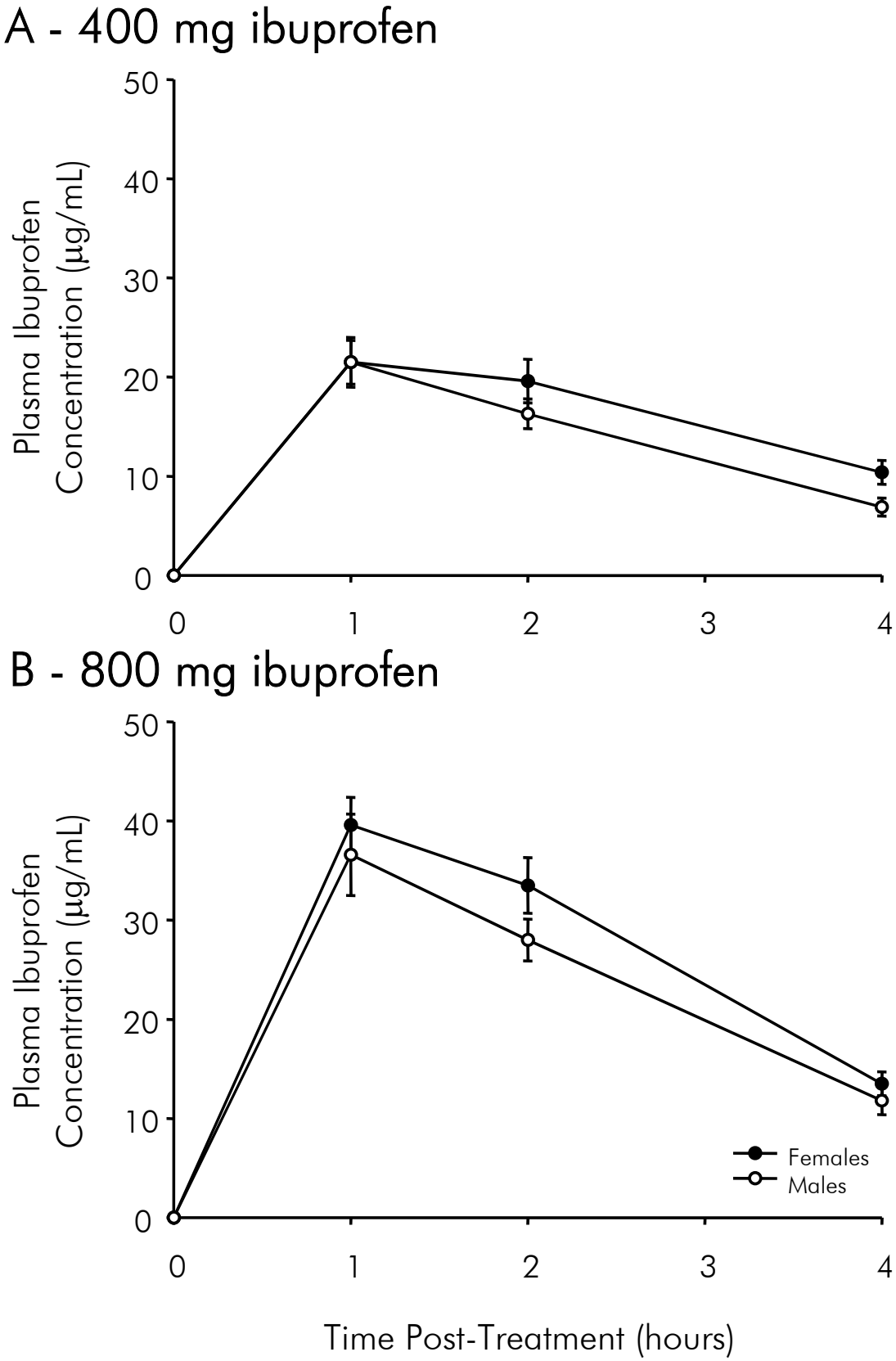


Figure 2.4: *Plasma concentration-time curves for females (filled circles) and males (unfilled circles) following administration of A ibuprofen 400mg p.o. and B ibuprofen 800mg p.o. as a single dose.*

2.3.3 Group Differences

To determine where differences in nociception and analgesic response might lie (if any were found), the data were repartitioned by group, and the analyses repeated.

Summary

- *No overall group differences in baseline pain variables (pain threshold, pain tolerance or VAS scores).*
- *Older subjects had higher VAS scores than younger subjects.*
- *No significant analgesic effect of any treatment (placebo, ibuprofen 400mg or ibuprofen 800mg) in older females taking exogenous hormones (EXH) and young females.*
- *Analgesia produced following all three treatments in young males.*
- *Analgesia produced following placebo and ibuprofen 800mg in older males.*
- *Analgesia produced following placebo in older females not taking exogenous hormones (NIL).*
- *Young males had significantly lower peak plasma concentrations of ibuprofen than young females following administration of ibuprofen 400mg.*
- *No other group differences in pharmacokinetic profile of ibuprofen.*

Baseline Pain

When the data were repartitioned by subject group, there were no differences in baseline pain measures (Table 2.6).

Table 2.6: Baseline pain threshold and tolerance levels in Volts, and baseline Visual Analogue Score (0-100) by subject group. Pain threshold and tolerance values are mean ± SEM, VAS scores are median [range].

Subject Group	Pain Threshold (Volts)	Pain Tolerance (Volts)	Visual Analogue Scale Score
Young Female (n=19)	12.7 ± 0.8	17.1 ± 0.8	30.8 [3.7 – 70.4]
Young Male (n=19)	13.4 ± 1.2	18.1 ± 1.3	38.1 [5.0 – 80.1]
Older Female EXH (n=10)	12.8 ± 1.2	17.2 ± 1.8	43.2 [8.3 – 64.5]
Older Female NIL (n=10)	14.1 ± 1.3	20.0 ± 2.1	42.1 [7.3 – 77.8]
Older Male (n=10)	12.4 ± 1.2	19.6 ± 2.1	58.7 [19.4 – 82.9]

The *P* values for between-group differences as calculated by repeated measures ANOVA were 0.90, 0.56 and 0.11 for pain threshold, pain tolerance and VAS scores respectively. Note that older subjects had higher pain intensity (VAS scores) ratings at the pain tolerance level than did young subjects (47.6 [7.3 – 82.9] *cf* 36.7 [3.7 – 80.1], $P < 0.05$, unpaired *t*-test).

Time-course of Pain Tolerance

The data was repartitioned according to group, and the time-course of pain tolerance following placebo, ibuprofen 400mg and ibuprofen 800mg determined (Figure 2.5). Once again, data were normalised to baseline to allow comparison between treatments and groups (Figure 2.6).

Importantly there was *no significant analgesic effect of any treatment* (placebo, ibuprofen 400mg or ibuprofen 800mg) in young females or older females taking hormone replacement therapy (Figure 2.6; $P > 0.05$, repeated measures ANOVA).

Young males, older males and older females not taking exogenous hormones (NIL), all exhibited statistically significant analgesia at some time-points to some treatments. In *young males*, placebo produced analgesia at time-points 1, 2 and 4; ibuprofen 400mg at time 4 only; and ibuprofen 800mg at times 2 and 4 ($P < 0.05$, repeated measures ANOVA with *post hoc t*-tests and Bonferroni correction). Thus in young males, placebo analgesia was present early, while analgesia following the drug became apparent halfway through the time course of the experiment. In older males, placebo-induced analgesia occurred at the 2nd hour only, while ibuprofen 800mg was analgesic over the entire time-course of the experiment ($P < 0.05$, repeated measures ANOVA with *post hoc t*-tests and Bonferroni correction). Finally, in older females not taking exogenous hormones

(NIL), analgesia was present only at the 4th hour post-placebo ($P < 0.05$, repeated measures ANOVA with *post hoc t*-tests and Bonferroni correction).

In young males, older males and older females not taking exogenous hormones (NIL), there were no significant differences in the magnitude of analgesia produced following placebo, ibuprofen 400mg, and ibuprofen 800mg ($P \approx 0.96$, 0.26 and 0.90 respectively, repeated measures ANOVA).

Pharmacokinetics

The plasma concentration-time profiles for young females, young males, older females taking exogenous hormones (EXH), older females not taking exogenous hormones (NIL) and older males following administration of dosages of 400mg or 800mg of ibuprofen are shown in Figure 2.7, and the calculated pharmacokinetic parameters in Table 2.7.

Young males had significantly lower peak plasma ibuprofen concentrations following administration of ibuprofen 400mg than young females ($P < 0.05$, one-way ANOVA with *post hoc t*-tests and Bonferroni correction). There were no other group differences in plasma ibuprofen concentration time profile, or any other pharmacokinetic variable ($P > 0.05$, one-way ANOVA).

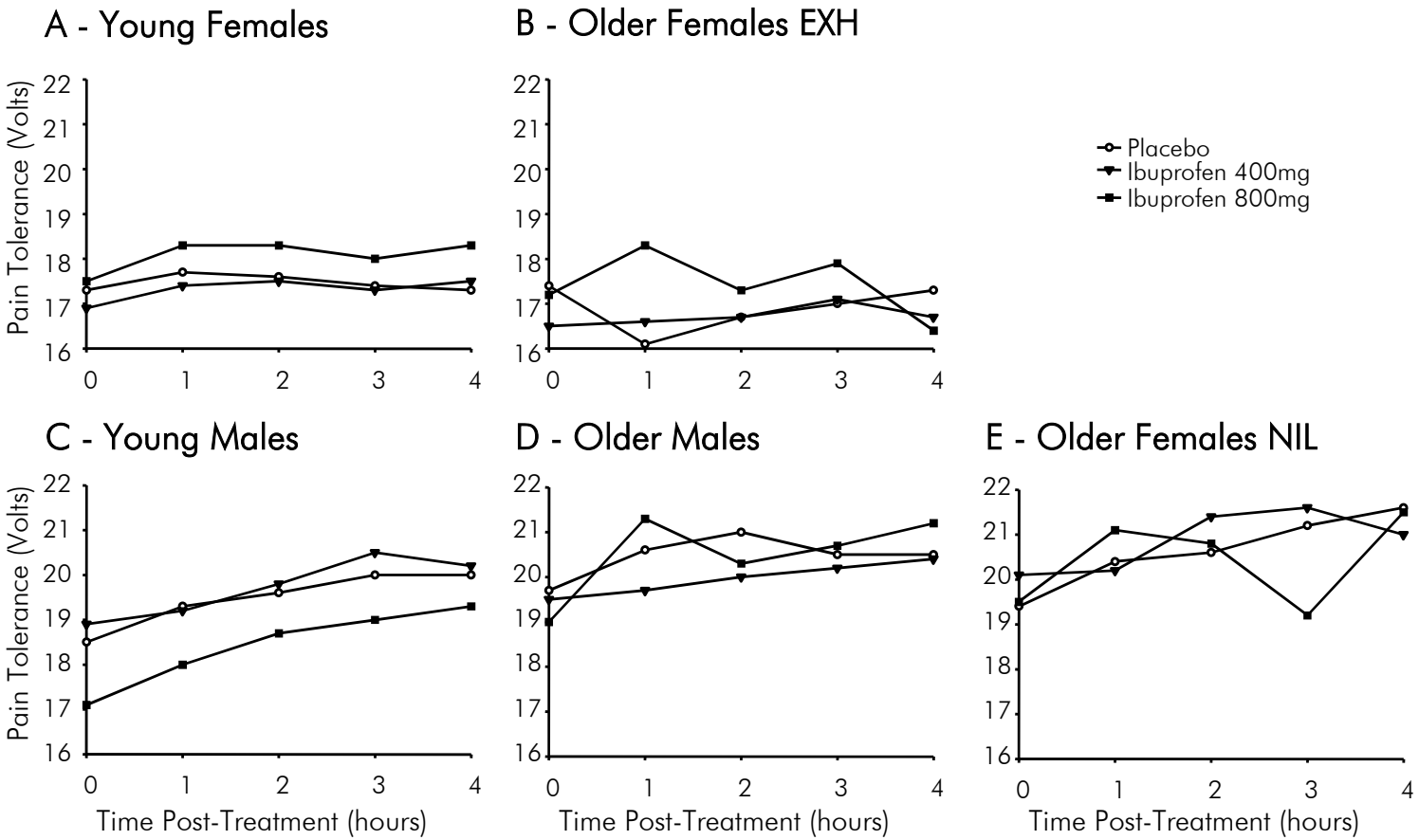


Figure 2.5: *Time-course of pain tolerance in* ***A*** *young females,* ***B*** *older females taking exogenous hormones (EXH),* ***C*** *young males,* ***D*** *older males, and* ***E*** *older females not taking exogenous hormones (NIL); following administration of placebo (open circles), ibuprofen 400mg (closed triangles), and ibuprofen 800mg (closed squares)* p.o. *as a single dose. For clarity standard errors have been omitted, however, the standard errors of a single observation as calculated from the ANOVA matrix were 0.3, 0.6, 0.5, 0.7, and 0.7 Volts for young females, older females EXH, young males, older males and older females NIL respectively.*

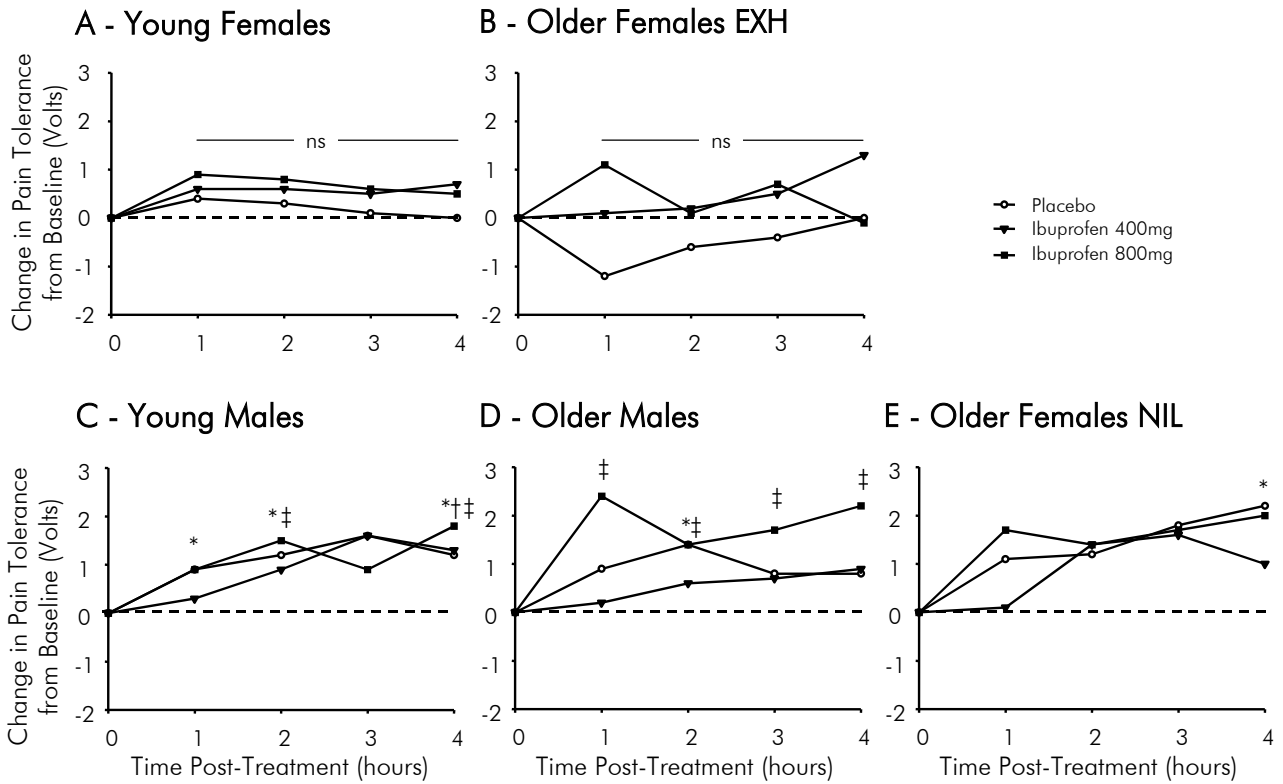


Figure 2.6: Time-course of baseline corrected pain tolerance in A young females, B older females taking exogenous hormones (EXH), C young males, D older males, and E older females not taking exogenous hormones (NIL); following administration of placebo (open circles), ibuprofen 400mg (closed triangles), and ibuprofen 800mg (closed squares) p.o. *as a single dose. For clarity standard errors have been omitted, however, the standard errors of a single observation as calculated from the ANOVA matrix were 0.1, 0.2, 0.1, 0.2, and 0.2 Volts for young females, older females EXH, young males, older males and older females NIL respectively.*
** denotes significant difference from placebo baseline (*$P < 0.05$*, repeated measures ANOVA); † denotes significant difference from ibuprofen 400mg baseline (*$P < 0.05$*, repeated measures ANOVA); and ‡ denotes significant difference from ibuprofen 800mg baseline (*$P < 0.05$*, repeated measures ANOVA); ns – not significantly different from baseline.*

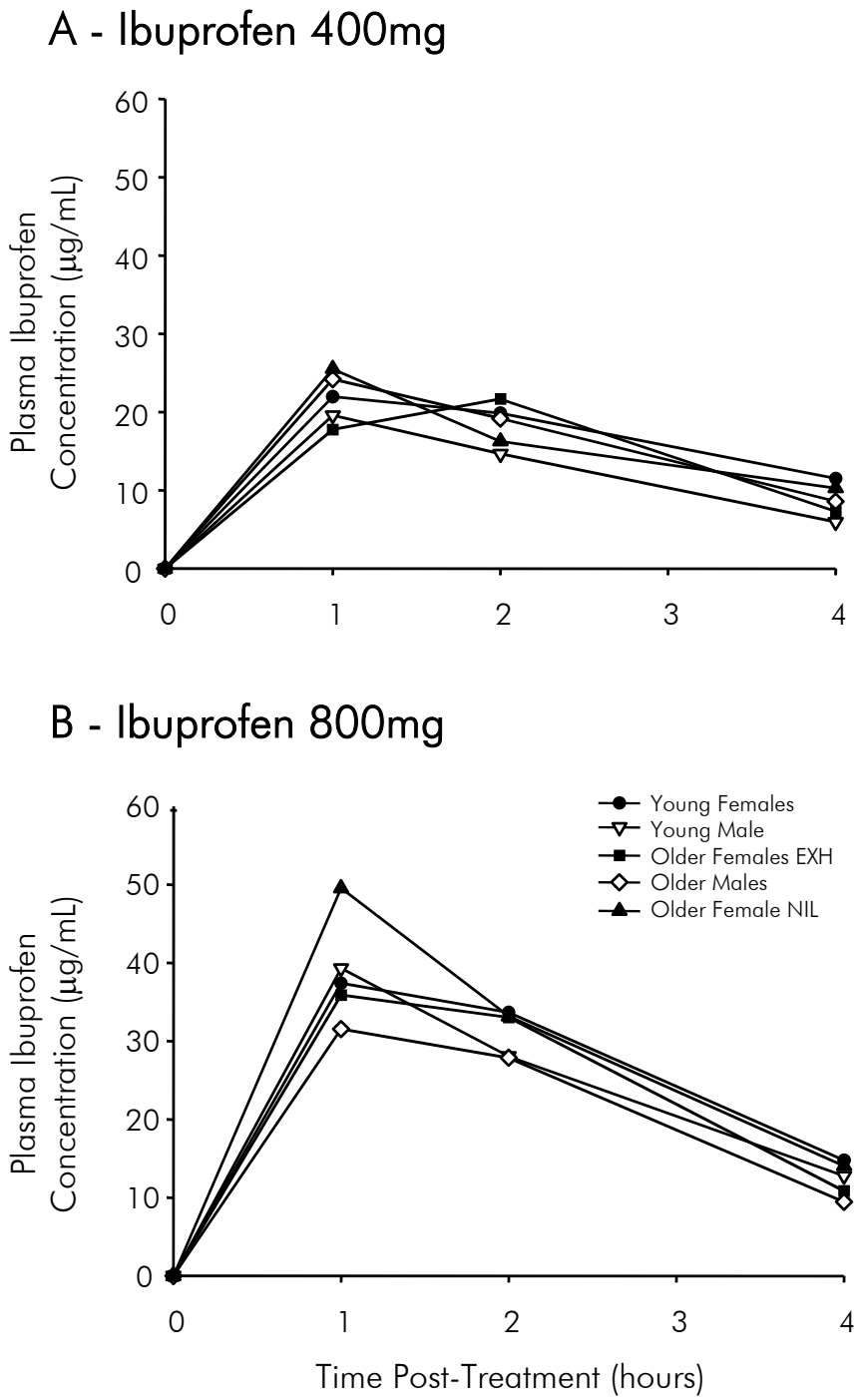


Figure 2.7: Plasma concentration-time curve for young females (•), young males (∇), older females taking HRT (EXH, ■), older males (◊), and older females not taking HRT (NIL, ▲) following A ibuprofen 400mg p.o. and B ibuprofen 800mg p.o. as a single dose. For clarity standard errors have been omitted, but the standard error of a single observation calculated from the ANOVA matrix was 0.9µg/mL for ibuprofen 400mg, and 1.1µg/mL for ibuprofen 800mg.

Table 2.7: Mean pharmacokinetic parameters of ibuprofen according to group and dose; A: Ibuprofen 400mg; B: Ibuprofen 800mg.

A - Ibuprofen 400mg Parameter	Young Female (n = 11)	Older Female EXH (n = 4)	Older Female NIL (n = 2)	Young Male (n = 10)	Older Male (n = 8)
C_{max} ($\mu g.mL^{-1}$)	27.5 ± 2.4 (n = 15)	24.6 ± 5.3 (n = 4)	25.5 (n = 2)	18.9 ± 1.6* (n = 14)	22.0 ± 1.2 (n = 8)
AUC ($\mu g.mL^{-1}.h^{-1}$)	99.4 ± 11.5	85.8 ± 16.8	87.6	74.5 ± 6.3	99.2 ± 15.6
Half-Life (h)	1.79 ± 0.17	1.58 ± 0.27	1.77	2.03 ± 0.38	1.64 ± 0.15
Clearance ($L.h^{-1}$)	5.12 ± 1.02	5.27 ± 1.03	4.98	6.86 ± 1.34	4.67 ± 0.60
Clearance ($mL.min^{-1}.kg^{-1}$)	1.27 ± 0.22	1.34 ± 0.23	1.24	1.49 ± 0.29	0.92 ± 0.12
Volume of Distribution (L)	14.3 ± 2.1	15.1 ± 3.6	14.7	18.9 ± 4.4	15.0 ± 2.2
Volume of Distribution ($L.kg^{-1}$)	0.22 ± 0.06	0.23 ± 0.05	0.22	0.25 ± 0.06	0.17 ± 0.02
B - Ibuprofen 800mg Parameter	**Young Female (n=12)**	**Older Female EXH (n=7)**	**Older Female NIL (n=6)**	**Young Male (n=12)**	**Older Male (n=6)**
C_{max} ($\mu g.mL^{-1}$)	42.0 ± 3.2 (n = 16)	39.2 ± 3.7 (n = 7)	47.4 ± 6.9 (n = 7)	38.7 ± 4.2 (n = 14)	30.7 ± 3.5 (n = 7)
AUC ($\mu g.mL^{-1}.h^{-1}$)	152.8 ± 16.3	153.3 ± 17.0	158.6 ± 31.6	138.3 ± 14.3	114.2 ± 10.5
Half-Life (h)	1.93 ± 0.35	1.66 ± 0.27	1.49 ± 0.11	1.62 ± 0.16	1.38 ± 0.18
Clearance ($L.h^{-1}$)	5.93 ± 0.62	5.67 ± 0.68	6.14 ± 1.13	6.38 ± 0.57	7.32 ± 0.72
Clearance ($mL.min^{-1}.kg^{-1}$)	1.63 ± 0.16	1.44 ± 0.19	1.62 ± 0.30	1.45 ± 0.13	1.40 ± 0.15
Volume of Distribution (L)	17.5 ± 1.9	15.1 ± 3.5	16.1 ± 3.2	17.9 ± 2.0	18.7 ± 1.4
Volume of Distribution ($L.kg^{-1}$)	0.29 ± 0.03	0.23 ± 0.05	0.25 ± 0.05	0.25 ± 0.03	0.21 ± 0.00

C_{max} = maximal plasma concentration, AUC = area under the plasma concentration-time curve.

** denotes significant difference from young female C_{max} following administration of ibuprofen 400mg (one-way ANOVA with post hoc t-tests and Bonferroni correction)*

2.3.4 Sex Hormone Status Differences

The analysis of data based on group (Section 2.3.3) highlighted the importance of sex hormone status in predicting response to both placebo and drug. Thus data were therefore repartitioned according to each subject's presumed female sex hormone status. Subjects' with a presumed high level of female sex hormones (young females and older females taking exogenous hormones) were grouped together and called "+SH"; whilst the others (young males, older males, and older females not taking exogenous hormones) were grouped together and called "-SH".

Summary

- *No sex hormone status differences in baseline variables (pain threshold, pain tolerance or VAS scores).*
- *No analgesia following any treatment (placebo, ibuprofen 400mg, ibuprofen 800mg) in the +SH group.*
- *Significant persistent analgesia following all treatments (placebo, ibuprofen 400mg, ibuprofen 800mg) in the –SH group.*
- *Higher maximal plasma ibuprofen concentrations in +SH group following administration of ibuprofen 400mg.*
- *No other pharmacokinetic differences between +SH and –SH groups.*
- *Ibuprofen 800mg analgesic only during menses phase in young females*
- *No analgesia following any treatment (placebo, ibuprofen 400mg or ibuprofen 800mg) in older females taking either ERT or HRT. Hyperalgesia 2 hours after ibuprofen 800mg treatment in HRT females.*

Baseline Pain

The baseline pain threshold, pain tolerance and visual analogue scale scores are summarised in Table 2.8.

Table 2.8: Baseline pain threshold and tolerance levels in Volts, and baseline Visual Analogue Score (0-100) by sex hormone status. Pain threshold and tolerance values are mean ± SEM, VAS scores are median [range].

Subject Group	Pain Threshold (Volts)	Pain Tolerance (Volts)	Visual Analogue Scale Score
+SH (n = 29)	12.8 ± 0.7	17.2 ± 0.8	40.7 [3.7 – 70.4]
-SH (n = 39)	13.3 ± 0.7	19.0 ± 1.0	47.4 [5.0 – 82.9]

There were no statistically significant differences as a function of sex hormone status in pain threshold, pain tolerance or visual analogue scale scores ($P \approx 0.61$, 0.16 and 0.06 respectively, repeated measures ANOVA), although it should be noted that the difference in VAS scores closely approached statistical significance (P approached 0.05; although it is still unlikely to be of clinical significance).

Time-course of Pain Tolerance

As before, the data were partitioned by sex hormone status, and the time-courses of pain tolerances following administration of placebo, ibuprofen 400mg and ibuprofen 800mg were examined (Figure 2.8). Once again, to allow easy comparison between sex hormone status groups, and between treatments, data were normalised to baseline (Figure 2.9).

The *+SH* group did not exhibit any analgesia following any treatment (placebo, ibuprofen 400mg, ibuprofen 800mg; $P > 0.05$ repeated measures ANOVA).

Following placebo, and ibuprofen 800mg, subjects in the *–SH* group exhibited statistically significant analgesia throughout the entire time-course of the experiment ($P < 0.05$ repeated measures ANOVA with *post hoc t*-tests and Bonferroni

correction). Significant analgesia developed at the 2nd hour following administration of 400mg dosages of ibuprofen ($P < 0.05$ repeated measures ANOVA with *post hoc t*-tests and Bonferroni correction). Thus in the –SH group, once analgesia developed, it was persistent over the entire time-course of the experiment.

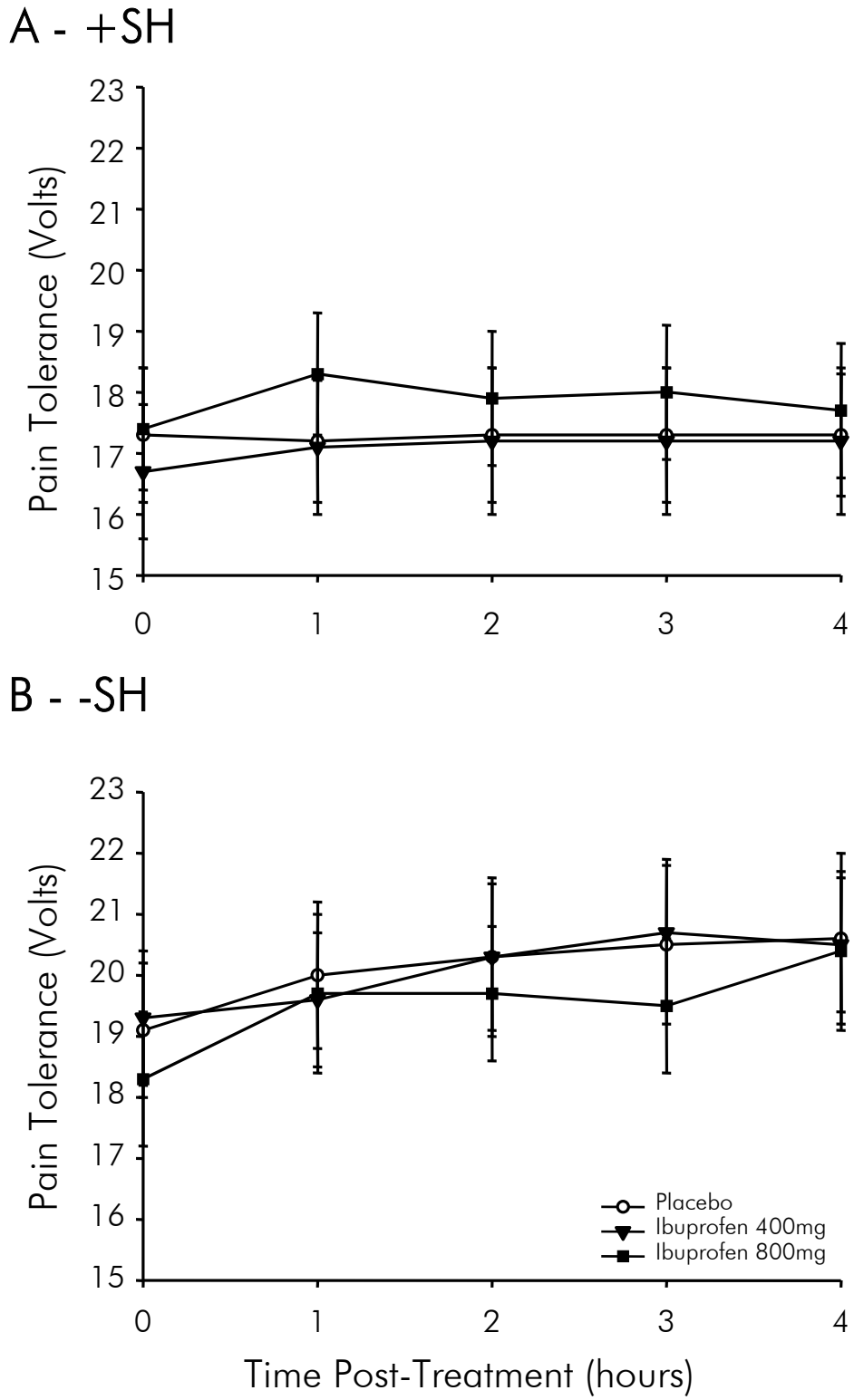


Figure 2.8: Time-course of pain tolerance in A +SH and B –SH groups following placebo (open circles), ibuprofen 400mg (closed triangles), and ibuprofen 800mg (closed squares) p.o. *as a single dose.*

The standard error of a single observation was 0.3V for both +SH and –SH groups.

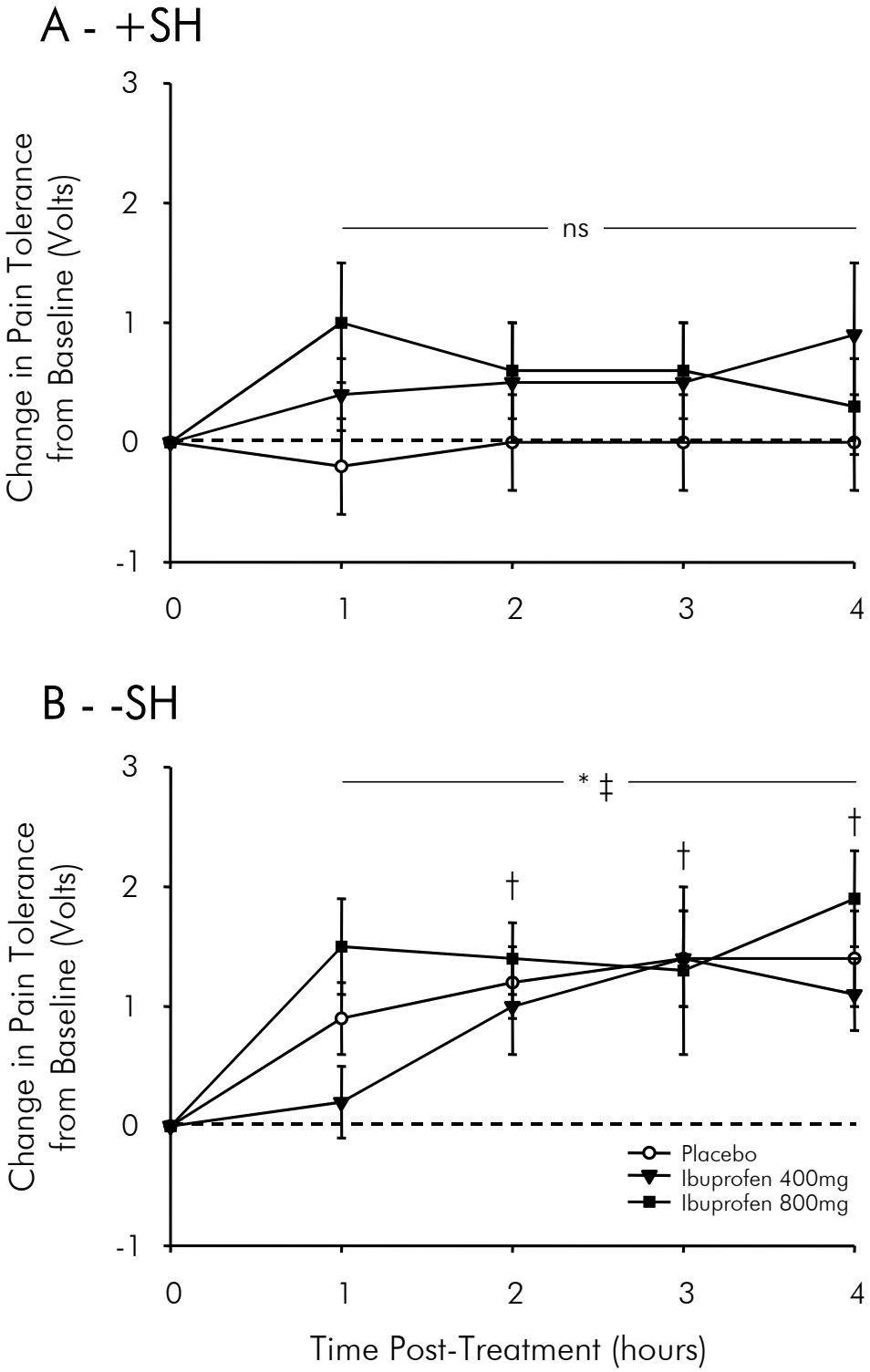


*Figure 2.9: Time-course of baseline corrected pain tolerance in **A** +SH and **B** –SH groups following placebo (open circles), ibuprofen 400mg (closed triangles), and ibuprofen 800mg (closed squares)* p.o. *as a single dose. * denotes significant difference from placebo baseline (P < 0.05, repeated measures ANOVA); † denotes significant difference from ibuprofen 400mg baseline (P < 0.05, repeated measures ANOVA); and ‡ denotes significant difference from ibuprofen 800mg baseline (P < 0.05, repeated measures ANOVA); ns – not significantly different from baseline. The standard error of a single observation was 0.1V for both +SH and –SH groups.*

Pharmacokinetics

The plasma ibuprofen concentration-time profiles for +SH and –SH are shown in Figure 2.10, and the calculated pharmacokinetic parameters in Table 2.9. Subjects in the +SH group had significantly higher peak plasma concentrations of ibuprofen following the administration of ibuprofen 400mg, than did subjects in the –SH group ($P < 0.05$, unpaired *t*-test). No other differences in pharmacokinetic variables could be attributed to the subject's sex hormone status.

It also appeared that subjects in the +SH group had right shifted plasma ibuprofen concentration-time curves (by approximately ½ hour), although this was not statistically significant.

Table 2.9: Mean pharmacokinetic parameters of ibuprofen according to sex hormone status and dose. C_{max} = maximal plasma concentration, AUC = area under the plasma concentration-time curve.

	-SH		+SH	
Parameter	400mg ibuprofen (n = 21)	800mg ibuprofen (n = 25)	400mg ibuprofen (n = 15)	800mg ibuprofen (n = 19)
C_{max} ($\mu g.mL^{-1}$)	21.0 ± 1.8 (n = 24)	37.8 ± 2.9 (n = 28)	26.9 ± 2.2* (n = 19)	41.2 ± 2.5 (n = 23)
AUC ($\mu g.mL^{-1}.h^{-1}$)	83.8 ± 7.3	135.6 ± 10.7	95.8 ± 9.4	153.0 ± 11.8
Half-Life (h)	1.78 ± 0.16	1.51 ± 0.09	1.74 ± 0.14	1.83 ± 0.24
Clearance ($L.h^{-1}$)	5.92 ± 0.72	6.63 ± 0.42	5.16 ± 0.78	5.84 ± 0.45
Clearance ($mL.min^{-1}.kg^{-1}$)	1.24 ± 0.16	1.48 ± 0.10	1.29 ± 0.17	1.56 ± 0.12
Volume of Distribution (L)	17.4 ± 2.3	17.9 ± 1.3	14.5 ± 1.7	16.6 ± 1.7
Volume of Distribution ($L.kg^{-1}$)	0.22 ± 0.03	0.24 ± 0.02	0.22 ± 0.02	0.27 ± 0.03

** denotes significant difference from male 400mg ibuprofen C_{max} value ($P < 0.05$, unpaired t-test).*

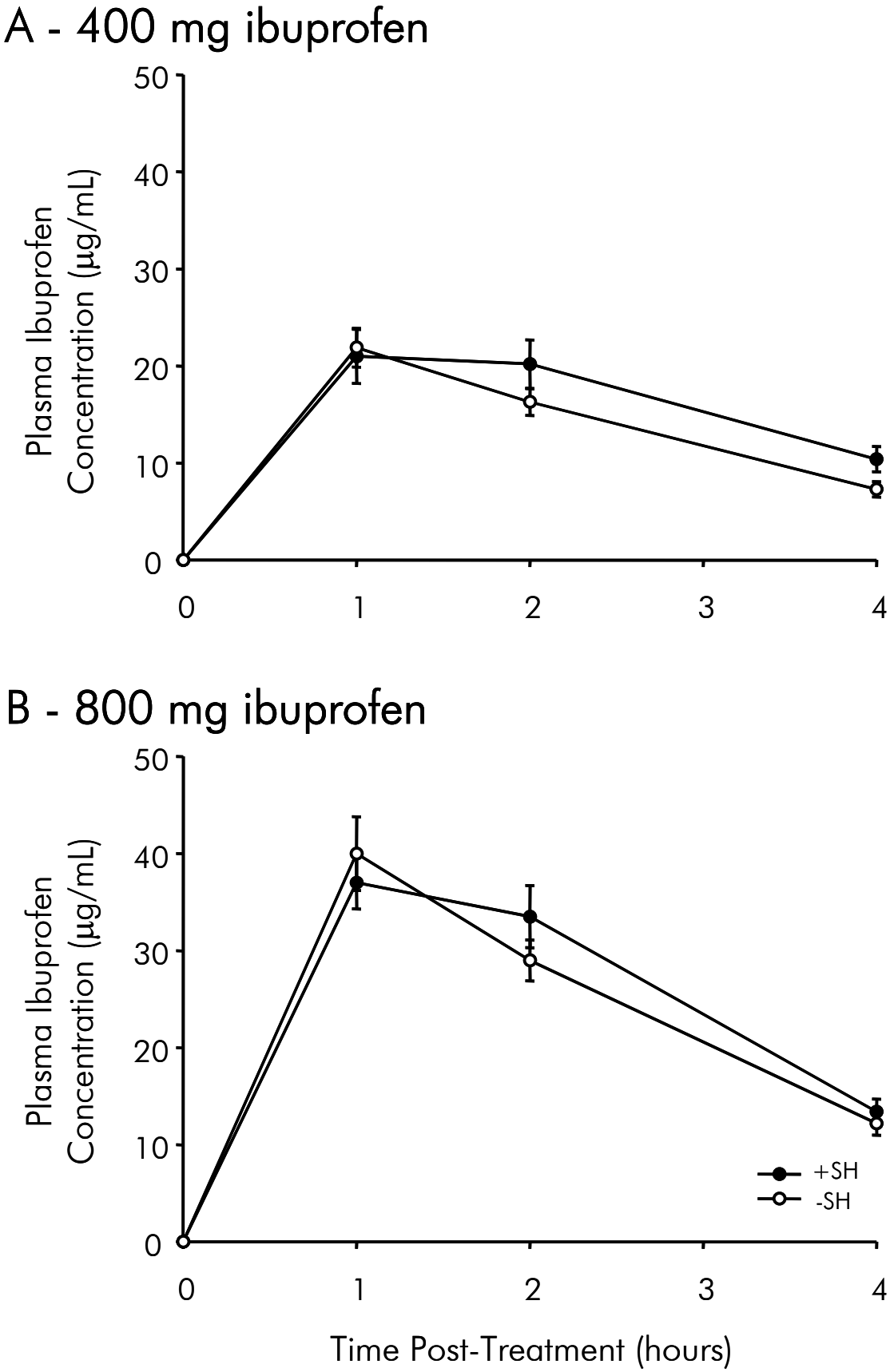


Figure 2.10: Plasma ibuprofen concentration-time curve for +SH (filled circles) and -SH (unfilled circles) following A ibuprofen 400mg p.o. *and B ibuprofen 800mg* p.o. *as a single dose.*

2.3.5 Effect of Endogenous and Exogenous Hormones on Nociception and Analgesic Response

Summary

- *No differences in baseline variables (pain threshold, pain tolerance or VAS scores) in young females between those taking oral contraceptive preparations, and those cycling physiologically.*
- *No difference in baseline pain variables amongst different phases of the menstrual cycle.*
- *Those older women taking combined oestrogen and progesterone therapy(HRT) had lower baseline pain thresholds and tolerances than those taking oestrogen alone (ERT).*
- *No placebo or ibuprofen induced analgesia in young females during the follicular or luteal phases, or in older women taking oestrogen and progesterone therapy (HRT).*
- *Analgesia 1 hour post-800mg ibuprofen in young females during the menses phase.*
- *Hyperalgesia at 1 hour post-800mg ibuprofen in those taking the active component of their oral contraceptive preparation; and 2 hours post-800mg ibuprofen in older women taking oestrogen replacement (ERT).*

Section 2.3.4 highlighted the importance of a subject's sex hormone status in determining analgesic response to both placebo and ibuprofen. In order to determine whether variations in the levels of female hormones could be important in predicting response, the data from young females and older females taking exogenous hormones were more closely examined.

Baseline Pain

The baseline pain variables in young females by phase of the menstrual cycle in: normally cycling women (NOC); active or inactive phases of the oral contraceptive pill in oral contraceptive users (OC); and older women taking exogenous hormones by hormone replacement type (oestrogen replacement therapy, ERT or oestrogen and progesterone replacement therapy, HRT), are shown in Table 2.10.

*Table 2.10: Baseline Pain levels in young females and older females by menstrual phase or exogenous hormone replacement type. * denotes significant difference from ERT value (P < 0.05, unpaired t test).*

Normally Cycling Young Females (NOC)	Pain Threshold (Volts) Mean ± SEM	Pain Tolerance (Volts) Mean ± SEM	VAS Score Median [range]
Follicular (n = 13)	12.5 ± 1.3	16.3 ± 1.6	28.4 [4.2 – 57.0]
Luteal (n = 14)	13.4 ± 1.0	17.9 ± 1.1	27.6 [3.6 – 73.8]
Menses (n = 7)	10.5 ± 1.8	14.2 ± 2.4	24.6 [5.3 – 43.8]
Young Females taking Oral Contraceptives (OC)			
Inactive Oral Contraceptive (Menses, n = 6)	14.4 ± 1.4	19.9 ± 1.4	49.8 [3.3 – 55.2]
Active Oral Contraceptive (n = 10)	14.1 ± 1.8	18.5 ± 1.5	43.5 [3.1 – 56.0]
Older Females taking Exogenous Hormones			
ERT (n = 12)	15.0 ± 1.3	20.4 ± 1.8	40.9 [21.7 – 58.8]
HRT (n = 17)	11.3 ± 0.8*	14.6 ± 1.3*	45.1 [7.7 – 66.4]

All the *young females'* (*i.e.* NOC and OC) values for each variable were examined using one-way ANOVA. There were no significant differences in baseline pain values (pain threshold, pain tolerance or VAS scores) amongst the young women irrespective of whether they were cycling physiologically (NOC) or were taking oral contraceptive preparations (OC).

All the *older women taking exogenous hormones'* values (ERT and HRT) were examined using unpaired *t*-tests. Women taking combined oestrogen and progesterone replacement therapy had lower pain threshold and tolerance levels compared with the women taking oestrogen replacement therapy alone ($P < 0.05$, unpaired *t*-test, Table 2.10).

When all data were pooled into an analysis of variance matrix – there were no significant differences in baseline pain threshold, pain tolerance or VAS scores ($P > 0.05$, one-way ANOVA).

Time-Course of Pain Tolerance

The intriguing differences in the time-course of pain tolerances from the analyses by sex hormone status highlighted the importance of female sex hormones in predicting response to placebo and ibuprofen: *i.e.* females appeared to have no analgesic response to any treatment, whilst males did. The obvious question was then: *does this lack of response occur always, or does response change according to changes in levels of female sex hormones?* Thus the *young females'* pain tolerance time-course data were further divided according to the menstrual phase of the subject (Figure 2.11, and baseline corrected data in Figure 2.12). As this was effectively a *post hoc* analysis, subject numbers in some menstrual cycle treatment groups are very low.

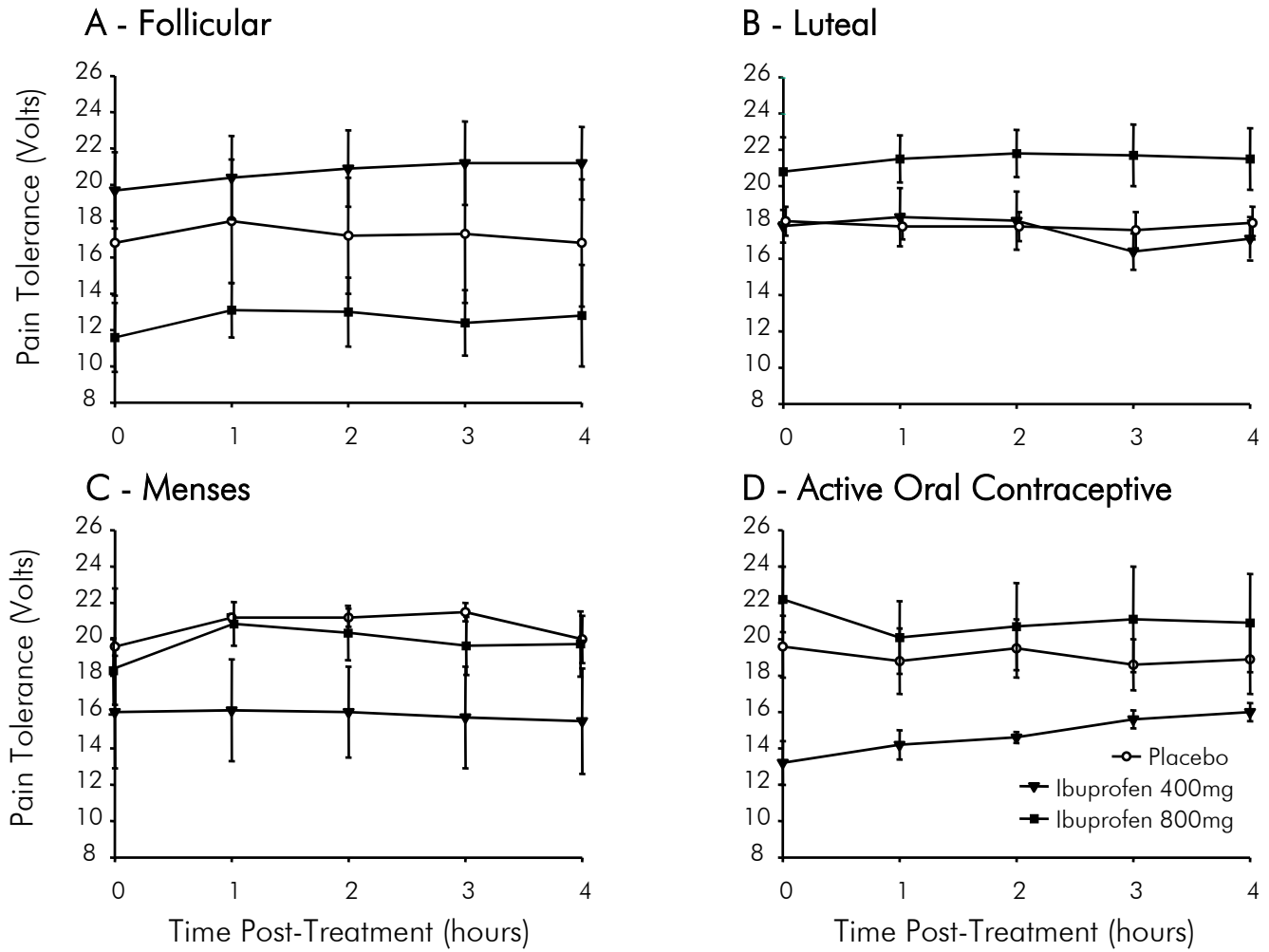


*Figure 2.11: Time-courses of pain tolerances in young females following administration of placebo (circles), ibuprofen 400mg (triangles) and ibuprofen 800mg (squares) during **A** follicular phase (n = 4, 5, 2), **B** luteal phase (n = 5, 4, 4), **C** menses (n = 2, 4, 6), **D** active OC phase (n = 4, 3, 3).*

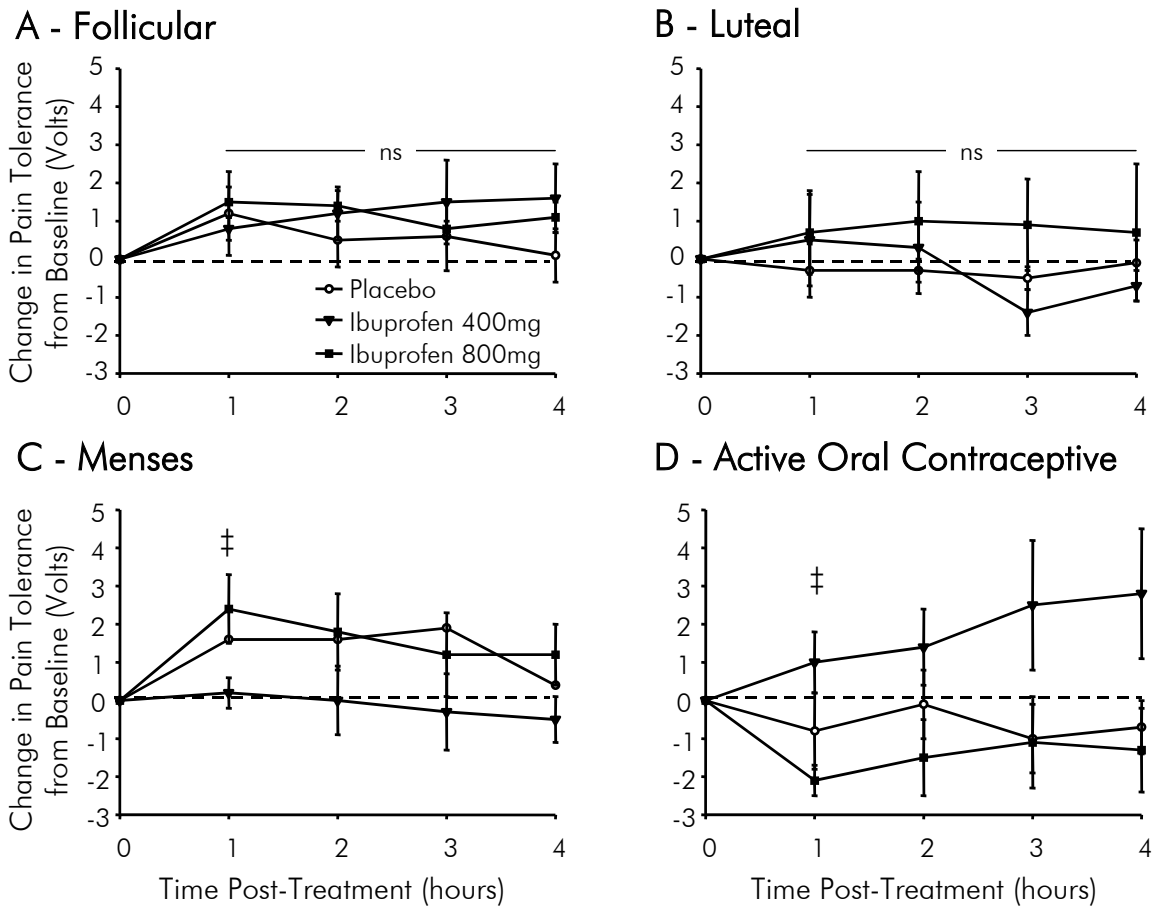


*Figure 2.12: Time-courses of baseline corrected pain tolerances in young females following administration of placebo (circles), ibuprofen 400mg (triangles) and ibuprofen 800mg (squares) during **A** follicular phase (n = 4, 5, 2), **B** luteal phase (n = 5, 4, 4), **C** menses (n = 2, 4, 6), **D** active OC phase (n = 4, 3, 3). ‡ denotes significant difference from ibuprofen 800mg baseline (P < 0.05, repeated measures ANOVA); ns – not significantly different from baseline.*

This data was difficult to interpret, due to the very low sample numbers. In both follicular and luteal phases (panels A and B respectively, Figure 2.12), no significant analgesia was observed following any treatment (placebo, ibuprofen 400mg, ibuprofen 800mg). During the menses phase, ibuprofen 800mg produced a statistically significant increase in pain tolerance levels from baseline at 1 hour post-treatment (Panel C; $P < 0.05$, repeated measures ANOVA with *post hoc t*-tests and Bonferroni correction), whilst during the active component of the oral contraceptive pill, 800mg ibuprofen produced a statistically significant *decrease* in pain tolerance levels from baseline at 1 hour post-treatment (Panel C; $P < 0.05$, repeated measures ANOVA with *post hoc t*-tests and Bonferroni correction), no other treatment (placebo or ibuprofen 400mg) had any statistically significant effect.

To determine the effects of different types of exogenous hormone replacement in *older females*, the data from the OFEXH group were partitioned into subjects taking oestrogen replacement therapy (ERT; n=4) and those taking combined oestrogen and progesterone replacement therapy (HRT; n=6). The time-courses of pain tolerances following administration of placebo, ibuprofen 400mg and ibuprofen 800mg are shown in Figure 2.13. Baseline corrected pain tolerances are shown in Figure 2.14. There was no significant analgesic effect of any treatment (placebo, ibuprofen 400mg or ibuprofen 800mg) in older women taking HRT ($P > 0.05$, repeated measures ANOVA). In older females taking ERT, neither placebo nor ibuprofen were analgesic ($P > 0.05$, repeated measures ANOVA), and ibuprofen 800mg induced hyperalgesia 2 hours after its administration ($P < 0.05$, repeated measures ANOVA with *post hoc t*-tests and Bonferroni correction).

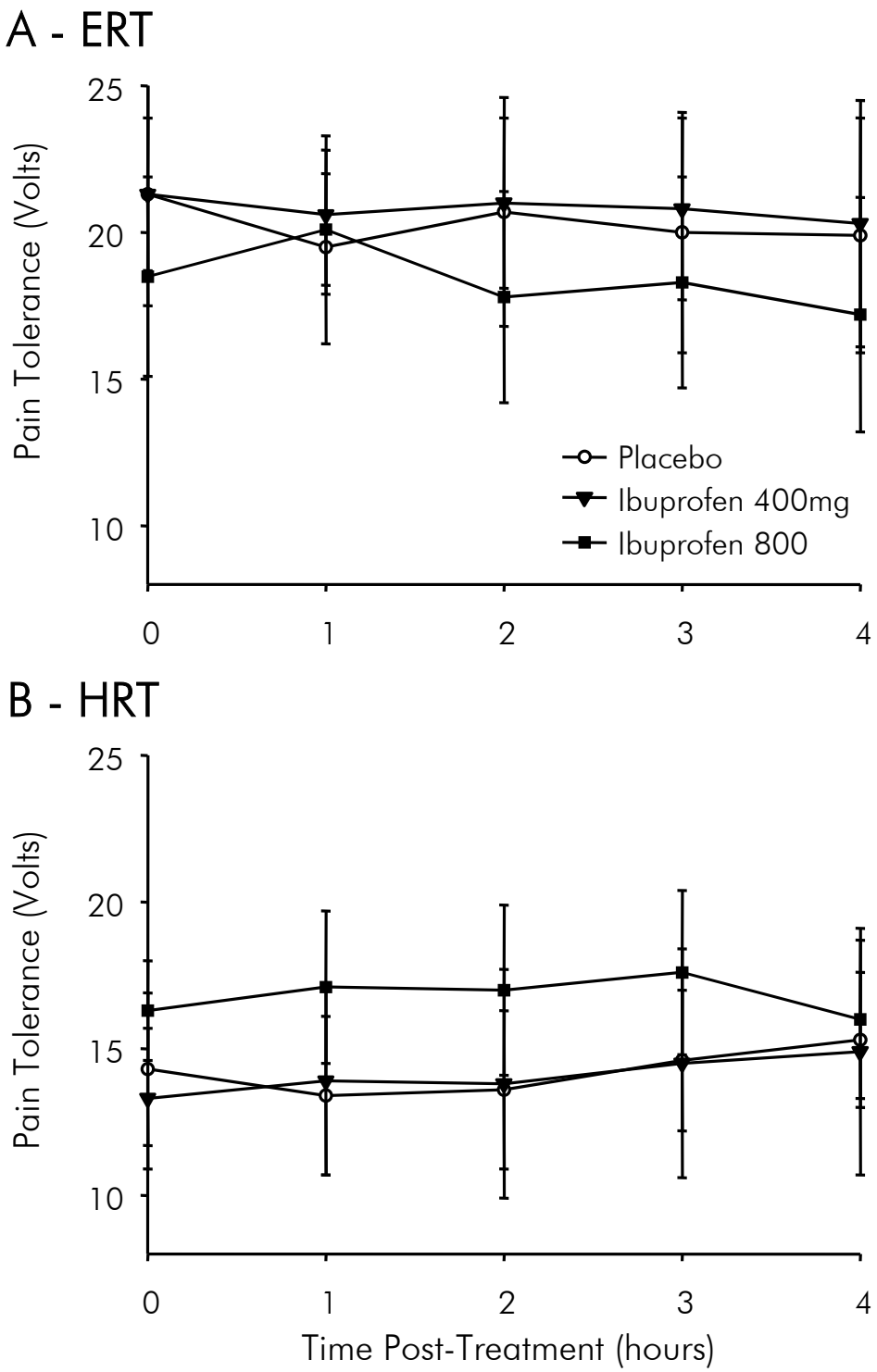


Figure 2.13: Time-course of pain tolerances in older females taking exogenous hormones following placebo (circles), ibuprofen 400mg (triangles) and ibuprofen 800mg (squares). A ERT (n = 4, 4, 4) and B HRT (n = 5, 6, 6). The SEM of a single observation as calculated from the ANOVA matrix was 1.0V for the ERT females and 0.7V for the HRT females.

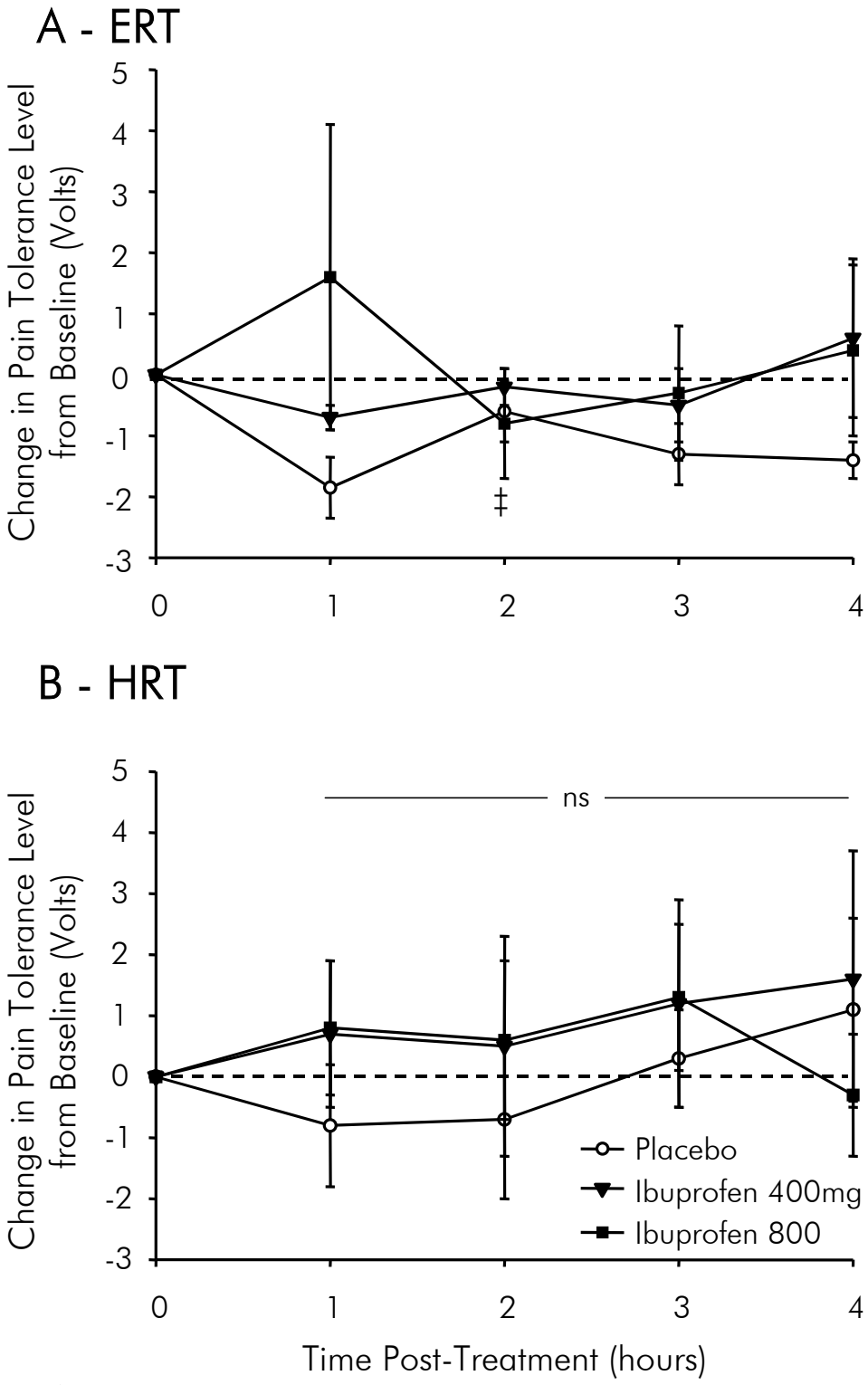


Figure 2.14: Time-course of baseline corrected pain tolerances in older females taking exogenous hormones following placebo (circles), ibuprofen 400mg (triangles) and ibuprofen 800mg (squares). A ERT (n = 4, 4, 4) and B HRT (n = 5, 6, 6). The SEM of a single observation as calculated from the ANOVA matrix was 0.3V for the ERT females and 0.4V for the HRT females.

2.4 DISCUSSION

The technique used in this study had the advantage that the stimulation to the earlobe via surface electrodes has previously been shown to give reproducible pain endpoints without the discomfort of using subcutaneous electrodes, and in addition is sensitive to the analgesic effects of the nonsteroidal anti-inflammatory drug ibuprofen (Walker *et al.*, 1993).

It is important to note, however, that the present experimental paradigm has a fundamental difference from the clinical pain situation: that is, the production of pain *without* concomitant inflammation. Clinical pain usually has an inflammatory component, which activates a class of "sleeping nociceptors" (Schaible & Schmidt, 1988), that are different from the physiologically operational polymodal C-nociceptors (*e.g.* they have lower conduction velocities; Schmidt *et al.*, 1995). Thus – the sex differences and sex hormonal influences outlined in the present experiments pertain to differences in *pain* and *analgesia* only and not *inflammation* and *antiinflammation*.

2.4.1 Baseline Pain

No differences in baseline (*i.e.* prior to treatment) pain threshold, pain tolerances or VAS scores were found as a function of *sex* ($P \approx 0.94, 0.56, 0.08$ respectively, repeated measures ANOVA) *or* sex hormone status ($P \approx 0.61, 0.16, 0.06$ respectively, repeated measures ANOVA). Although, if one examines the *P* values arising from the statistical analyses related to sex hormone differences in pain tolerance and VAS scores from the present experiments ($P \approx 0.16$ and 0.06, respectively), one can see that *sex hormone status* may be important in determining

pain tolerance and VAS scores in a larger subject population. This issue will be explored further in Chapter 4.

There were also no differences found between various stages of the menstrual cycle, or between those taking oral contraceptives and those who were not. However, older women taking combined oestrogen and progesterone therapy (HRT) has significantly lower pain thresholds and tolerances than those who were taking oestrogen alone (ERT).

Sex and Sex Hormone Status Differences

The lack of sex or sex hormonal differences were somewhat surprising, considering the multitude of literature reports which find that there are sex differences in pain threshold, pain tolerance and pain report (for review see Introduction and Giles & Walker, 1999; Giles & Walker, 2000). For example, a previous report from Walker & Carmody (1998), using an identical noxious stimulation technique, reported that male subjects have higher pain threshold and tolerance levels than female subjects (although Walker and Carmody used a slightly different endpoint – their subjects were asked to endure pain until they could no longer tolerate it). However, a re-examination of Walker and Carmody's raw data revealed erroneous statistical analysis, which resulted in reduced standard errors (in their study data from all days was pooled, resulting in standard errors of the mean that were approximately half what they should have been). In a reanalysis using repeated measures ANOVA, the hypothesised sex difference in pain threshold was *not* significant ($P \approx 0.07$), the hypothesised sex difference in pain tolerance was *not* significant ($P \approx 0.10$), and the hypothesised sex difference in VAS scores (not reported in the paper) was also *not* significant ($P \approx 0.67$). It should be noted

however, that in the Walker and Carmody study both pain threshold and pain tolerance level differences approached the arbitrary level of significance (*i.e.* they approached $P < 0.05$).

Lautenbacher & Rollman (1993) found reduced pain threshold and tolerance levels in females compared with males following noxious electrical stimulation (1.6mA *vs.* 2.2mA; 2.1mA *vs.* 4.6mA respectively). However, in that experiment, a constant voltage electrical noxious stimulation paradigm was used (*cf* the constant current paradigm in the experiment outlined in this chapter. Constant current paradigms have been shown to be more reproducible than constant voltage paradigms (Walker *et al.*, 1993).

Endogenous and Exogenous Hormones

One of the difficulties in menstrual cycle analyses is the confirmation of cycle stage. Some studies have simply counted the days starting with the first day of menses (as in the present research according to customary practice; for example Giamberardino *et al.* (1997) and Hapidou & Rollman (1998)), while others have monitored basal body temperatures or used ovulation tests to confirm that ovulation has occurred (Pfleeger *et al.*, 1997). The best method is to monitor plasma sex hormone levels, as it allows relationships between plasma hormone levels and pain sensitivity to be determined (Fillingim *et al.*, 1997). In any case, generalisations can be made between the three menstrual phases: menses, follicular and luteal.

Several reports have indicated that sex hormones influence pain responses in humans (Fillingim *et al.*, 1997; Giamberardino *et al.*, 1997; Pfleeger *et al.*, 1997; Hapidou & Rollman, 1998). Stimulation method seems to be an important factor

in determining the direction of the differences: most stimulation methods find the greatest pain thresholds during the follicular phase (*e.g.* ischaemia (Fillingim *et al.*, 1997; Pfleeger *et al.*, 1997) and cold pressor (Hapidou & de Catanzaro, 1988)); while electrical stimulation produces the highest pain thresholds during the luteal phase of the human menstrual cycle (Giamberardino *et al.*, 1997; for review see Riley *et al.*, 1999). This issue is discussed at length in Chapter 4.

In terms of exogenous hormone use, the study outlined in this chapter suggests that progesterone is important in reducing pain threshold and tolerance levels in older women (as pain thresholds and tolerances were reduced in women taking HRT compared to those taking ERT); while the differences in baseline pain responses between men and women are strongly influenced by oestrogen level.

Fillingim & Edwards (2001) have reported that exogenous hormone in post-menopausal women use causes a reduction in heat pain threshold and tolerance levels compared to those of men and post-menopausal women not taking hormone replacement (pain threshold ~42.5°C, 45°C, 47°C; and pain tolerance ~46.5°C, 49°C, 50°C respectively). Unfortunately, in their study no distinction was made between those taking oestrogen replacement therapy and those taking combined oestrogen and progesterone.

2.4.2 Analgesia Data

There are several important issues arising from the experiments outlined in this chapter. There are the differences in placebo effect and in response to ibuprofen, and these will be discussed in turn.

Placebo Effect

Ever since Beecher's pivotal paper in 1955, which reported that placebos have an average significant effectiveness of approximately 35%, the issues of placebo and placebo responders have been controversial. Despite claims that the placebo is powerless (Hróbjartsson & Gøtzsche, 2001), the present experiment demonstrates a clear response to placebo, which was limited to those subjects with low levels of oestrogen (–SH).

The mechanism responsible for eliciting the placebo effect has been controversial. Several hypotheses have been put forward, including classical conditioning, expectancy, and through the endogenous opioid peptides such as the endorphins, especially β-endorphin. In the current experiment, it is hypothesised that the placebo response is mediated by the endorphins. The idea that endorphins mediate placebo response is not new, it was first proposed by Levine and colleagues in 1978 (Levine *et al.*, 1978). Much debate surrounded their hypothesis, especially as there were suggestions that Levine's experimental method was flawed (Goldstein & Grevert, 1978). Since then, several experimental and clinical studies have strengthened Levine's hypothesis by showing that naloxone (an endorphin antagonist) antagonises placebo analgesia (Grevert *et al.*, 1983; Levine & Gordon, 1984; Benedetti *et al.*, 1995). More detail regarding placebo can be found in Chapter 3.

The significant finding from the present experiments was that sex hormone status was an important factor in determining placebo-mediated analgesia, such that subjects with high levels of female hormones (+SH) did not exhibit placebo analgesia, while subjects with low levels of female hormones (-SH) did. As placebo

analgesia is hypothesised to be mediated by endorphins, a possible mechanism for the differential placebo effects could be via sex hormonal regulation of β-endorphin. In animal studies, oestradiol has been shown to be toxic to β-endorphin-containing neurones in the arcuate nucleus (Desjardins *et al.*, 1993), and also decreases the levels of β-endorphin in the hypothalamus and plasma (Wardlaw *et al.*, 1982; Forman *et al.*, 1985). While the exact relevance of these effects to the pain system is yet to be determined (for example, it seems unreasonable that endogenous oestrogen would be neurotoxic in humans), one might postulate that subjects with high levels of oestrogen (*i.e.* +SH) might be expected to have reduced placebo responses compared to those with lower levels (-SH). This was evident in the present study (Figure 2.9). The hypothesis is strengthened as there was no placebo response in young females, regardless of menstrual cycle stage *or* in older females taking exogenous hormones, regardless of their exogenous hormone therapy type (Figures 2.12 and 2.14 respectively).

While many other studies have attempted to identify factors that predict placebo response, most have proved fruitless (for review see Pearce, 1995). Characteristics studied have included age, sex (Averbuch & Katzper, 2001), ethnicity, education level, IQ and various other psychosocial and psychobiological factors (for review see Richardson, 1994). Thus, the suggestion that female sex hormones may modulate placebo response is especially exciting, and one that requires further study.

Ibuprofen Response

Response to ibuprofen *only* consistently occurred in subjects with low levels of female sex hormones (-SH; Figure 2.9). No effect of ibuprofen was seen in older

females taking exogenous hormones (Figure 2.14), and doses of 800mg of ibuprofen were transiently effective in young women *only* during the menses phase of their cycle (2.11). Paradoxically, administration of ibuprofen 800mg caused hyperalgesia in subjects taking the active component of their oral contraceptive preparation and in those women taking oestrogen replacement (Figures 2.12 and 2.13 respectively). Thus response to ibuprofen was dependent upon a subject's sex hormone status, and not their sex *per se*, as was previously hypothesised (Walker & Carmody, 1998). One other study has refuted the concept of sex differences in ibuprofen *analgesia*. However, in that study, a mixed inflammatory-nociceptive clinical pain model was used, in contrast to our purely nociceptive stimulus Averbuch & Katzper (2000); and previous reports have shown that there are no sex differences in the anti-inflammatory effect of ibuprofen (Walker *et al.*, 1994).

The ibuprofen-induced analgesia in the –SH subjects was prolonged, despite declining plasma concentrations of the drug. It is therefore hypothesised that ibuprofen *either* persists at a higher level in an extra-circulatory functional compartment *or* it produces an analgesic mediator with duration of action longer than ibuprofen itself.

Thus it is postulated that ibuprofen analgesia is mediated, at least in part, by β-endorphin. This idea is not novel. Oral diclofenac has been shown to increase plasma β-endorphin in humans (Martini *et al.*, 1984). β-endorphin in turn, is influenced by the female sex hormone oestrogen (see placebo section above). If this hypothesis is correct, then it might be expected that subjects with high levels of female sex hormones will also have reduced ibuprofen responses. This was certainly true in the present study.

There are alternate explanations for why ibuprofen is ineffective at producing analgesia in subjects with high levels of female sex hormones – and all relate to the (postulated) mechanisms of action of nonsteroidal anti-inflammatory drugs. It is now widely accepted that NSAIDs exert their *analgesic* actions via a centrally mediated action. While the exact mechanism of this action remains controversial, several molecules or receptors have been suggested to be of importance; these include: β-endorphin (discussed above), 5-hydroxytryptamine (5-HT), nitric oxide (NO), glutamate, aspartate and the *N*-methyl-D-aspartate (NMDA) receptors (Martini *et al.*, 1984; Malmberg & Yaksh, 1992a; Malmberg & Yaksh, 1992b; Björkman, 1995). The central inhibition of prostaglandin synthesis via the cyclooxygenase pathway is also an area of theoretical importance. Sex hormonal influences on any of these systems would produce the differences in pharmacodynamic effect of ibuprofen seen in the present study, but these experiments remain to be done.

Pharmacokinetics

Peak plasma concentrations, and pharmacokinetic parameters were comparable to literature values (Lee *et al.*, 1985; Walker & Carmody, 1998; Davies, 1998).

Despite the distinct differences in the pharmacodynamic effects of ibuprofen as a function of sex hormone status, there was no pharmacokinetic explanation for these differences. Paradoxically, subjects in the +SH group had *higher* peak plasma ibuprofen concentrations compared to their –SH counterparts and still showed no analgesia. The higher peak plasma concentration in +SH subjects is likely to reflect a higher milligram per kilogram dosage administered to subjects in the +SH group, due to their lower mean body weight.

The lack of a pharmacokinetic basis for the sex hormone status differences in pharmacodynamic effect of ibuprofen, confirms Walker & Carmody's earlier report of the same.

2.5 FUTURE DIRECTIONS

The experiment outlined in this chapter has raised several important issues that need to be further explored. These include:

- *The determination of baseline pain threshold and tolerance levels in a large population of young normally cycling women, whose menstrual cycle phase has been confirmed hormonally.*
- *Determination of baseline pain threshold and tolerance levels in a large population of older women taking exogenous hormones (both ERT and HRT).*
- *Examination of the role of oestrogen, and other sex hormones on β-endorphin.*
- *Discovery of the role of expectancy on placebo response in men and women (see Chapter 3).*
- *Determination of the role of the sex hormones oestrogen and progesterone on ibuprofen response (see Chapter 5).*
- *Discovery of the role of endogenous neurotransmitters on ibuprofen analgesia, and how these might be modulated in the presence of oestrogen and progesterone.*

2.6 FINAL COMMENTS

The data presented here provide new evidence for the role of sex hormones in nociception and in analgesic response to ibuprofen. These hormonal effects are important in the light that NSAIDs are so frequently prescribed, and taken as over the counter medications, by both men and women. However, one must be cautious in the interpretation of this data, as the experiments completed here examine *only* the *analgesic* response to ibuprofen – and clinical pain often has an *inflammatory* component. Nonetheless, the fact that such sex hormonal modulation of analgesia occurs for ibuprofen warrants the widespread re-evaluation of analgesic drugs for sex hormonal effects, and does provide the basis for a predictor for response to analgesic drugs.

Chapter 3

INFLUENCE OF SEX ON IBUPROFEN AND PLACEBO EXPECTANCY

3.1 INTRODUCTION

> *"Placebo: an inactive substance administered to a patient who insists on receiving medication or who would benefit by the psychological deception"*[1]

The definition of placebo is as elusive as the mechanisms mediating its effects. Often conjuring a sense of "quackery", the placebo has been a component of treatment through the history of medicine (Hahn, 1985), and is mandatory in most clinical trials (there are notable exceptions, for example those trials where there cannot be a placebo leg on ethical or observational grounds, and in those studies where an existing therapy has some efficacy). In medicine, the placebo effect is viewed as the response that cannot be directly attributed to the pharmacological or physiological effects of treatment (Benedetti & Amanzio, 1997). The aim of placebo use is to differentiate the effects due to a drug's pharmacological effects (*specific effects*) from other unspecified "placebo effects" (*non-specific effects*). However, these non-specific effects may nevertheless form an integral part of the treatment of a patient (Ross & Buckalew, 1985). The routine inclusion of a control group receiving an inert or non-specific medication

[1] McLeod, W.T. ed. (1991) *The New Collins Dictionary and Thesaurus in One Volume*, Harper Collins Publishers Glasgow.

(such as a placebo), implicitly recognises that the interaction among the doctor, the patient and the drug taking ritual may have powerful effects on response to medication and treatment (Evans, 1985).

Placebo responses have been observed in many studies of pharmacological agents (for example, in Chapter 2). However, the efficacy of placebo is variable, to the extent that patients have been categorized as either *placebo responders* or *placebo non-responders*. The issue of determining if a subject is a placebo responder has perplexed scientists for many years, especially in light of Henry Beecher's famous 1955 article, which claimed that approximately 35% of people are placebo responders (Beecher, 1955). Since then, many researchers have attempted to discover factors that may predispose a person to be a "placebo responder". While many factors have been postulated, none has been able to fully account for the inter-individual differences in placebo response seen experimentally and clinically. This raises an interesting theoretical question – is a person consistently a *placebo responder* – or does response depend upon quite specific assertions in the placebo administration process? For example, in Pavlovian conditioning, a bell might cause a dog to salivate, but a buzzer will not. Perhaps response also depends on the specific ailment being treated.

In any case, the exact mechanism responsible for the elicitation of placebo response is not understood, but several major theories have been proposed: (i) classical conditioning, (ii) expectancy and (iii) endogenous opioids. It is unlikely that these theories are mutually exclusive – they all probably combine to produce the placebo effect seen clinically and experimentally, although testing the relative contributions of each part would be extremely difficult.

(i) *Classical conditioning* involves the Pavlovian concept of association between previous ameliorative effects of active treatment with ingestion of a placebo. The classical conditioning hypothesis states that a "neutral (unconditioned) stimulus may acquire the capability of producing an improvement (placebo effect) after repeated associations with an unconditioned stimulus" (Benedetti & Amanzio, 1997). For example, if a patient regularly takes an active pharmacological preparation (*e.g.* ibuprofen; unconditioned stimulus) for pain relief (unconditioned response) then eventually the patient may start to associate the shape, colour or taste of the tablets (conditioned stimulus) with pain relief. Therefore when the patient is given an inactive preparation with the same shape, colour or taste of the active tablet, their pain may decrease also. Thus classical conditioning requires learning associations between treatment and effect. Therefore differences in placebo effect amongst individuals can be explained by different learning history.

(ii) It is also possible that the placebo effect is a *direct* response to the subject's faith or hope in the medication – that is their *expectation* that the treatment will be effective or ineffective (depending on whether *positive* or *negative* expectancies are involved). According to expectancy theory, expectation leads to a cognitive readjustment of appropriate behaviour (for review see Benedetti & Amanzio, 1997). This was important in the present experiment, as it was hypothesised that the different written instructions would produce different analgesic responses.

(iii) More recently the role of *endogenous opioids* and *cholecystokinin (CCK)* in placebo analgesia has been demonstrated. Endogenous opioid act peripherally, spinally and supraspinally (*e.g.* at the nucleus raphe magnus and periaqueductal grey). The nucleus raphe magnus and periaqueductal grey project to the spinal cord through descending inhibitory pathways to produce analgesia under the influence of endogenous opioids. There are several neurotransmitters that are important in the spinal and supraspinal levels. These include noradrenaline, serotonin, enkephalins, substance P and GABA. Several pieces of experimental evidence support the role of endogenous opioids in placebo analgesia: primarily because naloxone (an opioid antagonist) can block placebo analgesia (*e.g.* Abbott & Melzack, 1983; Grevert *et al.*, 1983; Levine & Gordon, 1984). The non-opioid peptide cholecystokinin also appears to have a role in modulating placebo analgesia: proglumide (a CCK-A antagonist) potentiates placebo analgesia (Benedetti, 1996). Some researchers have even suggested that CCK has the function of an anti-opioid peptide, and that the distribution of CCK in the brain matches that of μ-opioids (for review see Benedetti & Amanzio, 1997). In addition, CCK receptors can be found both pre- and post-synaptically to C-fibres – mirroring the μ-opioid receptor distribution (for review see Benedetti & Amanzio, 1997). Thus cholecystokinin may play an important role in the modulation of placebo analgesia.

The three theories are probably not mutually exclusive – the psychological effects on drug response probably occur through physiological mechanisms, such as through endogenous opioids.

The previous chapter examined the influence of sex hormone status on pain and analgesic response to ibuprofen – with an intriguing result: males and post-menopausal women who were not taking exogenous hormones (*-SH*) had far greater placebo responses than young females and post-menopausal females who were taking exogenous hormones (*+SH*). This difference may have followed from differences in expectancies since no subject was told prior to that experiment that a placebo would be administered. However, it is important to note that this was not purely a *sex* difference since postmenopausal women not taking exogenous hormones essentially responded like males. It seems relevant, therefore, to ask whether *sex hormone status* influences expectancy.

The primary hypothesis was that young, normally-cycling females would exhibit less placebo and ibuprofen analgesia than age-matched males. The secondary hypothesis was that in males, and not females, placebo and ibuprofen analgesia would be influenced by expectancy.

3.2 METHODS

3.2.1 Subjects

Subjects were recruited from within The University of New South Wales and its surrounds using posters, flyers and newspaper advertisements, as per Section 2.2.1. They were healthy, pain free volunteers between 18 and 45 years of age, who had provided written, informed and free consent, in accordance with institutional guidelines (Approval Number HREC 99062, Human Research Ethics Committee, The University of New South Wales, Sydney, Australia). The subjects were given modest payment for their participation and were free to withdraw at any time. A total of 20 subjects completed the trial (10 female and 10 male).

As described earlier, the subjects underwent a medical examination prior to entry into the trial Briefly, this involved a check of general health, heart sounds, lung sounds, and a check for gastrointestinal problems. A relevant clinical and family history was also taken. All potential subjects were subjected to the same exclusion criteria as detailed in Section 2.2.1. All of the females were cycling normally, and if oral contraceptives were being taken, this information was noted upon entry to the trial.

3.2.2 Equipment Familiarisation and Testing Procedure

The equipment familiarisation and testing procedures were identical to those in Section 2.2.2.

3.2.3 Electrical Stimulation

The electrical stimulation methodology was identical to that reported in Section 2.2.3.

3.2.4 Drug Supply and Dosing

A two-by-two factorial design known as the "balanced placebo design" (Marlatt & Rohsenow, 1980) was utilised in this trial, so that subjects received ibuprofen 800mg (4 tablets, 200mg Actiprofen™, GlaxoSmithKline) twice and placebo twice (4 tablets of identical appearance). Subjects were randomly assigned to start in one of four expectancy states:

1. Subject told they were receiving ibuprofen, and received ibuprofen *(positive expectancy)*.
2. Subject told they were receiving ibuprofen, and received placebo *(positive expectancy)*.
3. Subject told they were receiving placebo, and received ibuprofen *(negative expectancy)*.
4. Subject told they were receiving placebo, and received placebo *(negative expectancy)*.

At the same time as the subjects were given their tablets, they were also given a sealed envelope containing information about whether they were to receive ibuprofen or placebo that. They were also told not to alert the experimenter to this information, to reduce possible experimenter bias. This design had the advantage that it included combinations of all relevant expectancy/drug treatments.

3.2.5 Statistical Considerations

All data were tested for normality and equal variances. Data are expressed as mean ± SEM for normally distributed data, and as median [range] for data that did not fit the normal distribution.

Pain threshold, pain tolerance and VAS scores for each time-point were calculated as the average of the last 3 stimulus trials. This was performed because previous experience with this technique has shown that the voltage measurements in the first train of each stimulus block are always lower than subsequent readings as the subject becomes familiar with the procedure (Walker *et al.*, 1993). Therefore, in all measurements the results from the first train are rejected.

Baseline pain threshold, pain tolerance and VAS values for each subject were determined from the average of the three pre-treatment blocks of stimuli from each day. These baseline pain measures were later used to calculate baseline corrected analgesic response (see below). Differences in baseline nociception, which may have followed from differences in sex hormone status, were explored using repeated measures ANOVA.

Analgesia was defined as *a statistically significant increase in the pain tolerance voltage level at any post-treatment time compared with baseline*. The effects of treatment were analysed using single-factor (time), repeated-measures ANOVA with treatment type as covariate. *Post hoc* analyses were performed on pre-planned comparisons (sex and expectancy) using the Bonferroni correction (Wallenstein *et al.*, 1980).

All statistical analyses were performed using the statistical program SPSS version 11 [SPSS Inc, Chicago, Illinois, USA]. Values of $P < 0.05$ were considered statistically significant.

3.3 RESULTS

3.3.1 Subject Recruitment and Demographics

As in the previous experiment, subject recruitment was a major part of this trial. A total of 56 people responded to the advertisement for subjects. Following initial telephone screening, 21 entered the trial (11 females and 10 males), with 20 completing all four days of the trial (10 females and 10 males; 1 female discontinued prior to her third visit owing to time constraints, and her incomplete data were excluded from the analysis). Of the 10 females, 4 were taking oral contraceptives (subject F3 biphasic: dosage varied twice during the 21 day active phase; subjects F5, F8, F10 monophasic: dosage did not vary during the 21 day active phase); the other 6 were cycling physiologically. The demographics of each are shown in Table 3.1:

Table 3.1: Subject demographics by sex (mean ± SEM).

Group	Age (years)	Weight (kg)	Ear Thickness (cm)
Females (n=10)	26.2 ± 2.3 (range 21 – 45)	62.3 ± 3.1	0.43 ± 0.03
Males (n=10)	28.3 ± 1.9 (range 22 – 43)	75.2 ± 2.4*	0.50 ± 0.03

* *denotes significant difference from female group (*$P < 0.05$*, unpaired* t-test*)*

Although there was no difference in the mean age of subjects or their ear thickness, males were significantly heavier than female subjects ($P < 0.05$, unpaired *t*-test).

3.3.2 Baseline Pain

Summary

- *No sex differences in baseline pain threshold or tolerance levels (Table 3.2).*
- *Males had greater VAS scores than females (Table 3.2).*

The mean baseline pain measures of male and female subjects' are shown in Table 3.2. There were no significant sex differences in baseline pain threshold or tolerances ($P > 0.05$, repeated measures ANOVA), but males had greater VAS scores than females ($P < 0.05$, repeated measures ANOVA).

Table 3.2: Baseline pain measures by sex

Group	Pain Threshold (Volts)	Pain Tolerance (Volts)	VAS Score (0-100)
Females (n=10)	12.9 ± 1.4	15.8 ± 1.6	23.2 [4.2 - 57.7]
Males (n=10)	14.9 ± 2.2	18.9 ± 2.7	43.7 [13.2 – 72.6]*

** denotes significant difference from female VAS scores (P < 0.05, repeated measures ANOVA).*

3.3.3 Time-course of Analgesia

Summary

- *When data partitioned by sex alone, no significant analgesic effect of ibuprofen or placebo in men or women (Figure 3.1).*
- *When data partitioned by sex and expectancy, no analgesic effect in women (Figure 3.2).*
- *When data partitioned by sex and expectancy, analgesia in males only during positive expectancy states, at 2, 3, and 4 hours post-placebo, and 1 and 2 hours post-ibuprofen (Figure 3.4).*

The time-course of analgesia in all subjects following pooled ibuprofen or placebo treatments is depicted in Figure 3.1. There was no significant analgesic effect of either ibuprofen or placebo ($P > 0.05$, repeated measures ANOVA).

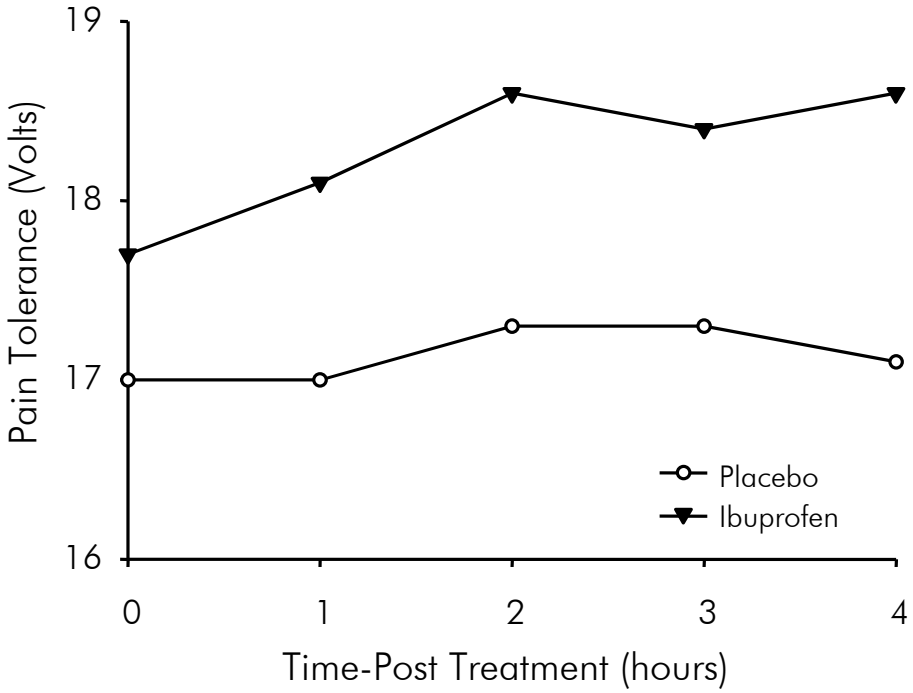


Figure 3.1: Time-course of pain tolerance in pooled subject group following ibuprofen 800mg (n = 20 x 2 observations) or placebo (n = 20 x 2 observations).

For clarity, error bars have been omitted, but the standard error of the mean of a single observation as calculated from the ANOVA matrix was 0.7 Volts for placebo and 0.5 Volts for ibuprofen.

To test the primary hypothesis (that females would exhibit less placebo and ibuprofen analgesia than males), the data were partitioned by sex. The time course of analgesic response to an 800mg dosage of ibuprofen or to placebo, by sex, is shown in Figure 3.2. There was no statistically significant analgesic effect of ibuprofen or placebo in either men or women when this was done ($P < 0.05$, repeated measures ANOVA) although males' pain tolerance levels tended to increase following administration of placebo or ibuprofen.

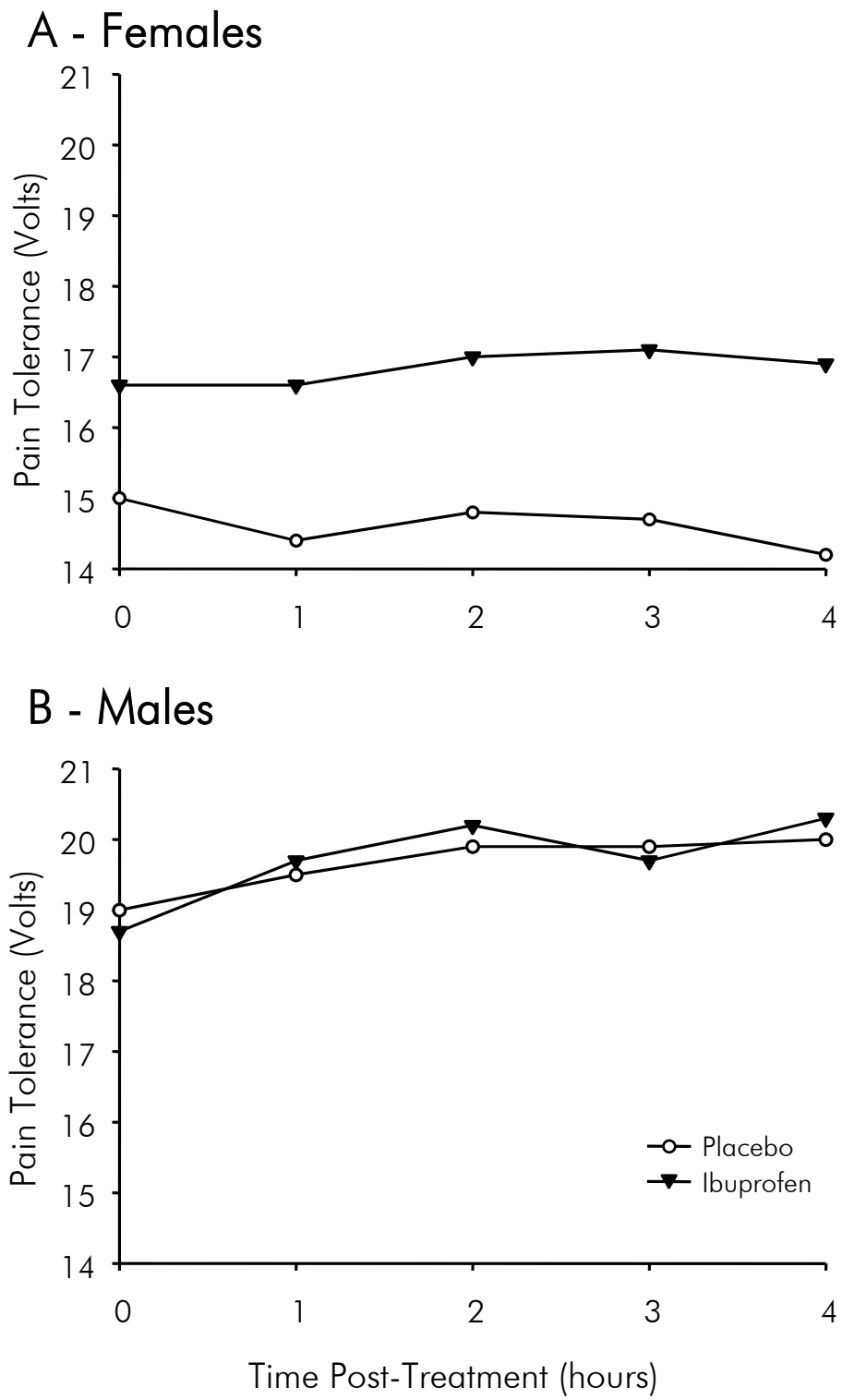


Figure 3.2: *Time-course of pain tolerance in A females (n=10 with 2 observations for each dose) and B males (n=10 with 2 observations for each dose) following ibuprofen 800mg or placebo.*

For clarity, error bars have been omitted, but the standard error of the mean of a single observation as calculated from the ANOVA matrix was 0.7V for placebo and 0.5V for ibuprofen for the females and 1.1V for placebo and 0.9V for ibuprofen for the males.

To test the second hypothesis (that in males, and not females, placebo and ibuprofen analgesia would be influenced by expectancy), the data were partitioned according to expectancy and treatment type. The time-course of analgesic response is shown in Figure 3.3. Here there are clear differences in the patterns of analgesic response in men and women dependent upon treatment and expectancy. However, because of the difficulty of comparison between expectancy and dose regimes owing to different baseline pain tolerance levels (especially in the females), data were normalised to baseline by subtracting the baseline pain tolerance voltage from every pain tolerance voltage. Thus all baseline voltages became zero, and the subsequent pain tolerance voltages became the change in voltage from baseline. Figure 3.4 shows the time-course of baseline corrected analgesic response in women and men by expectancy and treatment type. The extremely different patterns between men and women should be noted.

In *females* no dosage or expectancy regime resulted in statistically significant analgesia ($P \approx 0.52$, repeated measures ANOVA, Figure 3.4). In *males*, however, analgesia developed *only* following positive expectancy treatments (with either placebo or ibuprofen; $P < 0.01$, repeated measures ANOVA). It is also of interest that the time-course of this analgesia was dependent upon treatment. That is, ibuprofen-induced analgesia developed within one hour and was sustained only until the second hour post-treatment ($P < 0.05$, repeated measures ANOVA). In contrast, placebo-induced analgesia did not develop until the second hour post-treatment, and was sustained for the rest of the experimental period ($P < 0.05$, repeated measures ANOVA). Despite the

apparent decrease in pain tolerance following negative expectancy placebo treatment, this was not statistically significant.

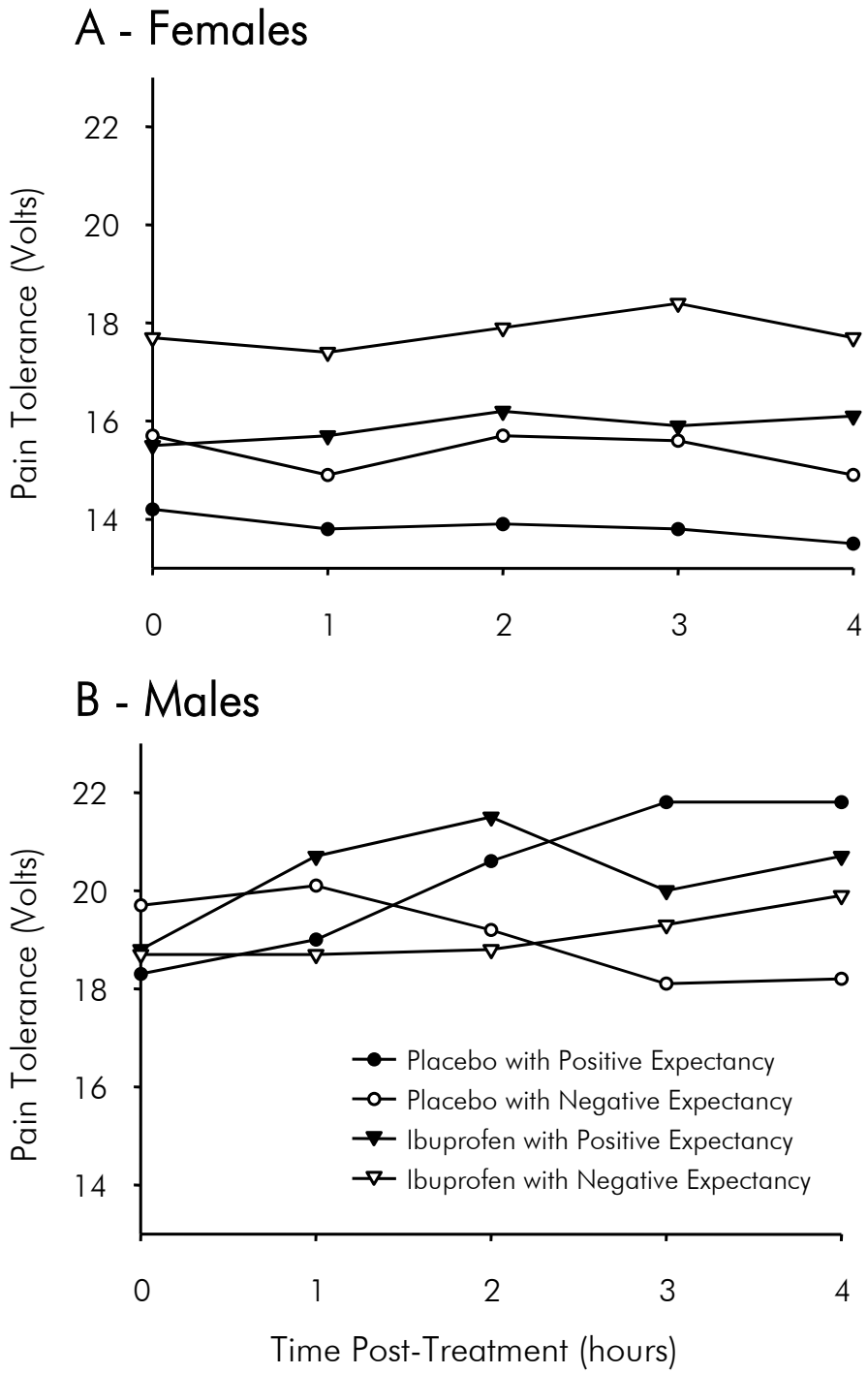


Figure 3.3: Time-course of pain tolerance in A women (n = 10) and B men (n = 10) by expectancy and treatment type. Positive expectancy denoted by closed symbols; negative expectancy by open symbols. Placebo denoted by circles, Ibuprofen 800mg by triangles. For clarity error bars are not shown, however the standard error of a single observation as calculated from the ANOVA matrix was 0.4V for females and 0.3V for males.

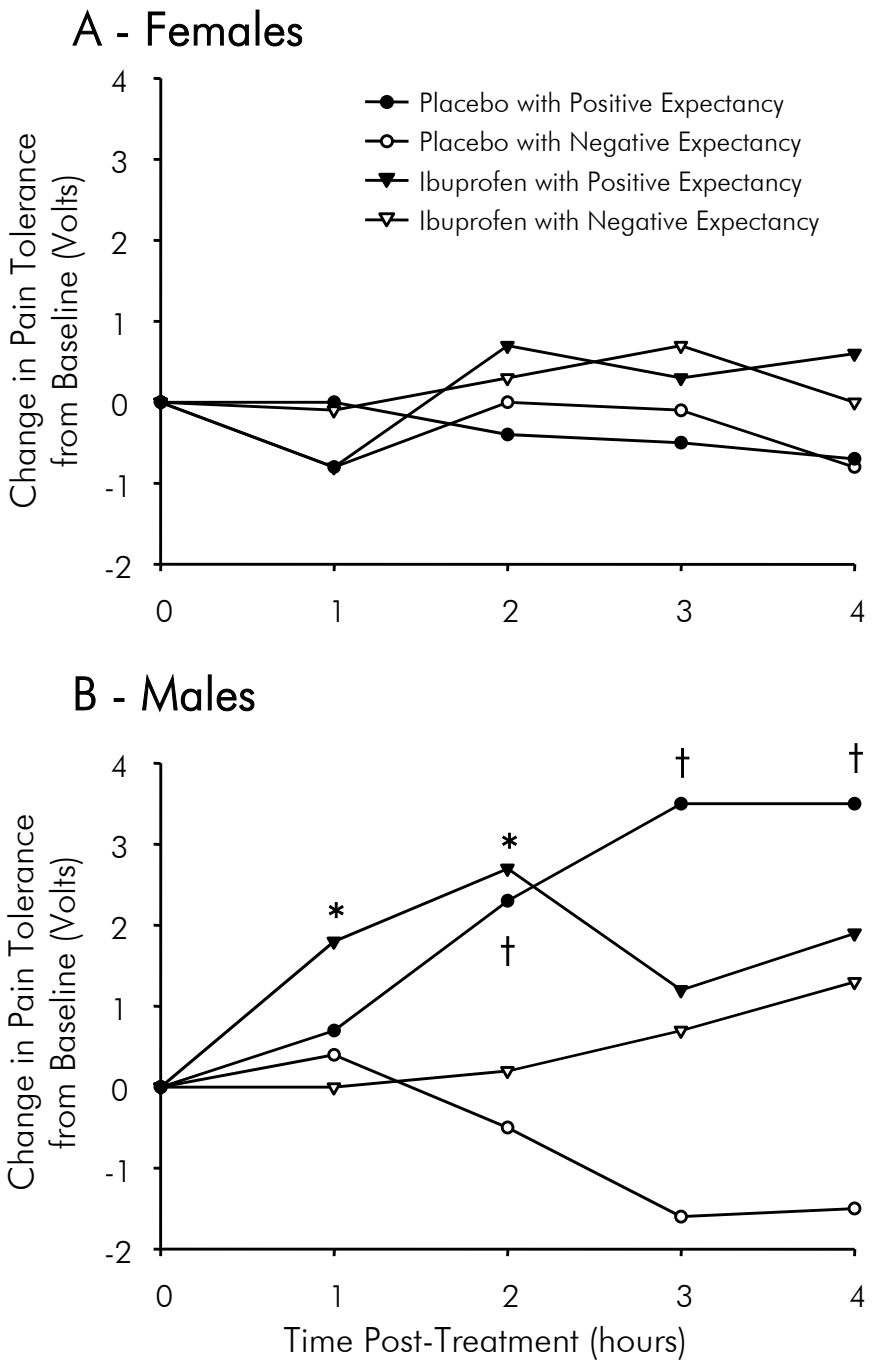


Figure 3.4: Baseline corrected time-course of analgesic response in A females (n =10) and B males (n = 10) following a single dose of placebo (circles) or ibuprofen 800mg (triangles) with positive expectancy (filled symbols) and negative expectancy (open symbols). For clarity, error bars have been omitted, but the standard error of a single observation as calculated from the ANOVA matrix was 0.1 V for females, and 0.2 V for males.

† denotes significant difference from placebo baseline (P < 0.05, repeated measures ANOVA with post hoc t-tests and Bonferroni correction).
** denotes significant difference from ibuprofen baseline (P < 0.05, repeated measures ANOVA with post hoc t-tests and Bonferroni correction).*

3.4 DISCUSSION

3.4.1 Baseline Pain

There were no differences in baseline pain measures between men and women in the present experiment. A previous report from Walker & Carmody (1998), using identical noxious stimulation techniques had suggested that male subjects had greater pain threshold and tolerance levels (although the experimenters used a slightly different endpoint where subjects were asked to endure the pain for as long as possible). However, a re-examination of Walker and Carmody's original data reveals that erroneous statistical analysis was utilised, resulting in reduced standard error of the means (all data in their study were pooled, giving SEMs that were approximately half of what they should have been). This error resulted in significance being claimed where it should not have been, *i.e.* in their experiments there were really no sex differences in baseline pain threshold ($P \approx 0.35$) or baseline pain tolerance levels ($P \approx 0.07$). Another study has also reported reduced pain threshold and tolerance levels in females compared to males (median pain threshold 1.6 *vs.* 2.2 mA, and median pain tolerance 2.1 *vs.* 4.6mA, respectively; Lautenbacher & Rollman, 1993). However, in that study, a variable current paradigm was used (*cf* constant-current in the study presented in this chapter), involving electrodes placed slightly proximal to the base joints of the subjects' finger (cathode) and thumb (anode). Thus, the physical distance of the cathode and anode may have varied from subject to subject, and produced inherent sex differences, as female subjects would most likely have had smaller hands than their male counterparts. In addition, one study has shown that the

constant-current methodology allows subjects to more reliably identify pain endpoints when surface electrodes are involved (Walker *et al.*, 1993).

3.4.2 Analgesia Data

Rohsenow and Marlatt recognized the important contribution of subjects' expectancies to their reactions (Rohsenow & Marlatt, 1981). Accordingly, they expressed concern about traditional drug trials where, when a subject has been informed that a placebo will be given (as is often an ethical requirement), the effects of the drug "uncontaminated" by the subject's belief of what is being administered is not assessed. The balanced placebo design, as used in the present study, allows the differentiation between expectancy effects (non-specific effects) and pharmacological action (specific effects). On theoretical note, it would be interesting to determine whether the magnitude of this expectancy was dependent upon the potency of the analgesic involved. Would expectancy be less, for example in ibuprofen, where the analgesic effect is relatively small compared to its anti-inflammatory effect, in contrast to a drug with a much greater analgesic potency, for example morphine?

Several questions arise from the experiment outlined in this chapter. Does placebo response vary by sex? Does ibuprofen response vary by sex? Does expectancy differ by sex? The results suggest that the answer to all these questions is YES. For example, no placebo or ibuprofen response was seen in females, while there was a marked placebo and ibuprofen response in males during their positive expectancy state (Figure 3.4). These results indicate that not only do responses to treatments vary by sex, but that expectancies do as well.

The clinical implications of this are enormous: if a large component of analgesic action is dependent upon both sex and expectancy, then the widespread re-evaluation of currently marketed analgesic drugs is paramount.

The experiment outlined in this chapter confirms the finding of (Walker & Carmody, 1998), that 800mg doses of ibuprofen are ineffective in producing analgesia in young females – with the additional important information that this ineffectiveness in females was regardless of the subject's expectancy state. In the male subjects, both placebo and ibuprofen responses were dependent upon expectancy: **that is when the subject was told they were to receive an analgesic substance – analgesia resulted**. On the other hand, if the subject was told to expect an inactive substance, no analgesia resulted. These are significant and important findings: however, they must be interpreted with caution: *these findings pertain only to the analgesic effects of ibuprofen and not the anti-inflammatory effect*. The issue of sex (and sex hormone status) and expectancy on the anti-inflammatory effect of ibuprofen needs to be further examined.

In males, the time-courses of placebo and ibuprofen analgesia in the positive expectancy states were different (Figure 3.4). Analgesia did not develop until the second hour post-placebo (*cf* first hour post-ibuprofen), and it was then sustained for the remainder of the experimental time-course (*cf* ibuprofen where analgesia persisted only until the second hour). This delayed response suggests that placebo causes the synthesis and subsequent release of an endogenous mediator – and it is hypothesised that this substance is β-endorphin. There is reasonable experimental evidence that placebo analgesia is mediated, at least in part, by endogenous opioids (Levine *et al.*, 1978; Grevert *et al.*, 1983; Amanzio &

Benedetti, 1999). The issue of the role of β-endorphin in placebo and NSAID analgesia will be further discussed below.

In males, with ibuprofen, analgesia was already present at 1 hour post-treatment, but persisted only until the second hour post-treatment. This time-course of analgesic action is what is to be expected considering the pharmacokinetic properties of ibuprofen, where peak plasma concentrations occur at approximately one hour post-treatment, and the half-life of elimination is approximately one and a half hours (see Section 2.3.2); but is different to what has been seen previously using this methodology (see Chapter 2 of this thesis and Walker & Carmody, 1998). In contrast to the current experiment's expectancy paradigm, in the aforementioned experiments, male subjects were *unaware* of which treatment (ibuprofen or placebo) they were to be given. Indeed in both cases they were unaware a placebo was involved at all, and in those studies analgesia was prolonged over the time-course of the experiment. It is therefore likely that the ibuprofen analgesia seen in those previous experimental designs combines a true pharmacological component with an added expectancy (or placebo) component. So in those previous experiments, the initial analgesia (1 –2 hours post-treatment) was probably due to the true pharmacological properties of ibuprofen, while the later component (2+ hours) was likely to be due to the expectancy-mediated component of treatment. Both the specific and non-specific effects of placebo and ibuprofen are dependent upon the subject's sex (no drug or placebo effects were noted in women), and both are important in determining a subject's response to that treatment.

Furthermore, the non-specific component of a drug's effect is likely to be mediated by endogenous opioids.

Amanzio and colleagues designed an experiment to asses the role of endogenous opioids in ketorolac (an NSAID) response using an expectancy methodology (Amanzio *et al.*, 2001). In their study, experimental ischaemic pain in the arm was used to assess analgesic response to ketorolac following open and hidden injections. Open injections were performed in full view of the subject, who was told that a powerful analgesic was being administered; *i.e.* positive expectancy; while in the hidden injections, the injection was performed by a pre-programmed machine which infused the substance into the intravenous line; *i.e.* negative expectancy. While there was a decrease in pain intensity following both injections, there was a greater reduction in analgesic response (as measured by the number of minutes a subject could tolerate the ischaemic condition) in subjects who received ketorolac under positive expectancy conditions (increase in ischaemic tolerance was 9.0 *cf* 4.7 minutes). The magnitude of the reduction in pain intensity following hidden injection of ketorolac could be mimicked by giving a hidden pre-injection of naloxone prior to open injection of ketorolac (increase in ischaemic tolerance was 4.7 minutes without naloxone and 5.7 minutes with naloxone; Amanzio *et al.*, 2001). Amanzio and colleagues concluded that the reduced effectiveness of ketorolac following hidden naloxone in their experimental paradigm was due to the blockage of an opioid mediated component of placebo (Amanzio *et al.*, 2001).

Therefore, it seems reasonable to conclude, as it is from the experiments outlined in this chapter, that when an analgesic is given to a subject under positive

expectancy conditions, both specific and/or non-specific drug effects are involved.

One of the questions raised in this chapter has been whether sex differences in placebo response do occur. This question has also been recently examined by (Averbuch & Katzper, 2001). In their study the analgesic effects of placebo were examined in a dental model (third molar extraction) of acute pain. No sex difference was found. However, in their study, several problems can be identified.

Firstly, the subject population was pooled from a number of different studies, with different experimenters, and presumably, slightly different techniques. For example, was a standardised set of instructions given to each participant in the study as to the pain endpoints etc? Was the surgical technique identical?

Secondly, a greater fraction of female subjects required rescue medication (thus excluding them from further study) – indicating placebo was less efficacious in women. For example, at 3 hours post-medication about 70% of males had utilised rescue medication, compared to about 79% of females. Furthermore, rescue medication in females was requested earlier than in males, albeit only slightly (90 minutes *cf* 93 minutes, respectively).

Thirdly, a fairly insensitive efficacy endpoint was used (only a 4-point numerical scale for pain intensity, and 5-point categorical scale for pain relief), which did not allow the examination of smaller effects between the sexes.

Fourthly, no standard deviations or standard errors in the pain intensity or pain relief scores were provided to allow the reader to easily examine differences in

the variability between the sexes. This is a crucial omission which casts doubt on the validity of the whole experiment.

Finally, the experimental model used involves a great deal of concomitant inflammation, so placebo-induced analgesia cannot be easily separated from placebo-induced anti-inflammation. Therefore Averbuch & Katzper (2001) have not allowed the reader to draw any conclusions from their experiments. Thus their assertion that there are no sex differences in placebo response is viewed with great scepticism.

Another important question arising from the experiment outlined in this chapter is why males display expectancy while females do not. While it could be asked why females lack this effect, probably the more relevant question is why men *do* have this expectancy response.

Several issues need to be addressed in order to answer this question. *Firstly*, and probably most importantly in terms of this thesis, is whether the difference in expectancy effect seen in the experiment outlined in this chapter was influenced by *sex* or *sex hormone status*. While this issue has not been directly addressed in the present experiment, it is hypothesised that while the pharmacological effect of ibuprofen is determined by *sex hormone status* (see Chapter 2), the psychological effect of expectancy is determined by *sex*. This hypothesis was made on the basis that it seems unlikely expectancy would change in women pre- and post-menopause. Further experiments are required in this regard.

Secondly, was the increase in analgesia during positive expectancy state caused by a reduction in anxiety? Placebo effects are often attributed to anxiety

reduction – and in the present study the noxious electrical stimulation paradigm may have induced anxiety in normally non-anxious subjects. For example, several studies have shown that during ischaemic pain in the arm, if the placebo can decrease anxiety, pain tolerance will increase (for review see Evans, 1985). The question then becomes: why do men benefit from the reduction in anxiety, while women do not. There are several possible answers to this question. Firstly, women may not have been anxious in the first place – thus the placebo was of no benefit; secondly, women did not believe that the substance they were to be given was analgesic at all (while this is theoretically possible, on the basis of previously published research, the experimenter was very careful not to alert the subjects to this information); finally, males may be more anxious when facing pain situations.

Another possibility is that men are more likely to be affected by psychological deception or persuasion. This study was carefully designed, so that the experimenter was kept unaware of the treatment information given to the subject. This may have fuelled the subject's belief in the plausibility of the treatment. That is the subjects believed that when they were told they received ibuprofen, they believed they received ibuprofen. The experimenter could not have validated this assumption, because she was blinded to the treatment administered. In addition, during debriefing, female subjects were more likely to state they thought psychological deception was involved, whilst male subjects tended to express surprise at the experimental design.

3.5 FUTURE DIRECTIONS

This chapter has highlighted several important issues that need further consideration. These include:

- *To determine the effect of expectancy (using the balanced placebo design) on the anti-inflammatory effects of ibuprofen in male and female subjects.*
- *To discover whether, by blocking endogenous opioids with naltrexone, the placebo response in analgesic paradigms in males is mediated by β-endorphins.*
- *To elucidate whether the magnitude of analgesia in positive expectancy states is dependent upon the perceived or actual analgesic potency (e.g. a weak NSAID compared to morphine).*
- *To determine if expectancy is dependent upon sex or sex hormone status, by analysing pre- and post-menopausal women. Also to discover the effect of the menstrual cycle on expectancy responses.*
- *To find out the role of anxiety in men, to determine whether the placebo-induced analgesia resulted from a reduction in anxiety.*

3.6 FINAL COMMENTS

This study has confirmed that dosages of 800mg of ibuprofen are ineffective in women regardless of their expectations. In men, analgesia is dependent upon expectancy – with positive expectancies resulting in analgesia, regardless of treatment (placebo *vs.* ibuprofen). As the time-course of analgesic effect of ibuprofen in males was different to that observed previously in a non-expectancy paradigm, it is hypothesised that ibuprofen analgesia produced by a combination of specific pharmacological effects and a non-specific β-endorphin-mediated placebo effect.

Regardless of the mechanism responsible for the analgesic response seen in males, this research should re-emphasise, for everyone in the clinical pain context, the importance of psychological factors in determining drug response. It also shows that these factors can differ between men and women. Therefore the contribution of psychological factors on analgesia needs to be widely evaluated.

Chapter 4

SEX AND SEX HORMONAL DIFFERENCES IN BASELINE PAIN

4.1 INTRODUCTION

Chapters 2 and 3 both examined the effect of sex and sex hormone status on baseline pain and analgesic response to ibuprofen. This chapter is designed to more closely scrutinize the baseline pain variables (pain threshold, pain tolerance and visual analogue scale (VAS) scores), by using the combined subject pool from Chapters 2 and 3.

The hypothesis had four aspects, *firstly*, that there would be differences in baseline pain threshold, pain, tolerance and visual analogue scale scores between males and females; *secondly,* that there would be differences in baseline pain responses between +SH and –SH subjects (as defined in Chapter 2); *thirdly*, that there would be differences in baseline pain responses in oral contraceptive users versus non users; and *finally*, that there would be influences of the menstrual cycle on baseline pain measures.

4.2 METHODS

The baseline pain measurements (pain threshold, pain tolerance and VAS scores) from the young females (n=19), young males (n=19), older females taking exogenous hormones (EXH, n=10), older females not taking exogenous hormones (NIL, n=10) and older males (n=10) as determined using the methods outlined in Section 2.2 were combined with those obtained from the young male (n=10) and female (n=10) subjects in the experiments of Chapter 3 (as per methods Section 3.2). Of the total pool of young females, 10 were taking oral contraceptives (35%), while the other 19 were cycling physiologically (65%). In the age range of 20 to 44, approximately 23% have been reported to take oral contraceptives, but this rate goes up to around 33% in women aged 20-24 (Wreje *et al.*, 1997). Baseline data from all three experimental days (Chapter 2) or four days (Chapter 3) were entered into a repeated measures ANOVA matrix.

For the examination of *sex differences* the data were partitioned according to sex, so that there were forty-nine females and thirty-nine males.

When *sex hormone status differences* were analysed, the subjects were grouped according to their presumed levels of female sex hormones, thus the older males, young males and older females not taking exogenous hormones (NIL) from chapter 2, were combined with the males from chapter 3, and called '-SH' (meaning female sex hormones minimal; n=49). The young females and older females taking exogenous hormones from Chapter 2 were combined with the

females from Chapter 3 (all of whom were pre-menopausal) and called '+SH' (meaning that a greater level of female sex hormones were present, n=39). The justification of this methodology can be found in Section 2.2.6.

4.2.1 Statistical Considerations

All data were tested for normality and equal variances. Data are expressed as mean ± SEM for normally distributed data and median [range] for data that did not fit the normal distribution.

Differences in baseline pain threshold, pain tolerance and VAS scores which may have arisen as a result of sex differences; *or* sex hormone status differences; *or* differences arising from the used of oral contraceptives in young females, were all explored using repeated measures ANOVA with *post hoc t*-tests and Bonferroni correction. Variability in baseline pain threshold, pain tolerance and VAS scores that may have arisen as a result of differences in menstrual cycle stage in young females were explored using one-way ANOVA with *post hoc t*-tests and Bonferroni correction for pain threshold and tolerance levels, and Kruskal-Wallis one-way ANOVA for VAS scores, as the data proved to differ from the normal distribution.

All Analyses were performed using the statistical program SPSS [SPSS Inc, Chicago, Illinois, USA]; with values of $P < 0.05$ considered statistically significant.

4.3 RESULTS

4.3.1 Sex Differences

Summary

- *No significant sex difference in baseline pain threshold (Figure 4.1).*
- *No significant sex differences in baseline pain tolerance (Figure 4.2).*
- *Males had significantly greater VAS scores than females (Figure 4.3).*

A total of 88 people were included in this analysis: 49 females and 39 males. The demographics of the subjects by sex are shown in Table 4.1. Males were significantly heavier than female subjects ($P < 0.0001$, unpaired *t*-test), but there were no significant differences in age or ear thickness.

Table 4.1: Subject demographics by sex.

Group	Age (years)	Weight (kg)	Ear Thickness (cm)
Females (n=49)	38.9 ± 2.4	64.0 ± 1.4	0.49 ± 0.02
Males (n=39)	36.2 ± 2.3	76.9 ± 2.3*	0.48 ± 0.02

** denotes significant difference from female weight (P < 0.0001, unpaired t-test)*

The baseline pain thresholds, pain tolerances and visual analogue scale (VAS) scores of males and females are shown in Figures 4.1, 4.2 and 4.3 respectively. While there was no significant difference in pain threshold or tolerance levels between men and women ($P \approx 0.61$ and 0.31 respectively, repeated measures ANOVA), the males had significantly higher VAS scores than the females ($P < 0.05$, repeated measures ANOVA).

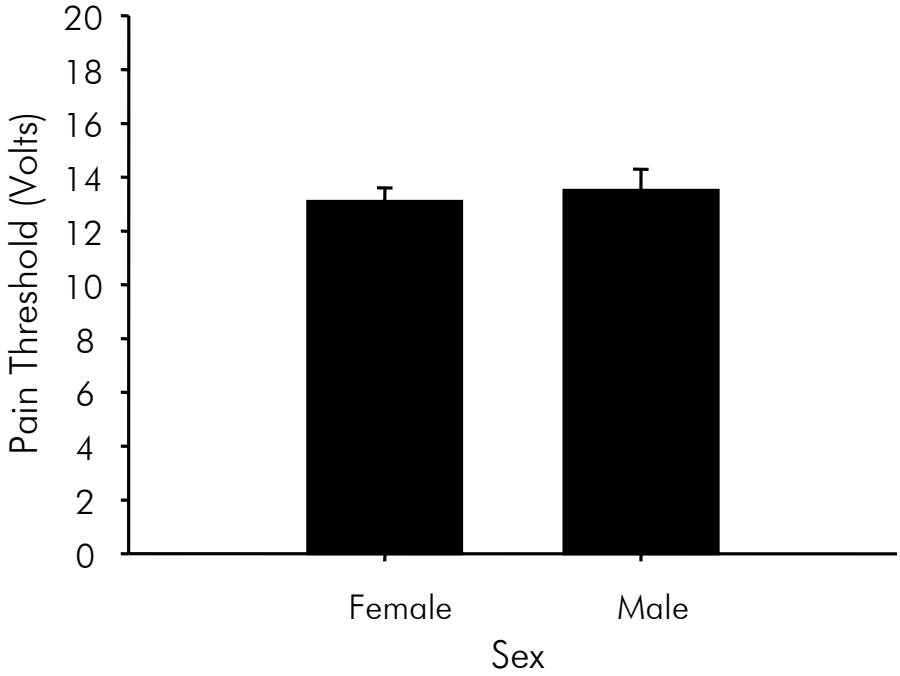


Figure 4.1: Baseline pain threshold level in Volts of all female (n=49) and male subjects (n=39).

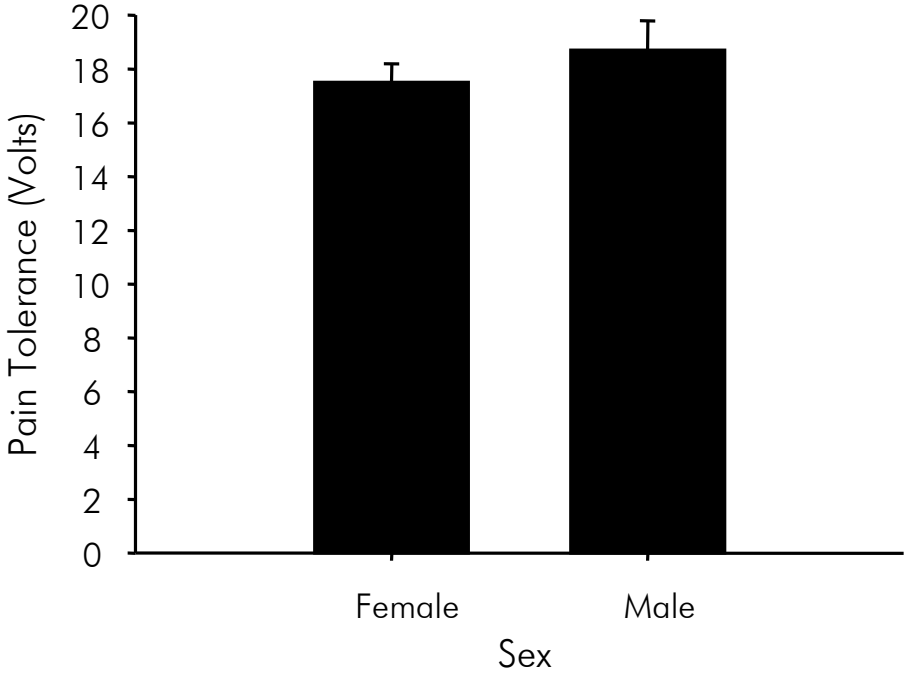


Figure 4.2: Baseline pain tolerance levels in Volts of all females (n=49) and males (n=39).

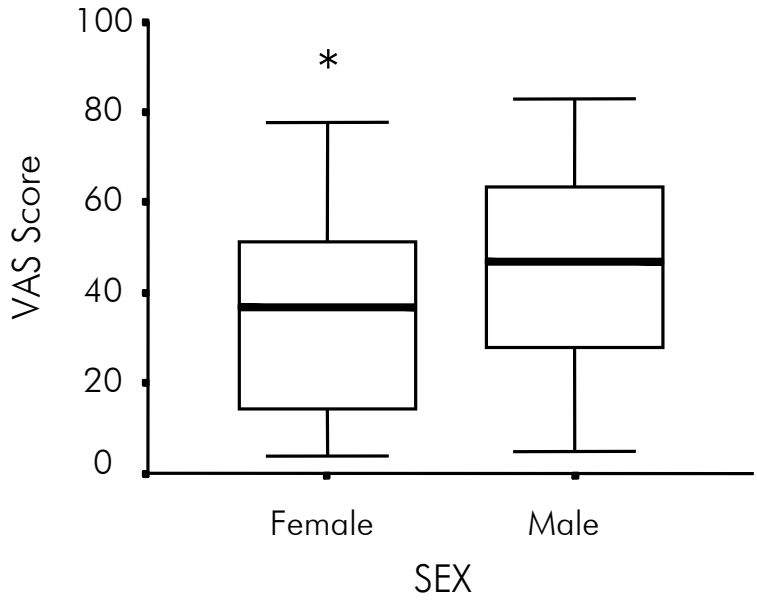


*Figure 4.3: Box-plot of Baseline VAS scores at the pain tolerance level in females (n=29) and males (n=29). Dark line represents median value; each box represents the inter-quartile range (i.e. covering 50% of all responses); the lower whisker represents minimum score; the upper whisker represents maximum score. * denotes significant difference from male VAS score (P < 0.05; repeated measures ANOVA).*

4.3.2 Sex Hormone Status Differences

Summary

- *No significant sex hormone status difference in baseline pain threshold voltage (Figure 4.4).*
- *The sex hormone status differences in baseline pain tolerance voltage approached significance ($P \approx 0.07$; Figure 4.5).*
- *-SH subjects had significantly greater VAS scores than +SH subjects (Figure 4.6).*

To test the secondary hypothesis (that there would be differences in baseline pain responses between +SH and –SH), the subjects were repartitioned according to their sex hormone status. The demographic variables by sex hormone status are shown in Table 4.2. Subjects in the –SH group were significantly heavier and older than those in the +SH group ($P < 0.05$, unpaired *t*-test), but there was no significant differences in ear thickness.

Table 4.2: Subject demographics by sex hormone status

Group	Age (years)	Weight (kg)	Ear Thickness (cm)
+SH (n=39)	34.3 ± 2.5	62.8 ± 1.6	0.47 ± 0.02
-SH (n=49)	40.3 ± 2.2*	75.2 ± 1.9†	0.49 ± 0.02

** denotes significant difference from +SH age (P < 0.05, unpaired t-test).*
† denotes significant difference from +SH weight (P < 0.0001, unpaired t-test).

The baseline pain thresholds, pain tolerances and visual analogue scale (VAS) scores of +SH and –SH subjects are shown in Figures 4.4, 4.5 and 4.6 respectively. There were no significant differences in baseline pain thresholds that may have arisen from differences in sex hormone status ($P \approx 0.35$, repeated measures ANOVA). However, the difference between +SH and –SH subjects in baseline pain tolerance level closely approached the conventionally chosen level

of significance ($P \approx 0.07$, repeated measures ANOVA). –SH subjects had significantly greater VAS scores than +SH subjects ($P < 0.01$, repeated measures ANOVA).

Figure 4.4: Baseline pain threshold levels in Volts for +SH (n=39) and –SH subjects (n=49).

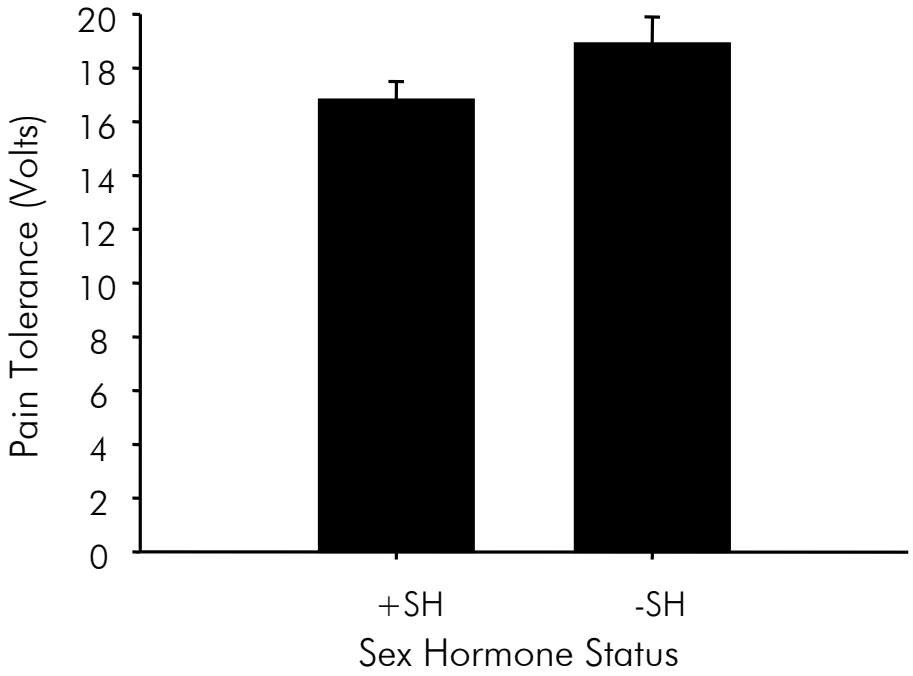


Figure 4.5: Baseline pain tolerance levels in Volts for +SH (n=39) and –SH subjects (n=49).

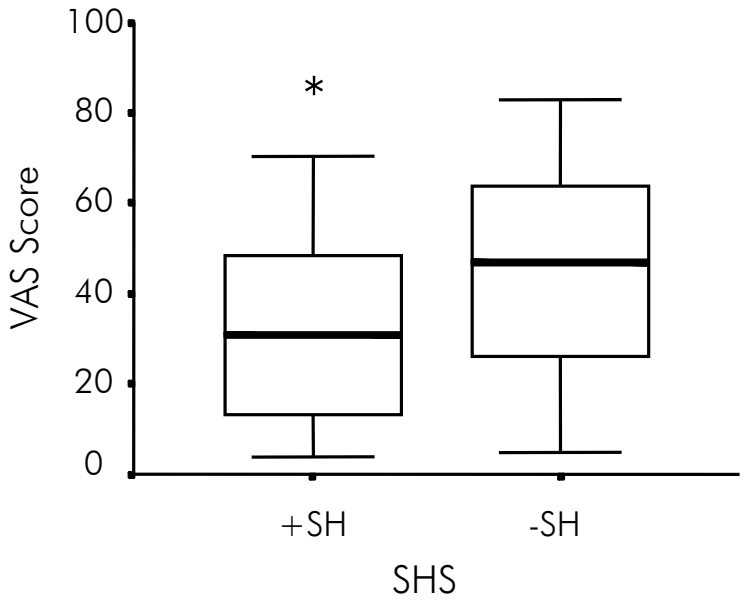


Figure 4.6: Box-plot of Baseline VAS scores in +SH (n=39) and -SH (n=49). Dark line represents median value; box represents inter-quartile range (covering 50% of all responses); lower whisker represents minimum score; upper whisker represents maximum score.
** denotes significant difference from male VAS score (P < 0.05; repeated measures ANOVA).*

4.3.3 Effect Of Oral Contraceptives In Young Females

Summary

- *No significant difference in baseline pain levels (pain threshold, pain tolerance or VAS scores) between subjects taking oral contraceptive preparations and those who were not (Table 4.4).*

To determine whether exogenous hormones influence baseline pain response, the young females' data were then further partitioned into those taking oral contraceptives (n=10) and those who were not (n=19; Table 4.3). Those taking oral contraceptive preparations were significantly younger than those young females who were not taking oral contraceptive preparations ($P < 0.05$, unpaired *t*-test), but there were no differences in weight or ear thickness.

Table 4.3: Subject demographics by oral contraceptive use in young females

Group	Age (years)	Weight (kg)	Ear Thickness (cm)
Taking Oral Contraceptives (OC; n=10)	22.2 ± 1.1	62.1 ± 1.5	0.51 ± 0.03
Not Taking Oral Contraceptives (NOC; n=49)	28.1 ± 2.0*	61.5 ± 2.9	0.48 ± 0.02

** denotes significant difference from those taking oral contraceptive preparations ($P < 0.05$, unpaired t-test).*

There were no differences in baseline pain threshold, pain tolerance or VAS scores between those who took oral contraceptives, and those who did not ($P \approx$ 0.83, 0.71 and 0.75 respectively, repeated measures ANOVA, Table 4.4).

Table 4.4: Baseline pain levels in young females by oral contraceptive use.

Group	Baseline Pain Threshold (Volts)	Baseline Pain Tolerance (Volts)	Visual Analogue Scale Score
Non-Oral Contraceptive Users (n=19)	13.3 ± 0.6	16.9 ± 0.7	24.7 [3.6 – 73.8]
Oral Contraceptive Users (n=19)	12.1 ± 0.8	16.0 ± 1.0	28.6 [3.1 – 56.0]

4.3.4 Effect of Menstrual Cycle on Baseline Pain Responses in Young Females

Summary

- *Those taking the active component of their oral contraceptive preparation had lower pain thresholds than those cycling physiologically in their luteal or follicular phases (Figure 4.7), and lower pain tolerances than those in the luteal phase (Figure 4.8).*
- *No differences in VAS scores were found as a function of menstrual cycle phase (Figure 4.9).*

To elucidate whether there were menstrual cycle influences on baseline pain responses, the young females' data were partitioned by menstrual phase. Those cycling physiologically were divided into menses, follicular and luteal phases, whilst those taking oral contraceptive therapy were divided into menses and active components. The results obtained when the subjects were in their menses phase were combined, as there was no difference between those taking oral contraceptive preparations and those who were not, see Section 4.3.3, and in both users and non-users, the levels of oestrogen and progesterone are low. Baseline pain threshold, pain tolerance and visual analogue scale scores by menstrual phase are shown in Figures 4.7, 4.8 and 4.9 respectively.

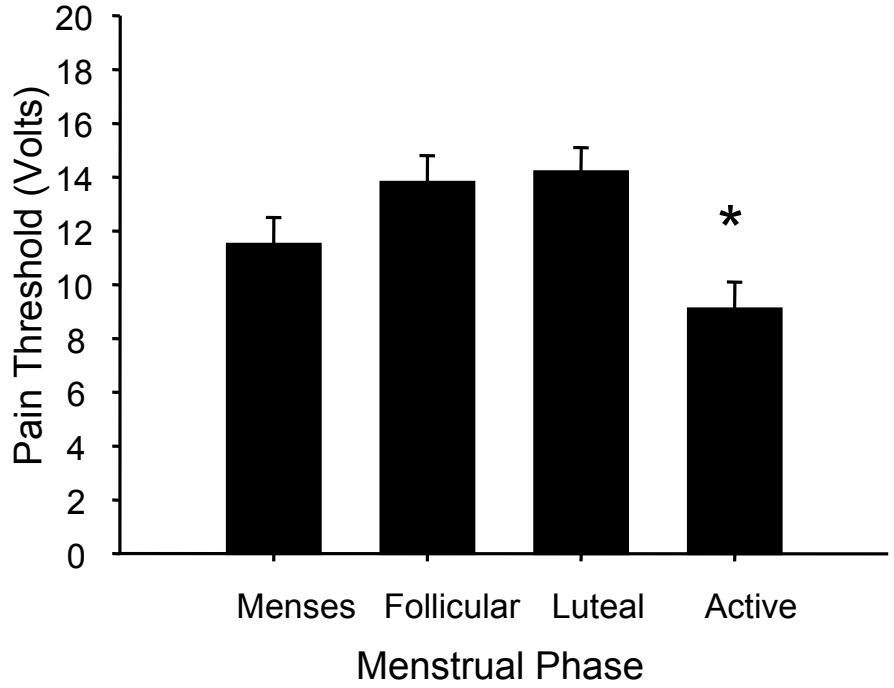


Figure 4.7: Baseline Pain Threshold levels in young women by menstrual phase (menses n=15; follicular n=23; luteal n=20; active n=11).
** denotes significant difference from subjects in the luteal and follicular phases (P < 0.05; one-way ANOVA with post-hoc Bonferroni correction).*

Those taking the active component of the oral contraceptive pill had significantly lower pain threshold levels than those cycling physiologically in the luteal or follicular phases ($P < 0.05$, one-way ANOVA with post-hoc Bonferroni correction; Figure 4.7). They also had significantly lower pain tolerance levels compared to those in the luteal phase ($P < 0.05$, one-way ANOVA with post-hoc Bonferroni correction; Figure 4.8). No differences in VAS scores were found amongst the different menstrual phases ($P > 0.05$, Kruskal-Wallis one-way ANOVA; Figure 4.9).

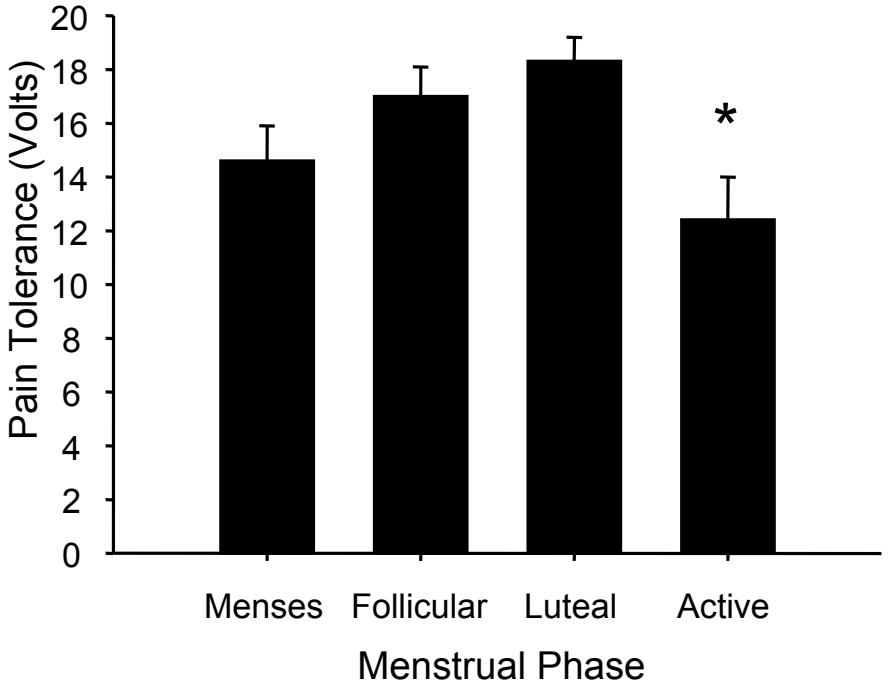


Figure 4.8: Baseline Pain Tolerance levels in young women by menstrual phase (menses n=15; follicular n=23; luteal n=20; active n=11).
** denotes significant difference from subjects in the luteal and follicular phases. (P < 0.05; one-way ANOVA with post-hoc Bonferroni correction).*

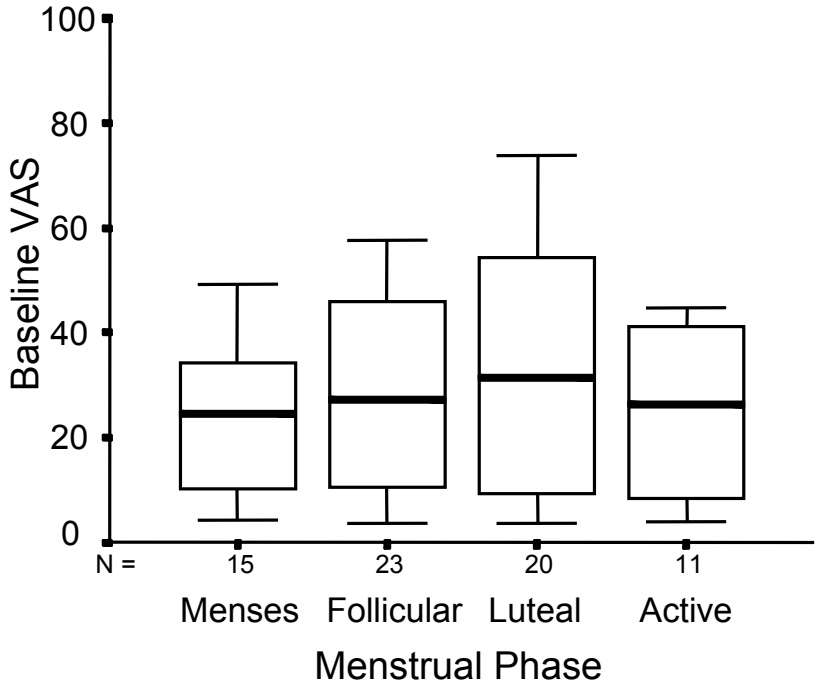


Figure 4.9: Box-plot of Baseline VAS scores in young women by menstrual phase (menses n=15; follicular n=23; luteal n=20; active n=11). Dark line represents median value; box represents inter-quartile range (covering 50% of all responses); lower whisker represents minimum score; upper whisker represents maximum score.

4.4 DISCUSSION

Many researchers have attempted to determine whether there are sex differences in baseline pain responses (for review see Section 1.5 of the Introduction). However, the issue remains controversial, as methodological differences have hampered easy comparison between studies. It is, however, generally considered that males have higher pain threshold and tolerance levels, and lower pain ratings. Importantly, almost all studies have examined only sex differences *i.e.* male data vs. female data, without considering that sex hormonal status may also be the crucial influence. However, the results of Chapter 2 suggested that female sex hormone status is an important factor affecting nociceptive and analgesic drug response.

4.4.1 Sex and Sex Hormone Status Differences

No significant differences in baseline pain threshold ($P \approx 0.61$) or tolerances ($P \approx 0.31$) were found between men and women (Figures 4.1 and 4.2), although, as stated earlier, male values were numerically higher for both measures. Males had significantly higher VAS scores than females in this subject population (Figure 4.3).

This raises an important question: is the conventionally chosen level of significance of 5% meaningful in such psychophysical studies? The arbitrary cut-off of $P < 0.05$ was used prior to computer-assisted statistical analyses, and statistical tables were the only method of determining probabilities. With the advent of computer statistical packages, *P* values can be determined more

precisely. This prevents investigators from concluding that a result is significant when $P = 0.05$, but not when $P = 0.06$ – which is a dubious distinction. Indeed, considering the wide variability, and the number of confounding variables involved, a cut-off significance of 10% might be more readily justifiable in psychobiosocial studies.

In a previous study, using an identical noxious stimulation protocol (with the exception that the endpoint was pushed further, until no further increase in stimulus was acceptable to subjects), Walker & Carmody (1998) reported sex differences in pain threshold and pain tolerance voltages; with males (n=10) having higher voltages than females (n=10) in both cases. However, a re-examination of the raw data revealed erroneous statistical analyses – producing standard errors of the mean that were at least twofold smaller than they should have been. Re-analysis of these results using repeated measures ANOVA (with a significance level of $P < 0.05$), found no significant differences between males and females for pain threshold ($P \approx 0.10$), pain tolerance ($P \approx 0.07$) or visual analogue scale scores (this measure was not reported in the original paper, $P \approx 0.67$). Although if the new criterion of $P < 0.10$ is used, then the differences in pain threshold and tolerance remain. This was not the case in the present experiment however, where no difference in pain threshold or pain tolerance were found ($P \approx 0.61$ and 0.31 respectively).

There are several possible explanations for the difference in results; *firstly* the subject population was older in the present experiment than that used in the Walker and Carmody study (37.7 ± 1.9 *vs.* 21.6 ± 0.7); *secondly*, the training

of subjects may also have been different between the two studies, in Walker and Carmody's study, a slightly different endpoint was used, with subjects asked to push the endpoint further until they could not accept any further increase in the stimulus (*c.f.* in the present study, subjects were asked to terminate the stimulus when it became deep and burning); and *finally,* Walker and Carmody's study may really reflect *sex hormone status differences* rather than *sex differences:* a point which they did not consider. In the study presented in this chapter, the females were both pre- and post-menopausal, *i.e.* both +SH and –SH: in Walker and Carmody's study, all females were pre-menopausal (*i.e.* all +SH).

Once reanalysis of the results on the basis of sex hormone status was completed, no differences in pain threshold voltages ($P \approx 0.35$) were found (Figure 4.4), but differences in pain tolerance ($P \approx 0.07$) voltages were found (based on the criteria of $P < 0.10$; Figure 4.5). Furthermore those in the –SH group had significantly higher VAS scores than those in the +SH group (Figure 4.6). Thus *sex hormone status* appears to be an important predictor for determining basal pain tolerance – and this difference would presumably become statistically significant at the arbitrary 0.05 level in larger subject populations.

The pain experience is sculpted by biological, sociocultural and psychological factors. Sex may influence any one of these factors to produce the differences in pain that are seen in the literature. Several explanations have been proposed for the sex differences that have emerged in studies of both clinical and experimental pain (for reviews see Introduction; Unruh, 1996; Berkley, 1997;

Fillingim & Ness, 2000; Fillingim, 2000; Giles & Walker, 2000). These have included *neurophysiological* factors such as: sex hormones; genes; and differences in endogenous pain inhibition; and *psychosocial* factors such as gender-role expectations, cognitive and affective factors and social learning (Fillingim, 2000). However the distinction between psychosocial and neurophysiological factors is made purely for convenience and neglects that the two are inextricably co-dependent, such that psychosocial factors produce their effects via neurophysiological mechanisms, and neurophysiological mechanisms affect psychosocial processes (Fillingim, 2000). Thus, the approach of attempting to delineate the mechanisms of sex differences into neurophysiological or psychosocial factors is simplistic. Rather it is important to understand that the pain experience is complex, and that it encompasses dynamic interactions between biological, sociocultural and psychological factors, and it is not so relevant to determine which factor is the most important, but rather, to discover the *relative contributions* of each factor in the overall scheme. A brief overview of each factor is provided below.

Biological Considerations

In terms of biological differences, the literature reports that men have higher pain thresholds and tolerances (for review see Riley *et al.*, 1998; Giles & Walker, 2000): however, most of the research examining sex differences in pain response has studied young males and pre-menopausal females, who would have different levels of female sex hormones (for example Feine *et al.*, 1991; Lautenbacher & Rollman, 1993; Fillingim & Maixner, 1996; Koutantji *et al.*,

1998; Robinson *et al.*, 1998; Walker & Carmody, 1998; Fillingim *et al.*, 1999). Thus without their authors appearing to recognise or acknowledge it, these studies, have in fact examined *sex hormone status differences*. The differences in baseline pain levels between males and females (and therefore –SH and +SH) have been explained using the suggestion that males have better endogenous pain inhibition than women. The importance of the descending inhibitory control of pain is increasingly recognised. Indeed, some investigators have proposed that certain forms of chronic pain (temporomandibular disorder and fibromyalgia) reflect a failure of inhibitory systems rather than excessive excitatory ascending input (Maixner *et al.*, 1995; Lautenbacher & Rollman, 1997). The fact that epidemiological studies have shown both of these conditions to be more prevalent in women (Unruh, 1996), supports the postulation that males have better endogenous pain inhibition than women.

There are several levels of the nervous system where sex hormones may be important in affecting nociception. Because of the evidence above, it is likely that the inhibitory modulations of pain signals are the relevant area: perhaps by way of sex hormonal modulation of relevant neurotransmitters such as Substance P, and GABA. For example, Substance P levels have been suggested to alter with the oestrus cycle in rats (Duval *et al.*, 1996a; Duval *et al.*, 1996b), but not with the menstrual cycle in humans (Mohysi *et al.*, 1998); while there is a definite association between sex hormones and the action of GABA (for review see Berkley, 1997) – indeed several groups have shown that both oestradiol and progesterone and its metabolites can increase pain thresholds, probably by the

modulation of GABAergic activity (Frye & Duncan, 1994; Frye & Duncan, 1996; Goodchild *et al.*, 2000; Nadeson & Goodchild, 2000; Nadeson & Goodchild, 2001; Goodchild *et al.*, 2001).

Sociocultural and Psychological Explanations

Sociocultural and psychological expectations influence pain perception and pain behaviour (for review see Unruh, 1996). For example, gender role expectancies can influence sex differences in pain. In a carefully designed study of 391 subjects, (Robinson *et al.*, 2001) have shown that both men and women rate men as less willing to report pain, and women as more sensitive and less enduring of pain than men. They stated that sex accounted for 46% of the variance in subjects' perceptions of gender-stereotyped willingness to report pain, while it accounted for only 15% of the variance of gender-stereotyped pain endurance and 2.4% of the variance of gender-stereotyped pain sensitivity (Robinson *et al.*, 2001). It is therefore postulated that sociocultural and psychological factors can influence pain report (whilst only being marginally involved in pain endurance and pain sensitivity). Therefore, in the experiment outlined in this chapter, the VAS scores are postulated to be affected by sex differences in sociocultural and psychological factors.

The question is now: why is the direction of pain report (VAS scores) in the present study (males higher than females) different to that commonly reported in the literature (females higher than males, for example Feine *et al.*, 1991; Wise *et al.*, 2002)? Was it because a female experimenter was used in the present

study? The difficulty in answering this question is that many (indeed most) studies *fail to specify whether a male or female experimenter was employed*.

Alternately, is the directional difference because sex differences in pain report, while statistically significant, are not clinically relevant? One well designed experiment has produced scales of pain and pain relief, and found that when mild differences are involved (either in pain or pain relief), changes in VAS score of greater than 20% (*i.e.* 20/100) are involved (Wallenstein, 1984). If the sex differences in pain report (as determined using the VAS) are not clinically relevant, some might suggest that this casts aspersions on the numerous clinical and experimental studies that have used this tool. This is not the intention. The VAS has an indispensable role (it is even considered the "gold standard") for clinical pain situations. It has been repeatedly scrutinized and proven useful in the analysis of analgesic action in clinical pain populations (for review see Jensen & Karoly, 1992). In the clinical context that VAS scores need to reduce by at least 20% following mild analgesic treatment (as outlined above), one might consider a VAS difference of 20% between men and women would indicate a true, clinically relevant difference in pain perception. In the present study that difference in VAS score was only 16% (males > females), while other experimental studies have reported 10% (female > male; Wise *et al.*, 2002) and 8% (female > male; Feine *et al.*, 1991) differences: differences which one might consider clinically irrelevant.

That said, there is another possible explanation: could the discrepancy be due to sex differences (perhaps influenced by gender role expectancies) *in the scaling of*

pain report tools, such as the VAS? This idea is very important, especially in the light that subjects in the experiment outlined in this chapter were asked to rate their pain on the VAS at the pain tolerance level. One would assume that at the pain tolerance level, pain perception would be the same in men and women, and therefore that VAS scores would be the same. This was not the case in the present experiment (male *vs.* female, 47 vs. 37; -SH vs. +SH, 47 vs. 31). Another researcher has found that "women's tolerance for electrical stimuli was at a point they themselves described as 5 [out of 10] on the scale (moderate) whereas men went to nearly 7 [out of 10]" (Rollman, 1997). According to the 20% criterion this is just clinically relevant. Unfortunately, the full details of this study have never been published, so a critique of the exact methodology is not possible. However, the investigator (Rollman, 1997), is well respected, and has previously demonstrated a rigorous approach to this type of research, and on this basis, the results are trusted. Taken together, there may be male *vs*. female scaling differences in pain report. This finding should be further explored experimentally: possibly by presenting male and female subjects with stimuli (*e.g.* electrical noxious stimulation) at their pain tolerance voltage level and then asking them to rate the painful sensation on the VAS. Following this, discrete voltage levels could be chosen (say ¼, ½ and ¾ of this voltage), and ask the subjects to rate each level on the VAS. The plots of voltage versus VAS score in males and females could then be compared.

4.4.2 Influence of Oral Contraceptives and of the Menstrual Cycle

There was no difference in baseline pain measures between women taking oral contraceptives and those who were not. However, menstrual cycle influence on pain threshold and tolerance measures were found (*i.e.* those taking the active component of their oral contraceptive, were more pain sensitive than those cycling physiologically).

Giamberardino and colleagues have reported that during constant-current electrical stimulation of the skin via surface electrodes the highest pain thresholds occur during the luteal phase (days 17-22), and the lowest during the peri-ovulatory phase (days 12-16; Giamberardino *et al.*, 1997). Interestingly in that study menstrual variations in pain thresholds depended on the tissue being tested (skin, subcutis, and muscle), although this may have been due to the type of electrode used (needle electrodes were used for subcutis and muscle measurements), and their physical distance from each other (1cm for skin, 1.5cm for subcutis and muscle). Needle electrodes cause significant discomfort to subjects, in the absence of any additional noxious stimulation (Walker *et al.*, 1993): possibly increasing peripheral sensitisation, which in turn may be potentiated by sex hormones. Surface electrodes might not have this effect. In any case, in all tissues, the highest pain threshold (*i.e.* lowest sensitivity) was found during the luteal phase (Giamberardino *et al.*, 1997). This can also be seen in the experiments outlined in this chapter, although it was not statistically significant (Figures 4.7 and 4.8). Several other studies have suggested that the greatest pain thresholds occur during the follicular phase of the menstrual cycle;

however these studies used different stimulus modalities *e.g.* cold pressor (Hapidou & de Catanzaro, 1988), heat (Pfleeger *et al.*, 1997), and ischaemia (Fillingim *et al.*, 1997; Pfleeger *et al.*, 1997). It is intriguing why electrical stimulation produces the greatest thresholds during the luteal phase, whilst other stimulation types tend to produce the greatest thresholds during the follicular phase.

Presumably this is due to the differences in the dimensions of pain stimuli of electrical, thermal and ischaemic nature, and the influence of sex hormones on these dimensions. For example, while electrical stimulation is brief, producing acute pain that disappears upon termination of the stimuli (Fillingim & Ness, 2000). There may also be an anxiety component associated with electrical methods as some associate it with electrocution or electric shock. Ischaemic methods produce sub-acute tonic pain sensations that stimulate deeper structures such as muscle (Fillingim & Ness, 2000). They can be considered inescapable, as the sensation is slow to develop, and continues for some time after the tourniquet is released. Furthermore, ischaemic methods may produce tissue damage, and they certainly have an inflammatory component, which sensitises the "sleeping nociceptors" (Fillingim & Ness, 2000), all of which are minimal in electrical methods. Thermal tests are generally acute cutaneous sensations that involve minimal inflammation. Because these stimulus modalities are so different, it is not surprising that there are different menstrual cycle influences on them. Further research is required to determine the exact influence of the menstrual cycle on these different stimulation methodologies.

The present study found no difference in VAS scores between women taking oral contraceptives and those who were not. Furthermore, no menstrual cycle influence on pain report was found. This suggests that pain report is independent of biological factors such as sex hormonal fluctuations in young women. It is therefore hypothesised that pain report is influenced by other factors, such as sociocultural and psychological factors, as discussed in Section 4.4.1.

The present results must be interpreted with caution: *firstly*, only a relatively small number of subjects are included in the menstrual cycle analyses; *secondly*, one of the difficulties with this type of research is defining and correctly determining the menstrual cycle phases, and a limitation of this study was that no hormonal confirmation of menstrual cycle phase was possible. In this study, the menstrual cycle phases were calculated retrospectively: menses (all days of bleeding), follicular (from the end of bleeding to about day 14) and luteal (the 14 days prior to bleeding). For subjects with cycles longer than 28 days, the extra days were assigned to their follicular phase, following customary practice (Hapidou & Rollman, 1998).

4.5 FUTURE DIRECTIONS

It is now clear that men and women (pre- and post-menopausal) should be used to all pain research, both clinical and experimental. Several areas of importance have emerged from this study which require further attention:

1. *Large studies, using many different noxious methodologies, are required to determine the influence of sex hormone status on baseline nociception.*
2. *Menstrual cycle effects on baseline pain responses need to be carefully examined, preferably with hormonal confirmation of cycle stage.*
3. *The effects of sex hormones (oestrogen, progesterone and testosterone) on neurotransmitters of the pain system need to be determined.*
4. *The potential scaling differences in VAS between men and women require rigorous exploration.*

4.6 FINAL COMMENTS

The experiments outlined in this chapter have provided a possible mechanism for the reported sex differences in nociception between men and women: differing levels of female sex hormones. This hypothesis is strengthened by the finding that these parameters are influenced by the female menstrual cycle, further implying that the documented sex differences are partially accounted for by active biological differences: that is differences in the levels of female sex hormones. Further experiments are required to rigorously test this hypothesis (see Section 4.5).

While a statistically significant difference in pain report (VAS score) between men and women was found, it is hypothesised that while the difference is not clinically relevant as the magnitude was so small, there may be scaling differences in VAS between men and women. Furthermore, as there was no menstrual cycle influence on VAS scores, or strengthening of the male-female VAS difference as a function of sex hormone status, this factor is probably not influenced by the female sex hormones.

In conclusion, there are sex differences in baseline pain tolerance levels that are influenced by sex hormones. The identification of specific mechanisms for these differences remains elusive, but researchers should focus their attention on areas where sex hormonal influence is probable.

Chapter 5

INFLUENCE OF SEX HORMONES ON NSAID ANALGESIA IN RATS

5.1 INTRODUCTION

> *"We are who we are because of hormones. We are the result of our chemistry"*[1]

In both humans and animals many physiological and clinical responses, including those to analgesic drugs, are influenced by sex (see Introduction). While it is inappropriate to assume that all sex differences which vary with oestrus or menstrual stage result specifically from the actions of sex hormones (Magos, 1988; Reisert & Pilgrim, 1991), given the substantial qualitative and quantitative changes in sex hormones that *do* occur over the ovarian cycle, it is important to study their effects on analgesic response, especially given the results of the experiments outlined in Chapters 2 to 4.

It is hypothesised that the sex difference observed in the previous study of Walker & Carmody (1998) was attributable to differences in sex hormone status of the subjects rather than their sex *per se* (see Chapters 2 and 3). Therefore, the aim of the present study was to determine the role of the sex hormones oestrogen and

[1] Pease A. & Pease B. (1999) *Why men don't listen and women can't read maps – how we're different and what to do about it.* Pease Training International, Mona Vale, Australia. Pg 60.

progesterone in nociception and analgesic response to ibuprofen, in an animal model.

5.2 METHODS

One hundred female Sprague-Dawley rats (150-200g, University of NSW Biological Resources Centre) were housed according to ethical guidelines: in large cages (20 x 40 x 65.5cm with 4 to 5 rats per cage) containing thick bedding, nesting material (shredded paper) and hiding spaces (cardboard boxes). Food [Rat Chow, Gordon's Specialty Stockfeeds, Yanderra, NSW, Australia] and water were provided *ad libitum*. The animals were handled for 30 minutes daily by the experimenter and subjected to a 12 hour light-12 hour dark cycle (lights on at 0600) for one week after delivery.

They were then anaesthetised with a ketamine-xylazine mixture and ovariectomized (OVX) via the dorsal route [ketamine 50mg/kg *i.p.*, Parnell Ketamine Injection (100 mg/mL), Parnell Laboratories (Aust) Pty Ltd., Alexandria, NSW, Australia; xylazine 5 mg/kg *i.p.*, Xylazil-20 (20mg/mL), Troy Laboratories Pty Ltd., Smithfield, NSW, Australia]. The animals were allowed to recover in individual cages for 18-21 days and their condition was monitored daily. This timing was to allow washout of circulating sex hormones and restabilization of nociceptive baselines (Drury & Gold, 1978; Frye *et al.*, 1992). During the first postoperative week, the antibiotic enrofloxacin [0.125mg/mL Baytril 25 (25mg/mL), Bayer Australia Ltd., Pymble, NSW, Australia] was administered in the drinking water. After the 18-21 days, the rats were re-housed in groups of 4-5 for the analgesic (n=80), and pharmacokinetic (n=20) studies and the hormone treatment begun.

5.2.1 Analgesic Testing

The animals were divided into 8 groups of 10 rats each, according to the hormonal and NSAID regimes. These groups are detailed in Table 5.1. The ibuprofen dose was chosen following preliminary experiments in intact female rats: it was the lowest dose that produced analgesia in this model; higher doses (greater than 80 mg/kg) caused the animals to show signs of acute toxicity. Hormone treatments were given at 1400 hours on days 1, 2, 3 and 4 of the experimental period. This timing was chosen as it is the commonly reported experimental time period used in nociceptive assays (for example Kest *et al.*, 1999; Mogil *et al.*, 1999): it was near mid-phosphatase - reducing the circadian variability (Kavaliers & Hirst, 1983), and was also used in the other experimental project outlined in Chapter 6.

Table 5.1: The eight experimental rat groups

Ibuprofen Group (70 mg/kg *i.p.* in Phosphate Vehicle Buffer)	Control Group (Phosphate Vehicle Buffer 1 mL/kg *i.p.*)
Oestradiol (50 μg/kg/day s.c., E)	Oestradiol (50 μg/kg/day s.c., E)
Progesterone (5 mg/kg/day s.c., P)	Progesterone (5 mg/kg/day s.c., P)
Oestradiol (50 μg/kg/day s.c.) plus Progesterone (5 mg/kg/day s.c., EP)	Oestradiol (50 μg/kg/day s.c.) plus Progesterone (5 mg/kg/day s.c., EP)
Olive Oil (1 mL/kg s.c., O)	Olive Oil (1 mL/kg s.c., O)

Hormone treatment was given daily for four days. Oestradiol [oestradiol benzoate 5 mg/mL and benzyl alcohol 0.01 mg/mL, Intervet (Australia) Pty Ltd., Castle Hill, NSW, Australia]; Progesterone [Jurox Progesterone Injection, progesterone 25 mg/mL, Jurox Pty Ltd., Rutherford, NSW, Australia, diluted to 5 mg/kg in olive oil]

All experiments were conducted using a blinded, parallel protocol, and the nociceptive testing was completed between 1400 and 1630 hours (near mid-phosphatase). This was to eliminate circadian effects on pain sensitivity (Kavaliers

& Hirst, 1983) or drug effects (Halsas *et al.*, 1999). Control and ibuprofen-treated rats were evaluated on day 5 for pain threshold at -20, -10, 15, 30, 60, and 120 minutes post-ibuprofen or vehicle treatment, using the Tail Pinch method (Takagi *et al.*, 1966). This involved applying a plastic coated alligator clip (closure force 12 N) to the base of the animal's tail and measuring the latency for reflex biting of the clip. If the animal did not respond within 20 seconds, the clip was removed to reduce the risk of damage to the tail; in such cases, response time was recorded as 20s.

One of the difficulties in NSAID research is finding an ethically acceptable pain model that is sensitive to the analgesic effects of the NSAID in question. Such popular tests as the tail flick test and hot-plate test are not sensitive to NSAIDs (Björkman, 1995), and electrical stimulation was ethically unacceptable to the University's Ethics Committee. Furthermore an important aspect of this experiment was to characterise differences in *analgesic* response to the drug and not differences in the *anti-inflammatory* effect, so it was important to use a test that was unequivocally painful, without producing inflammation - this excluded such valuable tests as the formalin and abdominal constriction tests. Thus the tail pinch test, which satisfies the ethical, sensitivity, and nociceptive criteria, was chosen. It also has the advantage of not causing overt damage to the animal.

At the end of day 5 of the experiment, the animals were sacrificed with pentobarbitone [60mg *i.p.*; Lethobarb, Virbac (Australia) Pty Ltd., Peakhurst, NSW, Australia].

5.2.2 Pharmacokinetic Study

Twenty rats were ovariectomized, allowed to recover from surgery (as described above), and allocated to 4 groups (n=5) for hormone replacement (as above). On day 4 of hormone replacement, they were anaesthetised with the ketamine-xylazine mixture and a jugular cannula implanted [polyethylene tubing tipped with Silastic, internal diameter 0.50mm]. Unmodified physiological saline was used to keep the line open since heparin interferes with the binding of acidic drugs to plasma proteins (Sato *et al.*, 1980). The rats were allowed to recover overnight. On the next day (day 5), ibuprofen was administered at 1400 hours [70 mg/kg, *i.p.* Sigma Chemical Co.], and venous blood samples (0.5mL) were collected at 0, 15, 30, 60, 120 and 240 minutes post-treatment, for HPLC analysis of ibuprofen concentration in serum. Technical difficulties prevented blood collection in two of the twenty rats (one oestrogen-treated and one-progesterone treated). All animals were then euthanased as before. These experiments were approved and conducted according to institutional ethical guidelines [Animals Care and Ethics Committee, UNSW, Sydney, NSW, Australia, Approval No. ACEC 99/38].

Ibuprofen Assay

Blood samples were centrifuged (4,800rpm, 5 minutes), the serum transferred to micro centrifuge tubes and stored at –20°C until analysis. Serum concentrations of ibuprofen were determined using HPLC as in the human study, except that the volumes were reduced. Briefly, an aliquot of internal standard (10μL 0.5mM naproxen) was added to serum (100μL) and the mixture acidified with 1M hydrochloric acid (100μL) and extracted with dicholoromethane (1mL). The solvent layer was then evaporated until dry [SpeedVac® Plus SC210A, Thermo Savant,

Holbrook, NY, USA] and the residue redissolved in sodium dihydrogen orthophosphate buffer (40μL 0.025M NaH_2PO_4, with orthophosphoric acid to pH 3.4). The assay then proceeded as described in the human study (Section 2.2.5).

5.2.3 Data Analysis

Pharmacodynamic Analysis

All data were tested for normality and equal variances. Possible sex-hormonal differences in baseline nociception were analysed using one-way ANOVA with post-hoc *t*-tests and Bonferroni correction (Wallenstein *et al.*, 1980). Analgesia was defined simply as *a statistically significant increase in tail pinch latency following treatment compared to baseline*. To assess ibuprofen or vehicle-induced analgesia, the results were analysed with repeated-measures ANOVA, with hormone and drug treatments as covariates.

All analyses were completed using the statistical program SPSS for Windows v11.0 [SPSS Inc., Chicago, Illinois, USA], with values of $P < 0.05$ considered statistically significant. All data are presented as mean ± SEM.

Pharmacokinetic Analysis

To assess possible hormonal influences on serum ibuprofen levels, data were analysed using repeated-measures ANOVA. Serum-concentration data for each rat was used to derive pharmacokinetic parameters as per the human study (Section 2.2.6). One parameter was observed (maximal serum concentration C_{max}), while the others were calculated: apparent serum clearance (CL), apparent volume of distribution (V_d), and terminal half-life ($t_{1/2}$). Sex hormonal differences in apparent clearance, apparent volume of distribution, terminal half-life and maximum serum concentration were explored using one-way ANOVA with post-

hoc *t* tests and Bonferroni correction (Wallenstein *et al.*, 1980). All analyses were completed using the statistical program SPSS for Windows v11.0 [SPSS Inc., Chicago, Illinois, USA], with values of $P < 0.05$ considered significant. Data are presented as mean ± SEM.

5.3 RESULTS

5.3.1 Nociception

Summary

- *No statistically significant differences in baseline tail pinch latency as a function of hormone replacement type (Table 5.2).*

Baseline nociception was measured 10 minutes prior to drug administration, and the results are shown in Table 5.2 as tail-pinch latency (seconds).

Table 5.2: Baseline Tail Pinch Latency for the four hormone groups, OVX, post hormone treatment and pre-drug/vehicle treatment.

Hormone Replacement	Tail Pinch Latency (s) Mean ± SEM
Oestrogen (n= 20)	6.9 ± 1.1
Progesterone (n= 20)	7.1 ± 1.2
Oestrogen + Progesterone (n= 20)	9.5 ± 1.2
Oil (n= 20)	6.4 ± 1.1

There were no statistically significant differences in baseline nociception by hormone treatment ($P > 0.05$, one-way ANOVA with Bonferroni correction).

5.3.2 Analgesic Time-Course

Summary

- *When all rats were observed (n=80), vehicle-treatment (n=40) caused significant hyperalgesia, which was reversed by ibuprofen treatment (n=40; Figure 5.2).*
- *Significant hyperalgesia occurred following vehicle treatment in oestrogen, progesterone and oestrogen+progesterone pre-treated rats (Figure 5.4).*
- *Hyperalgesia reversed in oestrogen pre-treated rats only (Figure 5.4).*
- *No difference in peak serum ibuprofen concentrations between the four hormone-treated groups (Figure 5.5).*
- *Oestrogen pre-treated rats tended to have quicker ibuprofen half-lives of elimination, and progesterone pre-treated rats had significantly reduced clearances (Table 5.3).*
- *The volume of distribution of ibuprofen varied widely across the four hormone treated groups (Table 5.3).*

The time-courses of tail-pinch latency in all ibuprofen and vehicle-treated rats are shown in Figure 5.1. Because of the difficulties of comparing ibuprofen and vehicle treated rats across the different hormone groups due to differing baselines, data were normalised to baseline by subtracting baseline tail pinch latency from every recorded tail pinch latency for each rat (Figure 5.2). Vehicle treatment caused significant hyperalgesia ($P < 0.05$, repeated measures ANOVA), which was reversed by ibuprofen treatment ($P < 0.05$, repeated measures ANOVA; Figure 5.2).

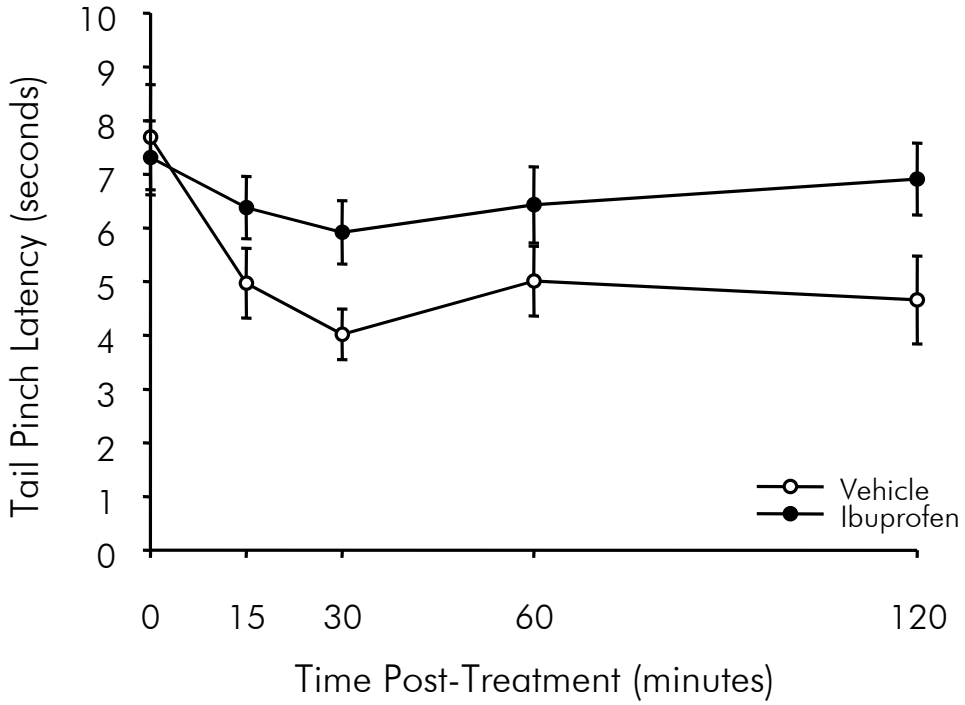


Figure 5.1: Tail Pinch Latency (seconds) in vehicle treated (○, n = 40) and ibuprofen treated 70 mg/kg i.p., ●, n = 40) rats over the time-course of the experiment.

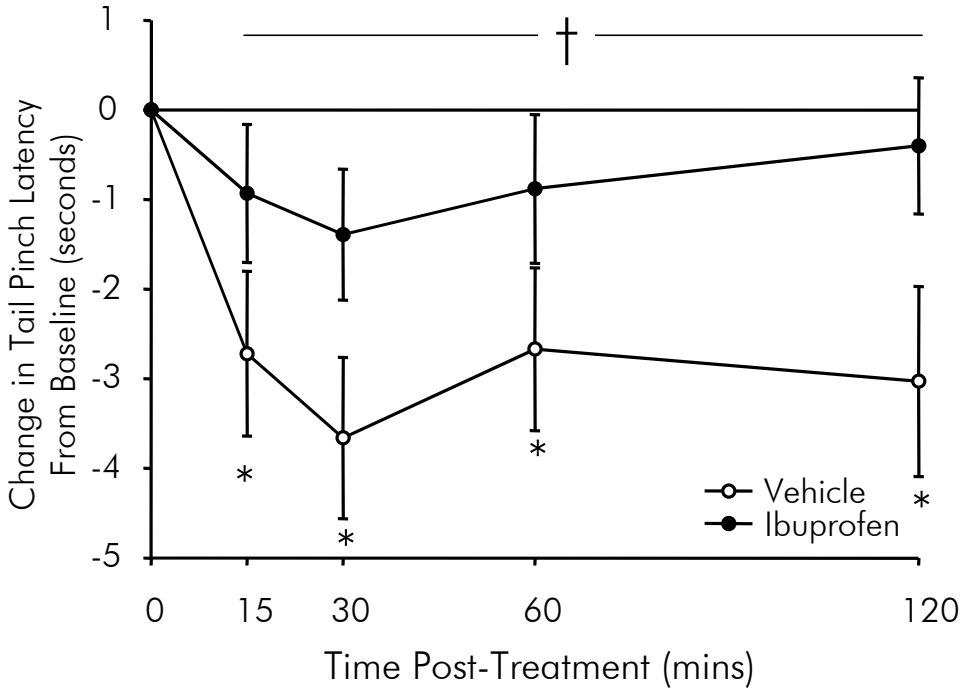


Figure 5.2: Baseline corrected Tail Pinch Latency (seconds) in vehicle treated (○, n = 40) and ibuprofen treated 70 mg/kg i.p., ●, *n = 40) rats over the time-course of the experiment. * denotes significant difference from baseline (time 0) vehicle change in tail pinch latency (P < 0.05, repeated measures ANOVA). † denotes significant difference between vehicle and ibuprofen treatment (P < 0.05, repeated measures ANOVA).*

The time-courses of tail-pinch latencies in the hormone treatment groups are shown in Figure 5.3, and baseline corrected latencies in Figure 5.4. Rats that were pre-treated with oestrogen and progesterone (either alone or in combination) showed significant hyperalgesia following vehicle-treatment ($P < 0.05$, repeated measures ANOVA; Figure 5.4, panels A-C), but no hyperalgesia was present in Oil pre-treated, vehicle-treated rats (Figure 5.4, panel D). Ibuprofen was able to reverse this hyperalgesia *only* in rats who had oestrogen as part of their pre-treatment (Figure 5.4, panels A and C). This effect was significant in oestrogen-treated rats at the 60 and 120 minute time points ($P < 0.05$, repeated measures ANOVA), and approached significance for the 15 and 30 minute time points in oestrogen+progesterone treated rats ($P \approx 0.08$, repeated measures ANOVA).

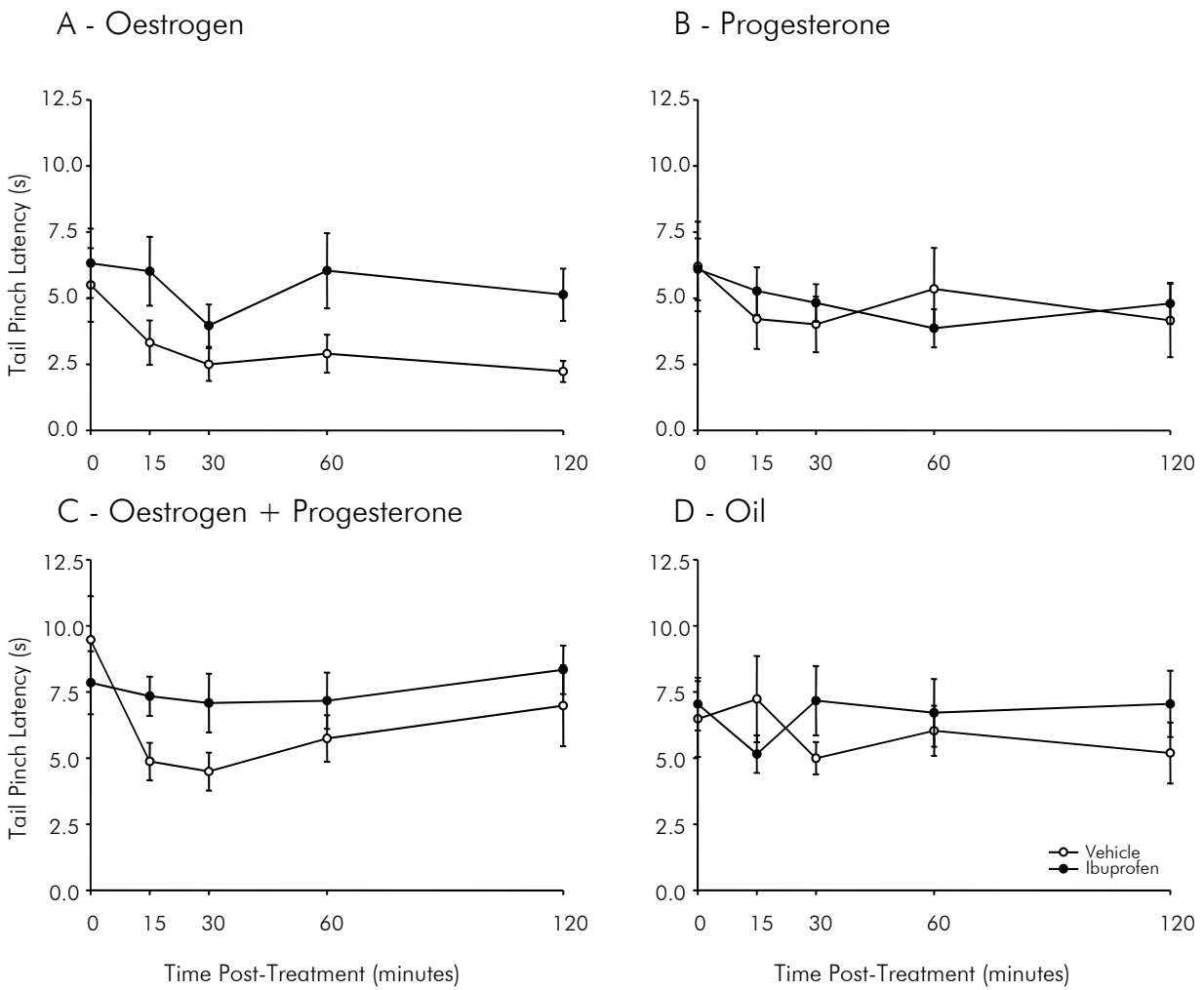


Figure 5.3: Time-course of tail pinch latency following ibuprofen (70 mg/kg i.p.*, ●) or vehicle control (○) in OVX female rats.* ***A*** *Oestrogen treated;* ***B*** *Progesterone treated;* ***C*** *Oestrogen + Progesterone treated and* ***D*** *Oil treated.*

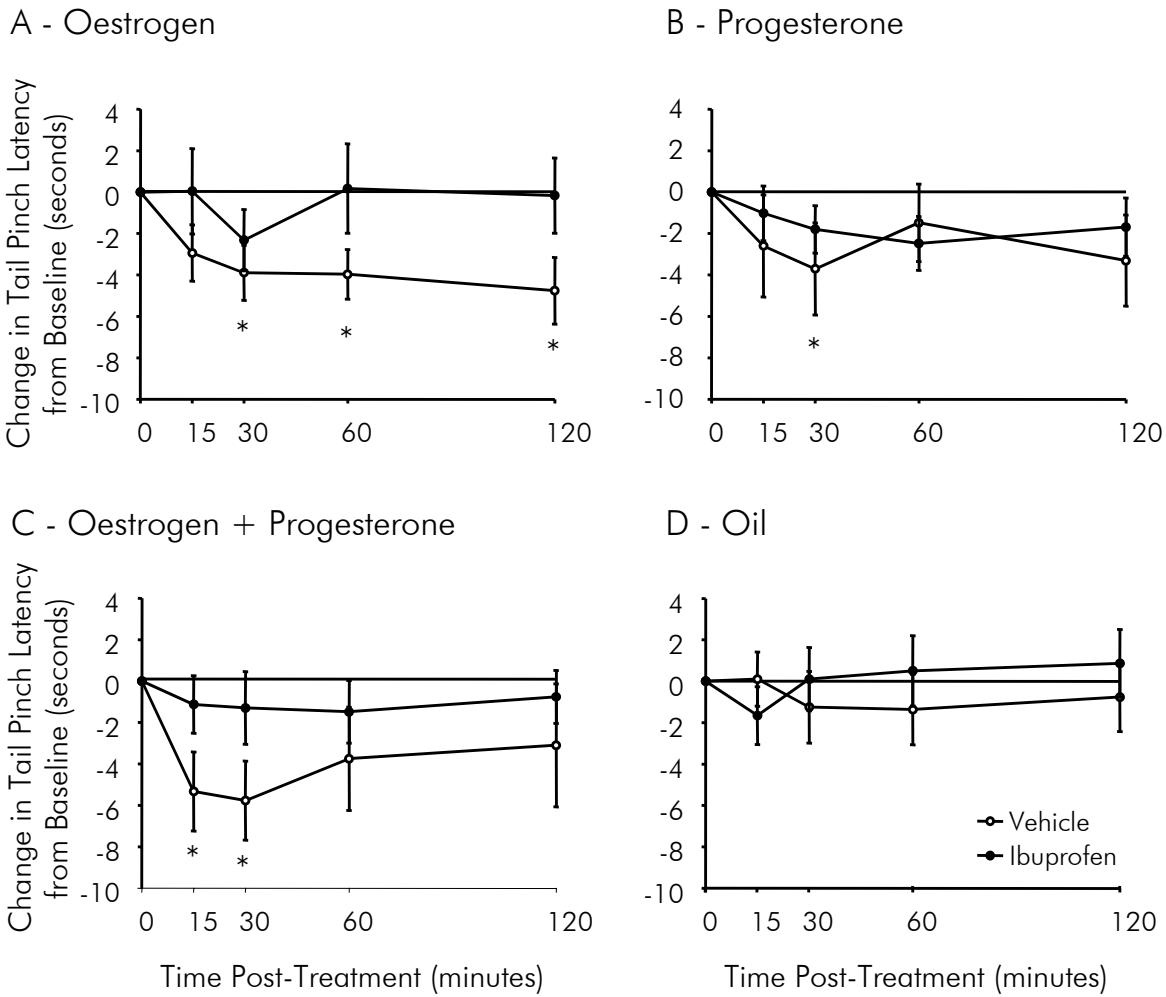


Figure 5.4: Time-course of baseline corrected tail pinch latency following ibuprofen (70 mg/kg i.p., ●*) or vehicle control (○) in OVX female rats.* **A** *Oestrogen treated;* **B** *Progesterone treated;* **C** *Oestrogen + Progesterone treated and* **D** *Oil treated.*
** denotes significant difference from vehicle-treated baseline (*$P < 0.05$*, repeated measures ANOVA).*

5.3.3 Pharmacokinetics

Figure 5.5 shows the relationship between serum concentration of ibuprofen and time for each hormone treatment group. These profiles are very similar despite differences in hormone pre-treatment ($P \approx 0.35$, ANOVA). Peak serum concentrations occurred at the 15 minute sampling point (importantly, they were not delayed with any treatment). The oestrogen pre-treated and progesterone pre-treated rats appeared to have faster rates of decline, but they were not significantly different from those of the oestrogen+progesterone or the oil pre-treated rats ($P > 0.05$, one-way ANOVA, see half-lives in Table 5.3). At the 120 minute time-point, progesterone treated rats had significantly higher serum ibuprofen concentrations than the oestrogen + progesterone treated animals, but there were no other differences between the four hormone treatments ($P < 0.05$, repeated-measures ANOVA with post-hoc *t* tests and Bonferroni correction).

The calculated pharmacokinetic parameters (Table 5.3) were more variable than in the human study (Table 2.5), perhaps because the sample volume represented a greater proportion of the drug dose and thus the serum sampling was a greater stress on homeostasis, and because the sample size in the present study was much smaller. Variation in the amount of absorption from the peritoneal cavity may have also influenced the consistency of these results - in particular, less than complete absorption would lead to inflated values of apparent clearance and volume of distribution. There was no difference amongst the C_{max} values, but clearance was lower in the P animals than in the other groups ($P < 0.05$, one-way ANOVA) and half-life ($t_{1/2}$) was lowest in the E animals (but not significantly so).

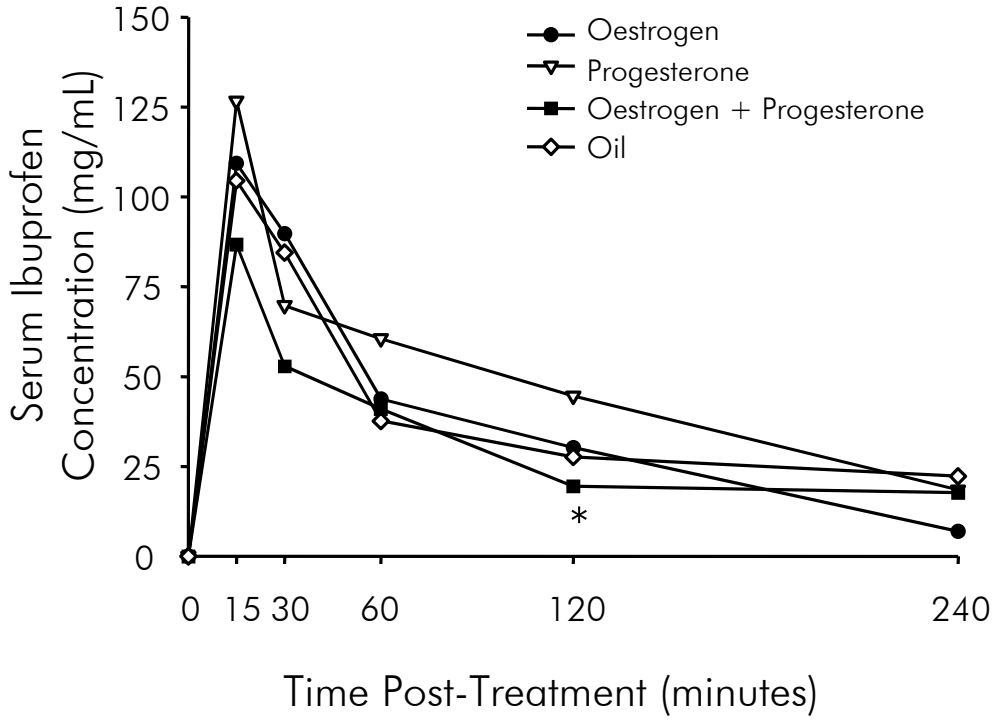


Figure 5.5: Mean ibuprofen serum concentration-time profiles in OVX rats receiving Oestrogen (filled circles, n=4 animals); Progesterone (open triangles, n=4 animals); Oestrogen + Progesterone (filled squares, n=5 animals); and Oil (open diamonds, n=5 animals) following ibuprofen (70mg/kg i.p.) treatment. For reasons of clarity, error bars are not shown: the SEM of a single observation calculated from the ANOVA matrix was 3.9μg/mL.
** denotes significant difference between progesterone-treated and oestrogen + progesterone-treated rats at the 120 minute time-point (P < 0.05, repeated-measures ANOVA with post-hoc t tests and Bonferroni correction).*

Table 5.3: Mean Pharmacokinetic parameters of ibuprofen 70 mg/kg i.p. *according to hormone replacement in ovariectomized rats.*

Parameter	Oestrogen (n = 4)	Progesterone (n = 4)	Oestrogen + Progesterone (n = 5)	Oil (n = 5)
C_{max} ($\mu g.mL^{-1}.kg^{-1}$)	109.4 ± 9.5	134.5 ± 25.7	108.1 ± 51.3	105.4 ± 22.0
CL ($mL.min^{-1}.kg^{-1}$)	7.83 ± 0.75	3.70 ± 0.15*	9.36 ± 2.34	7.21 ± 1.35
$T_{1/2}$ (min)	55.7 ± 6.8	99.6 ± 24.8	101.4 ± 23.5	118.7 ±7.9
VD ($mL.kg^{-1}$)	697.4 ± 95.1	557.7 ± 196.0	1568.9 ± 698.4	1722.3 ± 5.2

** denotes significant difference from all other hormone groups (P < 0.05, Kruskal-Wallis z Test).*

5.4 DISCUSSION

The present experiment shows that in female rats, oestradiol has significant influences on the analgesic efficacy of ibuprofen. This is an important finding because the genesis of these experiments was the quest for an explanation of the well-known variability in the clinical response to NSAIDs, in particular, the possibility that a patient's sex might be an important factor.

5.4.1 Baseline Pain

In the experiments outlined in this chapter, there were no differences in baseline tail-pinch latency by hormone treatment. Previous reports examining sex hormonal effects on basal nociception have been contradictory – possibly because of the different methods used to induce pain (for review see Fillingim & Ness, 2000). Different stimulus modalities have separate physiological mechanisms (Mogil *et al.*, 1996), probably due to differences in location, duration and intensity of the assay. These dimensions may be variably affected by the sex hormones. For example, oestrogen may be able to peripherally sensitise nociceptive neurons (for review see Fillingim & Ness, 2000), especially in the presence of inflammation. Therefore those assays with an inflammatory component may be especially affected by changes in oestrogen level, compared to other methods.

In the animal literature reporting acute pain stimuli in ovariectomized animals with hormone replacement (Drury & Gold, 1978; Forman *et al.*, 1989; Ratka & Simpkins, 1991; Frye *et al.*, 1992), two studies were found where *oestrogen treatment* increased pain sensitivity (Drury & Gold, 1978; Ratka & Simpkins, 1991), one reported no difference (Frye *et al.*, 1992), while a fourth recorded a decrease

in pain sensitivity with the tail-flick test, but hyperalgesia with the hot-plate test (Forman *et al.*, 1989).

Hormone treatment regimes seem to be important, with the attainment of steady state likely to be a factor: Drury and Gold (1978) gave 5μg oestradiol subcutaneously, and then performed nociceptive testing (electric footshock) three hours later; Ratka and Simpkins implanted single 100mg pellets containing 0.5% or 5% oestradiol, and performed the hot-plate test after two or fourteen days; Frye and colleagues (1992) administered 10μg oestradiol only once, with baseline nociceptive testing (tail-flick test) two days later; while Forman and colleagues (1989) treated their rats daily for three weeks with 5μg/day oestradiol and tested at the end of the first, second and third weeks using the tail withdrawal and hot plate tests; while in the experiment outlined in this chapter, rats had four days' treatment with oestradiol 50μg/kg/day, prior to tail-pinch testing on day five. Analysing these different regimes finds that high levels of oestrogen are required to produce the increase in pain sensitivity – in Frye *et al.* (1992), the 10μg dosage of oestradiol had probably significantly reduced by the time that nociceptive testing was performed two days later. The results of Forman *et al.* (1989) are a little difficult to interpret, while they stated that oestradiol treatment increased tail-withdrawal latencies at all testing times (at the end of one, two and three weeks of hormone replacement), oestradiol only caused the decrease in hot-plate latencies after three weeks of treatment. One possible explanation for this variable effect is the different stimulus type used, as the rest of the method was identical. Perhaps stress-induced analgesia was elicited in animals undergoing the tail withdrawal test, which was

performed in animals that were "mildly restrained", and this SIA was enhanced by oestradiol.

When only *progesterone* was administered, both Drury and Gold (1978) and Ratka and Simpkins (1991) found an increase in pain sensitivity. Drury and Gold administered 5mg of progesterone subcutaneously, while Ratka and Simpkins implanted 10% or 75% progesterone in 100mg pellets. The experiment outlined in this chapter used 5mg/kg/day, but found no difference in pain sensitivity compared to control ovariectomized animals.

Of these four, Frye and colleagues (1992) were the only ones to co-administer *oestrogen and progesterone* (10μg oestradiol, 0.5mg or 1mg progesterone). They found a marginal 11% increase in nociception with 1mg progesterone dosages.

While no hormonal differences in baseline nociception were found in the present study, this was probably because it was not optimally designed to address this issue. That is, it did not allow comparisons between tail-pinch latencies in ovariectomized animals pre- and post-hormone. Nonetheless, if both oestrogen and progesterone (both singly and together) produce increases in nociceptive sensitivity (as was the general trend in the studies reviewed above), then it would have been expected that oil-treated rats in the present experiment would have lower nociceptive sensitivity (longer tail-pinch latencies) than their hormone treated counterparts, but this was certainly not the case. Perhaps if increased animal numbers were used, a clearer picture of the effect of exogenous hormones on basal nociceptive sensitivity could have been determined.

5.4.2 Time-course of Tail-Pinch Latencies

Vehicle Treatment

The time-courses of tail-pinch latencies following vehicle treatment were dependent upon hormone pre-treatment. For example, following vehicle treatment in animals that were pre-treated with oestrogen, there was sustained hyperalgesia at 30 minutes post-vehicle, which persisted for the full time-course of the experiment. In progesterone pre-treated animals, the hyperalgesia was present *only* at 30 minutes post-vehicle, while those pre-treated with combined oestrogen and progesterone showed hyperalgesia at both 15 and 30 minutes post-vehicle (Figure 5.4). No hyperalgesia was observed in the oil pre-treated animals. It is difficult to determine why hormone pre-treatment influences tail pinch latency following vehicle-treatment. Sex steroids have been reported to influence afferent fibres arising from the uterus: for example, for the hypogastric nerve, sensitivity is greatest during proestrus and oestrus, while the pelvic nerve sensitivity is greatest during proestrus (Robbins *et al.*, 1992). Therefore it is possible that sex steroids influence nociceptive sensitivity at the level of the primary afferent fibre via the sensitisation of peripheral sleeping nociceptors.

Alternatively, the sex steroid influences on the repeated nociceptive testing paradigm may reflect differences in "learning". Learning is the phenomenon that occurs when animals learn the behaviour that terminates the nociceptive assay, causing artificial reductions in the nociceptive latencies. It is a particular problem in repeat-nociceptive testing paradigms that do not involve reflexive behaviours (*e.g.* hot-plate test).

Another possible area for sex steroid influence on nociceptive sensitivity is at the level of descending inhibition of pain. Endogenous opioid peptides (such as β-endorphin) are able to attenuate painful noxious stimulation through the activation of descending inhibitory pain pathways (Fillingim & Ness, 2000). Indeed, noxious stimulation itself causes the release of β-endorphin in rat brain (Zangen *et al.*, 1998), and perhaps this endogenous opioid peptide is responsible for modulating the nociceptive input. β-endorphin may also regulate baseline of pain sensitivity in repeated nociceptive testing situations – producing a steady basal nociceptive sensitivity. Such a level baseline can be seen in oil pre-treated rats in the current experiment (Figure 5.4, panel D). Therefore it is of interest that there are reciprocal interactions between gonadal hormones and endogenous opioid systems. For example, a negative correlation between circulating levels of oestradiol and during the follicular phase in humans and μ-opioid receptor binding in the amygdala and hypothalamus has been reported (Smith *et al.*, 1998). Furthermore, in female rats, oestrogen replacement (10μg 17β-oestradiol s.c.) after ovariectomy increases μ-opioid receptor mRNA levels in the ventromedial nucleus and the arcuate nucleus (Quiñones-Jenab *et al.*, 1997). While these results seem contradictory, they show that oestrogen can have a modulatory effect on the endogenous opioid system. Therefore, it is postulated that in the present study the female sex hormone oestrogen decreases in endogenous pain relief to the extent that oestradiol treated animals have increased nociceptive sensitivity compared to their oil-treated counterparts.

Ibuprofen Treatment

Importantly, ibuprofen did not produce any analgesia of its own (according to the original definition of analgesia, see Section 5.2.3; *i.e.* ibuprofen did not increase tail-pinch latencies from baseline levels) - rather it *prevented the hormonal-dependent hyperalgesia produced by vehicle treatment* (Figure 5.2). Furthermore, this anti-hyperalgesic effect was only present in animals where oestrogen formed a part of their pre-treatment (*i.e.* panels A and C, Figure 5.4). It is also of interest that the magnitude of this effect was reduced somewhat in the oestrogen+progesterone pre-treated group compared with the oestrogen pre-treated group. Perhaps then, oestrogen is essential for producing this anti-hyperalgesic effect of ibuprofen, while progesterone dampens the effect.

The differences in pharmacodynamic effect of ibuprofen seen in this experiment were despite comparable serum ibuprofen levels in all of the four hormone treatment groups (Figure 5.5). The dissociation between drug concentration and effect has previously been reported in the scientific literature (Walker & Carmody, 1998), and is also evident in the experiments outlined in Chapters 2 and 3.

It could be considered paradoxical that oestrogen is necessary for ibuprofen to exert its anti-hyperalgesic effect in rats, while in humans (Chapters 2 and 3), oestrogen *prevents ibuprofen from producing analgesic effects*. The important differences between the rodent and human studies are: *firstly*, different species were involved, and *secondly* different stimulus modalities (mechanical pressure *vs.* electrical) were used.

While the mechanism for the observed sex hormone-dependent ibuprofen analgesic effect is unclear, it is hypothesised that oestrogen (rather than

progesterone) is involved. This hypothesis is based on the requirement for oestrogen to be present if ibuprofen is to elicit an analgesic effect. The alternate hypothesis, that progesterone inhibits ibuprofen analgesia, is unlikely because progesterone (and its metabolites) have been shown to potentiate analgesia. For example, progesterone potentiates sufentanil analgesia in progesterone pre-treated rats (10-40μg, *i.t.*; Jayaram & Carp, 1993), and its metabolites are currently being explored as analgesic agents (Goodchild *et al.*, 2000; Nadeson & Goodchild, 2000; Nadeson & Goodchild, 2001; Goodchild *et al.*, 2001).

As the analgesic effects of ibuprofen observed in this chapter are considered to stem from its analgesic rather than its antiinflammatory actions, the oestrogen influences probably occur centrally rather than through the peripheral inhibition of the prostaglandin synthesis pathway, even though oestrogen has been shown to influence enzymes and products of this pathway in other areas (e.g. vascular system, pregnancy). As in previous chapters, a possible central nervous system mechanism is sought, for example through oestrogen's effects on the neurotransmitters of the pain system (see Chapters 2 and 3).

5.4.3 Pharmacokinetics

The pharmacokinetic parameters reported in this chapter for ibuprofen in rats from the four different hormone treatment groups are similar to previously published data (Knihinicki *et al.*, 1990; Satterwhite & Boudinot, 1991). This was important, as it ruled out any kinetically relevant effects of the second anaesthesia given to these animals when their jugular cannulas were implanted, and any effects of ovariectomy on ibuprofen kinetics.

The pharmacokinetic parameters are essentially comparable with each other except for a slower clearance in the progesterone-treated rats and a shorter half-life in oestrogen-treated rats. It should be pointed out that those differences, if they were relevant, might be expected to induce opposite pharmacodynamic results to those which we observed - that is progesterone pre-treated rats should have exhibited anti-hyperalgesia. The variability in the calculated parameters for each group (greatest in the oestrogen+progesterone-treated group) could be due to the sample size (although this would not explain the inter-group variability) but may also be a result of incomplete or heterogenous absorption of the drug. Incomplete absorption may have arisen due to the sheer quantity of drug that was injected. This could have saturated absorption, while heterogenous absorption may have arisen because of slight difference in the location of intraperitoneal injection, or through sex hormonal influences on drug absorption itself. However, the differences in pharmacokinetic parameters between hormone treatments could not account for the pharmacodynamic findings.

5.5 FUTURE DIRECTIONS

There are several important issues arising from the experiments in this chapter that require further exploration:

- *Analysis of the effect of ovariectomy in female rats, and gonadectomy in male rats on basal pain responses.*
- *Determination of the effect of oestrogen and progesterone on basal nociceptive sensitivity by comparison of ovariectomized animals pre- and post-hormone treatment.*
- *Examination of the effect of testosterone replacement in female rats on baseline nociceptive sensitivity, and of oestrogen and progesterone replacement in gonadectomized male rats.*
- *Discovery of the role of oestrogen in the endogenous pain relief system.*
- *Elucidation of the mechanism responsible for the oestrogen-dependent analgesic effect of ibuprofen.*
- *Determination of the effect of sex hormones on the absorption of ibuprofen from the peritoneal cavity – perhaps by using radio labelled ibuprofen.*

5.6 FINAL COMMENTS

The results presented in this chapter lend further support to the theory that there are sex hormonal influences on ibuprofen analgesia in rodents. While the mechanism responsible for this effect is unknown, it is postulated that it is via oestrogen-dependent modulation of central nociceptive processes, and not through sex-hormonal modulation of the pharmacokinetics of ibuprofen. Further experimental work is required to determine why there are species differences in this sex hormonal modulation, as oestrogen enables ibuprofen to produce analgesia in rodents, while suppressing it in humans.

Chapter 6

PARACETAMOL ANALGESIA IS DEPENDENT UPON GENETIC MAKE-UP IN MICE

6.1 INTRODUCTION

There have been several advances in our knowledge of pain and analgesia (for example the discovery of pain-modulatory systems, and nervous system plasticity following exposure to noxious stimuli), but there remains substantial unexplained inter-individual variability. Chapters 2 to 4 have focussed on differences in nociception and analgesic response that may have arisen from sex or sex hormone status. Sex differences (and the ensuing sex hormone status differences) are probably the simplest genetic differences to observe. It is not unreasonable to presume that other genetic differences also influence nociception and analgesic response. This chapter seeks to determine whether genetics influence nociception and analgesic response to paracetamol.

Different nociceptive assays are mediated by separable physiological mechanisms: there are at least nine different dimensions that can differ from nociceptive assay to nociceptive assay (from Mogil *et al.*, 1996a):

1. Type of noxious stimulation (*e.g.* thermal *vs.* chemical).
2. Stimulus duration (*e.g.* acute/phasic *vs.* tonic).
3. Stimulus location (*e.g.* cutaneous, subcutaneous or visceral).
4. Stimulus intensity
5. Intensity-time relationship (increasing *vs.* consistent).
6. Presence/absence of tissue damage and/or inflammation.
7. Stimulus escapability.
8. Response characteristics (reflexive *vs.* operant).
9. Level of nociceptive processing (spinal *vs.* supraspinal).

Given these differences, it is not surprising that there are different ascending mechanisms of nociceptive transmission, and perhaps separate descending modulatory mechanisms inhibiting the different stimulus modalities (Mogil *et al.*, 1996a). Furthermore, it is not unlikely that there may be different genetic mediation of sensitivities to the various pain modalities (Mogil *et al.*, 1996a). Despite this, genetic approaches to pain have been largely overlooked in favour of pharmacological interventions (Mogil *et al.*, 1996b).

With the improvements in molecular techniques, several laboratories have helped to characterise strain differences in nociception and analgesic response in mice (Belknap *et al.*, 1990; Mogil *et al.*, 1999a; Mogil *et al.*, 1999b). Furthermore, sex-specific quantitative trait loci (QTLs) on chromosome 4, which determine the expression of systems that mediate sensitivity to acute, thermal nociception as measured on the hotplate test (Mogil *et al.*, 1997) perhaps provide a genetic explanation for sex differences in pain and analgesia.

The study of p*harmacogenetics* (the study of monogenic variants *i.e.* the effect of variation of specific proteins on drug response) and *pharmacogenomics* (the study

of multigenic variation) are an emerging area of importance. These sciences have developed in order to help explain interindividual differences in some physiological responses, for example pain and analgesia (Kalow, 2001). Genetic variability in the coding for some enzymes can explain differences in response to many drugs including isoniazid, alcohol, morphine, cocaine, amphetamine, caffeine and codeine (for review see Kalow, 2001). For example, there is a genetic variability in the human expression of the P450 enzyme CYP2D6 that is involved in the metabolism of codeine. In patients with reduced CYP2D6 enzyme activity (for frequencies see Table 6.1), codeine is an ineffective analgesic. In addition, these patients are less tolerant to pain, presumably due to defective β-endorphin synthesis (Mogil *et al.*, 1996b).

Table 6.1: Some of the common dysfunctional phenotypes of the CYP2D6 in humans and their genetic basis (from Wilcox & Owen, 2000).

			Allelic frequencies (% population)				
CYP2D6 allelic variant	Mutation	Functional Consequence	Caucasians	Asians	Negroid Africans	Ethiopians & Saudi Arabians	Australian Aborigine
2D6*4	Defective splicing	Inactive enzyme	12-21	1	2	1-4	1.5
2D6*5	Gene deletion	No enzyme	2-7	6	4	1-3	7.5
2D6*10	Pro34Ser, Ser486Thr	Unstable enzyme	1-2	51	6	3-9	0.8
2D6*17	Thr107Ile, Arg296Cys, Ser486Thr	Reduced affinity for substrates	0	Not known	34	3-9	0.2
2D6*2xN	Gene duplication or multi-duplication	Increased enzyme activity	1-5	0-2	2	10-16	0

The aim of the experiment outlined in this chapter was to determine if there are genetic differences in paracetamol induced analgesia in two strains of mouse, the C57BL/6j and the DBA/2j. Based upon previous experiments, it was hypothesised

that C57BL/6j mice would have shorter baseline hot-plate latencies. In addition, it was hypothesised that there would be strain differences in analgesic effect of paracetamol in the two chosen strains.

These studies were performed in collaboration with Prof. J.S. Mogil, Dr W. Lariviere and Ms S.G. Wilson from The Department of Psychology and Neuroscience Program, University of Illinois at Urbana-Champaign, Champaign, Illinois, USA.

6.2 METHODS

The behavioural component of this experiment was performed by these colleagues: Prof. Jeffrey Mogil, Dr Willliam Lariviere and Dr Sonya G. Wilson at the University of Illinois at Urbana-Champaign, Illinois, USA. The University of Illinois' Ethics Committee approved all experiments.

Twenty-four male C57BL/6j (11-23g) and twenty-four male DBA/2j (16-24g) mice were obtained from The Jackson Lab [Bar Harbor, ME, USA], and were housed in groups of 2-5 in a temperature controlled (22°C) environment. Food and water were provided *ad libitum*. The animals were subjected to a 12:12 hour light/dark cycle (lights on at 0600 h) for 1 week. These strains of mouse were chosen partly because of their divergent ancestry, which makes them among the most genetically dissimilar of existing inbred strains (Mogil *et al.*, 1997), and partly on the basis of previous experiments, which found strain differences in their sensitivity to hot-plate nociception (the relative responsiveness of DBA/2j being considered "average", while C57BL/6j were deemed "sensitive"; Mogil *et al.*, 1999a).

6.2.1 Analgesic Testing

After one week of acclimatisation, the analgesic testing was completed. All testing proceeded near mid-phosphatase (around 1400 – 1600) to reduce circadian effects on pain sensitivity (Kavaliers & Hirst, 1983). The mice were weighed and numbered and allowed to sit in the testing room for 30 minutes prior to the start of the experiment. The animals (n=24 per strain) baseline pain thresholds were determined by Sonya Wilson using the hotplate test. The hotplate test was based on that described by Eddy & Leimbach (1953) and Woolfe & Macdonald (1944)

and involved removing the mice from their cages and placing them on a flat aluminium surface that was maintained at 50.0 ± 0.2°C [Thermolyne Dri-Bath, Barnstead Thermolyne, Dubuque, Iowa, USA]. While nociceptive testing usually proceeds at temperatures around 54°C, it doesn't allow the analgesic effects of weak analgesics to be seen. Therefore in this experiment, the lowest temperature that produced a reliable nociceptive assay, and was sensitive to the effects of mild analgesics, was used (50°C). A 15cm high Plexiglass covering an area of 10 x 10cm limited ambulation of the mouse on the plate. Latency to respond (in seconds) to the heat stimulus with behaviour indicative of nociception (sustained paw lift, paw lick or paw shake/flutter) was measured with a stopwatch to the nearest 0.1s. Only hind-paw responses terminated the test since rearing and forepaw licking are components of normal behaviour and are therefore not reliable indicators of nociceptive behaviour (Hammond, 1989; Espejo & Mir, 1993). The first nocifensive behaviour of any type was used as the endpoint since there are strain differences in response to the hotplate test (Belknap *et al.*, 1990). For example, the DBA/2j strain is markedly slow to show hind-paw lick as a nocifensive response compared to the C57BL/6j strain (Belknap *et al.*, 1990). The maximum time on the hotplate was set at 150 seconds to avoid damage to the animal's paws.

Following the determination of baseline responses, the animals were injected with paracetamol (4-acetamidophenol in 25% propylene, heat-stirred for 15 mins, 400mg/kg s.c.) and re-tested on the hot-plate for pain threshold at 30, 60 and 120 minutes post-paracetamol (n=8 per strain per time-point).

6.2.2 Blood Collection

Immediately following the analgesic testing (at 30, 60 and 120 minutes), the animals were euthanased using isofluorane, decapitated, and trunk blood collected into a 2mL EDTA blood collection tube [containing 0.4mL of 7.5% buffered solution of EDTA, equivalent to 3.0mg EDTA, Sherwood Medical, St Louis, Missouri, USA]. The tubes were placed upon ice until centrifugation at high speed (10,000rpm, 10 minutes). Plasma aliquots (100μL) were freeze-dried [Freeze Drying 101, VirTis, Gardiner, New York, USA] according to the following protocol: chamber and condenser cooled to –40°C, samples added and vacuum and heat applied. Once the samples were dry, the temperature quickly increased to –5°C, and then climbed in 5°C increments over several days until it reached 20-25°C, at which time the samples were removed.

6.2.3 HPLC Analysis

Following freeze-drying, the samples were shipped to Australia and underwent high performance liquid chromatography (HPLC) analysis for paracetamol (conducted solely by Ms Belinda Giles). Plasma concentrations of paracetamol were determined by a methodology which was a refinement of published HPLC procedures (Granados-Soto *et al.*, 1993). Samples were prepared in the following manner: an aliquot (50μL) of the internal standard phenacetin (0.5mg/mL) was added to freeze-dried mouse plasma (100μL) followed by ethyl acetate (5mL). The solvent layer was evaporated until dry and the residue redissolved in 50μL methanol. Samples (10μL) were injected onto a HPLC system, which consisted of a C8 HPLC column [Platinum EPS C8 100Å, 5μm, 150x4.6mm; Alltech Associates

(Aust.) Pty. Ltd, Baulkham Hills, Sydney, NSW, Australia] and eluted with a mixture of 0.05M sodium acetate buffer (pH 4.0) and acetonitrile (92.5:7.5) at a constant flow of 1mL/min. The effluent from the column was detected by UV (254nm) using a UV-Vis Detector [SPD10Avp, Shimadzu Corporation, Kyoto, Japan]. Retention times for paracetamol and the internal standard were 3.4 and 11.3 minutes, respectively. The limit of determination of paracetamol was 0.5μg/mL, and the inter-assay coefficient of variation was 6% over the range of concentrations 0.5μg/mL to 500μg/mL.

6.2.4 Data Analysis

Belinda Giles performed the data analysis presented in this chapter.

Pharmacodynamic Analysis

All hotplate latency data were tested for normality and equal variances. Strain differences in baseline hotplate latency were determined using unpaired *t*-tests with the null hypothesis that there were no differences in baseline hotplate latency between strains. Hotplate latency was averaged by strain for each time-point, and these data were analysed using two-way ANOVA with post-hoc Bonferroni correction for two-factor (strain x time) differences. Analgesia was defined as a *statistically significant increase in hotplate latency from baseline hotplate latency following paracetamol treatment*.

The unpaired *t*-tests, ANOVA and subsequent multiple comparisons were all performed using the statistical program NCSS 2000 [Number Crunching Statistical Systems, Kaysville, Utah, USA], with values of $P < 0.05$ considered significant. All data are presented as mean ± SEM.

Population Pharmacokinetic Analysis

The present study was not optimally designed to examine the pharmacokinetics of paracetamol in the mouse (owing to insufficient concentration-time data, with only three observation times). Hence the estimates of the mean and variability of the clearance, volume of distribution and absorption rate constant for paracetamol can be viewed only as approximates. The study did however allow comparison between strains of mice since the data were collected using an identical study design. Furthermore, given the small total blood volume of a mouse it was not possible to conduct a traditional pharmacokinetic investigation where multiple samples are withdrawn from each mouse over the time course of the experiment, so population pharmacokinetic analysis was performed to characterise the pharmacokinetic behaviour of paracetamol in mice.

Concentration-time data were analysed using non-linear mixed effects modelling implemented in P-PHARM Ver 1.3 (Gomeni *et al.*, 1994). The population approach examines fixed (eg pharmacokinetic model parameters such as clearance and volume of distribution) and random (eg inter-animal variance of pharmacokinetic parameters and residual variability) effects (Whiting *et al.*, 1985; Aarons, 1991; Aarons, 1993).

Analysis of the pooled plasma concentration-time data for paracetamol indicated that a one-compartment pharmacokinetic model best described the disposition of this drug in the mouse. The parameters of the combined pharmacokinetic model were: the apparent volume of distribution (V/F; where F is the fraction of the dose that is absorbed; and was assumed to be unity), first order absorption rate constant (K_a) and apparent clearance (CL/F).

Random effects are considered to consist of inter-individual variability in each pharmacokinetic parameter with the remaining variability being termed the residual or unexplained variability within animals (Gomeni *et al.*, 1994). In this study the inter-animal variability in pharmacokinetic parameters was described using a normal distribution and the residual variability that encompasses measurement error and model misspecification was assumed to be constant across the study population.

The P-PHARM software generates the population mean of pharmacokinetic parameters and an estimate of the inter-animal variability in this parameter (expressed as a percent coefficient of variation). Posterior Bayesian parameter estimates for each animal were also generated, allowing the population concentration time profile in plasma to be determined despite only one sample being available in each animal (*i.e.* the analysis looks at the population of concentration-time points, and determines where a single observation fits – or is likely to fit – within that population, and then extrapolates other time-points based on that fit for the single observation).

Plasma paracetamol concentrations were averaged by strain for each time-point and these data were analysed with two factor (strain x time) ANOVA with post-hoc Bonferroni correction, using the statistical program NCSS 2000 [Number Crunching Statistical Systems, Kaysville, Utah, USA], with values of $P < 0.05$ considered significant. All data are presented as mean ± SEM.

6.3 RESULTS

6.3.1 Mean Weights and Baseline Nociception

Summary

- *C57BL/6j mice have shorter baseline hotplate latencies (and therefore reduced pain thresholds) than DBA/2j mice.*

There were no differences in normality or variances between the two strains for the weight and baseline hotplate latency measures. There was no difference in mean weight between the two groups of mice. However, the C57BL/6j mice had significantly shorter hotplate latencies than the DBA/2j mice (n=24 per strain, $P < 0.01$, unpaired *t*-test, Table 6.2).

Table 6.2: Mean weight and baseline hotplate latencies by strain.

Strain	Weight (grams)	Baseline Hotplate Latency (seconds)
C57BL/6j (n = 24)	21.0 ± 0.5	23.8 ± 1.5
DBA/2j (n = 24)	20.3 ± 0.4	31.4 ± 2.1*

** denotes significant difference from C57BL/6j mice (P < 0.05, unpaired t test)*

6.3.2 Time-Course of Hot-Plate Latencies

Summary

- *C57BL/6j mice have prolonged analgesia following paracetamol administration.*
- *DBA/2 mice exhibit mild analgesia only after 120 minutes.*

The analgesic time-courses in the two mouse strains following paracetamol are shown in (Figure 6.1).

There were marked strain differences in the analgesia produced by paracetamol. It induced a high level of sustained analgesia in C57BL/6j mice ($P < 0.0001$, one-way ANOVA), while only producing mild analgesia at 120 minutes in DBA/2j mice ($P < 0.05$, one-way ANOVA). Note also that the variability in C57BL/6j mice after paracetamol were greater than those in the DBA/2j mice (the mean variance across the post-drug time-course were 3297 and 529 respectively); and were also much greater after paracetamol, than before it (the variances were 54 for C57BL/6j mice and 106 for DBA/2j mice prior to drug administration).

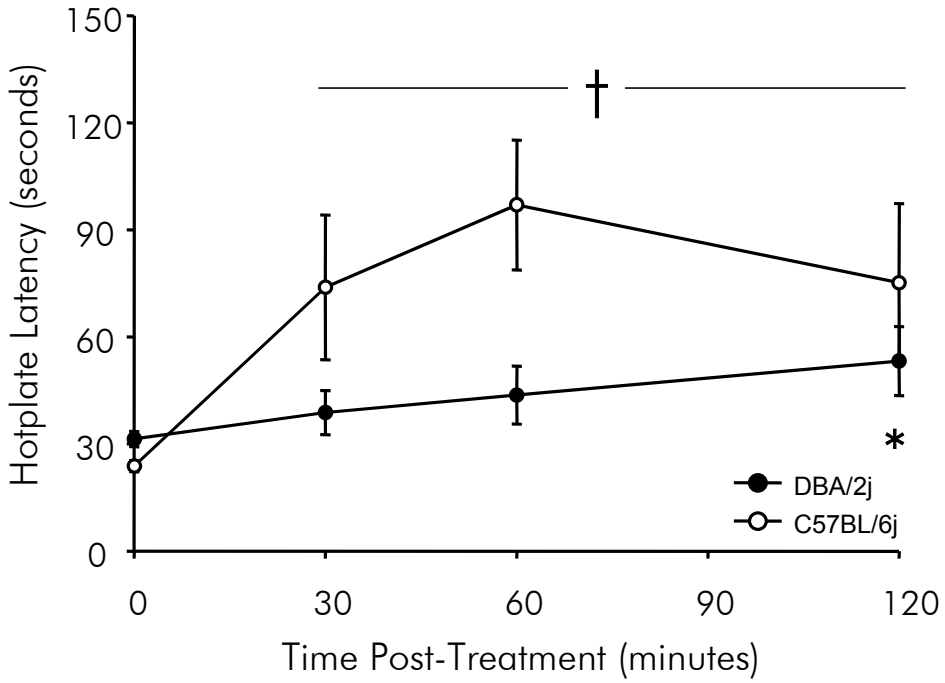


Figure 6.1: Time-course of hot-plate latencies in DBA/2j (filled circles) and C57BL/6j (open circles) mice following paracetamol (400mg/kg s.c.) administration, with the hotplate test.

† denotes significant difference in C57BL/6j hotplate latency from baseline (time 0) latency ($P < 0.05$, one-way ANOVA with post-hoc Bonferroni correction).

** denotes significant difference in DBA/2j hotplate latency from baseline (time 0) latency ($P < 0.05$, one-way ANOVA with post-hoc Bonferroni correction).*

6.3.3 Pharmacokinetics

Summary

- *C57BL/6j mice have right shifter plasma-concentration time curves, with lower plasma paracetamol concentrations than DBA/2j mice at 30 minutes post-treatment.*
- *C57BL/6j mice have smaller volumes of paracetamol distribution, and slower absorption rate constants than DBA/2j mice, but no difference in paracetamol clearance.*

The mean plasma concentration-time courses following paracetamol (400mg/kg s.c.) in the two mouse strains are shown in Figure 6.2.

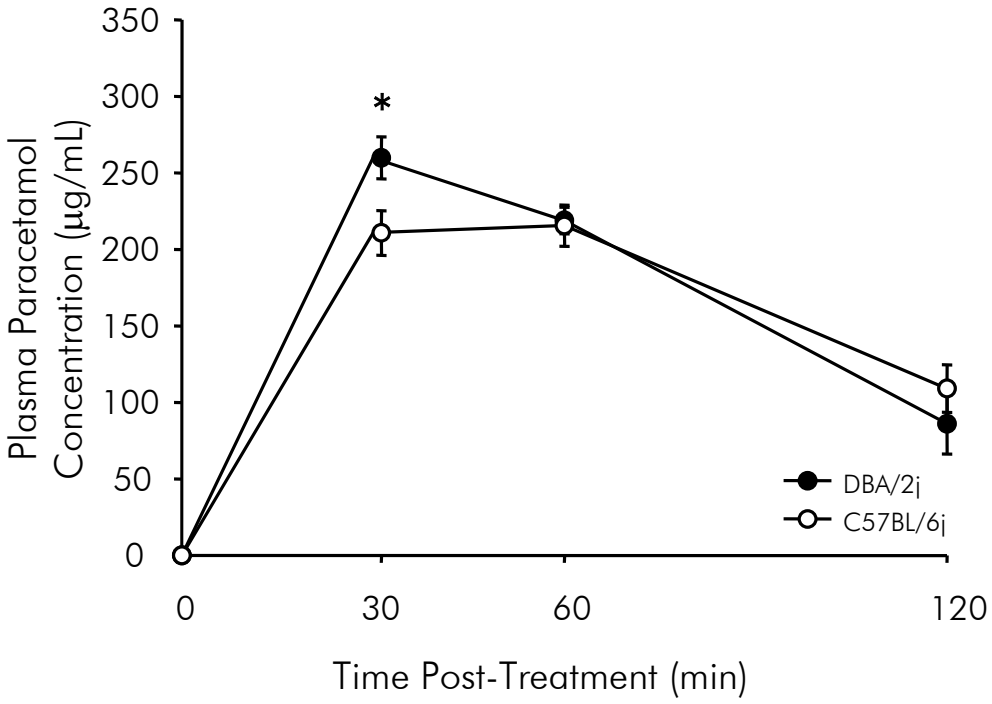


Figure 6.2: Time-courses of plasma paracetamol concentrations (µg/mL) in DBA/2j (filled circles) and C57BL/6j mice (unfilled circles) following paracetamol (400mg/kg s.c.).

** denotes significant difference from C57BL6 mice plasma concentration at 30 minutes (n=8 per group, P < 0.05,two-way ANOVA with Bonferroni correction).*

While the temporal profiles of the paracetamol concentrations in the two strains of mouse are fairly similar, it appears that the plasma concentration curve for C57BL/6j mice is somewhat right-shifted, which might explain the difference in peak plasma paracetamol concentrations at 30 minutes post-paracetamol ($P < 0.05$, two-way ANOVA).

The results of the pharmacokinetic modelling analysis are shown in Table 6.3. There were no strain differences in apparent clearance, but both apparent volume of distribution and the absorption rate constant were lower in C57BL/6j mice than in DBA/2j mice. It is interesting to note that the C57BL/6j mice also had greater intra-strain variability for all calculated parameters (*i.e.* their coefficients of variation (CV) were higher in all cases).

Table 6.3: Population mean pharmacokinetic parameters for DBA/2j (n=8) and C57BL/6j (n=8) mice following paracetamol (400mg/kg s.c). Data are expressed as mean ± SEM, with percent coefficient of variation (CV) shown in brackets.

Strain	Apparent Clearance ($mL.min^{-1}.kg^{-1}$)	Volume of Distribution ($L.kg^{-1}$)	Absorption Rate Constant (K_a, min^{-1})
DBA/2j (n=8)	14.1 ± 0.9 (CV = 18.5%)	0.87 ± 0.05 (CV = 15.4%)	0.067 ± 0.009 (CV = 36.1%)
C57BL/6j (n=8)	12.1 ± 1.2 (CV = 28.1%)	0.58 ± 0.08* (CV = 38.6%)	0.020 ± 0.003* (CV = 37.3%)

** denotes significant difference from DBA/2j value (P < 0.05, unpaired t-test).*

The lower volume of distribution in the C57BL/6j mice probably reflects incomplete absorption from the injection site.

6.4 DISCUSSION

While the other chapters have examined a different form of genetic difference (*i.e.* sex differences and their hormonal consequences), this chapter has looked at the non-sex linked genetic differences in basal nociception and analgesic response to paracetamol in two strains of mouse. The two strains of mouse (C57BL/6j and DBA/2j) were chosen on the basis of their divergent ancestry (Mogil *et al.*, 1997), and partly on the basis of previous experiments which have shown strain differences in their sensitivity to hot-plate stimulation (Mogil *et al.*, 1999a).

6.4.1 Baseline Pain

The nociceptive assay was chosen on the basis that it is a simple, reliable and ethically acceptable (to the University's Ethics Committee) method for inducing pain. It has also been used previously to demonstrate strain differences in nociceptive sensitivity (Mogil *et al.*, 1999b). While it might be criticised that this test is less reliable than other methods (*e.g.* the tail-flick test) when repeated testing paradigms are involved, each animal was only introduced to the hot-plate twice, and because of the variability in intra-strain basal nociception it was very important that each animal had a baseline and a post-drug measurement taken. Other methods, such as the tail flick test, are insensitive to the analgesic effects of weak analgesics such as paracetamol.

The baseline hot plate latencies determined in the experiment outlined in this chapter were significantly shorter in C57BL/6j mice compared to the DBA/2j strain, confirming the primary hypothesis. The significance of these results are strengthened by the use of a specific methodology that utilised the first nocifensive

behaviour as a cut-off. This more stringent methodology reduces the possibility that strain differences in analgesic efficacy were found *because of strain differences in nocifensive behaviour to noxious thermal stimuli* (Mogil *et al.*, 1997).

The differences found in the experiments outlined in this chapter concur with other reports that DBA/2 mice consistently display higher basal hotplate latencies than C57BL/6j (for example 18s *vs.* 11s at a hot-plate temperature of 54°C (Mogil *et al.*, 1996a); 19.3s *vs.* 28.2s at a hot-plate temperature of 53°C (Mogil *et al.*, 1999a)). One review has reported that C57BL/6 mice have the greatest nociceptive sensitivity to thermal nociceptive assays (hot-plate, Hargreaves' test of thermal nociception and tail withdrawal assays), compared to approximately 8-10 different mouse strains (for review see Mogil, 1999).

Other nociceptive tests show different strain sensitivities (for review see Mogil *et al.*, 1999b). For example, in other acute stimulus modalities, C57Bl/6 mice were more sensitive than DBA/2 mice in the Hargreaves' test and the tail withdrawal test (both are thermal tests), while DBA/2 mice were more sensitive to magnesium sulphate induced abdominal constriction, the early component of the formalin test, and the Von Frey filament test (the first two are chemical tests, while the last is a mechanical test; Mogil *et al.*, 1999b). In tests producing pain of sub-acute duration, such as the acetic acid induced abdominal constriction test and the late component of the formalin test, there was a marginal difference (C57BL/6 less sensitive than DBA/2) in the acetic acid abdominal constriction test, while C57BL/6 were more sensitive than DBA/2 in the late component of the formalin test. Finally looking at chronic stimuli, for example autotomy following hind-limb denervation, C57BL/6 mice were more sensitive to pain, while using the Chung model of

peripheral nerve injury, DBA/2 mice were more sensitive to painful thermal and mechanical stimulation. These differences reflect genetic differences in the separable physiological mechanisms responsible for mediating the different nociceptive assay types (see Introduction).

6.4.2 Time-Course of Hot-Plate Latencies

The time-courses of hot-plate latencies were very different in the two strains of mouse. While C57BL/6j mice showed considerable prolonged analgesia over the time-course of the experiment, DBA/2j mice only showed mild analgesia at 120 minutes post-treatment (Figure 6.1), despite very high dosages of paracetamol being administered (400mg/kg *s.c.* compared to the recommended human dosage of between 7 and 14mg/kg *p.o.*). It is of considerable interest that these responses are *so* different, and would suggest that there are genetic differences in paracetamol-induced analgesia between the two strains, independently of pharmacokinetic differences (see Section 6.4.3). The intra-strain differences in hot-plate latency following paracetamol were approximately six times higher in C57BL/6j mice than in DBA/2j mice. While the inter-strain variability can be accounted for on the basis of allelic variation at a reasonably small number of genetic loci, the intra-strain variation cannot. Thus, variation among members of the inbred strain must arise from *environmental* sources, or through an interaction of genotype and environment, suggesting this interaction is different in the C57BL/6j and DBA/2j mice.

Physiological differences between the two strains may account for the difference in pharmacodynamic effect observed. It would be of interest to determine whether there are strain differences anatomical differences in brain, liver, or fat content,

which might produce differences in central antinociceptive processing, drug hepatic elimination, or volume of distribution respectively. Also differences in hormone secretions (in particular sex hormones) or β-endorphin levels that also may influence the anti-nociceptive effect produced by paracetamol (via the mechanisms suggested in Chapters 2 and 3) should be determined. Differences in corticosteroid levels may also be important as anxiety plays a big role in determining basal nociception and response to analgesic drugs (as suggested in Chapters 2 and 3). If these anatomical or physiological differences were found between the two strains of mice, these may produce differences in dose-response, *i.e.* the C57BL/6j mice may have lower effective dosages than DBA/2j mice (assuming a concentration-effect relationship can be determined for paracetamol in these strains of mouse).

While no other published studies have examined strain differences in paracetamol analgesia, several studies have shown that genetic differences influence morphine analgesia in mice (for review see Mogil, 1999). Several studies have found DBA/2 mice to be more sensitive to the analgesic effects of morphine than C57BL/6 mice (for example Mogil's laboratory has used morphine (10mg/kg, *i.p.*) and a 54°C hot-plate to show the difference (Mogil *et al.*, 1996a, for review see Mogil, 1999). As morphine shows the opposite strain sensitivities, the reported high analgesia/low analgesia strains (Panocka *et al.*, 1986; Belknap *et al.*, 2002) are dependent upon the strains examined, the nociceptive assay, and the analgesic drug used.

6.4.3 Pharmacokinetics

The plasma concentration-time curves of paracetamol in the two strains of mouse indicated that C57BL/6j mice might have a right shifted concentration curve compared to the DBA/2j mice (Figure 6.2). This would suggest reduced absorption of paracetamol in the C57BL/6j strain compared to the DBA/2j, possibly due to physiological differences between these two strains. In particular, the C57BL/6 mice may have lower local blood flow, or slower paracetamol diffusion through the tissue at the site of injection.

The strain differences in pharmacokinetics are also reflected in the calculated population pharmacokinetic parameters (Table 6.3), where the volume of distribution is smaller in C57BL/6j mice, and the absorption rate constant is also slower in this strain of mouse. The smaller volume of distribution in the C57BL/6j mice probably reflects the reduced (and delayed) absorption of paracetamol in this strain. However, the strain differences in pharmacokinetic parameters could not account for the differences in pharmacodynamic finding (*i.e.* that paracetamol is a more effective analgesic in C57BL/6j), because the differences in pharmacokinetics, if they were relevant, would cause a *reduction* in analgesic efficacy in C57BL/6j mice.

Others have found no differences in the pharmacokinetic profile of paracetamol following intraperitoneal injections of 400mg/kg doses of paracetamol (Lubek *et al.*, 1988). This published experiment has the advantage of repeated plasma sampling in each mouse over a greater number of time-points (0, 15, 30, 45, 60, 90, 120, 240 and 480 minutes post-paracetamol). Peak plasma concentrations in that study (~570 μg/mL) were achieved rapidly (at the 15 minute time-point), and

the concentrations declined rapidly so that there were minimal detectable levels within 120 minutes. Thus the calculated K_a from the experiments outlined in this chapter may be inaccurate because a 15 minute sampling time was not included.

In any case, the differences in pharmacokinetics are unlikely to have contributed to the differences in analgesic sensitivity of the two strains of mouse, and it is more likely that there are differences in pharmacodynamic component of paracetamol between the two strains, raising the possibility that there are pharmacodynamically relevant genes.

6.5 FUTURE DIRECTIONS

Several questions arise from the present research. Firstly, the daunting tasks of determining which genes participate in basal nociceptive sensitivity, and which are responsible for paracetamol analgesic sensitivity. Secondly, the re-assessment of analgesic drugs needs to be broadened beyond morphine and paracetamol, to determine whether all analgesic drugs are influenced by genotype. Finally, this type of research needs to be completed in human subjects, in order to discover the relevance to the human situation, eventually allowing the development of novel analgesic strategies, and the improved, individualised use of conventional therapies to treat pain.

6.6 FINAL COMMENTS

The sex and sex hormonal differences reported elsewhere in this thesis (Chapters 2 to 5) are one type of "genetic difference" that are easily observable. The experiments outlined in this chapter have examined the possibility that other, non-sex linked genes might be important in predicting response to mild non-opioid analgesics. Strain differences in paracetamol analgesia were found between C57BL/6j and DBA/2j mice, and these differences were independent of differences in paracetamol pharmacokinetics between the two strains. This is an important finding which suggests that there are pharmacodynamically relevant genes that determine analgesic response to paracetamol using the hotplate test. If these genes could be located, and the experiments replicated in humans, then it is possible that gene-alteration or specially targeted analgesic drugs could be used in the treatment of severe, intractable pain in human patients – thus attaining the ultimate goal of pain relief rapidly and with the minimum of side effects.

Chapter 7

GENERAL DISCUSSION

> *"There is growing appreciation that the delineation of the body into distinct systems is too simplistic to reflect its cohesiveness of function"*[1]

Evidence for an interest in pain research dates back to the ancient Egyptian, Indian, Chinese, and Mesopotamian kingdoms (for review see Todd, 1999). Despite this long interest, real developments in terms of understanding the pathophysiology of pain have been relatively recent (in the last 100 years or so), for example, the understanding that different people (and animals) respond differently to pain, and also to the analgesic drugs used to treat that pain. Thus modern pain research is of the utmost importance if better methods of pain management are to be devised.

7.1 SUMMARY OF RESULTS

The aim of this thesis was to determine whether subjects' sex, sex hormone status, or genetics could predict basal nociceptive sensitivity or analgesic response to ibuprofen and paracetamol. Two major approaches were taken. The *first* was the examination of one type of genetic difference: *sex* and the consequences of a subject's sex - the *sex hormones* - on nociception, expectancy and analgesic response to ibuprofen in both humans and animals (Chapters 2 to 5). The *second* was an examination of non-sex-linked genetic differences in mice that may have produced variability in response to paracetamol (Chapter 6).

[1] Michelle Ting UNSW, personal communication 2001

7.1.1 Basal Nociception

The experiments outlined in this thesis found that **sex** was a good predictor for response for pain report (VAS score, males > females), while ***sex hormone status*** was a good predictor for pain tolerance levels (–SH subjects > +SH subjects; Chapter 4). Neither sex nor sex hormone status could adequately predict baseline pain threshold levels however (Chapter 4). In addition, neither the human menstrual cycle *per se* (Chapter 4) nor exogenous hormone treatment in rats (Chapter 5) appeared to alter baseline nociception, although it is postulated that this result was more likely due to an insufficient sample size, and therefore reduced power in the statistical analyses, rather than an actual lack of effect. In humans, the active component of oral contraceptives reduced both pain thresholds and pain tolerances compared to women cycling physiologically in their luteal phase (Chapter 4). In mice, ***genetics*** could predict baseline nociception, with C57BL/6j mice more sensitive than DBA/2j mice (Chapter 6).

7.1.2 Analgesic Response

As mentioned in Chapter 1, achieving pain relief rapidly, consistently and with minimal side effects is the ultimate goal of pain management, and the genesis of this thesis was to determine some predictors for response to NSAIDs, in order to account for the clinically troublesome variability in response to these agents.

This thesis has shown that ibuprofen response is dependent upon **sex hormone status** and not **sex** *per se*, as was previously thought (Walker & Carmody, 1998). Indeed in humans, oestrogen abolishes analgesic response to ibuprofen, and ibuprofen analgesia in males is dependent upon **expectancy** (Chapters 2 and 3).

In males, analgesic response to placebo and ibuprofen only occurs when they believe that an analgesic drug is to be given (Chapter 3). No effect of expectancy was seen in females.

There also appear to be menstrual cycle, and exogenous sex hormone effects on ibuprofen analgesia in humans and rodents - to the extent that in humans ibuprofen is only analgesic during the menses phase of the young females' menstrual cycle, and paradoxically only during oestrogen treatment in rodents (Chapters 2 and 5).

Additionally, non-sex-linked genotype was important in determining response to paracetamol in a mouse population, with DBA/2j mice being refractory to the effects of paracetamol 400mg/kg (Chapter 6).

7.2 RELEVANCE TO LITERATURE

This thesis has altered the way in which the literature should be viewed. Three pertinent points deserve mention in this brief recapitulation of the thesis.

When the work of this thesis was begun, it was thought that differences in basal nociception would be determined solely by biological differences, such as a subject's sex (or sex hormone status). Numerous other reports have attributed differences in basal nociceptive sensitivity to biological differences, such as a subject's sex (for review, see Introduction, and Riley *et al.*, 1998). However, while biological differences in sex hormone levels have been useful in predicting basal pain tolerance, they have not been helpful in predicting differences in pain report (VAS scores). Thus, the results of this thesis have strengthened Fillingim's

hypothesis that the sex differences in pain are sculpted by multiple factors, including biological, psychological and sociocultural ones (Fillingim, 2000).

Firstly, biological differences. A stimulus to this thesis was the need to ascertain whether there were any predictors that would help to determine basal nociception or analgesic response. While this suggests a rather wide scope, the underlying motivation was to determine whether the biological difference of sex hormone status could predict both nociceptive and analgesic response – and it can.

This thesis has shown that in humans, sex hormones influence pain tolerance levels - subjects with high levels of oestrogen have reduced pain tolerance levels compared with those subjects with low levels of oestrogen. In addition, those with high levels of oestrogen showed no analgesic response to ibuprofen. This provides a possible explanation for the reported sex differences in pain tolerance in the literature, especially as most studies have looked at premenopausal women and young men, and therefore are ***really*** studies of sex hormone differences. The argument that sex hormone levels determine analgesic response is also important. However, there appear to be species differences in the particular effect of the hormones (see Chapters 2 and 5), perhaps reflecting the different noxious stimulation methods used. It would be interesting to determine whether these species differences disappear when the same noxious stimulus modality is employed, which is a question for future experiments. In any case, sex hormones may also produce the sex differences in other analgesics, such as morphine – especially as there are sex hormonal influences on opioid receptor systems (for example Candido *et al.*, 1992; Cicero *et al.*, 1996). Further work is required to

determine the mechanisms responsible for sex hormone influences on both basal nociceptive sensitivity, and analgesic drug response.

The other potential biological predictor that was examined in this thesis was the effect of strain (or genetics) on nociceptive sensitivity and response to paracetamol. The issue of genetics determining drug response is an area of enormous potential. If accurate predictors of analgesic response could be determined from a simple genetic screen, the treatment of pain would be revolutionised. While this is a long way off because the issue is complex, and it would appear that several genes are involved, the advancements reported in this thesis in addition to those already published in the literature (for review see Mogil, 1999), have enhanced our understanding of genetic differences in analgesic sensitivity. In addition, while one gene may be important in determining paracetamol response, it may have no role in determining ibuprofen response. A great body of further work is required to understand the role of genetics on pain and its inhibition.

Secondly, sociocultural factors. Several previous studies have proposed that sex differences in pain report are due to differences in sex-role expectancies. For example, one study has found that males report significantly less pain in front of a female experimenter than a male experimenter (Levine & de Simone, 1991). Another group found that 46% of the variance in willingness to report pain could be accounted for by differences in subject sex, and that both men and women thought men would report less pain (Robinson *et al.*, 2001). *This finding was not replicated in any of the experiments outlined in Chapters 2-4 of this thesis: indeed women reported less pain (i.e. lower VAS scores) in all these cases.* Either sociocultural factors play no role in pain report, *or* they are not important in the

experimental situation described in this thesis. It is hypothesised that the latter (that sociocultural factors are not important) better reflects the situation in this thesis. Another researcher has found that "women's [pain] tolerance for electrical stimuli was at a point they themselves described as 5 [out of 10] on the scale (moderate) whereas men went to nearly 7 [out of 10]" (Rollman, 1997). Perhaps the fact that no sex-role expectancies were found in the experiments in Chapters 2-4, reflects sex differences in the scaling properties of tools such as the VAS. This is an important question, which must be further examined.

Finally, psychological factors. Probably the most important finding of this thesis was the role of expectancy in analgesic response. For the first time, sex differences in expectancy-produced analgesia have been shown. The experimental design used in Chapter 3 does not allow one to determine whether **sex** or **sex hormone status** is important, but it seems most unlikely that post-menopausal women would change their expectancies depending on whether they were taking exogenous hormones or not. One other study has shown that positive expectancy increases the analgesic potency of an NSAID, ketorolac (Amanzio *et al.*, 2001). In the experimental part of that study, both males and females were used – but regrettably their results were not analysed by sex. In addition, only a small number of subjects were studied (6-8 depending on the expectancy condition), so the statistical power to detect sex differences would have been small. In any case, it would be valuable if Amanzio and colleagues were to revisit their original data to determine whether any sex differences existed in the expectancies of their subjects.

7.3 FUTURE DIRECTIONS AND RECOMMENDATIONS

This thesis has raised several important questions that need to be pursued. Future experiments might involve:

- *Determination of the effect of exogenous hormones on baseline pain and ibuprofen response in a larger subject population, and with other pain methodologies.*
- *Determination of the effect of changing levels of endogenous sex hormone during the menstrual phase on baseline pain and ibuprofen response in a larger subject population.*
- *Determination of the effect of sex hormones (both endogenous and exogenous) on the efficacy of other known analgesic drugs, especially opioids.*
- *Discovery of the mechanisms responsible for the sex hormonal influences on baseline pain and analgesic response, with specific focus on substance P, NMDA and AMPA and their possible role in NSAID analgesia.*
- *Determination of whether sex or sex hormone status predicts expectancy response in both pre- and post-menopausal women.*
- *Evaluation of the effects of expectancy on other known analgesic drugs both the NSAID and opioid classes.*
- *Determination of whether expectancy is dependent upon perceived potency of the analgesic drug (for example, whether morphine is more influenced by expectancy than ibuprofen).*
- *Determination of the role of oestrogen, progesterone, testosterone, follicle stimulating hormone, and luteinizing hormone on baseline nociceptive sensitivity and ibuprofen response in rodent models using both male and female gonadectomized rats.*
- *Determination of the effect of oestrus cycle on ibuprofen response in female rats.*
- *Determination of the effect of murine strain on analgesic response to a wide variety of pharmacological analgesic agents (e.g. ibuprofen, naproxen, and opioids etc).*

This thesis has highlighted the importance of sex, sex hormone status and genetics on basal nociceptive sensitivity and analgesic response. Therefore, several recommendations are made for future pain research. *Firstly*, as sex hormones are important predictors of response, future pain research must examine the responses

of both men and women, of varying ages, and sex hormone status when determining analgesic response. *Secondly*, the effect of genes must be elucidated, perhaps by the development of gene banks from chronic pain patients.

7.4 CONCLUDING COMMENTS

This thesis has contributed significant evidence about the role of sex and sex hormone status, as well as genetic constitution on basal nociceptive sensitivity and response to the NSAIDs ibuprofen and paracetamol. The influence of sex on psychological conditioning, such as the expectancy paradigm, has also been highlighted. These results are important, and potentially highly clinically relevant.

This thesis has also shown that while biological differences such as differences in sex steroid levels can account for some of the sex differences seen in the literature (and the experiments of this thesis), they do not account for all of them. For example, pain report seems to be a function of sex and in the clinical situation may be influenced by sex-role expectancies, or familial history of pain (although further experiments are required in this regard). Hormonal effects on pain response (threshold and tolerances) appear to be of a cyclical nature, considering the menstrual cycle influences seen in Chapter 4.

The ultimate goal of pain physicians is the rapid relief of pain, with minimal side effects. While much further work needs to be done, especially with regard to the mechanisms responsible for eliciting the effects seen in the experiments outlined in this thesis, clinicians now need to consider the patient's sex and sex hormonal status when prescribing medications for pain relief. The results of this thesis should convince clinicians and experimenters alike that sex, sex hormone status and genes play an integral role in determining basal nociceptive sensitivity and the response to analgesic drugs.

Appendix A

INCLUSION AND EXCLUSION CRITERIA

The following is a comprehensive list of inclusion and exclusion criteria used for the experiments outlined in Chapters 2 and 3.

A.1 INCLUSION CRITERIA

- Male or female aged 18 to 45 (for Chapter 3) or to 65 (Chapter 2).
- Be in general good health
- Agree to sign a consent form

A.2 EXCLUSION CRITERIA

- Pregnancy
- NSAID sensitive asthma
- Peptic ulcer
- NSAID allergy
- Heart problems
- Liver problems
- Stomach problems
- Blood pressure problems
- Arthritis
- Clinically significant illness within the last 4 weeks
- Surgery within the last 3 months

Appendix B

HAEMATOLOGICAL AND BIOCHEMICAL TESTS

The following tests were used in screening subjects in Chapters 2 and 3.

- Full blood count
- Electrolytes
- Urea
- Creatinine
- Liver Function Tests
- Serum Oestrogen
- Serum Progesterone
- Plasma Testosterone

Appendix C

PHARMACOKINETIC EQUATIONS

The following equations were used to calculate pharmacokinetic parameters in Chapters 2 and 3. The fraction absorbed (F) was assumed to be one.

1. Area under the curve (AUC, μg.hr.mL^{-1}) $AUC = \int_0^\infty C.\delta t$

2. Half-Life ($t_{1/2}$, hours) Time taken for plasma concentration to drop by half in the terminal phase of elimination.

3. Elimination rate constant (K_e, hours^{-1}) $K_e = \frac{t_{1/2}}{0.693}$

4. Clearance (CL, L.hr^{-1}) $CL = \frac{dosexF}{AUC}$

   (CL, mL.min^{-1}.kg^{-1}) $CL = \frac{Fxdose/kg}{AUCx60}$

5. Volume of Distribution (V_D, L) $V_D = \frac{t_{1/2}xCL(L.hr^{-1})}{0.693}$

Appendix D

RAW DATA

The raw data produced in this thesis can be found on the accompanying CD-ROM found inside the back cover of this thesis.

Notes : Raw Data in the CD have been included in the end of this PDF file.

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Ch2data

CODE	SEX	GROUP	AGE	WEIGHT	MENSTRUAL PHASE	EAR THICKNESS (cm)	TAKING OC PILL?	SEX HORMONE STATUS	DOSE (mg)	PAIN THRESHOLD @ BASELINE	PAIN THRESHOLD @ 1hr	PAIN THRESHOLD @ 2 hrs	PAIN THRESHOLD @ 3 hrs	PAIN THRESHOLD @ 4 hrs
yt1	Female	1	26	62	3	.44	0	+SH	0	5.0	6.0	5.0	5.0	5.0
yt1	Female	1	26	62	1	.44	0	+SH	400	6.2	6.0	7.3	6.3	6.0
yt1	Female	1	26	62	2	.44	0	+SH	800	5.6	8.0	6.6	6.3	6.3
yt2	Female	1	36	48	2	.45	0	+SH	400	15.5	14.3	16.0	17.6	16.3
yt2	Female	1	36	48	3	.45	0	+SH	800	16.9	16.6	15.3	15.6	14.6
yt2	Female	1	36	48	1	.45	0	+SH	0	12.3	14.6	14.0	16.3	15.0
yt3	Female	1	19	62	2	.38	0	+SH	800	12.3	12.6	13.3	13.3	13.3
yt3	Female	1	19	62	3	.38	0	+SH	400	14.2	22.3	21.7	17.3	18.3
yt4	Female	1	19	65	4	missing	1	+SH	400	8.2	9.3	10.6	11.0	11.0
yt4	Female	1	19	65	4	missing	1	+SH	0	19.4	15.0	16.6	15.3	16.0
yt4	Female	1	19	65	1	missing	1	+SH	800	19.0	20.0	21.6	19.6	20.3
ym1	Male	2	21	72	5	missing	2	-SH	0	18.0	20.0	17.3	17.6	14.3
ym1	Male	2	21	72	5	missing	2	-SH	800	8.2	8.3	10.0	10.0	11.3
ym1	Male	2	21	72	5	missing	2	-SH	400	13.4	14.0	15.3	15.0	12.3
ym2	Male	2	23	82	5	.60	2	-SH	0	12.8	13.3	13.3	14.0	14.0
ym2	Male	2	23	82	5	.60	2	-SH	800	12.6	12.6	13.6	13.3	13.0
ym2	Male	2	23	82	5	.60	2	-SH	400	14.6	14.3	14.3	14.0	15.0
yt5	Female	1	37	55	3	.56	0	+SH	0	15.6	16.0	14.3	15.0	15.0
yt5	Female	1	37	55	1	.56	0	+SH	800	19.0	20.0	20.0	19.6	21.0
yt5	Female	1	37	55	2	.56	0	+SH	400	12.7	13.6	15.6	16.0	16.3
yt6	Female	1	21	63	1	.53	1	+SH	400	14.1	15.5	16.0	15.3	15.0
yt6	Female	1	21	63	4	.53	1	+SH	0	21.4	20.0	21.3	18.6	19.6
yt6	Female	1	21	63	4	.53	1	+SH	800	21.9	21.0	21.6	22.3	22.3
ym3	Male	2	20	73	5	.46	2	-SH	400	13.7	12.3	15.3	16.0	15.7
ym3	Male	2	20	73	5	.46	2	-SH	0	12.2	23.3	15.3	15.0	17.0
ym3	Male	2	20	73	5	.46	2	-SH	800	18.9	19.7	19.7	21.3	19.0
yt7	Female	1	19	58	1	missing	0	+SH	800	4.0	3.3	3.7	4.0	3.3
yt7	Female	1	19	58	2	missing	0	+SH	0	10.1	10.0	10.0	9.7	10.0
yt7	Female	1	19	58	3	missing	0	+SH	400	11.6	12.0	12.0	12.0	10.7
ym4	Male	2	31	78	5	.48	2	-SH	400	16.1	14.0	15.0	15.0	14.0
ym4	Male	2	31	78	5	.48	2	-SH	800	10.7	9.7	10.3	11.7	11.3
ym4	Male	2	31	78	5	.48	2	-SH	0	15.3	15.3	15.0	14.7	17.0
yt8	Female	1	25	72	3	.47	0	+SH	400	12.8	13.0	11.3	12.3	12.7
yt8	Female	1	25	72	1	.47	0	+SH	800	11.4	15.3	12.7	11.0	10.0
yt8	Female	1	25	72	2	.47	0	+SH	0	10.3	11.0	12.0	11.3	11.0
ym5	Male	2	45	0	5	.62	2	-SH	0	11.2	13.0	12.0	12.7	13.3
ym5	Male	2	45	0	5	.62	2	-SH	800	12.9	13.0	13.3	13.0	13.3
ym5	Male	2	45	0	5	.62	2	-SH	400	11.2	11.0	10.0	10.7	10.3
yt9	Male	1	24	61	4	missing	1	+SH	400	9.3	10.7	11.0	12.3	12.0
yt9	Female	1	24	61	4	missing	1	+SH	0	17.1	29.0	35.3	31.7	39.0
yt9	Female	1	24	61	1	missing	1	+SH	800	13.0	15.7	17.0	16.7	14.7
om1	Male	5	57	96	5	.66	2	-SH	400	7.7	7.3	8.0	7.3	8.0
om1	Male	5	57	96	5	.66	2	-SH	0	7.8	10.3	9.3	9.3	9.3
om1	Male	5	57	96	5	.66	2	-SH	800	11.0	11.3	10.7	11.0	12.0
yt10	Female	1	43	100	1	.54	0	+SH	800	8.7	10.3	12.0	11.3	10.0

CODE	SEX	GROUP	AGE	WEIGHT	MENSTRUAL PHASE	EAR THICKNESS (cm)	TAKING OC PILL?	+SH HORMONE STATUS	DOSE (mg)	PAIN THRESHOLD @ BASELINE	PAIN THRESHOLD @ 1hr	PAIN THRESHOLD @ 2 hrs	PAIN THRESHOLD @ 3 hrs	PAIN THRESHOLD @ 4 hrs
yt10	Female	1	43	100	2	.54	0	+SH	400	11.0	10.3	11.3	9.3	10.3
yt10	Female	1	43	100	3	.54	0	+SH	0	9.7	11.0	11.3	10.0	9.7
otnil1	Female	4	54	69	5	.56	0	-SH	400	7.9	7.7	9.3	8.7	10.0
otnil1	Female	4	54	69	5	.56	0	-SH	0	8.2	8.7	8.7	9.3	9.7
otnil1	Female	4	54	69	5	.56	0	-SH	800	10.9	11.7	11.7	11.7	13.3
ym6	Male	2	26	100	5	.48	2	-SH	800	12.2	14.0	13.0	13.7	14.0
ym6	Male	2	26	100	5	.48	2	-SH	400	16.2	17.7	17.3	17.7	16.0
ym6	Male	2	26	100	5	.48	2	-SH	0	14.7	15.3	14.7	15.6	14.7
yt11	Female	1	27	54	5	.39	0	+SH	0	8.6	10.3	10.0	10.0	9.7
yt11	Female	1	27	54	5	.39	0	+SH	400	9.2	9.7	8.7	8.7	8.7
yt11	Female	1	27	54	5	.39	0	+SH	800	14.9	14.0	15.0	14.7	14.0
yt12	Female	1	22	53	5	missing	0	+SH	400	20.1	22.0	21.7	22.0	23.0
ym7	Male	2	42	69	5	.56	2	-SH	400	7.8	10.3	8.0	8.3	10.0
ym7	Male	2	42	69	5	.56	2	-SH	800	4.2	4.0	5.3	5.7	5.0
ym7	Male	2	42	69	5	.56	2	-SH	0	5.7	5.0	4.7	6.3	7.0
yt13	Female	1	20	62	1	.61	1	+SH	0	17.2	17.7	17.7	17.7	18.3
yt13	Female	1	20	62	4	.61	1	+SH	800	18.1	16.0	14.0	16.7	16.3
yt13	Female	1	20	62	4	.61	1	+SH	400	9.9	10.0	10.3	9.3	8.7
yt14	Female	1	22	60	1	.53	1	+SH	400	9.0	7.7	9.0	11.7	9.3
yt14	Female	1	22	60	4	.53	1	+SH	800	9.5	8.7	9.0	9.3	8.7
yt14	Female	1	22	60	4	.53	1	+SH	0	8.0	5.7	10.3	11.7	8.0
ym8	Male	2	21	75	5	.45	2	-SH	0	18.4	19.0	20.3	19.7	20.3
ym8	Male	2	21	75	5	.45	2	-SH	800	14.0	15.3	15.7	16.3	17.3
ym8	Male	2	21	75	5	.45	2	-SH	400	17.8	17.0	15.7	19.3	17.7
ym9	Male	2	21	73	5	.49	2	-SH	400	10.7	11.7	10.0	10.0	7.7
ym9	Male	2	21	73	5	.49	2	-SH	800	12.3	13.0	12.0	11.7	11.0
ym9	Male	2	21	73	5	.49	2	-SH	0	12.9	13.7	13.3	13.0	13.3
otnil2	Female	4	57	68	5	.62	2	-SH	0	16.1	15.0	15.3	13.3	13.3
otnil2	Female	4	57	68	5	.62	2	-SH	400	14.4	13.3	14.0	15.3	14.0
otnil2	Female	4	57	68	5	.62	2	-SH	800	10.7	12.7	12.0	10.3	11.7
ym10	Male	2	22	74	5	.46	2	-SH	0	6.2	4.7	4.7	5.0	4.0
ym10	Male	2	22	74	5	.46	2	-SH	800	7.9	8.0	7.0	6.7	7.0
ym10	Male	2	22	74	5	.46	2	-SH	400	8.8	10.0	9.7	9.7	9.0
otnil3	Female	4	54	64	5	.55	2	-SH	800	8.0	9.0	11.3	13.0	10.0
otnil3	Female	4	54	64	5	.55	2	-SH	400	15.4	18.3	19.7	18.7	19.0
otnil3	Female	4	54	64	5	.55	2	-SH	0	15.0	16.7	18.3	17.7	18.3
om2	Male	5	61	80	5	.39	2	-SH	800	8.0	9.7	9.7	10.3	11.3
om2	Male	5	61	80	5	.39	2	-SH	400	8.1	8.3	8.3	8.3	8.3
om2	Male	5	61	80	5	.39	2	-SH	0	6.7	7.0	7.0	6.7	6.7
ym11	Male	2	31	70	5	.47	2	-SH	800	9.8	11.0	14.3	17.3	14.3
ym11	Male	2	31	70	5	.47	2	-SH	400	10.1	9.3	9.7	11.3	11.0
ym11	Male	2	31	70	5	.47	2	-SH	0	11.6	17.0	18.0	14.3	14.7
yt15	Female	1	20	70	3	.52	0	+SH	0	15.7	14.3	16.7	16.0	15.7
yt15	Female	1	20	70	2	.52	0	+SH	400	18.9	19.3	20.0	18.7	20.3
yt15	Female	1	20	70	3	.52	0	+SH	800	21.2	20.3	20.3	21.0	19.3

CODE	SEX	GROUP	AGE	WEIGHT	MENSTRUAL PHASE	EAR THICKNESS (cm)	TAKING OC PILL?	+SH HORMONE STATUS	DOSE (mg)	PAIN THRESHOLD @ BASELINE	PAIN THRESHOLD @ 1hr	PAIN THRESHOLD @ 2 hrs	PAIN THRESHOLD @ 3 hrs	PAIN THRESHOLD @ 4 hrs
yt16	Female	1	30	50	3	.60	0	+SH	800	12.6	14.7	15.0	13.3	13.0
yt16	Female	1	30	50	3	.60	0	+SH	0	12.8	11.7	11.7	12.0	11.0
yt16	Female	1	30	50	3	.60	0	+SH	400	14.0	14.7	15.0	14.0	14.0
ym12	Male	2	22	70	5	.33	2	-SH	0	24.2	23.3	24.0	25.0	24.7
ym12	Male	2	22	70	5	.33	2	-SH	400	23.1	2.3	22.0	20.0	20.7
ym12	Male	2	22	70	5	.33	2	-SH	800	27.4	27.0	27.3	26.7	26.0
om3	Male	5	53	85	5	.70	2	-SH	0	5.2	5.0	5.7	5.7	6.0
om3	Male	5	53	85	5	.70	2	-SH	800	5.4	8.0	7.7	7.7	7.0
om3	Male	5	53	85	5	.70	2	-SH	400	7.8	8.0	9.0	10.3	9.0
otnil4	Female	4	57	58	5	.48	2	-SH	400	12.2	14.3	16.3	21.3	16.7
otnil4	Female	4	57	58	5	.48	2	-SH	0	13.2	16.0	18.3	18.7	18.0
otnil4	Female	4	57	58	5	.48	2	-SH	800	14.9	17.3	18.3	18.3	19.3
otexh1	Female	3	58	54	7	.45	2	+SH	400	10.7	9.7	10.7	9.0	10.0
otexh1	Female	3	58	54	7	.45	2	+SH	0	21.7	22.3	22.3	27.3	26.0
otexh1	Female	3	58	54	7	.45	2	+SH	800	14.1	14.3	16.0	15.7	14.0
otexh2	Female	3	58	67	6	.69	2	+SH	0	9.4	10.0	9.7	9.0	9.0
otexh2	Female	3	58	67	6	.69	2	+SH	800	8.7	8.3	8.0	8.3	8.7
otexh2	Female	3	58	67	6	.69	2	+SH	400	12.0	11.0	10.7	10.7	10.0
om4	Male	5	59	75	5	.54	2	-SH	0	12.9	14.3	17.0	16.0	20.3
om4	Male	5	59	75	5	.54	2	-SH	800	12.7	11.3	12.3	15.3	13.7
om4	Male	5	59	75	5	.54	2	-SH	400	14.6	15.7	18.7	13.3	14.7
yt17	Female	1	20	57	2	.64	0	+SH	800	7.0	10.3	11.0	10.0	missing
yt17	Female	1	20	57	2	.64	0	+SH	0	12.1	14.7	13.0	12.7	12.3
yt17	Female	1	20	57	2	.64	0	+SH	400	8.5	10.3	16.0	9.0	15.7
om5	Male	5	55	114	5	.46	2	-SH	400	11.0	8.3	9.3	3.0	9.3
om5	Male	5	55	114	5	.46	2	-SH	0	12.6	14.7	14.0	13.3	14.0
om5	Male	5	55	114	5	.46	2	-SH	800	14.0	17.5	13.7	13.3	15.0
yt18	Female	1	23	66	2	.56	0	+SH	800	7.3	7.0	7.0	7.3	6.7
yt18	Female	1	23	66	3	.56	0	+SH	0	11.2	12.0	12.0	11.0	11.7
yt18	Female	1	23	66	1	.56	0	+SH	400	11.6	11.7	10.7	11.0	14.0
yt19	Female	1	22	55	4	.60	1	+SH	400	10.8	10.0	10.3	11.0	10.3
yt19	Female	1	22	55	4	.60	1	+SH	0	14.3	13.3	13.0	13.0	13.3
yt19	Female	1	22	55	1	.60	1	+SH	800	14.2	14.0	15.0	15.3	15.3
ym13	Male	2	40	84	5	.60	0	-SH	400	7.7	9.3	8.7	8.0	7.7
ym13	Male	2	40	84	5	.60	0	-SH	800	11.2	11.0	11.3	10.3	10.7
ym13	Male	2	40	84	5	.60	0	-SH	0	12.4	20.3	11.0	1.3	10.3
om6	Male	5	63	75	5	.55	0	-SH	800	12.3	12.7	13.0	12.7	12.0
om6	Male	5	63	75	5	.55	0	-SH	0	12.4	17.7	16.7	18.7	15.0
om6	Male	5	63	75	5	.55	0	-SH	400	15.7	17.0	20.0	20.3	17.3
otexh3	Female	3	59	67	6	.57	0	+SH	0	15.3	17.7	20.0	21.7	21.7
otexh3	Female	3	59	67	6	.57	0	+SH	400	14.0	16.3	18.3	17.7	missing
otexh3	Female	3	59	67	6	.57	0	+SH	800	17.6	17.0	18.3	18.0	missing
otnil5	Female	4	58	77	5	.39	0	+SH	400	9.8	13.3	14.0	12.7	13.7
otnil5	Female	4	58	77	5	.39	0	-SH	0	16.4	18.3	18.7	18.7	18.7
otnil5	Female	4	58	77	5	.39	0	-SH	800	14.0	17.7	15.3	missing	17.7

CODE	SEX	GROUP	AGE	WEIGHT	MENSTRUAL PHASE	EAR THICKNESS (cm)	TAKING OC PILL?	SEX HORMONE STATUS	DOSE (mg)	PAIN THRESHOLD @ BASELINE	PAIN THRESHOLD @ 1hr	PAIN THRESHOLD @ 2 hrs	PAIN THRESHOLD @ 3 hrs	PAIN THRESHOLD @ 4 hrs
om7	Male	5	68	65	5	.60	0	-SH	0	20.0	16.3	17.7	17.0	18.7
om7	Male	5	68	65	5	.60	0	-SH	800	14.2	14.3	11.3	10.7	17.3
om7	Male	5	68	65	5	.60	0	-SH	400	16.0	14.0	13.3	15.7	14.7
otnil6	Female	4	49	75	5	missing	0	-SH	400	7.7	8.0	6.7	8.0	8.7
otnil6	Female	4	49	75	5	missing	0	-SH	0	9.1	7.3	10.0	9.3	9.3
otnil6	Female	4	49	75	5	missing	0	-SH	800	12.3	11.3	11.0	12.0	11.7
otexh4	Female	3	46	68	7	missing	0	+SH	0	9.7	6.3	7.3	8.0	9.7
otexh4	Female	3	46	68	7	missing	0	+SH	400	10.3	7.3	9.0	8.0	8.0
otexh4	Female	3	46	68	7	missing	0	+SH	800	12.0	12.0	10.3	11.3	10.0
ym14	Male	2	27	80	5	missing	0	-SH	800	12.2	13.0	16.0	11.7	15.7
ym14	Male	2	27	80	5	missing	0	-SH	400	14.9	15.3	15.0	13.3	14.3
ym14	Male	2	27	80	5	missing	0	-SH	0	12.8	14.3	17.3	15.0	13.0
yf20	Female	1	47	56	3	.51	0	+SH	800	14.1	17.7	18.0	17.0	17.0
yf20	Female	1	47	56	2	.51	0	+SH	400	21.3	18.7	19.7	23.7	21.7
yf20	Female	1	47	56	2	.51	0	+SH	0	18.4	20.3	23.7	23.0	20.7
ym15	Male	2	47	98	5	missing	0	-SH	0	9.2	10.3	9.0	13.3	13.0
ym15	Male	2	47	98	5	missing	0	-SH	400	16.0	15.7	15.3	15.3	16.3
ym15	Male	2	47	98	5	missing	0	-SH	800	14.0	17.0	18.0	16.0	15.3
om8	Male	5	59	80	5	missing	0	-SH	800	10.9	12.7	11.7	12.3	12.3
om8	Male	5	59	80	5	missing	0	-SH	0	13.7	13.3	14.7	14.0	14.0
om8	Male	5	59	80	5	missing	0	-SH	400	18.7	19.3	18.0	18.3	20.3
otexh5	Female	3	63	61	7	.31	0	+SH	0	15.0	18.0	18.7	17.3	20.0
otexh5	Female	3	63	61	7	.31	0	+SH	800	16.1	16.0	16.0	16.0	15.3
otexh5	Female	3	63	61	7	.31	0	+SH	400	9.0	9.3	9.3	9.7	9.7
om9	Male	5	50	80	5	.55	0	-SH	800	17.2	18.7	15.3	17.7	18.3
om9	Male	5	50	80	5	.55	0	-SH	0	16.9	18.3	16.3	16.0	17.0
om9	Male	5	50	80	5	.55	0	-SH	400	17.0	15.0	14.7	13.7	14.0
otexh6	Female	3	58	82	7	.29	0	+SH	800	12.3	11.0	13.0	13.7	12.7
otexh6	Female	3	58	82	7	.29	0	+SH	0	5.0	6.3	6.3	7.0	6.7
otexh6	Female	3	58	82	7	.29	0	+SH	400	5.7	5.0	5.0	6.0	6.3
ym16	Male	2	34	98	5	.30	0	-SH	400	15.0	17.3	18.7	20.3	18.7
ym16	Male	2	34	98	5	.30	0	-SH	0	12.2	17.0	17.3	17.0	17.7
ym16	Male	2	34	98	5	.30	0	-SH	800	18.3	18.3	18.7	19.3	18.0
ym17	Male	2	23	65	5	.30	0	-SH	800	19.0	19.0	16.0	19.0	16.7
ym17	Male	2	23	65	5	.30	0	-SH	0	25.3	26.0	29.3	29.3	35.0
ym17	Male	2	23	65	5	.30	0	-SH	400	30.6	29.3	30.3	31.7	30.0
otnil7	Female	4	58	59	5	.47	0	-SH	800	10.4	12.7	13.7	13.0	11.7
otnil7	Female	4	58	59	5	.47	0	-SH	0	11.8	13.0	12.3	12.7	13.3
otnil7	Female	4	58	59	5	.47	0	-SH	400	9.2	9.7	9.0	10.0	9.7
ym18	Male	2	31	53	5	.30	0	-SH	400	14.2	14.0	14.3	13.0	15.0
ym18	Male	2	31	53	5	.30	0	-SH	0	15.0	6.3	9.0	6.0	6.3
ym18	Male	2	31	53	5	.30	0	-SH	800	7.4	9.3	9.7	6.7	missing
otnil8	Female	4	58	75	5	missing	0	-SH	800	19.0	21.0	21.7	21.7	22.0
otnil8	Female	4	58	75	5	missing	0	-SH	400	22.8	25.0	25.3	24.7	25.0
otnil8	Female	4	58	75	5	missing	0	-SH	0	13.3	18.0	18.3	21.0	21.0

CODE	SEX	GROUP	AGE	WEIGHT	MENSTRUAL PHASE	EAR THICKNESS (cm)	TAKING OC PILL?	SEX HORMONE STATUS	DOSE (mg)	PAIN THRESHOLD @ BASELINE	PAIN THRESHOLD @ 1hr	PAIN THRESHOLD @ 2 hrs	PAIN THRESHOLD @ 3 hrs	PAIN THRESHOLD @ 4 hrs
otnil9	Female	4	64	83	5	.56	0	-SH	0	13.0	14.0	12.7	12.7	13.0
otnil9	Female	4	64	83	5	.56	0	-SH	800	24.3	22.3	24.3	24.3	23.7
otnil9	Female	4	64	83	5	.56	0	-SH	400	23.9	25.3	27.3	25.7	25.0
otexh7	Female	3	63	75	7	.26	0	+SH	400	14.3	13.7	11.7	13.3	14.0
otexh7	Female	3	63	75	7	.26	0	+SH	0	13.1	13.0	14.0	14.0	14.3
otexh7	Female	3	63	75	7	.26	0	+SH	800	14.0	14.7	15.3	16.0	12.7
otexh8	Female	3	55	66	6	missing	0	+SH	0	10.3	8.7	10.3	13.0	11.7
ym19	Male	2	25	74	5	.42	0	-SH	800	4.1	4.3	4.3	4.7	4.0
ym19	Male	2	25	74	5	.42	0	-SH	0	6.9	7.0	6.7	6.7	7.0
ym19	Male	2	25	74	5	.42	0	-SH	400	5.9	5.7	5.7	5.0	6.0
ym20	Male	2	24	0	5	missing	0	-SH	400	24.8	25.0	31.0	25.7	39.3
ym20	Male	2	24	0	5	missing	0	-SH	0	38.3	29.7	41.3	48.6	43.3
ym20	Male	2	24	0	5	missing	0	-SH	800	47.0	40.7	43.7	45.0	43.3
otnil10	Female	4	57	60	5	.37	0	-SH	400	26.3	20.3	20.0	20.0	20.7
otnil10	Female	4	57	60	5	.37	0	-SH	0	18.3	14.0	11.7	11.3	13.0
otnil10	Female	4	57	60	5	.37	0	-SH	800	13.2	15.0	13.7	12.0	13.7
otexh9	Female	3	59	54	7	.42	0	+SH	800	8.8	10.0	9.7	9.0	7.3
otexh9	Female	3	59	54	7	.42	0	+SH	0	14.6	13.7	10.7	15.0	10.7
otexh9	Female	3	59	54	7	.42	0	+SH	400	8.1	9.7	6.0	8.7	8.7
om10	Male	5	50	76	5	.34	0	-SH	400	9.4	9.3	10.0	10.0	9.3
om10	Male	5	50	76	5	.34	0	-SH	0	16.3	15.0	15.0	13.7	13.0
om10	Male	5	50	76	5	.34	0	-SH	800	15.1	17.0	17.3	16.3	15.7
otexh10	Female	3	61	57	6	.37	0	+SH	400	12.8	19.0	17.3	16.7	22.0
otexh10	Female	3	61	57	6	.37	0	+SH	0	15.1	15.0	16.3	16.0	16.3
otexh10	Female	3	61	57	6	.37	0	+SH	800	10.8	11.0	10.3	11.0	14.7
otexh11	Female	3	51	65	6	.39	0	+SH	800	21.9	20.7	19.7	20.3	22.7
otexh11	Female	3	51	65	6	.39	0	+SH	400	21.0	21.3	19.7	22.0	21.0
otexh11	Female	3	51	65	6	.39	0	+SH	0	21.2	20.3	20.0	19.7	20.0

1 = YF; 2 = YM; 3 = OFEXH; 4 = OFNIL; 5 = OM

1 = menses, 2 = follicular, 3 = luteal, 4 = active OC, 5 = N/A, 6 = ERT, 7 = HRT

0 = No; 1 = Yes; 2 = N/A

CODE	PAIN TOLERANCE @ BASELINE	PAIN TOLERANCE @ 1 hr	PAIN TOLERANCE @ 2 hr	PAIN TOLERANCE @ 3 hr	PAIN TOLERANCE @ 4 hr	VAS @ BASELINE	VAS @ 1 hr	VAS @ 2 hr	VAS @ 3 hr	VAS @ 4 hr	KEEP DATA?	KEEP MENSTRUAL DATA?	BASELINE CORRECTED PAIN TOLERANCE @ BASELINE	BASELINE CORRECTED PAIN TOLERANCE @ 1 hr
yt1	6.2	7.0	6.0	6.0	6.0	9.9	10.0	8.6	7.0	5.0	Yes	0	.0	.8
yt1	7.2	8.0	8.6	7.3	7.0	5.3	4.0	4.0	3.0	3.6	Yes	1	.0	.8
yt1	6.6	9.0	7.6	7.3	7.3	10.1	13.3	10.3	11.6	7.6	Yes	1	.0	2.4
yt2	18.0	18.6	19.0	20.6	20.6	4.6	4.3	9.3	8.6	10.6	Yes	1	.0	.6
yt2	19.6	19.6	20.0	19.6	19.6	12.3	11.6	11.6	14.6	13.6	Yes	1	.0	.0
yt2	16.3	21.0	20.6	22.0	18.6	9.6	10.6	10.0	10.0	11.3	Yes	1	.0	4.7
yt3	15.6	16.0	16.3	16.0	16.3	6.6	5.6	6.0	8.3	6.6	Yes	1	.0	.4
yt3	18.4	22.3	21.6	17.6	18.6	5.9	6.0	5.7	7.3	5.7	Yes	1	.0	3.9
yt4	11.5	12.6	14.0	16.6	16.6	4.6	3.3	4.6	2.3	2.6	Yes	2	.0	1.1
yt4	19.4	21.0	21.3	20.0	20.3	3.1	3.0	2.0	3.0	3.0	Yes	2	.0	1.6
yt4	24.8	26.0	27.0	25.6	25.6	3.3	2.6	2.0	3.3	2.6	Yes	2	.0	1.2
ym1	19.3	21.6	18.6	19.6	15.6	2.9	3.6	3.3	3.3	3.6	Yes	0	.0	2.3
ym1	9.9	9.3	11.0	12.5	13.6	5.4	6.3	7.0	6.5	7.3	Yes	0	.0	-.6
ym1	14.5	15.3	16.0	16.0	14.0	6.8	6.3	7.6	8.3	9.3	Yes	0	.0	.8
ym2	14.6	15.0	15.0	15.6	15.6	26.7	21.0	20.3	24.3	21.6	Yes	0	.0	.4
ym2	14.1	14.3	15.0	15.0	14.0	23.0	21.0	22.3	29.6	23.3	Yes	0	.0	.2
ym2	14.4	16.6	17.0	17.0	17.0	23.4	24.0	30.0	28.6	23.7	Yes	0	.0	2.2
yt5	17.9	17.3	17.0	16.6	17.0	37.3	34.3	34.7	30.0	27.0	Yes	1	.0	-.6
yt5	21.1	22.0	21.3	22.0	23.0	26.6	26.7	27.7	25.3	28.7	Yes	1	.0	.9
yt5	14.1	15.3	17.6	18.3	18.3	28.4	25.0	26.0	27.3	26.0	Yes	1	.0	1.2
yt6	16.6	17.5	18.3	18.6	17.6	19.0	17.5	18.3	20.0	20.5	Yes	2	.0	.9
yt6	24.5	22.0	22.3	21.3	23.0	17.7	21.3	21.3	23.3	24.3	Yes	2	.0	-2.5
yt6	25.0	23.6	25.3	26.3	26.0	18.2	19.3	17.3	18.3	18.6	Yes	2	.0	-1.4
ym3	21.5	18.6	21.6	24.6	24.3	59.8	61.0	62.0	61.7	62.7	Yes	0	.0	-2.9
ym3	17.9	28.0	21.3	21.0	28.3	62.8	63.3	64.0	64.3	64.3	No	0	.0	10.1
ym3	22.6	24.6	26.6	25.6	24.6	67.0	65.0	67.3	67.3	66.3	Yes	0	.0	2.0
yt7	5.1	5.0	5.0	5.0	5.0	12.6	13.3	15.3	17.0	15.7	Yes	0	.0	-.1
yt7	13.2	12.3	11.6	12.0	11.6	11.0	13.0	13.7	15.0	14.3	Yes	1	.0	-.9
yt7	15.1	14.6	14.3	13.6	13.6	13.6	14.0	13.7	12.0	12.0	Yes	1	.0	-.5
ym4	20.5	19.6	19.6	20.3	19.6	31.1	28.7	33.7	31.0	36.4	Yes	0	.0	-.9
ym4	12.6	13.0	13.0	14.0	13.3	36.4	37.7	37.7	38.3	39.7	Yes	0	.0	.4
ym4	18.6	19.0	19.0	19.0	19.3	38.4	38.3	38.3	38.7	38.7	Yes	0	.0	.4
yt8	19.4	17.6	17.0	16.3	18.3	17.8	14.7	15.3	19.7	3.3	Yes	1	.0	-1.8
yt8	15.9	19.0	16.3	14.0	14.3	24.6	22.3	17.0	13.7	17.5	Yes	1	.0	3.1
yt8	13.2	13.3	13.7	13.0	13.3	27.1	29.3	28.0	35.7	36.3	Yes	1	.0	.1
ym5	15.9	17.3	17.3	18.7	18.3	62.2	61.7	63.0	67.0	65.7	Yes	0	.0	1.4
ym5	19.3	20.0	20.7	2.0	21.3	64.1	64.7	64.0	64.7	64.3	Yes	0	.0	.7
ym5	16.1	16.7	16.0	17.3	17.3	64.9	66.0	65.3	65.7	66.0	Yes	0	.0	.6
yt9	12.4	14.7	14.7	15.3	16.3	46.8	54.3	49.7	59.0	44.7	Yes	2	.0	2.3
yt9	23.6	34.7	47.0	39.0	50.0	42.9	30.7	32.3	41.7	30.3	No	0	.0	11.1
yt9	16.5	21.0	20.3	19.7	20.3	52.5	45.3	46.3	47.0	45.3	Yes	2	.0	4.5
om1	10.8	9.3	10.0	9.7	10.0	45.8	42.7	47.7	45.7	37.3	Yes	0	.0	-1.5
om1	10.9	12.0	12.7	12.7	12.3	46.3	44.3	45.7	41.7	45.3	Yes	0	.0	1.1
om1	14.3	15.0	15.0	15.0	15.0	48.1	44.6	45.3	46.7	46.0	Yes	0	.0	.7
yt10	12.3	17.3	17.7	17.3	15.0	41.9	42.7	33.0	41.3	44.3	Yes	1	.0	5.0

CODE	PAIN TOLERANCE @ BASELINE	PAIN TOLERANCE @ 1 hr	PAIN TOLERANCE @ 2 hr	PAIN TOLERANCE @ 3 hr	PAIN TOLERANCE @ 4 hr	VAS @ BASELINE	VAS @ 1 hr	VAS @ 2 hr	VAS @ 3 hr	VAS @ 4 hr	KEEP DATA?	KEEP MENSTRUAL DATA?	BASELINE CORRECTED PAIN TOLERANCE @ BASELINE	BASELINE CORRECTED PAIN TOLERANCE @ 1 hr
yt10	17.0	16.7	16.0	15.0	16.0	44.2	45.3	45.3	39.7	43.7	Yes	1	.0	-.3
yt10	16.7	19.0	17.3	17.0	18.7	45.0	37.7	30.6	34.0	39.3	Yes	1	.0	2.3
otnil1	9.3	10.3	12.0	11.0	11.7	7.0	6.7	6.3	8.3	9.3	Yes	0	.0	1.0
otnil1	10.0	10.0	11.0	12.0	11.7	6.8	6.7	6.7	6.7	7.0	Yes	0	.0	.0
otnil1	12.4	13.0	12.7	12.7	15.0	8.8	6.7	5.7	6.0	6.7	Yes	0	.0	.6
ym6	16.7	18.7	17.0	18.0	18.0	52.4	49.7	52.0	52.3	55.3	Yes	0	.0	2.0
ym6	21.4	26.0	21.7	24.3	22.3	54.4	53.0	51.0	51.0	49.0	Yes	0	.0	4.6
ym6	20.6	21.0	20.7	21.7	20.7	55.6	55.3	53.7	54.0	56.3	Yes	0	.0	.4
yt11	12.7	15.3	14.3	15.7	15.0	29.4	27.7	25.3	26.3	26.7	Yes	0	.0	2.6
yt11	13.6	14.0	13.3	13.3	13.7	17.8	19.7	20.3	21.3	18.3	Yes	0	.0	.4
yt11	19.9	19.7	20.0	19.7	19.3	19.9	21.0	22.3	23.7	23.0	Yes	0	.0	-.2
yt12	24.0	26.3	26.0	28.0	30.3	57.1	55.7	53.3	51.3	48.3	No	0	.0	2.3
ym7	13.2	16.0	16.0	15.0	16.0	10.4	10.0	9.3	10.7	10.5	Yes	0	.0	2.8
ym7	10.0	8.7	10.7	11.7	10.7	10.7	10.5	10.4	10.7	10.3	Yes	0	.0	-1.3
ym7	7.9	7.3	6.3	9.3	9.3	11.3	11.0	12.0	11.3	11.7	Yes	0	.0	-.6
yt13	22.8	21.3	21.7	21.0	21.3	55.2	55.0	54.3	55.3	55.7	Yes	2	.0	-1.5
yt13	22.7	20.0	19.7	20.7	20.0	54.9	53.3	55.0	55.3	54.3	Yes	2	.0	-2.7
yt13	12.0	13.3	14.7	23.0	60.2	60.2	58.3	60.0	56.0	57.7	No	0	.0	1.3
yt14	18.4	18.3	17.3	17.7	17.3	47.0	50.7	41.3	45.7	51.0	Yes	2	.0	-.1
yt14	18.8	16.7	17.0	16.3	16.7	45.2	37.7	44.3	39.0	41.7	Yes	2	.0	-2.1
yt14	16.3	14.0	15.3	14.7	14.0	41.7	42.0	39.3	40.0	41.3	Yes	2	.0	-2.3
ym8	29.4	28.3	30.7	30.0	31.3	7.3	9.0	9.0	8.7	8.7	Yes	0	.0	-1.1
ym8	20.6	21.5	22.0	22.7	23.0	8.4	9.0	10.0	8.7	8.7	Yes	0	.0	.9
ym8	24.6	23.0	22.3	23.7	22.7	8.6	8.0	8.0	8.7	8.7	Yes	0	.0	-1.6
ym9	16.9	18.3	16.3	16.0	14.7	8.9	8.3	8.0	10.0	10.7	No	0	.0	1.4
ym9	16.1	16.7	16.3	16.0	15.3	14.1	12.7	13.3	14.0	13.0	Yes	0	.0	.6
ym9	15.8	18.0	17.7	18.0	17.7	14.3	12.7	12.3	11.7	14.0	Yes	0	.0	2.2
otnil2	22.4	23.3	24.0	23.0	21.7	27.9	30.5	31.0	23.0	21.7	Yes	0	.0	.9
otnil2	21.9	23.7	27.0	26.3	24.0	28.6	26.3	25.0	21.3	20.7	Yes	0	.0	1.8
otnil2	18.8	22.7	22.0	21.3	22.0	35.7	26.7	24.3	27.3	28.7	Yes	0	.0	3.9
ym10	9.4	9.0	7.7	7.7	7.7	51.2	53.0	56.0	51.3	52.7	Yes	0	.0	-.4
ym10	12.1	9.0	7.7	8.0	8.7	50.6	46.7	42.0	48.0	48.0	No	0	.0	-3.1
ym10	13.3	11.7	11.7	12.0	12.7	52.9	53.3	47.7	46.7	46.3	Yes	0	.0	-1.6
otnil3	11.3	11.7	15.7	17.7	13.7	39.5	47.0	52.7	51.7	50.7	Yes	0	.0	.4
otnil3	20.7	20.7	21.7	21.7	22.0	53.3	52.3	52.3	54.3	53.7	Yes	0	.0	.0
otnil3	21.2	22.7	23.7	23.7	23.3	49.4	47.7	46.7	50.7	46.5	Yes	0	.0	1.5
om2	22.3	23.0	24.0	25.0	25.7	51.1	50.0	51.7	51.3	51.0	Yes	0	.0	.7
om2	22.8	20.7	20.7	20.7	19.7	50.7	51.3	50.7	50.3	50.7	Yes	0	.0	-2.1
om2	20.2	18.3	19.0	18.0	18.3	51.2	50.7	51.0	50.3	50.0	Yes	0	.0	-1.9
ym11	12.6	15.3	18.0	24.3	18.0	33.4	32.0	22.3	22.3	22.0	Yes	0	.0	2.7
ym11	12.9	12.0	13.0	14.0	13.7	27.9	24.7	19.7	22.7	23.0	Yes	0	.0	-.9
ym11	17.9	20.3	20.7	17.3	17.3	17.6	15.3	8.7	11.0	13.7	Yes	0	.0	2.4
yt15	21.2	20.0	21.0	21.3	20.7	3.6	3.7	3.0	3.7	4.0	Yes	1	.0	-1.2
yt15	23.4	24.3	25.0	23.7	25.0	4.2	4.3	2.3	3.7	3.7	Yes	1	.0	.9
yt15	26.4	24.7	24.7	25.7	24.0	3.7	4.0	3.7	4.7	4.0	Yes	1	.0	-1.7

CODE	PAIN TOLERANCE @ BASELINE	PAIN TOLERANCE @ 1 hr	PAIN TOLERANCE @ 2 hr	PAIN TOLERANCE @ 3 hr	PAIN TOLERANCE @ 4 hr	VAS @ BASELINE	VAS @ 1 hr	VAS @ 2 hr	VAS @ 3 hr	VAS @ 4 hr	KEEP DATA?	KEEP MENSTRUAL DATA?	BASELINE	CORRECTED PAIN TOLERANCE @ BASELINE	BASELINE CORRECTED PAIN TOLERANCE @ 1 hr
yt16	18.3	19.0	19.0	18.3	17.7	73.8	69.3	68.7	69.0	65.0	Yes	1	.0	.7	
yt16	16.7	16.0	16.0	15.7	15.3	68.4	76.7	68.3	74.0	73.7	Yes	1	.0	-.7	
yt16	18.3	18.7	19.3	18.0	17.7	69.0	67.7	70.3	71.0	69.0	Yes	1	.0	.4	
ym12	28.9	27.7	28.7	29.0	28.3	43.0	45.3	44.0	45.7	43.7	Yes	0	.0	-1.2	
ym12	26.1	24.3	24.7	23.7	24.0	46.8	48.0	47.7	45.7	46.7	Yes	0	.0	-1.8	
ym12	31.8	31.3	32.0	31.0	32.0	42.8	44.0	45.0	45.7	44.0	Yes	0	.0	-.5	
om3	6.6	6.0	6.7	6.7	7.3	61.8	34.0	46.7	39.5	45.0	Yes	0	.0	-.6	
om3	7.3	10.0	10.0	9.7	8.7	41.6	43.0	37.0	39.0	41.3	Yes	0	.0	2.7	
om3	10.1	10.3	11.7	12.7	11.7	39.7	35.0	39.3	51.0	38.3	Yes	0	.0	.2	
otnil4	18.8	19.7	24.3	25.3	21.3	78.8	78.3	75.7	81.0	79.7	Yes	0	.0	.9	
otnil4	17.4	22.0	22.0	23.7	23.3	77.9	77.0	74.7	73.7	76.3	Yes	0	.0	4.6	
otnil4	18.4	20.0	20.3	20.7	22.0	76.7	76.3	75.7	76.7	79.0	Yes	0	.0	1.6	
otexh1	23.6	28.7	32.3	33.0	35.0	62.6	78.0	82.7	89.0	89.0	Yes	0	.0	5.1	
otexh1	45.3	50.3	58.3	56.0	58.3	52.3	75.3	79.7	76.7	70.0	No	0	.0	5.0	
otexh1	23.0	29.0	30.0	30.0	27.0	66.4	71.3	78.3	77.7	77.3	Yes	0	.0	6.0	
otexh2	12.3	11.3	11.3	10.3	10.0	41.2	40.7	40.7	40.0	39.3	Yes	0	.0	-1.0	
otexh2	10.9	20.0	9.7	9.3	10.0	40.0	38.3	39.0	37.3	38.3	Yes	0	.0	9.1	
otexh2	13.9	13.0	12.7	12.0	12.0	39.4	39.0	38.0	37.7	38.3	Yes	0	.0	-.9	
om4	30.1	30.7	31.3	29.7	30.7	57.8	53.7	45.3	41.0	46.7	Yes	0	.0	.6	
om4	27.0	28.3	26.7	25.7	27.0	67.4	70.0	71.0	71.0	75.3	Yes	0	.0	1.3	
om4	29.1	28.7	29.3	29.7	29.3	74.0	75.0	76.0	75.3	77.0	Yes	0	.0	-.4	
yt17	11.2	13.0	13.7	12.7	missing	44.7	44.7	36.7	35.7	missing	Yes	1	.0	1.8	
yt17	15.4	19.3	17.3	16.0	15.3	36.1	28.7	31.0	28.7	25.0	Yes	1	.0	3.9	
yt17	12.7	14.7	20.7	13.7	21.0	41.2	45.7	37.7	38.3	31.7	No	1	.0	2.0	
om5	17.6	19.7	20.3	20.3	21.7	58.1	70.0	70.3	73.0	74.3	Yes	0	.0	2.1	
om5	25.7	29.7	30.0	26.0	26.7	79.0	80.7	78.0	81.0	81.0	Yes	0	.0	4.0	
om5	29.3	40.0	30.3	33.0	38.0	83.0	81.3	82.7	84.3	82.7	Yes	0	.0	10.7	
yt18	12.9	14.3	14.3	13.7	14.7	41.3	44.3	44.3	37.0	40.7	Yes	1	.0	1.4	
yt18	17.8	16.7	17.7	17.3	18.3	44.2	46.7	43.7	43.0	45.7	Yes	1	.0	-1.1	
yt18	21.7	20.7	19.7	19.0	20.0	43.8	37.7	42.3	41.3	40.0	Yes	1	.0	-1.0	
yt19	15.6	15.3	15.0	15.0	15.0	56.0	58.0	58.7	54.7	52.3	Yes	2	.0	-.3	
yt19	18.3	18.3	19.0	18.3	18.3	49.9	50.0	47.7	47.7	49.0	Yes	2	.0	.0	
yt19	20.0	19.7	19.0	19.0	19.7	53.1	50.7	50.3	49.0	52.0	Yes	2	.0	-.3	
ym13	12.7	14.0	15.0	15.7	15.7	61.2	60.7	59.7	63.0	60.7	Yes	0	.0	1.3	
ym13	18.9	17.7	20.0	17.0	19.3	58.1	57.7	29.3	27.3	58.3	Yes	0	.0	-1.2	
ym13	19.0	19.3	20.0	20.0	20.0	57.8	57.3	56.7	58.7	58.3	Yes	0	.0	.3	
om6	16.9	18.3	19.3	18.0	17.7	74.1	68.7	68.3	69.0	69.0	Yes	0	.0	1.4	
om6	18.1	22.3	21.3	22.0	21.3	68.7	70.3	66.0	65.3	61.0	Yes	0	.0	4.2	
om6	20.1	21.7	23.7	24.0	22.7	63.9	70.7	62.3	60.7	65.0	Yes	0	.0	1.6	
otexh3	30.0	27.0	30.3	30.0	28.7	58.8	45.5	43.0	43.3	40.0	Yes	0	.0	-3.0	
otexh3	25.9	25.7	26.0	25.3	missing	57.6	50.0	850.0	51.3	missing	Yes	0	.0	-.2	
otexh3	23.7	23.0	25.3	25.7	missing	50.7	48.0	50.0	50.3	missing	Yes	0	.0	-.7	
otnil5	30.7	33.7	38.3	30.3	35.7	71.2	79.3	73.0	74.3	74.7	No	0	.0	3.0	
otnil5	35.0	35.0	36.0	38.7	43.3	70.0	71.0	69.0	70.3	69.7	Yes	0	.0	.0	
otnil5	36.8	45.7	38.7	missing	43.0	66.2	66.0	66.3	missing	61.3	Yes	0	.0	8.9	

CODE	PAIN TOLERANCE @ BASELINE	PAIN TOLERANCE @ 1 hr	PAIN TOLERANCE @ 2 hr	PAIN TOLERANCE @ 3 hr	PAIN TOLERANCE @ 4 hr	VAS @ BASELINE	VAS @ 1 hr	VAS @ 2 hr	VAS @ 3 hr	VAS @ 4 hr	KEEP DATA?	KEEP MENSTRUAL DATA?	BASELINE CORRECTED PAIN TOLERANCE @ BASELINE	BASELINE CORRECTED PAIN TOLERANCE @ 1 hr
om7	27.7	28.0	29.0	28.0	31.0	79.4	91.0	89.0	78.3	85.7	Yes	0	.0	.3
om7	25.8	24.3	28.7	22.0	30.0	85.3	83.7	92.7	83.3	87.7	No	0	.0	-1.5
om7	30.4	31.7	32.3	30.0	33.7	83.9	88.0	87.3	73.0	83.7	Yes	0	.0	1.3
otnil6	9.4	9.0	7.7	9.0	9.7	10.8	6.3	4.3	5.7	7.3	Yes	0	.0	-.4
otnil6	11.2	9.7	11.0	11.0	10.7	4.7	7.3	10.0	8.0	5.7	Yes	0	.0	-1.5
otnil6	13.3	13.0	12.0	13.0	12.3	6.4	9.0	10.3	10.0	8.0	Yes	0	.0	-.3
otexh4	12.0	8.0	9.0	10.3	16.7	59.9	60.7	63.7	57.3	32.0	Yes	0	.0	-4.0
otexh4	12.4	9.3	9.0	10.0	9.0	47.2	47.7	47.0	40.7	35.3	Yes	0	.0	-3.1
otexh4	13.6	13.3	11.7	12.3	11.7	51.6	40.3	36.7	31.0	19.8	Yes	0	.0	-.3
ym14	18.9	20.0	21.3	23.7	24.3	74.6	79.0	83.0	81.3	89.0	Yes	0	.0	1.1
ym14	21.9	21.7	24.0	25.0	25.3	79.0	86.7	89.7	88.7	87.3	Yes	0	.0	-.2
ym14	22.4	24.3	26.0	25.0	25.0	86.7	87.0	80.3	82.7	90.0	Yes	0	.0	1.9
yf20	18.9	22.7	23.3	23.3	24.7	62.6	60.3	63.3	63.3	62.7	Yes	1	.0	3.8
yf20	25.8	27.3	26.7	28.3	26.3	56.6	56.3	56.7	58.0	56.0	Yes	1	.0	1.5
yf20	25.2	27.0	26.3	28.3	27.0	57.0	60.3	60.3	60.0	61.3	Yes	1	.0	1.8
ym15	16.6	18.0	18.3	19.3	19.7	80.1	81.7	83.0	80.3	83.3	Yes	0	.0	1.4
ym15	24.8	29.0	31.0	32.7	29.7	81.7	83.7	82.3	83.3	82.3	Yes	0	.0	4.2
ym15	23.4	28.7	28.7	28.7	29.3	78.1	79.3	80.0	80.3	81.3	Yes	0	.0	5.3
om8	14.3	15.7	15.7	16.7	17.0	76.6	77.0	80.3	80.0	78.7	Yes	0	.0	1.4
om8	18.9	18.3	19.3	20.0	19.3	79.2	78.7	81.3	79.7	80.7	Yes	0	.0	-.6
om8	24.1	25.0	22.7	24.3	25.0	78.0	79.0	80.0	78.7	80.0	Yes	0	.0	.9
otexh5	21.1	22.3	23.0	22.0	22.3	35.2	52.7	46.3	41.7	57.3	Yes	0	.0	1.2
otexh5	19.4	18.3	19.3	20.3	19.3	53.7	51.0	57.7	59.3	49.7	Yes	0	.0	-1.1
otexh5	10.7	13.0	13.3	12.7	13.3	47.1	48.0	49.3	48.3	51.0	Yes	0	.0	2.3
om9	22.6	23.0	22.7	24.7	24.0	36.9	36.7	36.7	36.0	36.0	Yes	0	.0	.4
om9	21.4	24.7	24.3	26.0	23.0	35.2	34.7	34.3	34.7	33.7	Yes	0	.0	3.3
om9	20.1	19.7	20.0	20.0	20.0	33.7	33.3	32.0	34.7	33.3	Yes	0	.0	-.4
otexh6	15.1	13.3	14.0	15.3	13.7	9.4	7.7	7.7	6.3	4.7	Yes	0	.0	-1.8
otexh6	5.8	7.3	7.7	8.3	8.0	7.9	6.7	5.3	6.0	5.7	Yes	0	.0	1.5
otexh6	6.8	6.3	6.0	7.0	7.3	7.7	7.0	5.0	5.3	5.7	Yes	0	.0	-.5
ym16	18.1	21.7	22.7	24.3	23.0	40.7	43.7	41.3	42.7	40.7	Yes	0	.0	3.6
ym16	18.8	23.3	22.0	22.7	23.0	30.9	35.0	34.0	36.3	34.7	Yes	0	.0	4.5
ym16	22.8	23.0	24.0	25.0	24.7	32.0	30.3	32.3	32.0	31.0	Yes	0	.0	.2
ym17	22.6	23.3	20.0	23.0	22.0	41.7	42.7	35.0	40.5	38.7	Yes	0	.0	.7
ym17	30.2	31.3	37.3	40.0	36.9	36.2	38.7	35.7	36.7	35.0	Yes	0	.0	1.1
ym17	36.9	35.0	37.3	38.7	38.0	36.4	33.7	32.7	35.3	35.7	Yes	0	.0	-1.9
otnil7	15.8	18.7	19.3	19.7	20.0	73.1	74.3	70.0	73.0	73.3	Yes	0	.0	2.9
otnil7	21.2	25.0	24.0	23.7	23.3	69.8	71.7	71.3	68.0	73.3	Yes	0	.0	3.8
otnil7	16.0	17.7	17.3	18.0	18.0	65.6	65.7	65.0	63.7	61.7	Yes	0	.0	1.7
ym18	18.8	18.0	18.5	17.7	20.0	79.7	73.7	79.5	71.3	85.0	Yes	0	.0	-.8
ym18	20.8	14.3	14.0	10.7	11.7	61.0	68.0	52.0	51.3	59.3	No	0	.0	-6.5
ym18	10.2	13.3	14.3	8.3	missing	47.6	44.3	49.0	48.7	missing	Yes	0	.0	3.1
otnil8	23.1	23.3	23.3	23.3	23.0	57.8	60.3	69.7	63.0	61.3	Yes	0	.0	.2
otnil8	24.2	26.0	26.3	26.0	26.0	69.8	72.3	69.7	65.3	64.3	Yes	0	.0	1.8
otnil8	16.3	21.0	20.0	22.7	22.7	61.8	65.7	66.7	67.0	66.3	Yes	0	.0	4.7

CODE	PAIN TOLERANCE @ BASELINE	PAIN TOLERANCE @ 1 hr	PAIN TOLERANCE @ 2 hr	PAIN TOLERANCE @ 3 hr	PAIN TOLERANCE @ 4 hr	VAS @ BASELINE	VAS @ 1 hr	VAS @ 2 hr	VAS @ 3 hr	VAS @ 4 hr	KEEP DATA?	KEEP MENSTRUAL DATA?	BASELINE CORRECTED PAIN TOLERANCE @ BASELINE	BASELINE CORRECTED PAIN TOLERANCE @ 1 hr
otnil9	15.8	16.0	15.0	14.7	15.0	15.1	15.7	15.3	15.0	16.0	Yes	0	.0	.2
otnil9	28.2	26.0	28.0	28.7	27.7	15.1	15.7	15.3	15.0	15.0	Yes	0	.0	-2.2
otnil9	28.2	28.3	30.7	29.3	28.0	15.2	14.7	15.0	16.0	20.3	Yes	0	.0	.1
otexh7	16.1	15.3	14.0	14.7	15.0	44.3	55.7	48.0	63.7	68.3	Yes	0	.0	-.8
otexh7	14.8	14.3	15.3	16.0	16.3	45.1	45.7	59.3	67.7	58.0	Yes	0	.0	-.5
otexh7	15.9	16.7	15.7	16.7	16.0	64.2	55.0	58.0	60.7	61.3	Yes	0	.0	.8
otexh8	13.0	10.7	13.0	16.0	15.3	36.0	26.7	30.3	30.3	35.3	No	0	.0	-2.3
ym19	5.1	5.3	5.3	5.3	5.0	26.0	27.3	27.0	29.0	26.0	Yes	0	.0	.2
ym19	8.1	8.0	7.7	7.7	8.0	28.9	32.0	31.3	33.3	32.7	Yes	0	.0	-.1
ym19	8.1	6.7	8.3	7.0	8.3	32.9	31.7	32.3	30.7	32.0	Yes	0	.0	-1.4
ym20	29.6	28.3	36.3	40.0	45.3	55.2	51.0	68.0	64.3	67.0	No	0	.0	-1.3
ym20	45.5	34.3	48.0	54.3	50.3	54.0	23.3	53.0	38.3	32.0	No	0	.0	-11.2
ym20	49.3	43.0	45.7	47.3	46.0	56.4	65.0	63.3	64.3	58.7	No	0	.0	-6.3
otnil10	32.0	26.3	26.0	28.0	28.7	34.7	34.0	36.0	42.3	37.7	Yes	0	.0	-5.7
otnil10	23.4	19.7	19.0	18.3	20.7	36.7	36.0	43.0	34.3	36.3	Yes	0	.0	-3.7
otnil10	16.7	17.3	16.3	16.0	16.0	39.0	36.7	36.0	39.3	42.0	Yes	0	.0	.6
otexh9	11.0	12.0	11.0	11.0	8.3	36.1	35.3	33.7	33.7	37.3	Yes	0	.0	1.0
otexh9	17.6	15.3	13.0	16.3	13.3	25.4	25.7	22.0	20.7	20.3	Yes	0	.0	-2.3
otexh9	10.0	11.0	8.0	9.7	9.7	23.9	26.7	25.3	25.0	26.3	Yes	0	.0	1.0
om10	10.1	10.3	11.0	11.0	10.3	24.2	19.0	16.0	13.3	19.0	Yes	0	.0	.2
om10	17.3	16.0	17.0	15.7	15.3	17.2	17.3	18.0	16.0	17.7	Yes	0	.0	-1.3
om10	16.6	18.7	19.3	18.3	17.3	16.8	27.0	25.0	19.0	21.0	Yes	0	.0	2.1
otexh10	21.4	20.7	21.7	21.0	23.7	21.7	20.3	23.0	22.3	18.0	Yes	0	.0	-.7
otexh10	18.8	17.7	19.0	17.7	17.7	22.1	25.0	28.7	27.7	27.0	Yes	0	.0	-1.1
otexh10	14.7	14.7	13.7	16.0	18.0	27.9	27.7	18.7	20.0	27.3	Yes	0	.0	.0
otexh11	24.8	22.7	22.3	22.0	23.7	41.3	43.0	42.3	40.7	43.0	Yes	0	.0	-2.1
otexh11	23.9	23.0	23.7	24.7	25.3	41.4	42.3	40.7	41.3	42.7	Yes	0	.0	-.9
otexh11	23.9	22.0	22.0	22.0	23.0	40.6	41.7	42.3	42.3	44.3	Yes	0	.0	-1.9

0 = No; 1 = Yes; 2 = Yes (OC Pill)

CODE	BASELINE CORRECTED PAIN TOLERANCE @ 2 hr	BASELINE CORRECTED PAIN TOLERANCE @ 3 hr	BASELINE CORRECTED PAIN TOLERANCE @ 4 hr
yt1	-.2	-.2	-.2
yt1	1.4	.1	-.2
yt1	1.0	.7	.7
yt2	1.0	2.6	2.6
yt2	.4	.0	.0
yt2	4.3	5.7	2.3
yt3	.7	.4	.7
yt3	3.2	-.8	.2
yt4	2.5	5.1	5.1
yt4	1.9	.6	.9
yt4	2.2	.8	.8
ym1	-.7	.3	-3.7
ym1	1.1	2.6	3.7
ym1	1.5	1.5	-.5
ym2	.4	1.0	1.0
ym2	.9	.9	-.1
ym2	2.6	2.6	2.6
yt5	-.9	-1.3	-.9
yt5	.2	.9	1.9
yt5	3.5	4.2	4.2
yt6	1.7	2.0	1.0
yt6	-2.2	-3.2	-1.5
yt6	.3	1.3	1.0
ym3	.1	3.1	2.8
ym3	3.4	3.1	10.4
ym3	4.0	3.0	2.0
yt7	-.1	-.1	-.1
yt7	-1.6	-1.2	-1.6
yt7	-.8	-1.5	-1.5
ym4	-.9	-.2	-.9
ym4	.4	1.4	.7
ym4	.4	.4	.7
yt8	-2.4	-3.1	-1.1
yt8	.4	-1.9	-1.6
yt8	.5	-.2	.1
ym5	1.4	2.8	2.4
ym5	1.4	-17.3	2.0
ym5	-.1	1.2	1.2
yt9	2.3	2.9	3.9
yt9	23.4	15.4	26.4
yt9	3.8	3.2	3.8
om1	-.8	-1.1	-.8
om1	1.8	1.8	1.4
om1	.7	.7	.7
yt10	5.4	5.0	2.7

CODE	BASELINE CORRECTED PAIN TOLERANCE @ 2 hr	BASELINE CORRECTED PAIN TOLERANCE @ 3 hr	BASELINE CORRECTED PAIN TOLERANCE @ 4 hr
yt10	-1.0	-2.0	-1.0
yt10	.6	.3	2.0
otnil1	2.7	1.7	2.4
otnil1	1.0	2.0	1.7
otnil1	.3	.3	2.6
ym6	.3	1.3	1.3
ym6	.3	2.9	.9
ym6	.1	1.1	.1
yt11	1.6	3.0	2.3
yt11	-.3	-.3	.1
yt11	.1	-.2	-.6
yt12	2.0	4.0	6.3
ym7	2.8	1.8	2.8
ym7	.7	1.7	.7
ym7	-1.6	1.4	1.4
yt13	-1.1	-1.8	-1.5
yt13	-3.0	-2.0	-2.7
yt13	2.7	11.0	48.2
yt14	-1.1	-.7	-1.1
yt14	-1.8	-2.5	-2.1
yt14	-1.0	-1.6	-2.3
ym8	1.3	.6	1.9
ym8	1.4	2.1	2.4
ym8	-2.3	-.9	-1.9
ym9	-.6	-.9	-2.2
ym9	.2	-.1	-.8
ym9	1.9	2.2	1.9
otnil2	1.6	.6	-.7
otnil2	5.1	4.4	2.1
otnil2	3.2	2.5	3.2
ym10	-1.7	-1.7	-1.7
ym10	-4.4	-4.1	-3.4
ym10	-1.6	-1.3	-.6
otnil3	4.4	6.4	2.4
otnil3	1.0	1.0	1.3
otnil3	2.5	2.5	2.1
om2	1.7	2.7	3.4
om2	-2.1	-2.1	-3.1
om2	-1.2	-2.2	-1.9
ym11	5.4	11.7	5.4
ym11	.1	1.1	.8
ym11	2.8	-.6	-.6
yt15	-.2	.1	-.5
yt15	1.6	.3	1.6
yt15	-1.7	-.7	-2.4

CODE	BASELINE CORRECTED PAIN TOLERANCE @ 2 hr	BASELINE CORRECTED PAIN TOLERANCE @ 3 hr	BASELINE CORRECTED PAIN TOLERANCE @ 4 hr
yt16	.7	.0	-.6
yt16	-.7	-1.0	-1.4
yt16	1.0	-.3	-.6
ym12	-.2	.1	-.6
ym12	-1.4	-2.4	-2.1
ym12	.2	-.8	.2
om3	.1	.1	.7
om3	2.7	2.4	1.4
om3	1.6	2.6	1.6
otnil4	5.5	6.5	2.5
otnil4	4.6	6.3	5.9
otnil4	1.9	2.3	3.6
otexh1	8.7	9.4	11.4
otexh1	13.0	10.7	13.0
otexh1	7.0	7.0	4.0
otexh2	-1.0	-2.0	-2.3
otexh2	-1.2	-1.6	-.9
otexh2	-1.2	-1.9	-1.9
om4	1.2	-.4	.6
om4	-.3	-1.3	.0
om4	.2	.6	.2
yt17	2.5	1.5	#NULL!
yt17	1.9	.6	-.1
yt17	8.0	1.0	8.3
om5	2.7	2.7	4.1
om5	4.3	.3	1.0
om5	1.0	3.7	8.7
yt18	1.4	.8	1.8
yt18	-.1	-.5	.5
yt18	-2.0	-2.7	-1.7
yt19	-.6	-.6	-.6
yt19	.7	.0	.0
yt19	-1.0	-1.0	-.3
ym13	2.3	3.0	3.0
ym13	1.1	-1.9	.4
ym13	1.0	1.0	1.0
om6	2.4	1.1	.8
om6	3.2	3.9	3.2
om6	3.6	3.9	2.6
otexh3	.3	.0	-1.3
otexh3	.1	-.6	#NULL!
otexh3	1.6	2.0	#NULL!
otnil5	7.6	-.4	5.0
otnil5	1.0	3.7	8.3
otnil5	1.9	#NULL!	6.2

CODE	BASELINE CORRECTED PAIN TOLERANCE @ 2 hr	BASELINE CORRECTED PAIN TOLERANCE @ 3 hr	BASELINE CORRECTED PAIN TOLERANCE @ 4 hr
om7	1.3	.3	3.3
om7	2.9	-3.8	4.2
om7	1.9	-.4	3.3
otnil6	-1.7	-.4	.3
otnil6	-.2	-.2	-.5
otnil6	-1.3	-.3	-1.0
otexh4	-3.0	-1.7	4.7
otexh4	-3.4	-2.4	-3.4
otexh4	-1.9	-1.3	-1.9
ym14	2.4	4.8	5.4
ym14	2.1	3.1	3.4
ym14	3.6	2.6	2.6
yt20	4.4	4.4	5.8
yt20	.9	2.5	.5
yt20	1.1	3.1	1.8
ym15	1.7	2.7	3.1
ym15	6.2	7.9	4.9
ym15	5.3	5.3	5.9
om8	1.4	2.4	2.7
om8	.4	1.1	.4
om8	-1.4	.2	.9
otexh5	1.9	.9	1.2
otexh5	-.1	.9	-.1
otexh5	2.6	2.0	2.6
om9	.1	2.1	1.4
om9	2.9	4.6	1.6
om9	-.1	-.1	-.1
otexh6	-1.1	.2	-1.4
otexh6	1.9	2.5	2.2
otexh6	-.8	.2	.5
ym16	4.6	6.2	4.9
ym16	3.2	3.9	4.2
ym16	1.2	2.2	1.9
ym17	-2.6	.4	-.6
ym17	7.1	9.8	6.7
ym17	.4	1.8	1.1
otnil7	3.5	3.9	4.2
otnil7	2.8	2.5	2.1
otnil7	1.3	2.0	2.0
ym18	-.3	-1.1	1.2
ym18	-6.8	-10.1	-9.1
ym18	4.1	-1.9	#NULL!
otnil8	.2	.2	-.1
otnil8	2.1	1.8	1.8
otnil8	3.7	6.4	6.4

CODE	BASELINE CORRECTED PAIN TOLERANCE @ 2 hr	BASELINE CORRECTED PAIN TOLERANCE @ 3 hr	BASELINE CORRECTED PAIN TOLERANCE @ 4 hr
otnil9	-.8	-1.1	-.8
otnil9	-.2	.5	-.5
otnil9	2.5	1.1	-.2
otexh7	-2.1	-1.4	-1.1
otexh7	.5	1.2	1.5
otexh7	-.2	.8	.1
otexh8	.0	3.0	2.3
ym19	.2	.2	-.1
ym19	-.4	-.4	-.1
ym19	.2	-1.1	.2
ym20	6.7	10.4	15.7
ym20	2.5	8.8	4.8
ym20	-3.6	-2.0	-3.3
otnil10	-6.0	-4.0	-3.3
otnil10	-4.4	-5.1	-2.7
otnil10	-.4	-.7	-.7
otexh9	.0	.0	-2.7
otexh9	-4.6	-1.3	-4.3
otexh9	-2.0	-.3	-.3
om10	.9	.9	.2
om10	-.3	-1.6	-2.0
om10	2.7	1.7	.7
otexh10	.3	-.4	2.3
otexh10	.2	-1.1	-1.1
otexh10	-1.0	1.3	3.3
otexh11	-2.5	-2.8	-1.1
otexh11	-.2	.8	1.4
otexh11	-1.9	-1.9	-.9

Ch3data

CODE	SEX	AGE	WEIGHT	MENSTPHS	EARTHICK	OCPILL	DOSE	EXPECTANCY TYPE	DOSEXP	PAIN THRESHOLD @	PAIN THRESHOLD @ 1hr	PAIN THRESHOLD @ 2hr	PAIN THRESHOLD @ 3hr	PAIN THRESHOLD @ 4hr	PAIN TOLERANCE @	PAIN TOLERANCE @ 1hr	PAIN TOLERANCE @ 2hr
m1	Male	26	75	5	.42	2	0	Negative	1	5.4	6.3	5.0	4.7	4.0	6.6	7.3	6.3
m1	Male	26	75	5	.42	2	800	Negative	3	5.8	5.0	6.3	6.7	6.7	6.9	7.3	7.7
m1	Male	26	75	5	.42	2	800	Positive	4	8.2	7.3	6.0	9.7	8.0	10.2	12.0	8.0
m1	Male	26	75	5	.42	2	0	Positive	2	4.3	4.0	4.3	4.7	5.0	5.5	5.0	5.3
m2	Male	31	66	5	.65	2	800	Positive	4	14.9	20.7	22.7	25.7	19.3	19.4	24.0	27.0
m2	Male	31	66	5	.65	2	0	Positive	2	19.6	21.3	20.0	18.0	19.7	22.8	24.3	24.7
m2	Male	31	66	5	.65	2	0	Negative	1	22.7	21.0	22.0	21.7	22.3	29.7	27.0	28.0
m2	Male	31	66	5	.65	2	800	Negative	3	18.2	16.7	16.7	15.7	16.3	24.6	23.3	23.3
m3	Male	31	75	5	.49	2	800	Negative	3	12.7	18.3	18.7	19.3	27.7	15.2	19.3	20.3
m3	Male	31	75	5	.49	2	0	Negative	1	19.6	22.0	20.0	20.3	20.0	21.6	25.0	22.0
m3	Male	31	75	5	.49	2	800	Positive	4	20.1	21.7	21.7	21.0	20.0	22.0	23.0	23.0
m3	Male	31	75	5	.49	2	0	Positive	2	21.3	22.0	32.7	30.0	33.7	22.8	24.3	33.7
f1	Female	21	62	3	.36	0	0	Negative	1	15.8	15.0	15.7	15.7	13.3	19.0	18.7	19.7
f1	Female	21	62	1	.36	0	800	Positive	4	16.9	18.0	17.7	15.0	16.7	19.4	20.3	20.0
f1	Female	21	62	2	.36	0	800	Negative	3	13.6	13.2	14.3	19.7	14.3	16.3	16.0	17.7
f1	Female	21	62	2	.36	0	0	Positive	2	9.1	7.3	7.0	6.7	7.0	10.3	8.3	8.0
f2	Female	24	48	3	.48	0	0	Negative	1	6.3	6.0	6.0	5.3	6.3	8.6	7.7	8.7
f2	Female	24	48	2	.48	0	0	Positive	2	14.6	16.0	16.3	14.0	14.0	17.6	18.3	18.3
f2	Female	24	48	2	.48	0	800	Negative	3	14.1	16.0	14.7	14.7	14.3	15.2	17.0	16.0
f2	Female	24	48	3	.48	0	800	Positive	4	15.2	15.0	14.0	14.0	14.0	16.3	16.0	15.0
f3	Female	20	55	4	.42	1	0	Positive	2	4.6	5.0	5.0	5.0	5.0	6.0	6.3	6.0
f3	Female	20	55	1	.42	1	800	Negative	3	8.7	8.3	9.0	8.3	8.3	9.7	9.7	10.0
f3	Female	20	55	4	.42	1	800	Positive	4	9.1	10.3	10.3	11.0	11.0	10.9	12.3	11.7
f3	Female	20	55	4	.42	1	0	Negative	1	5.4	6.7	6.7	6.0	6.3	6.7	7.7	7.7
f4	Female	26	56	3	.51	0	800	Negative	3	15.7	15.7	13.3	14.7	13.7	19.9	18.7	17.7
f4	Female	26	56	1	.51	0	800	Positive	4	9.2	12.7	13.3	13.3	15.0	11.3	13.7	15.0
f4	Female	26	56	2	.51	0	0	Negative	1	13.8	11.7	12.7	13.7	14.3	16.0	13.7	15.7
f4	Female	26	56	2	.51	0	0	Positive	2	14.4	13.7	13.3	11.7	10.7	16.1	15.0	15.0
m4	Male	24	68	5	.46	2	800	Negative	3	23.0	22.7	23.0	21.0	22.7	35.4	33.7	31.3
m4	Male	24	68	5	.46	2	0	Negative	1	35.3	34.0	30.0	19.3	23.0	42.2	42.7	39.0
m4	Male	24	68	5	.46	2	800	Positive	4	24.9	34.3	36.0	24.3	26.3	30.8	40.3	46.0
m4	Male	24	68	5	.46	2	0	Positive	2	28.1	28.0	25.7	30.0	27.0	35.6	38.0	43.0
m5	Male	22	65	5	.49	2	0	Positive	2	12.4	11.3	12.0	13.3	12.0	17.4	15.3	16.3
m5	Male	22	65	5	.49	2	800	Positive	4	12.6	12.3	12.3	11.0	10.0	18.4	20.0	20.7
m5	Male	22	65	5	.49	2	800	Negative	3	9.8	9.0	9.7	9.3	8.7	15.2	15.3	16.0
m5	Male	22	65	5	.49	2	0	Negative	1	10.5	11.0	11.3	11.3	11.0	18.1	20.3	19.7
m6	Male	43	73	5	.35	2	0	Negative	1	11.3	11.3	10.3	11.3	12.0	13.3	13.0	12.0
m6	Male	43	73	5	.35	2	0	Positive	2	14.0	14.7	16.3	17.3	17.0	17.9	17.0	19.0
m6	Male	43	73	5	.35	2	800	Negative	3	15.9	14.3	16.0	15.7	17.0	22.6	22.3	23.0
m6	Male	43	73	5	.35	2	800	Positive	4	19.2	19.7	21.7	21.0	20.0	22.7	23.3	25.3

SUBJECT_CODE	SEX	AGE	WEIGHT	MENSTPHASE	EARTHICK	OCPILL	DOSE	EXPECTANCY TYPE	DOSEXP	PAIN THRESHOLD @	PAIN THRESHOLD @ 1hr	PAIN THRESHOLD @ 2hr	PAIN THRESHOLD @ 3hr	PAIN THRESHOLD @ 4hr	PAIN TOLERANCE @	PAIN TOLERANCE @ 1hr	PAIN TOLERANCE @ 2hr
t5	Female	23	65	1	.36	1	800	Positive	4	7.3	6.3	8.0	7.3	6.7	8.8	8.3	10.0
t5	Female	23	65	4	.36	1	0	Negative	1	8.4	4.7	5.7	7.0	5.7	11.1	5.7	7.7
t5	Female	23	65	4	.36	1	0	Positive	2	6.4	5.0	5.0	5.3	6.7	7.7	6.3	6.3
t5	Female	23	65	1	.36	1	800	Negative	3	13.7	10.0	14.0	13.3	15.7	16.4	12.0	18.3
t6	Female	21	47	2	.37	0	800	Negative	3	5.4	7.0	7.0	6.3	6.3	6.7	8.0	8.0
t6	Female	21	47	2	.37	0	800	Positive	4	4.8	5.0	4.0	5.0	5.0	5.6	6.0	5.0
m7	Male	22	76	5	.45	2	0	Positive	2	17.9	19.7	18.7	22.0	21.0	24.8	27.3	26.7
m7	Male	22	76	5	.45	2	0	Negative	1	23.2	21.0	22.7	23.0	20.3	29.0	27.0	28.0
m7	Male	22	76	5	.45	2	800	Positive	4	20.6	19.7	18.3	20.3	20.7	24.3	23.3	22.7
m7	Male	22	76	5	.45	2	800	Negative	3	20.0	19.7	19.0	20.0	21.0	24.3	24.3	24.7
t7	Female	26	56	2	.30	0	800	Negative	3	15.8	16.7	16.3	18.3	15.0	20.1	21.3	20.3
t7	Female	26	56	5	.30	0	0	Negative	1	13.4	16.0	17.3	16.7	15.3	17.8	21.0	22.3
t7	Female	26	56	5	.30	0	0	Positive	2	22.8	21.7	21.7	21.7	20.3	27.0	26.0	26.0
t7	Female	26	56	5	.30	0	800	Positive	4	19.2	18.7	18.3	18.0	16.3	24.8	24.0	23.7
t8	Female	20	65	4	.54	1	0	Positive	2	7.0	6.3	6.0	6.7	5.7	9.7	9.0	10.0
t8	Female	20	65	4	.54	1	800	Positive	4	7.6	8.3	9.0	9.7	9.3	10.4	13.0	13.0
t8	Female	20	65	1	.54	1	0	Negative	1	15.6	14.3	16.7	15.3	13.3	19.4	20.0	20.3
t8	Female	20	65	4	.54	1	800	Negative	3	15.6	14.0	15.0	14.7	15.0	20.4	19.0	20.7
t9	Female	45	63	3	.39	0	0	Negative	1	8.0	6.7	7.0	8.0	8.3	10.3	8.7	9.0
t9	Female	45	63	2	.39	0	800	Negative	3	13.3	14.0	15.3	16.3	15.3	15.8	17.3	18.3
t9	Female	45	63	1	.39	0	800	Positive	4	11.4	10.3	11.3	11.0	12.0	13.8	12.7	12.7
t9	Female	45	63	1	.39	0	0	Positive	2	6.1	7.3	7.3	8.3	8.3	7.1	8.3	8.3
m8	Male	58	86	5	.58	2	800	Negative	3	7.2	6.7	6.7	6.0	6.0	9.1	8.0	8.0
m8	Male	58	86	5	.58	2	0	Positive	2	4.9	6.0	5.3	5.7	5.7	5.3	7.3	6.7
m8	Male	58	86	5	.58	2	0	Negative	1	7.4	6.0	5.7	6.0	5.7	9.7	8.0	7.7
m8	Male	58	86	5	.58	2	800	Positive	4	7.6	9.3	8.7	7.7	7.0	9.3	11.3	11.0
m9	Male	30	85	5	.66	2	800	Positive	4	8.0	7.7	9.7	8.0	9.0	9.9	9.0	10.7
m9	Male	30	85	5	.66	2	800	Negative	3	11.3	11.3	11.7	8.3	12.0	13.5	13.7	13.0
m9	Male	30	85	5	.66	2	0	Positive	2	9.4	10.0	8.0	11.3	13.0	10.4	12.0	10.0
m9	Male	30	85	5	.66	2	0	Negative	1	8.0	12.7	9.0	7.7	6.7	10.1	14.3	11.3
m10	Male	26	83	5	.45	2	0	Negative	1	12.3	13.7	14.3	14.3	15.3	16.7	16.7	18.0
m10	Male	26	83	5	.45	2	800	Positive	4	17.4	17.3	17.0	16.7	16.3	21.1	20.3	20.3
m10	Male	26	83	5	.45	2	0	Positive	2	16.9	16.0	16.7	17.0	16.0	20.1	19.3	20.3
m10	Male	26	83	5	.45	2	800	Negative	3	14.7	16.0	16.3	16.3	14.7	19.7	19.7	21.0
t10	Female	31	70	4	.46	1	800	Positive	4	10.9	8.7	10.3	9.0	9.3	15.6	12.3	15.0
t10	Female	31	70	1	.46	1	0	Positive	2	13.8	13.3	10.7	10.7	10.7	18.6	17.0	16.3
t10	Female	31	70	4	.46	1	0	Negative	1	11.0	9.3	7.0	9.7	9.3	18.7	17.3	17.0
t10	Female	31	70	4	.46	1	800	Negative	3	13.8	12.3	12.3	13.0	11.7	19.6	19.0	18.0
t11	Female	26	67	3	.51	0	800	Positive	4	20.6	21.3	21.0	21.3	22.0	23.9	24.7	25.7
t11	Female	26	67	3	.51	0	800	Negative	3	19.9	21.0	19.0	18.3	18.7	23.1	24.3	22.0

CODE	SEX	AGE	WEIGHT	MENSTPH	EARTHICK	OCPILL	DOSE	EXPECTANCY TYPE	DOSEXP	PAIN THRESHOLD @	PAIN THRESHOLD @ 1hr	PAIN THRESHOLD @ 2hr	PAIN THRESHOLD @ 3hr	PAIN THRESHOLD @ 4hr	PAIN TOLERANCE @	PAIN TOLERANCE @ 1hr	PAIN TOLERANCE @ 2hr
111	Female	26	67	2	.51	0	0	Positive	2	20.0	20.3	20.3	23.0	22.3	22.1	23.3	24.3
11	Female	26	67	2	.5	0	0	Negative	1	26.8	25.3	25.7	24.3	24.7	29.8	28.7	29.3

1 = menses; 2 = follicular; 3 = luteal; 4 = active OC; 5 = N/A

0 = No; 1 = Yes; 2 = N/A

1 = negative placebo; 2 = positive placebo; 3 = negative drug; 4

CODE	PAIN TOLERANCE @ 3hr	PAIN TOLERANCE @ 4hr	VAS @ BASELINE	VAS @ 1hr	VAS @ 2hr	VAS @ 3hr	VAS @ 4hr	KEEP DATA?	CORRECTED PAIN TOLERANCE @ BASELINE	CORRECTED PAIN TOLERANCE @ 1hr	CORRECTED PAIN TOLERANCE @ 2hr	CORRECTED PAIN TOLERANCE @ 3hr	BASELINE CORRECTED PAIN TOLERANCE
m1	5.7	5.3	25.6	26.3	31.3	31.0	29.7	Yes	.0	.7	-.3	-.9	-1.3
m1	8.0	8.0	29.3	27.7	28.3	27.3	29.3	Yes	.0	.4	.8	1.1	1.1
m1	12.7	15.7	19.3	22.0	18.0	20.0	22.3	Yes	.0	1.8	-2.2	2.5	5.5
m1	7.0	6.0	31.0	31.7	30.0	24.3	28.0	Yes	.0	-.5	-.2	1.5	.5
m2	27.7	26.7	42.0	45.7	35.0	39.0	44.7	Yes	.0	4.6	7.6	8.3	7.3
m2	25.0	25.7	34.9	46.7	43.7	39.0	52.7	Yes	.0	1.5	1.9	2.2	2.9
m2	29.0	29.3	46.6	51.7	60.0	59.0	60.7	Yes	.0	-2.7	-1.7	-.7	-.4
m2	23.7	24.7	48.2	63.0	56.7	70.3	75.3	Yes	.0	-1.3	-1.3	-.9	.1
m3	21.3	30.0	37.7	39.3	41.7	41.3	42.0	Yes	.0	4.1	5.1	6.1	14.8
m3	23.0	22.0	45.6	45.7	46.0	46.0	47.0	Yes	.0	3.4	.4	1.4	.4
m3	23.3	22.0	47.6	47.7	46.3	44.7	47.0	Yes	.0	1.0	1.0	1.3	.0
m3	31.7	36.3	47.2	47.0	50.3	48.0	47.0	Yes	.0	1.5	10.9	8.9	13.5
f1	19.7	20.3	44.4	44.0	43.7	44.3	45.7	Yes	.0	-.3	.7	.7	1.3
f1	18.3	20.3	49.2	48.7	49.7	41.7	46.7	Yes	.0	.9	.6	-1.1	.9
f1	22.0	17.3	47.4	32.8	41.7	53.3	47.7	Yes	.0	-.3	1.4	5.7	1.0
f1	7.3	8.0	57.7	54.3	54.7	55.3	57.0	Yes	.0	-2.0	-2.3	-3.0	-2.3
f2	7.0	7.0	6.3	7.3	6.0	4.7	5.7	Yes	.0	-.9	.1	-1.6	-1.6
f2	17.7	16.0	3.6	5.0	4.0	2.3	3.7	Yes	.0	.7	.7	.1	-1.6
f2	15.3	15.3	4.7	5.0	4.3	5.7	5.3	Yes	.0	1.8	.8	.1	.1
f2	15.0	15.0	4.4	5.0	3.7	4.3	4.7	Yes	.0	-.3	-1.3	-1.3	-1.3
f3	6.0	6.0	42.6	52.0	52.0	52.3	54.7	Yes	.0	.3	.0	.0	.0
f3	9.3	9.3	16.1	23.7	19.0	12.3	14.3	Yes	.0	.0	.3	-.4	-.4
f3	12.0	12.0	30.8	31.0	30.0	28.7	27.7	Yes	.0	1.4	.8	1.1	1.1
f3	7.7	7.7	26.4	14.7	8.0	28.7	15.7	Yes	.0	1.0	1.0	1.0	1.0
f4	19.0	16.7	14.4	13.3	15.0	14.3	15.0	Yes	.0	-1.2	-2.2	-.9	-3.2
f4	16.0	17.3	14.4	14.7	14.0	14.7	14.7	Yes	.0	2.4	3.7	4.7	6.0
f4	16.7	15.3	14.1	15.0	16.0	14.7	5.3	Yes	.0	-2.3	-.3	.7	-.7
f4	12.7	11.7	14.7	12.7	13.3	15.0	15.3	Yes	.0	-1.1	-1.1	-3.4	-4.4
m4	33.7	34.3	22.3	22.0	20.3	24.0	21.3	Yes	.0	-1.7	-4.1	-1.7	-1.1
m4	25.3	29.7	27.7	28.0	27.0	26.3	27.0	Yes	.0	.5	-3.2	-16.9	-12.5
m4	32.0	38.3	24.6	24.3	27.3	25.0	28.0	Yes	.0	9.5	15.2	1.2	7.5
m4	46.0	42.3	26.1	21.7	22.0	24.7	22.3	Yes	.0	2.4	7.4	10.4	6.7
m5	17.3	17.0	56.0	54.3	55.3	57.7	54.7	Yes	.0	-2.1	-1.1	-.1	-.4
m5	16.0	17.0	54.9	54.3	54.7	55.0	55.3	Yes	.0	1.6	2.3	-2.4	-1.4
m5	17.3	13.7	54.8	54.0	53.7	54.0	52.0	Yes	.0	.1	.8	2.1	-1.5
m5	20.3	22.3	53.8	54.3	54.0	54.0	55.3	Yes	.0	2.2	1.6	2.2	4.2
m6	13.3	13.0	13.4	13.7	14.0	14.0	13.7	Yes	.0	-.3	-1.3	.0	-.3
m6	21.7	22.0	13.1	13.0	11.7	12.3	12.3	Yes	.0	-.9	1.1	3.8	4.1
m6	21.7	22.3	12.0	11.0	12.0	10.3	12.3	Yes	.0	-.3	.4	-.9	-.3
m6	24.7	23.7	14.3	12.7	14.0	15.3	12.0	Yes	.0	.6	2.6	2.0	1.0

t5 CODE	PAIN TOLERANCE @ 3hr	PAIN TOLERANCE @ 4hr	VAS @ BASELINE	VAS @ 1hr	VAS @ 2hr	VAS @ 3hr	VAS @ 4hr	KEEP DATA?	CORRECTED PAIN TOLERANCE @ BASELINE	CORRECTED PAIN TOLERANCE @ 1hr	CORRECTED PAIN TOLERANCE @ 2hr	CORRECTED PAIN TOLERANCE @ 3hr	BASELINE CORRECTED PAIN TOLERANCE
t5	8.7	8.3	10.9	9.3	10.3	10.7	10.7	Yes	.0	-.5	1.2	-.1	-.5
t5	8.0	7.0	12.0	15.0	16.0	14.0	14.7	Yes	.0	-5.4	-3.4	-3.1	-4.1
t5	7.0	8.3	13.2	10.0	8.7	6.3	5.0	Yes	.0	-1.4	-1.4	-.7	.6
t5	16.3	19.3	7.7	8.0	5.0	7.7	7.0	Yes	.0	-4.4	1.9	-.1	2.9
t6	7.3	7.7	29.0	32.3	29.7	29.7	29.0	No	.0	1.3	1.3	.6	1.0
t6	5.7	5.7	33.2	29.3	28.3	29.3	29.0	No	.0	.4	-.6	.1	.1
m7	29.7	28.0	53.1	46.0	51.7	59.7	50.7	Yes	.0	2.5	1.9	4.9	3.2
m7	28.3	26.0	51.0	50.0	54.7	56.3	53.7	Yes	.0	-2.0	-1.0	-.7	-3.0
m7	24.3	23.7	54.6	53.0	58.7	54.7	50.7	Yes	.0	-1.0	-1.6	.0	-.6
m7	26.3	26.0	54.4	56.0	56.0	62.0	56.3	Yes	.0	.0	.4	2.0	1.7
t7	22.0	20.0	22.7	20.0	20.3	21.0	20.3	Yes	.0	1.2	.2	1.9	-.1
t7	21.0	19.0	24.7	25.7	26.3	25.0	24.3	Yes	.0	3.2	4.5	3.2	1.2
t7	26.0	24.7	17.1	17.0	18.0	16.7	16.0	Yes	.0	-1.0	-1.0	-1.0	-2.3
t7	22.7	20.0	19.9	19.3	20.7	17.7	20.0	Yes	.0	-.8	-1.1	-2.1	-4.8
t8	10.3	10.3	40.4	38.0	36.0	37.3	34.7	Yes	.0	-.7	.3	.6	.6
t8	13.3	13.3	41.6	35.7	39.7	40.7	41.3	Yes	.0	2.6	2.6	2.9	2.9
t8	20.3	18.7	42.1	42.0	40.7	41.7	41.3	Yes	.0	.6	.9	.9	-.7
t8	20.0	20.0	44.8	43.0	45.3	48.0	46.3	Yes	.0	-1.4	.3	-.4	-.4
t9	10.0	10.3	25.4	24.7	24.3	24.0	23.3	Yes	.0	-1.6	-1.3	-.3	.0
t9	19.0	19.0	25.9	25.7	25.7	26.0	26.7	Yes	.0	1.5	2.5	3.2	3.2
t9	13.0	13.3	25.3	26.0	26.0	26.3	26.3	Yes	.0	-1.1	-1.1	-.8	-.5
t9	9.7	9.7	24.7	24.7	25.0	24.7	24.0	Yes	.0	1.2	1.2	2.6	2.6
m8	7.7	7.0	37.6	37.7	37.7	38.0	39.7	Yes	.0	-1.1	-1.1	-1.4	-2.1
m8	7.0	6.7	37.8	37.0	37.3	34.7	37.0	Yes	.0	2.0	1.4	1.7	1.4
m8	8.3	7.7	35.4	35.3	35.0	36.7	35.3	Yes	.0	-1.7	-2.0	-1.4	-2.0
m8	10.0	9.3	38.9	34.7	35.0	36.7	36.3	Yes	.0	2.0	1.7	.7	.0
m9	9.3	11.0	72.6	66.7	79.3	78.3	70.0	Yes	.0	-.9	.8	-.6	1.1
m9	12.3	13.0	68.0	68.7	78.0	66.3	70.3	Yes	.0	.2	-.5	-1.2	-.5
m9	12.3	15.0	70.2	74.3	69.3	75.0	70.7	Yes	.0	1.6	-.4	1.9	4.6
m9	9.7	8.3	70.1	70.0	70.0	70.0	72.3	Yes	.0	4.2	1.2	-.4	-1.8
m10	18.3	18.3	69.3	64.7	63.7	61.7	63.7	Yes	.0	.0	1.3	1.6	1.6
m10	20.0	19.7	61.0	63.7	64.0	63.3	64.3	Yes	.0	-.8	-.8	-1.1	-1.4
m10	20.0	19.0	60.9	59.7	62.3	61.7	61.7	Yes	.0	-.8	.2	-.1	-1.1
m10	21.0	20.3	63.9	61.3	63.3	61.3	62.3	Yes	.0	.0	1.3	1.3	.6
t10	13.7	15.0	4.6	5.3	4.3	4.0	3.7	Yes	.0	-3.3	-.6	-1.9	-.6
t10	15.7	15.7	4.1	3.7	4.7	4.0	4.7	Yes	.0	-1.6	-2.3	-2.9	-2.9
t10	17.7	15.7	4.1	3.7	3.0	3.7	5.0	Yes	.0	-1.4	-1.7	-1.0	-3.0
t10	18.7	18.0	4.0	3.7	3.0	3.7	3.0	Yes	.0	-.6	-1.6	-.9	-1.6
t11	26.0	26.3	55.1	55.0	55.7	55.0	56.7	Yes	.0	.8	1.8	2.1	2.4
t11	22.0	22.0	53.7	56.0	53.7	53.0	53.7	Yes	.0	1.2	-1.1	-1.1	-1.1

CODE	PAIN TOLERANCE @ 3hr	PAIN TOLERANCE @ 4hr	VAS @ BASELINE	VAS @ 1hr	VAS @ 2hr	VAS @ 3hr	VAS @ 4hr	KEEP DATA?	CORRECTED PAIN TOLERANCE @ BASELINE	CORRECTED PAIN TOLERANCE @ 1hr	CORRECTED PAIN TOLERANCE @ 2hr	CORRECTED PAIN TOLERANCE @ 3hr	BASELINE CORRECTED PAIN TOLERANCE
t11	25.3	25.0	55.9	53.7	54.7	54.7	57.0	Yes	.0	1.2	2.2	3.2	2.9
t11	28.3	28.0	55.0	54.3	55.0	56.0	54.7	Yes	.0	-1.1	-.5	-1.5	-1.8

Ch4data

CODE	SEX	SEX HORMONE	TAKING OC PILL?	PAIN THRESHOLD VISIT 1	PAIN THRESHOLD VISIT 2	PAIN THRESHOLD VISIT 3	PAIN THRESHOLD VISIT 4	MEAN PAIN THRESHOLD	PAIN TOLERANCE VISIT	PAIN TOLERANCE VISIT	PAIN TOLERANCE VISIT	PAIN TOLERANCE VISIT	MEAN PAIN TOLERANCE	VAS VISIT 1	VAS VISIT 2	VAS VISIT 3
1m	Male	-SH	2	10.3	14.3	16.7	13.7	13.8	10.7	20.3	16.7	26.7	18.6	11.0	26.0	21.0
2m	Male	-SH	2	21.3	21.1	24.0	26.2	23.2	29.8	28.0	33.5	32.5	31.0	45.8	43.8	39.3
3m	Male	-SH	2	11.3	9.7	15.0		12.0	19.8	11.3	21.2		17.4	65.0	69.3	56.4
4m	Male	-SH	2	16.2	15.3	11.4	15.2	14.5	19.6	18.0	16.6	17.8	18.0	64.6	62.6	64.6
5m	Male	-SH	2	13.9	14.8	17.6	20.7	16.8	15.1	17.6	20.6	23.7	19.3	74.8	79.5	81.2
6m	Male	-SH	2	16.3	10.0	18.0	21.7	16.5	37.3	19.0	37.3	21.7	28.8	52.0	46.0	47.0
7m	Male	-SH	2	19.5	19.5	30.3	29.8	24.8	24.3	26.5	42.5	30.0	30.8	44.7	79.3	49.8
8m	Male	-SH	2	17.3	15.4	18.5	16.3	16.9	19.8	19.4	24.3	18.9	20.6	43.5	43.8	46.3
9m	Male	-SH	2	16.2	21.8	28.0	33.2	24.8	21.4	37.0	46.0	45.0	37.4	71.0	24.7	79.5
10m	Male	-SH	2	19.7	20.9	20.3	29.1	22.5	22.1	27.7	27.7	31.9	27.4	49.0	46.7	31.0
1f	Female	+SH	0	10.3	19.8	20.2	21.8	18.0	16.5	25.0	25.3	29.8	24.2	90.0	87.0	87.0
2f	Female	+SH	0	12.8	24.4	23.2	22.1	20.6	21.7	28.5	28.2	28.5	26.7	49.2	46.6	47.0
3f	Female	+SH	0	12.3	14.7	16.6	16.2	15.0	21.2	18.3	20.6	22.2	20.6	40.7	47.2	46.4
4f	Female	+SH	0	14.5	27.3	28.2	24.6	23.7	31.0	32.2	33.2	29.3	31.4	22.5	19.0	13.3
5f	Female	+SH	0	9.4	10.7	20.8	11.3	13.1	14.7	13.3	25.4	12.7	16.5	79.0	77.0	77.3
6f	Female	+SH	0	10.9	11.3	10.6	10.8	10.9	15.3	18.6	17.4	18.0	17.3	34.4	34.9	28.4
7f	Female	+SH	0	10.3	14.1	15.2	17.7	14.3	14.3	17.0	18.1	22.7	18.0	24.0	23.3	26.8
8f	Female	+SH	0	15.2	9.3	14.6	16.5	13.9	22.8	12.7	19.0	23.5	19.5	54.0	68.8	71.7
9f	Female	+SH	0	9.9	15.2	15.2	13.8	13.5	14.6	25.8	20.8	16.0	19.3	74.7	73.0	75.1
10f	Female	+SH	0	9.7	9.0	11.0	19.6	12.3	14.0	11.3	19.0	24.4	17.2	83.2	85.3	86.7
m1	Male	-SH	1	5.4	5.8	8.2	4.3	5.9	6.6	6.9	10.2	5.5	7.3	25.6	29.3	19.3
m2	Male	-SH	1	14.9	19.6	22.7	18.2	18.9	19.4	22.8	29.7	24.6	24.1	42.0	34.9	46.6
m3	Male	-SH	1	12.7	19.6	20.1	21.3	18.4	15.2	21.6	22.0	22.8	20.4	37.7	45.6	47.6
m4	Male	-SH	1	23.0	35.3	24.9	28.1	27.8	35.4	42.2	30.8	35.6	36.0	22.3	27.7	24.6
m5	Male	-SH	1	12.4	12.6	9.8	10.5	11.3	17.4	18.4	15.2	18.1	17.3	56.0	54.9	54.8
m6	Male	-SH	1	11.3	14.0	15.9	19.2	15.1	13.3	17.9	22.6	22.7	19.1	13.4	13.1	12.0
m7	Male	-SH	1	17.9	23.2	20.6	20.0	20.4	24.8	29.0	24.1	24.3	25.6	53.1	51.0	54.6
m8	Male	-SH	1	7.2	4.9	7.4	7.6	6.8	9.1	5.3	9.7	9.3	8.4	37.6	37.8	35.4
m9	Male	-SH	1	8.0	11.3	9.4	8.0	9.2	9.9	13.5	10.4	10.1	11.0	72.6	68.0	70.2
m10	Male	-SH	1	12.3	17.4	16.9	14.7	15.3	16.7	21.1	20.1	19.7	19.4	69.3	61.0	60.9
f1	Female	+SH	0	15.8	16.9	13.6	9.1	13.9	19.0	19.4	16.3	10.3	16.3	44.4	49.2	47.4
f2	Female	+SH	0	6.3	14.6	14.1	15.2	12.6	8.6	17.6	15.2	16.3	14.4	6.3	3.6	4.7
f3	Female	+SH	1	4.6	8.7	9.1	5.4	7.0	6.0	9.7	10.9	6.7	8.3	42.6	16.1	30.8
f4	Female	+SH	0	15.7	9.2	13.8	14.4	13.3	19.9	11.3	16.0	16.1	15.8	14.4	14.4	14.1
f5	Female	+SH	1	7.3	8.4	6.4	13.7	9.0	8.8	11.1	7.7	16.4	11.0	10.9	12.0	13.2
f7	Female	+SH	0	15.8	13.4	22.8	19.2	17.8	20.1	17.8	27.0	24.8	22.4	22.7	24.7	17.1
f8	Female	+SH	1	7.0	7.6	15.6	15.6	11.5	9.7	10.4	19.4	20.4	15.0	40.4	41.6	42.1
f9	Female	+SH	0	8.0	13.3	11.4	6.1	9.7	10.3	15.8	13.8	7.1	11.8	25.4	25.9	25.3
f10	Female	+SH	1	10.9	13.8	11.0	13.8	12.4	15.6	18.6	18.7	19.6	18.1	4.6	4.1	4.1
f11	Female	+SH	0	20.6	19.9	20.0	26.8	21.8	23.9	23.1	22.1	29.8	24.7	55.1	53.7	55.9
ym1	Male	-SH	2	18.0	8.2	13.4		13.2	19.3	9.9	14.5		14.6	2.9	5.4	6.8

CODE	SEX	SEX HORMONE	TAKING OC PILL?	PAIN THRESHOLD VISIT 1	PAIN THRESHOLD VISIT 2	PAIN THRESHOLD VISIT 3	PAIN THRESHOLD VISIT 4	MEAN PAIN THRESHOLD	TOLERANCE VISIT	TOLERANCE VISIT	TOLERANCE VISIT	PAIN TOLERANCE VISIT	MEAN PAIN TOLERANCE	VAS VISIT 1	VAS VISIT 2	VAS VISIT 3
ym2	Male	-SH	2	12.8	12.6	14.6		13.3	14.6	14.1	14.4		14.4	26.7	23.0	23.4
ym3	Male	-SH	2	13.7	12.2	18.9		14.9	21.5	17.9	22.6		20.7	59.8	62.8	67.0
ym4	Male	-SH	2	16.1	10.1	15.3		13.8	20.5	12.6	18.6		17.2	31.1	36.4	38.4
ym5	Male	-SH	2	11.2	12.9	11.2		11.8	15.9	19.3	16.1		17.1	62.2	64.1	64.9
ym6	Male	-SH	2	12.2	16.2	14.7		14.4	16.7	21.4	20.6		19.6	52.4	54.4	55.6
ym7	Male	-SH	2	7.8	4.2	5.7		5.9	13.2	10.0	7.9		10.4	10.4	10.4	11.3
ym8	Male	-SH	2	18.4	14.0	17.8		16.7	29.4	20.6	24.6		24.9	7.3	8.4	8.6
ym9	Male	-SH	2	10.7	12.3	12.9		12.0	16.9	16.1	15.8		16.3	8.9	14.1	14.3
ym10	Male	-SH	2	6.2	7.9	8.8		7.6	9.4	12.1	13.3		11.6	51.2	50.6	52.9
ym11	Male	-SH	2	9.8	10.1	11.6		10.5	21.6	12.9	17.9		17.5	33.4	27.9	17.6
ym12	Male	-SH	2	24.2	23.1	27.4		24.9	28.9	26.1	31.8		28.9	43.0	46.5	42.8
ym13	Male	-SH	2	7.7	11.2	12.4		10.4	1.7	18.9	19.0		13.2	61.2	58.1	57.8
ym14	Male	-SH	2	12.2	14.9	12.8		13.3	18.9	21.9	22.4		21.1	74.6	79.0	86.7
ym15	Male	-SH	2	9.2	16.0	14.0		13.1	16.6	24.8	23.4		21.6	80.1	80.7	78.1
ym16	Male	-SH	2	15.0	12.2	18.3		15.2	18.1	18.8	22.8		19.9	40.7	30.9	32.0
ym17	Male	-SH	2	19.0	25.3	30.6		25.0	22.6	30.2	36.9		29.9	41.7	36.2	36.4
ym18	Male	-SH	2	14.2	15.0	7.4		12.2	18.8	20.8	10.2		16.6	79.7	61.0	47.6
ym19	Male	-SH	2	4.1	6.9	5.9		5.6	5.1	8.1	8.1		7.1	26.0	28.9	32.9
yf1	Female	+SH	0	5.0	6.2	5.6		5.6	6.2	7.2	6.6		6.7	9.9	5.3	10.1
yf2	Female	+SH	0	15.5	16.9	12.3		14.9	18.0	19.6	16.3		18.0	4.6	12.3	9.6
yf3	Female	+SH	0	12.3	14.2			13.3	15.6	18.4			17.0	6.6	5.9	
yf4	Female	+SH	1	8.2		19.0		13.6	11.5	19.4	24.8		18.6	4.6	3.1	3.3
yf5	Female	+SH	0	15.6	19.0	12.7		15.8	17.9	21.1	14.1		17.7	37.3	26.6	28.4
yf6	Female	+SH	1	14.1	21.4	21.9		19.1	16.6	24.5	25.0		22.0	19.0	17.7	18.2
yf7	Female	+SH	0	4.0	10.1	11.6		8.6	5.1	13.2	15.1		11.1	12.6	11.0	13.6
yf8	Female	+SH	0	12.8	11.4	10.3		11.5	19.4	15.9	13.2		16.2	17.8	24.6	27.1
yf9	Female	+SH	1	9.3	17.1	13.0		13.1	12.4	23.6	16.5		17.5	46.8	42.9	52.5
yf10	Female	+SH	0	8.7	11.0	9.7		9.8	12.3	17.0	16.7		15.3	41.9	44.2	45.0
yf11	Female	+SH	0	8.6	9.2	14.9		10.9	12.7	13.6	19.9		15.4	29.4	17.8	19.9
yf13	Female	+SH	1	17.2	18.1	9.9		15.1	22.8	22.7	12.0		19.2	55.2	54.9	60.2
yf14	Female	+SH	1	9.0	9.5	8.0		8.8	18.4	18.8	16.3		17.8	47.0	45.2	41.7
yf15	Female	+SH	0	15.7	18.9	21.2		18.6	21.2	23.4	26.4		23.7	3.6	4.2	3.7
yf16	Female	+SH	0	12.6	12.8	14.0		13.1	18.3	16.7	18.3		17.8	73.8	68.4	69.0
yf17	Female	+SH	0	7.0	12.1	8.5		9.2	11.2	15.4	12.7		13.1	44.7	36.1	41.2
yf18	Female	+SH	0	7.3	11.2	11.6		10.0	12.9	17.8	21.7		17.5	41.3	44.2	43.8
yf19	Female	+SH	1	10.8	14.3	14.2		13.1	15.6	18.3	20.0		18.0	56.0	49.9	53.1
yf20	Female	+SH	0	14.1	21.3	18.4		17.9	18.9	25.8	25.2		23.3	62.6	56.6	57.0
otexh1	Female	+SH	2	10.7	100.0	14.1		12.4	23.6	100.0	23.0		23.3	62.6	100.0	66.4
otexh2	Female	+SH	2	9.4	8.7	12.0		10.0	12.3	10.9	13.9		12.4	41.2	40.0	39.4
otexh3	Female	+SH	2	15.3	14.0	17.6		15.6	30.0	25.9	23.7		26.5	58.8	57.6	50.7
otexh4	Female	+SH	2	9.7	10.3	12.0		10.7	12.0	12.4	13.6		12.7	59.9	47.2	51.6

CODE	SEX	+SEX HORMONE	TAKING OC PILL?	PAIN THRESHOLD VISIT 1	PAIN THRESHOLD VISIT 2	PAIN THRESHOLD VISIT 3	PAIN THRESHOLD VISIT 4	MEAN PAIN THRESHOLD	PAIN TOLERANCE VISIT	PAIN TOLERANCE VISIT	PAIN TOLERANCE VISIT	PAIN TOLERANCE VISIT	MEAN PAIN TOLERANCE	VAS VISIT 1	VAS VISIT 2	VAS VISIT 3
otexh5	Female	+SH	2	15.0	16.1	9.0		13.4	21.1	19.4	10.7		17.1	35.2	53.7	47.1
otexh6	Female	+SH	2	12.3	5.0	5.7		7.7	15.1	5.8	6.8		9.2	9.4	7.9	7.7
otexh7	Female	+SH	2	14.3	13.1	14.0		13.8	16.1	14.8	15.9		15.6	44.3	45.1	64.2
otexh9	Female	+SH	2	8.8	14.6	8.1		10.5	11.0	17.6	10.0		12.9	36.1	25.4	23.9
otexh10	Female	+SH	2	12.8	15.1	10.8		12.9	21.4	18.8	14.7		18.3	21.7	22.1	27.9
otexh11	Female	+SH	2	21.9	21.0	21.2		21.4	24.8	23.9	23.9		24.2	41.3	41.4	40.6
otnil1	Female	-SH	2	7.9	8.2	10.9		9.0	9.3	10.0	12.4		10.6	7.0	6.8	8.8
otnil2	Female	-SH	2	16.1	14.4	10.7		13.7	22.4	21.9	18.8		21.0	27.9	28.6	35.7
otnil3	Female	-SH	2	8.0	15.4	15.0		12.8	11.3	20.7	21.2		17.7	39.5	53.3	49.4
otnil4	Female	-SH	2	12.2	13.2	14.9		13.4	18.8	17.4	18.4		18.2	78.8	77.9	76.7
otnil5	Female	-SH	2	9.8	16.4	14.0		13.4	30.7	35.0	36.8		34.2	71.2	70.0	66.2
otnil6	Female	-SH	2	7.7	9.1	12.3		9.7	9.4	11.2	13.3		11.3	10.8	4.7	6.4
otnil7	Female	-SH	2	10.4	11.8	9.2		10.5	15.8	21.2	16.0		17.7	73.1	69.8	65.6
otnil8	Female	-SH	2	19.0	22.8	13.3		18.4	23.1	24.2	16.3		21.2	57.8	69.8	61.8
otnil9	Female	-SH	2	13.0	24.3	23.9		20.4	15.8	28.2	28.2		24.1	15.1	15.1	15.2
otnil10	Female	-SH	2	26.3	18.3	13.2		19.3	32.0	23.4	16.7		24.0	34.7	36.7	39.0
om1	Male	-SH	2	7.7	7.8	11.0		8.8	10.8	10.9	14.3		12.0	45.8	46.3	48.1
om2	Male	-SH	2	8.0	8.1	6.7		7.6	22.3	22.8	20.2		21.8	51.1	50.7	51.2
om3	Male	-SH	2	5.2	5.4	7.8		6.1	6.6	7.3	10.1		8.0	61.8	41.6	39.7
om4	Male	-SH	2	12.9	12.7	14.6		13.4	30.1	27.0	29.1		28.7	57.8	67.4	74.0
om5	Male	-SH	2	11.0	12.6	14.0		12.5	17.6	25.7	29.3		24.2	58.1	79.0	83.0
om6	Male	-SH	2	12.3	12.4	15.7		13.5	16.9	18.1	20.1		18.4	74.1	68.7	63.9
om7	Male	-SH	2	20.0	14.2	16.0		16.7	27.7	25.8	30.4		28.0	79.4	85.3	83.9
om8	Male	-SH	2	10.9	13.7	18.7		14.4	14.3	18.9	24.1		19.1	76.6	79.2	78.0
om9	Male	-SH	2	17.2	16.9	17.0		17.0	22.6	21.4	20.1		21.4	36.9	35.2	33.7
om10	Male	-SH	2	9.4	16.3	15.1		13.6	10.1	17.3	16.6		14.7	24.2	17.2	16.8

0 = No; 1 = Yes; 2 = N/A

CODE	VAS VISIT 4	MEAN VAS	EXPERIMENT
1m	25.0	20.8	1
2m	40.7	42.4	1
3m		63.6	1
4m	64.0	64.0	1
5m	85.1	80.2	1
6m	47.0	48.0	1
7m	65.5	59.8	1
8m	48.9	45.6	1
9m	75.4	62.7	1
10m	31.9	39.7	1
1t	84.0	87.0	1
2t	43.5	46.6	1
3t	49.0	45.8	1
4t	16.9	17.9	1
5t	74.5	77.0	1
6t	24.0	30.4	1
7t	29.0	25.8	1
8t	71.3	66.5	1
9t	74.8	74.4	1
10t	92.0	86.8	1
m1	31.0	26.3	3
m2	48.2	42.9	3
m3	47.2	44.5	3
m4	26.1	25.2	3
m5	53.8	54.9	3
m6	14.3	13.2	3
m7	54.4	53.3	3
m8	38.9	37.4	3
m9	70.1	70.2	3
m10	63.9	63.8	3
t1	57.7	49.7	3
t2	4.4	4.8	3
t3	26.4	29.0	3
t4	14.7	14.4	3
t5	7.7	11.0	3
t7	19.9	21.1	3
t8	44.8	42.2	3
t9	24.7	25.3	3
t10	4.0	4.2	3
t11	55.0	54.9	3
ym1		5.0	2

CODE	VAS VISIT 4	MEAN VAS	EXPERIMENT
ym2		24.4	2
ym3		63.2	2
ym4		35.3	2
ym5		63.7	2
ym6		54.1	2
ym7		10.7	2
ym8		8.1	2
ym9		12.4	2
ym10		51.6	2
ym11		26.3	2
ym12		44.1	2
ym13		59.0	2
ym14		80.1	2
ym15		79.6	2
ym16		34.5	2
ym17		38.1	2
ym18		62.8	2
ym19		29.3	2
yt1		8.4	2
yt2		8.8	2
yt3		6.3	2
yt4		3.7	2
yt5		30.8	2
yt6		18.3	2
yt7		12.4	2
yt8		23.2	2
yt9		47.4	2
yt10		43.7	2
yt11		22.4	2
yt13		56.8	2
yt14		44.6	2
yt15		3.8	2
yt16		70.4	2
yt17		40.7	2
yt18		43.1	2
yt19		53.0	2
yt20		58.7	2
otexh1		64.5	2
otexh2		40.2	2
otexh3		55.7	2
otexh4		52.9	2

CODE	VAS VISIT 4	MEAN VAS	EXPERIMENT
otexh5		45.3	2
otexh6		8.3	2
otexh7		51.2	2
otexh9		28.5	2
otexh10		23.9	2
otexh11		41.1	2
otnil1		7.5	2
otnil2		30.7	2
otnil3		47.4	2
otnil4		77.8	2
otnil5		69.1	2
otnil6		7.3	2
otnil7		69.5	2
otnil8		63.1	2
otnil9		15.1	2
otnil10		36.8	2
om1		46.7	2
om2		51.0	2
om3		47.7	2
om4		66.4	2
om5		73.4	2
om6		68.9	2
om7		82.9	2
om8		77.9	2
om9		35.3	2
om10		19.4	2

1 = Walker & Carmody (1998); 2 = Chapter 2 Study;

Ch5data

RAT NUMBER	HORMONE TREATMENT	DRUG TREATMENT	BASELINE	TIME15	TIME30	TIME60	TIME120	MAR15	MAR30	MAR60	MAR120
1	1	Vehicle	2.09	2.28	2.13	2.00	2.25	.19	.04	-.09	.16
2	1	Vehicle	6.69	.97	1.22	1.78	.56	-5.72	-5.47	-4.91	-6.13
3	1	buproten	.72	1.09	3.10	3.12	3.09	.37	2.38	2.40	2.37
4	1	buproten	1.47	1.56	1.06	2.91	1.22	.09	-.41	1.44	-.25
5	2	Vehicle	1.41	4.78	1.13	1.47	1.41	3.37	-.28	.06	.00
6	2	Vehicle	1.18	1.63	1.15	1.28	1.13	.45	-.03	.10	-.05
7	2	buproten	9.63	3.31	4.64	1.88	2.28	-6.32	-4.99	-7.75	-7.35
8	2	buproten	18.91	9.50	7.03	7.94	9.32	-9.41	-11.88	-10.97	-9.59
9	3	Vehicle	2.34	1.65	1.00	11.78	1.66	-.69	-1.34	9.44	-.68
10	3	Vehicle	20.00	4.94	7.50	5.46	1.94	-15.06	-12.50	-14.54	-18.06
11	3	buproten	6.70	3.03	3.25	6.16	5.35	-3.67	-3.45	-.54	-1.35
12	3	buproten	4.40	5.22	3.94	6.12	6.51	.82	-.46	1.72	2.11
13	4	Vehicle	15.44	11.15	9.31	10.44	16.91	-4.29	-6.13	-5.00	1.47
14	4	Vehicle	2.84	3.50	3.72	3.59	3.72	.66	.88	.75	.88
15	4	buproten	5.69	4.25	5.53	2.35	12.60	-1.44	-.16	-3.34	6.91
16	4	buproten	6.06	6.31	5.10	7.47	3.78	.25	-.96	1.41	-2.28
17	1	Vehicle	2.12	2.24	1.16	2.62	1.29	.12	-.96	.50	-.83
18	1	Vehicle	1.84	6.97	.95	.94	3.53	5.13	-.89	-.90	1.69
19	1	buproten	8.22	13.03	6.16	20.00	13.97	4.81	-2.06	11.78	5.75
20	1	buproten	20.00	10.31	9.09	9.88	13.56	-9.69	-10.91	-10.12	-6.44
21	2	Vehicle	20.00	1.09	1.13	10.16	.97	-18.91	-18.87	-9.84	-19.03
22	2	Vehicle	9.00	14.65	1.06	1.13	2.03	5.65	-7.94	-7.87	-6.97
23	2	buproten	7.06	1.49	8.10	4.47	.56	-5.57	1.04	-2.59	-6.50
24	2	Vehicle	17.19	7.18	12.44	20.00	20.00	-10.01	-4.75	2.81	2.81
26	3	Vehicle	11.90	5.28	4.10	7.31	6.59	-6.62	-7.80	-4.59	-5.31
27	3	buproten	9.04	8.85	12.44	8.72	16.31	-.19	3.40	-.32	7.27
28	3	buproten	14.34	8.50	10.88	5.29	10.35	-5.84	-3.46	-9.05	-3.99
29	4	Vehicle	4.78	1.41	.78	3.97	1.50	-3.37	-4.00	-.81	-3.28
30	4	Vehicle	1.28	7.97	5.78	5.47	7.34	6.69	4.50	4.19	6.06
31	4	buproten	5.90	2.00	11.09	5.50	7.44	-3.90	5.19	-.40	1.54
32	4	buproten	5.88	5.88	8.84	9.09	13.10	.00	2.96	3.21	7.22
17a	1	Vehicle	2.68	1.16	.90	.88	1.91	-1.52	-1.78	-1.80	-.77
18a	1	Vehicle	2.90	2.85	4.78	1.60	2.84	-.05	1.88	-1.30	-.06
21a	2	Vehicle	1.55	.84	1.31	2.72	1.59	-.71	-.24	1.17	.04
22a	2	Vehicle	6.90	3.09	1.75	1.19	.62	-3.81	-5.15	-5.71	-6.28
26a	3	Vehicle	12.53	3.16	2.69	3.81	20.00	-9.37	-9.84	-8.72	7.47
29a	4	Vehicle	1.59	2.44	2.31	3.32	1.68	.85	.72	1.73	.09
30a	4	Vehicle	3.00	8.15	5.18	1.56	1.44	5.15	2.18	-1.44	-1.56
17b	1	buproten	3.22	6.22	3.88	2.97	5.12	3.00	.66	-.25	1.90
18b	1	buproten	4.53	3.12	5.47	6.57	6.91	-1.41	.94	2.04	2.38

RAT NUMBER	HORMONE TREATMENT	DRUG TREATMENT	BASELINE	TIME15	TIME30	TIME60	TIME120	MAR15	MAR30	MAR60	MAR120
21b	2	Ibuproten	.72	3.31	1.10	1.00	10.34	2.59	.38	.28	9.62
22b	2	Ibuproten	1.32	1.87	.87	2.82	7.59	.55	-.45	1.50	6.27
26b	3	Ibuproten	6.91	7.25	9.75	3.19	5.22	.34	2.84	-3.72	-1.69
27b	3	Ibuproten	2.62	12.50	9.00	10.65	7.41	9.88	6.38	8.03	4.79
28b	3	Ibuproten	3.06	4.16	4.91	1.59	2.84	1.10	1.85	-1.47	-.22
29b	4	Ibuproten	1.19	4.41	2.75	5.76	1.09	3.22	1.56	4.57	-.10
30b	4	Ibuproten	3.88	3.13	4.87	5.47	7.41	-.75	.99	1.59	3.53
33	1	Ibuproten	3.10	16.13	6.88	9.75	7.78	13.03	3.78	6.65	4.68
34	1	Ibuproten	15.75	1.16	3.59	1.75	4.88	-14.59	-12.16	-14.00	-10.87
35	1	Vehicle	12.75	1.84	1.78	2.37	.69	-10.91	-10.97	-10.38	-12.06
36	1	Vehicle	6.57	.78	5.81	1.09	.83	-5.79	-.76	-5.48	-5.74
37	2	Ibuproten	11.37	4.68	5.97	.85	7.72	-6.69	-5.40	-10.52	-3.65
38	2	Ibuproten	3.56	6.47	5.22	9.79	2.97	2.91	1.66	6.23	-.59
39	2	Vehicle	7.28	10.85	10.03	13.31	10.37	3.57	2.75	6.03	3.09
40	2	Ibuproten	2.90	1.56	8.10	3.28	4.38	-1.34	5.20	.38	1.48
41	3	Ibuproten	5.38	7.72	1.71	7.62	5.06	2.34	-3.67	2.24	-.32
42	3	Ibuproten	17.03	14.04	6.31	20.00	16.03	-2.99	-10.72	2.97	-1.00
43	3	Vehicle	2.06	2.43	3.54	6.21	2.18	.37	1.48	4.15	.12
44	3	Vehicle	8.40	5.43	7.38	6.50	5.94	-2.97	-1.02	-1.90	-2.46
45	4	Ibuproten	5.91	4.37	.57	7.69	6.12	-1.54	-5.34	1.78	.21
46	4	Ibuproten	4.19	12.44	12.60	17.03	11.47	8.25	8.41	12.84	7.28
47	4	Vehicle	8.44	1.31	5.34	5.03	3.84	-7.13	-3.10	-3.41	-4.60
48	4	Vehicle	1.72	2.06	6.00	4.82	7.50	.34	4.28	3.10	5.78
49	1	Ibuproten	9.19	15.13	13.13	15.97	1.00	5.94	3.94	6.78	-8.19
50	1	Ibuproten	2.81	11.28	3.48	1.97	8.22	8.47	.67	-.84	5.41
51	1	Vehicle	20.00	12.56	9.25	11.34	6.06	-7.44	-10.75	-8.66	-13.94
52	1	Vehicle	7.56	6.38	1.28	6.31	2.63	-1.18	-6.28	-1.25	-4.93
53	2	Ibuproten	9.87	11.91	6.68	9.37	5.28	2.04	-3.19	-.50	-4.59
54	2	Ibuproten	1.82	6.62	1.78	1.38	2.06	4.80	-.04	-.44	.24
55	2	Vehicle	2.94	6.16	8.78	9.22	4.78	3.22	5.84	6.28	1.84
56	2	Ibuproten	7.78	7.15	6.03	3.93	7.46	-.63	-1.75	-3.85	-.32
57	3	Ibuproten	11.94	9.84	20.00	9.28	11.50	-2.10	8.06	-2.66	-.44
58	3	Ibuproten	7.66	9.72	9.41	9.44	8.30	2.06	1.75	1.78	.64
59	3	Vehicle	8.60	2.50	7.28	10.16	18.40	-6.10	-1.32	1.56	9.80
60	3	Vehicle	1.69	7.46	2.15	2.34	6.31	5.77	.46	.65	4.62
61	4	Ibuproten	4.63	2.94	6.72	1.53	2.22	-1.69	2.09	-3.10	-2.41
62	4	Ibuproten	16.06	10.21	18.40	20.00	20.00	-5.85	2.34	3.94	3.94
63	4	Vehicle	17.85	18.68	4.60	3.47	5.69	.83	-13.25	-14.38	-12.16
64	4	Vehicle	1.79	2.15	3.25	3.38	1.37	.36	1.46	1.59	-.42
66	1	Ibuproten	4.75	4.00	.91	14.56	2.87	-.75	-3.84	9.81	-1.88

RAT NUMBER	HORMONE TREATMENT	DRUG TREATMENT	BASELINE	TIME15	TIME30	TIME60	TIME120	MAR15	MAR30	MAR60	MAR120
68	1	Ibuproten	7.54	1.63	1.75	.69	20.00	-5.91	-5.79	-6.85	12.46
77	1	Ibuproten	9.72	6.85	2.31	3.15	4.66	-2.87	-7.41	-6.57	-5.06
78	1	Vehicle	10.51	5.38	3.62	1.12	.78	-5.13	-6.89	-9.39	-9.73
69	2	Ibuproten	7.41	14.01	7.93	6.11	6.10	6.60	.52	-1.30	-1.31
70	2	Ibuproten	7.03	1.19	.57	1.09	.62	-5.84	-6.46	-5.94	-6.41
71	2	Vehicle	12.78	4.00	4.32	4.94	4.25	-8.78	-8.46	-7.84	-8.53
72	2	Ibuproten	1.52	3.53	1.72	2.22	.53	2.01	.20	.70	-.99
73	3	Ibuproten	11.00	5.28	5.12	5.66	7.65	-5.72	-5.88	-5.34	-3.35
74	3	Ibuproten	17.28	6.54	3.75	4.37	5.00	-10.74	-13.53	-12.91	-12.28
75	3	Vehicle	20.00	10.25	5.21	8.06	5.63	-9.75	-14.79	-11.94	-14.37
76	3	Vehicle	13.09	4.25	2.10	1.50	.93	-8.84	-10.99	-11.59	-12.16
79	4	Ibuproten	7.28	2.37	1.78	.81	4.41	-4.91	-5.50	-6.47	-2.87
80	4	Ibuproten	14.44	2.90	3.00	4.37	1.75	-11.54	-11.44	-10.07	-12.69

1 = Oestrogen; 2 = Progesterone; 3 = Oestrogen +

Ch5pkda

Rat Number	Hormone Treatment	Ibuprofen Concentration 0mins	Ibuprofen Concentration15mins	Ibuprofen Concentration 30mins	Ibuprofen Concentration 60mins	Ibuprofen Concentration 120mins	Ibuprofen Concentration 240mins
e1	Oestrogen	0	116.8	117.01		20.2	8.8
e2	Oestrogen	0	120.79	77.66	25.17	43.13	2.69
e3	Oestrogen	0	90.5	74.61	62.32	21.65	9.25
e4	Oestrogen	0				36.03	
p1	Progesterone	0	110.82	38.83		38.8	4.99
p2	Progesterone	0	185.93	63.7	61.94	41.35	34.67
p4	Progesterone	0	83.31	106.77	59.11	53.61	27.67
p5	Progesterone	0					6.59
ep1	Oestrogen + Progesterone	0	259.76		65.36	5.33	34.73
ep2	Oestrogen + Progesterone	0	66.78	43.66	34.76	33.48	15.84
ep3	Oestrogen + Progesterone	0	7.65			27.42	10.23
ep4	Oestrogen + Progesterone	0	72.7	79.64		21.33	
ep5	Oestrogen + Progesterone	0	26.67	33.28	22.98	9.91	9.7
o1	Oil	0			33.37	27.12	17.87
o2	Oil	0					26.06
o3	Oil	0	62.33	71.81	33.48	18.24	16.48
o4	Oil	0	146.7	83.84	38.2		24.52
o5	Oil	0		97.66	45.6	37.66	26.47

Ch6data

Rat Number	Strain	Weight	Time	Hotplate Latency	Plasma Paracetamol
IB1	DBA2	18	0	31.4	0
IB1	DBA2	18	30	57.5	443.01
IB2	DBA2	16	0	21.2	0
IB2	DBA2	16	30	24.5	214.46
IB3	DBA2	19	0	48.3	0
IB3	DBA2	19	30	28.3	275.31
IB4	DBA2	20	0	18.1	0
IB4	DBA2	20	30	24.5	322.82
IB5	DBA2	24	0	28.3	0
IB5	DBA2	24	30	56.6	271.25
IB6	DBA2	19	0	22.1	0
IB6	DBA2	19	30	63.1	263.62
IB7	DBA2	23	0	58.4	0
IB7	DBA2	23	30	19.6	246.63
IB8	DBA2	22	0	38.9	0
IB8	DBA2	22	30	36	224.22
IIB1	C57BL6	21	0	28.8	0
IIB1	C57BL6	21	30	95.6	249.6
IIB2	C57BL6	21	0	28	0
IIB2	C57BL6	21	30	102	181.94
IIB3	C57BL6	11	0	42.6	0
IIB3	C57BL6	11	30	150	132.65
IIB4	C57BL6	23	0	41	0
IIB4	C57BL6	23	30	150	186.2
IIB5	C57BL6	21	0	21.9	0
IIB5	C57BL6	21	30	20.9	235.58
IIB6	C57BL6	23	0	15.1	0
IIB6	C57BL6	23	30	22.3	255.07
IIB7	C57BL6	24	0	21.2	0
IIB7	C57BL6	24	30	29.9	223.76
IIB8	C57BL6	23	0	19.3	0
IIB8	C57BL6	23	30	20.8	220.92
IC1	DBA2	19	0	19.6	0
IC1	DBA2	19	60	39.6	276.11
IC2	DBA2	22	0	38.8	0
IC2	DBA2	22	60	30.6	221.83
IC3	DBA2	20	0	19.2	0
IC3	DBA2	20	60	35.5	211.53
IC4	DBA2	21	0	32.6	0
IC4	DBA2	21	60	87.9	205.35
IC5	DBA2	20	0	33.3	0
IC5	DBA2	20	60	20.6	213.05
IC6	DBA2	21	0	37.7	0
IC6	DBA2	21	60	40.6	216.75
IC7	DBA2	19	0	29.9	0
IC7	DBA2	19	60	68	209.88
IC8	DBA2	22	0	30.8	0
IC8	DBA2	22	60	26.7	195.93
IIC1	C57BL6	21	0	21.6	0
IIC1	C57BL6	21	60	150	294.84
IIC2	C57BL6	22	0	25.9	0

Rat Number	Strain	Weight	Time	Hotplate Latency	Plasma Paracetamol
IIC2	C57BL6	22	60	40.5	185.92
IIC3	C57BL6	21	0	20.3	0
IIC3	C57BL6	21	60	36.5	166.64
IIC4	C57BL6	22	0	26.4	0
IIC4	C57BL6	22	60	36.5	214.56
IIC5	C57BL6	20	0	18.3	0
IIC5	C57BL6	20	60	150	217.69
IIC6	C57BL6	22	0	16.8	0
IIC6	C57BL6	22	60	98	196.67
IIC7	C57BL6	23	0	30.2	0
IIC7	C57BL6	23	60	133	229.15
IIC8	C57BL6	22	0	16.9	0
IIC8	C57BL6	22	60	131.6	218.55
IA1	DBA2	18	0	34.9	0
IA1	DBA2	18	120	84	56.39
IA2	DBA2	21	0	34.4	0
IA2	DBA2	21	120	65.8	109.97
IA3	DBA2	18	0	36.8	0
IA3	DBA2	18	120	29.8	70.54
IA4	DBA2	20	0	29.9	0
IA4	DBA2	20	120	23.8	98.91
IA5	DBA2	18	0	28.1	0
IA5	DBA2	18	120	40.3	93.55
IA6	DBA2	21	0	41.5	0
IA6	DBA2	21	120	98.7	104.29
IA7	DBA2	25	0	22.5	0
IA7	DBA2	25	120	31.6	89.59
IA8	DBA2	20	0	15.9	0
IA8	DBA2	20	120	52	64.13
IIA1	C57BL6	20	0	19.2	0
IIA1	C57BL6	20	120	150	167.53
IIA2	C57BL6	21	0	26.8	0
IIA2	C57BL6	21	120	17.9	97.77
IIA3	C57BL6	21	0	31.8	0
IIA3	C57BL6	21	120	21.9	111.4
IIA4	C57BL6	21	0	18.1	0
IIA4	C57BL6	21	120	31.6	89.97
IIA5	C57BL6	21	0	26.7	0
IIA5	C57BL6	21	120	150	159.24
IIA6	C57BL6	20	0	16.5	0
IIA6	C57BL6	20	120	28.4	24.69
IIA7	C57BL6	18	0	21.2	0
IIA7	C57BL6	18	120	150	114.57
IIA8	C57BL6	22	0	15.4	0
IIA8	C57BL6	22	120	51.8	106.76