Research
Based
Curricula

Your brain on estrogen

Key Stage 5

Biology/Psychology

2020

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For Students
Getting Started

RBC means Research-Based Curriculum,. Each RBC coursebook is written by a PhD student at a university about their cutting edge research.

Why complete an independent 'RBC' study pack?

RBC courses are challenge courses to sharpen your skills and resilience: finishing a RBC course is a major accomplishment to add to your academic CV. To get into the university, you must demonstrate that you are intellectually curious, and will make the most of the academic opportunities available to you. Completing a pack will allow you to gain invaluable experience to write about in your university application..

It allows you to:

- ✓ Build your subject experience to mention in your UCAS Personal Statement
- ✓ Sharpen your academic skills
- ✓ Experience what it's like to study beyond school and at university
- ✓ Better understand what you enjoy and don't
- ✓ Improve your overall subject understanding ahead of final exams

For Students
Getting Started

What's in this booklet?

Your RBC booklet is a pack of resources containing:

- ✓ More about how and why study this subject
- ✓ Six 'resources' each as a lesson with activities
- ✓ A final assignment to gauge learning
- ✓ Extra guidance throughout about the university skills you are building
- ✓ End notes on extra resources and where to find more information

Who should complete this pack?

Anyone interested in improving their academic skills or understanding what they should do at university. *This pack is especially great for anyone interested in studying* **Sciences,** *particularly* **Biology or Psychology,** *and want to understand how these link to each other.*

Even if you are unsure of where your interest in these subjects can take you, by completing this pack you will have a clearer idea of the variety of subjects that link to one another.

If you have any questions while you are using the resources in this pack, you can contact your teacher or email us directly at schools@access-ed.ngo.

Good luck with your journey to higher education!

For Students
University Skills

To complete this resource, you will have to demonstrate impressive academic skills. When universities are looking for new students, they will want young people who can study independently and go above and beyond the curriculum. All of these skills that you will see here will demonstrate your abilities as a university student – while you're still at school!

Every time you have to look something up, or write up a reference you are showing that you can work independently.

Every time that you complete a challenging problem or write an answer to a difficult question, you might demonstrate your ability to think logically or build an argument.

Every time that you evaluate the sources or data that you are presented with, you are showing that you can "dive deep" into an unfamiliar topic and learn from it!

Skills you will build for university:

independent research	your ability to work on your own and find answers online or in other books
creativity	your ability to create something original and express your ideas
problem solving	your ability to apply what you know to new problems
building an argument	your ability to logically express yourself
providing evidence	your ability to refer to sources that back up your opinions/ ideas
academic referencing	your ability to refer to what others have said in your answer, and credit them for their ideas
deep dive	your ability to go above and beyond the school curriculum to new areas of knowledge
source analysis	your ability to evaluate sources (e.g. for bias, origin, purpose)
data interpretation	your ability to discuss the implications of what the numbers show
active reading	your ability to engage with what you are reading by highlighting and annotating

Where can this subject take me?

Pathways

Studying Biology or Psychology can open the doors to many degrees and careers. It intersects with microbiology, chemistry, physiology, and sociology. Whatever interests you is likely to relate to biology in some way. See a snapshot of where studying Biology and Psychology can take you.

'Transferrable skills' from Biology to a career:

- research and data analysis
- problem-solving and creative thinking
- delivering successful projects
- communication, through report writing and presentations
- teamworking and collaboration
- the ability to work independently
- numeracy and maths
- IT and computer literacy

'Transferrable skills' from Psychology to a career:

- Empathy and interest in people
- analytical research
- problem solving
- the ability to work in teams.
- written and verbal communication; report writing and presenting
- information technology
- handling of data and statistics

What are some are the 'interdisciplinary' subjects in this course?

Interdisciplinary is a term you will hear used by higher education institutions. It's also how many professionals and academics in the real-world operate: they use multiple subjects, or disciplines, to achieve their work.

By thinking about which subjects you like, alongside maths, it can help you choose a career pathway later.

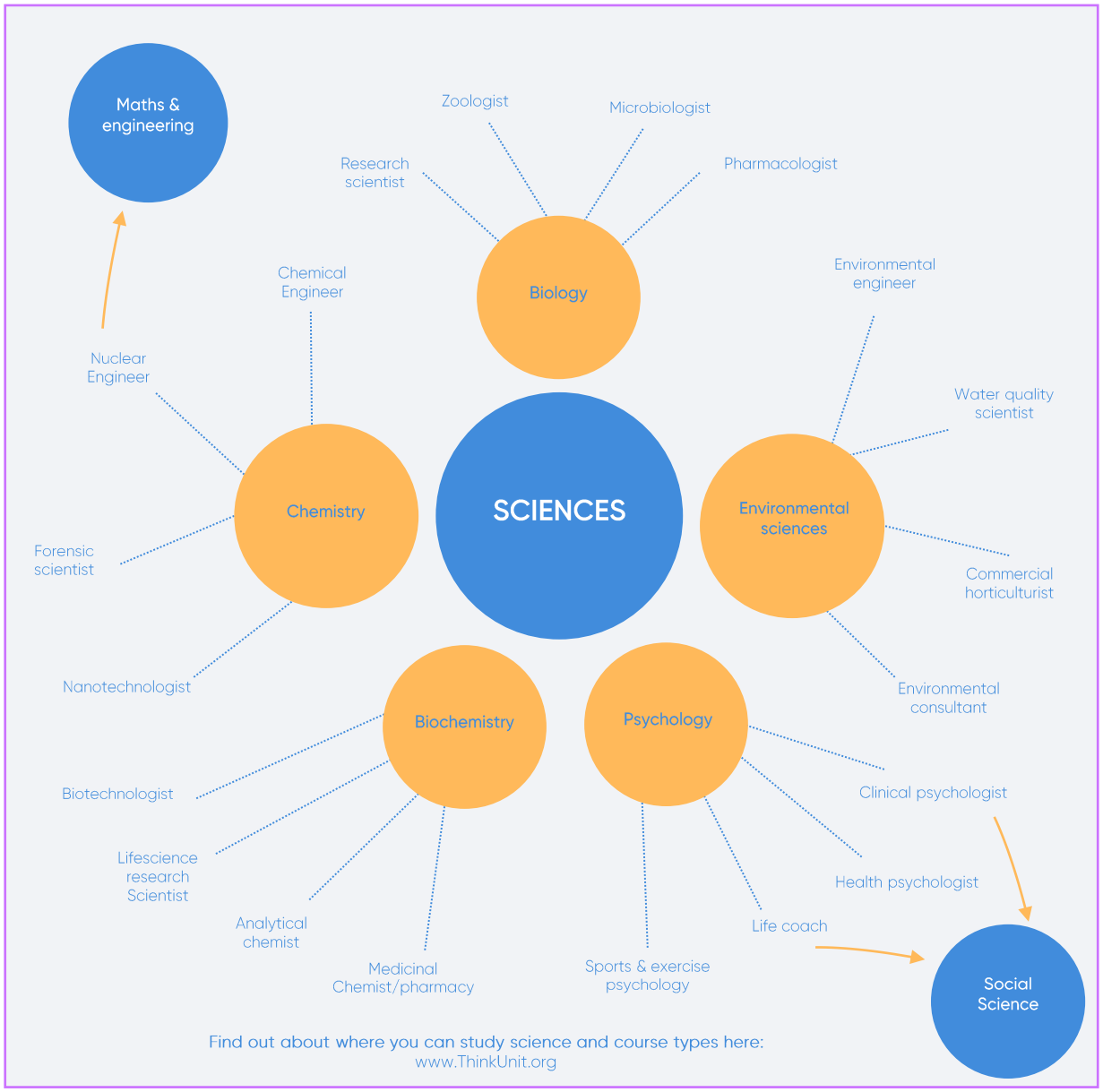
Read more about subject selection and careers pathways:

https://targetjobs.co.uk
https://www.prospects.ac.uk
https://thinkuni.org/

Subject map: Sciences

A degree in Sciences gives Students access to a large number of career choices. Many students who study sciences go on to pursue their Master's degree in Science. However, a significant portion of them also start looking out for jobs in the field of Cancer research, Stem Cell technology and other positions in this space.

Did you know? Being a scientist of any kind can open up may doors within any industries, from managing projects to labs to health policy teams with governments!



Find our about Science-related careers here:
PROSPECTS: https://www.prospects.ac.uk
TARGET JOBS: https://targetjobs.co.uk

For Teachers
RBC Guide

Learner aims

The Research-Based Curriculum aims to support student attainment and university progression by providing classroom resources about cutting-edge research at local universities. The resources are designed to:

- ✓ promote intellectual curiosity through exposure to academic research
- ✓ stretch and challenge students to think deeply about content that may be beyond the confines of the curriculum
- ✓ develop core academic skills, including critical thinking, metacognition, and written and verbal communication
- ✓ inform students about how subjects are studied at university, and provide information, advice and guidance on pursuing subjects at undergraduate level

Content

The programme represents a unique collaboration between universities and schools. Trained by AccessEd, PhD Researchers use their subject expertise to create rich resources that help bring new discoveries and debates to students.

The Research-Based Curriculum offers ten modules suitable for either KS4 or KS5 study. The modules span a range of disciplines, including EBacc and A-level subjects, as well as degree subjects like biochemistry. Each module includes six hours of teaching content, supported by student packs, teacher notes and slides. All modules are available online and free of charge for teachers at select schools.

Using the RBC pack

These resources are designed to be used flexibly by teachers. The resources can be completed by students individually or in groups, in or out of the classroom.

For Teachers
Using the RBC packs

Here are five examples of delivery options:

Extra-Curricular Subject Enrichment Clubs

The resources can be completed in small groups (4-8 pupils) across a series of weekly lunch clubs or after-school clubs. Groups can reflect on their learning by presenting a talk or poster on the subject matter at the end of the course.

University Access Workshops

The resources can be used by students to explore subjects that they are interested in studying at university. This can inform their decision making with regards to university degree courses, and allow students to write more effective personal statements by including reflections on the Research-Based Curriculum.

Research Challenge

The resources can be used to ignite curiosity in new topics and encourage independent research. Schools could hold a research challenge across a class or year group to submit a piece of work based on the resources. Pupils could submit individually or in small groups, with a final celebration event.

Summer Project

Resource packs can function as 'transition' projects over the summer, serving as an introduction to the next level of study between KS3 and KS4, or KS4 and KS5. Students could present their reflections on the experience in a journal.

Why offer these?

The Research-Based Curricula programme builds on the University Learning in Schools programme (ULiS), which was successfully delivered and evaluated through the London Schools Excellence Fund in 2015. The project was designed in a collaboration between Achievement for All and The Brilliant Club, the latter of which is the sister organisation of AccessEd. ULiS resulted in the design and dissemination of 15 schemes of work based on PhD research for teachers and pupils at Key Stage 3. The project was evaluated by LKMCo. Overall, pupils made higher than expected progress and felt more engaged with the subject content. The full evaluation can be found here: ULiS Evaluation.

Questions

For more information contact hello@access-ed.ngo

Introduction to Topic Behavioural neuroendocrinology

Neuroendocrinology combines the study of hormones and the endocrine system (endocrinology) with the brain (neuroscience). Relating this to behavioural outcomes makes my research interdisciplinary, combining biology with psychology.

The topics within this pack will include:

- Steroid hormones and their synthesis
- Modes of signalling via estrogen receptors
- Neuronal signalling and the synaptocrine signalling hypothesis of estrogens
- Estrogen and the sexually dimorphic brain
- Estrogen in sexually dimorphic reproductive behaviours
- Estrogen and mood

Estrogen is typically regarded as a female hormone mostly present in the ovaries where it is involved in menstruation and fertility. However, the functions of estrogen span well beyond the female sex organs, though most of these functions are not well understood. This is largely due to the fact that most research uses male animals to avoid having to standardise data according to the fluctuating hormone levels across the estrous cycle (the rodent equivalent of the human menstrual cycle). Therefore, my research is focused on understanding the function of estrogen and estrogen signalling in the female brain.

Estrogen is involved in the development of brain areas related to the expression of sex-typical behaviours in both males and females. After development, estrogen acts on these brain areas again, this time stimulating the expression of behaviours. The underlying mechanism for this is unknown. A novel estrogen receptor called G-protein-coupled estrogen receptor 1 (GPER1) has been identified in the brain and therefore could contribute to estrogen's actions on development and behaviour. Thus, part of my research aims to understand the function of GPER1 in the female brain.

Introduction to Topic Behavioural neuroendocrinology

The exploration of this topic is incredibly important. Biomedical research is beginning to highlight the presence of many sex differences (known as sexual dimorphisms) in human and animal physiology that go beyond differences in the sex organs. Understanding how estrogen contributes to the sexually dimorphic development of the brain could help us to better understand the mechanisms underlying psychopathologies which alter typical behaviours, as well as helping us to understand why neurodegenerative diseases affect men and women differently. This could then enable us to develop personalised therapies for neurodegenerative diseases and mood disorders.

Introduction to Topic Behavioural neuroendocrinology

Studying at University

Biomedical Science is often seen as the 'back up degree' for people who didn't get into Medicine. Though that is the case for many people (myself included), this is most certainly not the default case! Reasons for studying Biomedical Science and Medicine can overlap. Maybe you have an interest in bacteria and infectious diseases? Maybe you're interested in how the heart works and how you would go about treating cardiovascular disease? The major difference between Biomedical Science and Medicine is that in Biomedical Science you learn about the mechanisms behind normal function, disease, and treatment, whereas medics have to learn how to diagnose patients as well as being assessed on their bedside manner. I like to think of Biomedical Science as the 'behind-the-scenes' of medicine. Without Biomedical Scientists, we wouldn't be able to diagnose diseases in the first place because we wouldn't understand what is going wrong, let alone how we should go about treating it!

I studied Biomedical Science at Cardiff University. In my first year, we all followed a general structure of learning, including anatomy and physiology, bacteria and viruses, and genetics. After that, we were allowed to continue on the general course or 'specialise' in a certain topic, whether that be Anatomy, Physiology, Neuroscience, Genetics, or Microbiology. When you get to your second year, you undertake core modules (modules you have to take as part of your degree) and optional modules (modules you choose to take that may not be directly related to your degree but overlap with it). You spend a lot of time in lectures but also a lot of time in the lab. Here, you get to learn new techniques and see some pretty cool stuff like dissections of real human bodies and limbs! Some other cool stuff I did included measuring brain waves and almost passing out doing a (legal) VO2 max test for my friend's dissertation

Introduction to Subject Biomedical Science at University

Careers

Biomedical Science includes such a broad range of medical-related topics that it opens up a wide range of career options that aren't necessarily all lab-based. If being in a hospital-based lab is something that interests you, make sure you apply to courses that are accredited by the Health and Care Professions Council (HCPC) – you won't be able to get a job in a hospital without an accredited degree. If you don't do an accredited Biomedical Science degree, don't worry - you can do a top-up course at one of a few universities after you graduate. If you decide the lab isn't for you then you could work in medical sales, technical writing, data analysis to name a few.

After university, many people realise that they no longer want to pursue a career related to their degree topic, and that is perfectly fine (and really common!). Biomedical Science is a really challenging degree that doesn't just improve your scientific knowledge – you develop skills in critical thinking and analysis, logical thinking and planning, and spoken and written communication. These attributes are sought after in many sectors – the career prospects for STEM graduates are never-ending.

Meet the PhD Researcher Janine Dovey

If 10-year-old me knew what I was up to now, I wouldn't believe myself. I was always a good all-rounder in school, but throughout most of my school years I was into all things art – English literature, drama, singing, music… I even auditioned for a part in a Channel 4 TV series and was close to getting the part! After the rejection from the TV show, I started to really think about my career options. As much as I loved theatre and acting, I knew I couldn't cope with the constant rejections while waiting for my 'big break'. Because of my good academic record, when it came to picking my A-level subjects I was lucky enough to be in a position where I couldn't choose between them because I could have done any! So I thought about what I would like to do with my career, and worked backwards from there – what subject would I need to study at university? What A-levels do I need for that?

I pursued the idea of becoming a doctor (a medic, not a PhD). I loved human biology. I'm also the type of person that likes to know how things work and I like putting together puzzles to come up with an answer. Medicine seemed like the way to do that. I applied and after some rejections, got offered a place to study Medicine but I wasn't a huge fan of the university. I also got offered a place to study Biomedical Science, my back-up degree, at Cardiff University. I absolutely loved Cardiff, so much so that I applied there to Biomedical Science and Medicine. My application for Medicine at Cardiff got turned down, so I was left with my first-choice course at my second-choice university vs my second-choice course at my first-choice university.

Meet the PhD Researcher Janine Dovey

I wasn't sure how I would pick which course and university to study at but in the end, I didn't have to. I was just short of the grades I needed for Medicine and I went to Cardiff to study Biomedical Science instead.

I really enjoyed my degree. It was hard, and I got a little stressed out in my second year (as I think most people do – the jump from first year to second year of university is much harder than the jump from A-levels to university) but I'm so glad I did it. After my first year, I discovered I had a really keen interest for endocrinology, which mainly featured in the Physiology course. So when I came to specialise in my second year, I chose Physiology where I took optional modules in Neuroscience. This is where I first learnt about Neuroendocrinology. I then organised a placement year at the University of Bristol where I would have been working on a project looking at the effects of the stress hormone cortisol on brain function. Unfortunately, my funding didn't pull through, making the placement financially impossible. I didn't feel ready to graduate after my third year but luckily, I had applied for and been offered a place on the Integrated Masters course.

The Integrated Masters is technically an undergraduate course, but because you don't graduate between finishing your third year and starting the Masters, you just leave with a Masters degree. I didn't like any of the advertised Masters projects, so I went to my favourite lecturer (a neuroendocrinologist) and specifically asked him to write a Masters project for me instead. The project he came up with investigated the effects of feeding pattern (eating meals vs grazing) on eating habits and fat storage in male rats. This related back to the hunger hormone ghrelin, which was his hormone specialty.

Meet the PhD Researcher Janine Dovey

I loved my Masters. I had a brilliant supervisor and a great project – I even presented my research at two conferences that year, being the youngest presenter at both conferences. After this, I really got the bug for research and conferences and I wanted to do more, so I started applying for PhDs.

I'm now in my second year of a 3-year PhD programme. It's a lot of work but I make sure I take time to de-stress by doing the things I enjoy. I compete at a national level in Olympic Weightlifting and training is a huge mental outlet for me. I also really enjoy reading and travelling back to Wales to see my partner and friends.

I'm not sure what I'll do when I finish. I sometimes think about going back to do Medicine, but my interests have changed so much it wouldn't surprise me if they change again!

A-Level Subjects	Biology, Chemistry, Maths
Undergraduate	Integrated Masters in Biomedical Science (MBiomed)
Postgraduate	PhD in Neuroendocrinology

Glossary

Term	Definition
Action potential	An electrophysiological signal used by neurones in which voltage rapidly rises and falls in response to a stimulus.
Agonist/antagonist	A chemical that binds to a receptor to activate (agonist) or inhibit (antagonist) the receptor's activity.
All-or-nothing principle	The principle that no matter how strong the stimulus, as long as it reaches threshold potential an action potential will be initiated. This makes all action potentials the same, regardless of how strong the initial stimulus was.
Apoptosis	A highly regulated process of cell death.
Aromatase	One of the final enzymes involved in the steroidogenic pathway, which converts testosterone to estrogen by aromatisation.
Artificial cerebrospinal fluid (aCSF)	A buffer solution that mocks biological cerebrospinal fluid. It is used experimentally to incubate brain tissues and keep them at a constant pH and supply oxygen.
Depolarisation	A change in the voltage across the plasma membrane that results in positive charge moving into the neurone to make the inside less negative.
Dimer	A molecular complex consisting of two identical (homodimer) or different (heterodimer) molecules linked together.
Estrogen response element (ERE)	A sequence of DNA found in the promoter region of a gene that is specifically bound by estrogen receptors, driving estrogen-dependent gene transcription.
Genomic pathway	The 'classical' estrogen receptor signalling mechanism which involves nuclear estrogen receptors ERα and ERβ binding DNA and acting as transcription factors in response to estrogen over a time course of minutes to hours.
Gonadectomy	The surgical procedure that removes the gonads in either males or females. In females, it can otherwise be called ovariectomy (OVX; removal of the ovaries) or castration.

Glossary

Term	Definition
Hyperpolarisation	The 'overshoot' in positive charge that moves out of a neurone during repolarisation. This temporarily makes the neurone even more negative than it is at resting membrane potential, which prevents any other action potentials being fired.
Hypothalamic-pituitary-gonadal (HPG) axis	The relationship between the hypothalamus and pituitary with the gonads, by which hormone release from both the brain and gonads is tightly regulated.
Immunohistochemistry	A staining process performed on thin slices of tissue that allows you to visualise proteins when the sample is placed under a microscope.
Isoform	A variant of a protein that differs slightly in its characteristics. Many nuclear estrogen receptors have isoforms that are found in the cell membrane rather than the cytoplasm.
Knockout	The term used for an organism that has been genetically modified to no longer express a particular gene.
Lordosis	A female sexual behaviour characterised by arching the spine to show sexual receptivity towards a male.
Negative feedback	An important regulatory mechanism used by many hormones by which the production of the main product in turn reduces the stimulus of the process.
Neuroestrogen	Estrogen made in the brain.
Nongenomic pathway	A novel estrogen receptor signalling mechanism involving membrane estrogen receptors which, upon binding estrogen, stimulate a signalling cascade of other proteins which bring about a response in seconds to minutes.
Perinatal	The period of time including just before birth (prenatal) and just after birth (postnatal) of offspring.

Glossary

Term	Definition
Plasticity	The term given to the adaptability of neurones and their projections based on development and learning.
Repolarisation	The change in voltage across the plasma membrane following an action potential, where positive charge moves out of the cell to make the inside of the neurone more negative, thus returning it to resting membrane potential.
Resting membrane potential	The electrical difference (voltage, V) across the plasma membrane when a neurone is in a non-excited state. For most neurones, resting membrane potential is around -70 millivolts (mV).
Second messenger	Any protein or substance that is activated by a hormone binding a receptor. Second messengers make up the proteins in signalling cascades.
Sex steroid hormone	An organic molecule synthesised from cholesterol which facilitates the development of the sex organs and secondary sex characteristics.
Sexual dimorphism	Any sex difference between two sexes of the same species that goes beyond the sex difference in the gonads.
Spinogenesis	The development of dendritic spines in neurones.
Steroidogenic pathway	The synthetic pathway by which all steroid hormones are synthesised from cholesterol.
Synaptocrine	A neuromodulatory process involving the synthesis of a steroid hormone at the synaptic terminal.
Threshold potential	The critical level to which the membrane must be depolarised to initiate an action potential. Threshold potential for neurones is around -55mV.
Transcription factor	A protein that controls the transcription of a gene from the DNA code.

Glossary

Term	Definition
Vehicle	Often used as a control treatment when animals are injected with a drug. The vehicle is the solvent/oil that the drug was dissolved in for the injection.
Wildtype	The term used to describe an organism in its natural, non-mutated form.
3Rs	The framework in place to minimise animal usage and animal suffering in biomedical research.

Resource One Overview

Topic	Steroid hormones and their synthesis
A-level Modules	Biological molecules, proteins and enzymes; Role of the hypothalamus in regulating physiological processes
Objectives	By the end of this resource, you will be able to: ✓ Recall the three classes of sex steroid hormones, with examples for each, and identify the receptor they signal through. ✓ Describe how cholesterol is used to synthesise the sex steroid hormones and how they are linked in the steroidogenic pathway. ✓ Understand how the brain regulates hormone secretion.
Instructions	1. Read the data source 2. Complete the activities 3. Explore the further reading

Resource One
Data Source

Section A

What are steroids?

Steroids are organic molecules that are characterised by 4 rings of carbon atoms at the centre of their structure. All steroids are synthesised from cholesterol and are lipophilic. The lipophilic nature of steroids means that they can pass through lipid cell membranes easily, making them ideal cell signals. Steroids that act as cell signals are called steroid hormones. Because they are lipophilic, steroids can diffuse easily out of the cells that make them, meaning they can't be stored. Therefore, the production of steroids depends on the stimulation of the parent cell, which then converts cholesterol to an active steroid hormone as and when it is needed.

Resource One
Data Source

Section B

Synthesis of sex hormones

The sex hormones estrogen and testosterone are both examples of steroid hormones.

Active reading

The steroidogenic pathway is the synthetic pathway for steroid hormones and is shown in figure 1. The first step is the synthesis of pregnenolone which is initiated by the expression and activation of Steroidogenic Acute Regulatory protein (StAR). StAR is responsible for transporting cholesterol to the inner mitochondrial membrane; this is the rate-limiting step of steroid synthesis. Here, cholesterol is cleaved by the cytochrome P450 side chain cleavage (P450scc) enzyme to produce pregnenolone, which can be directly converted to the androgen DHEA, or it can be converted to progesterone. Both DHEA and progesterone act as the precursors to the other androgens whilst also having their own physiological effects.

Testosterone is the substrate for the estrogens, and this conversion involves the enzyme aromatase. The aromatisation of testosterone to estrogen is a really important step in many physiological functions, so understanding the characteristics of aromatase is of great interest for many scientists.

Testosterone can also form dihydrotestosterone (DHT). DHT is the only androgen that can't be aromatised to estrogen, so we can use it in experiments to distinguish between androgenic and estrogenic effects. For example, if we add

Resource One
Data Source

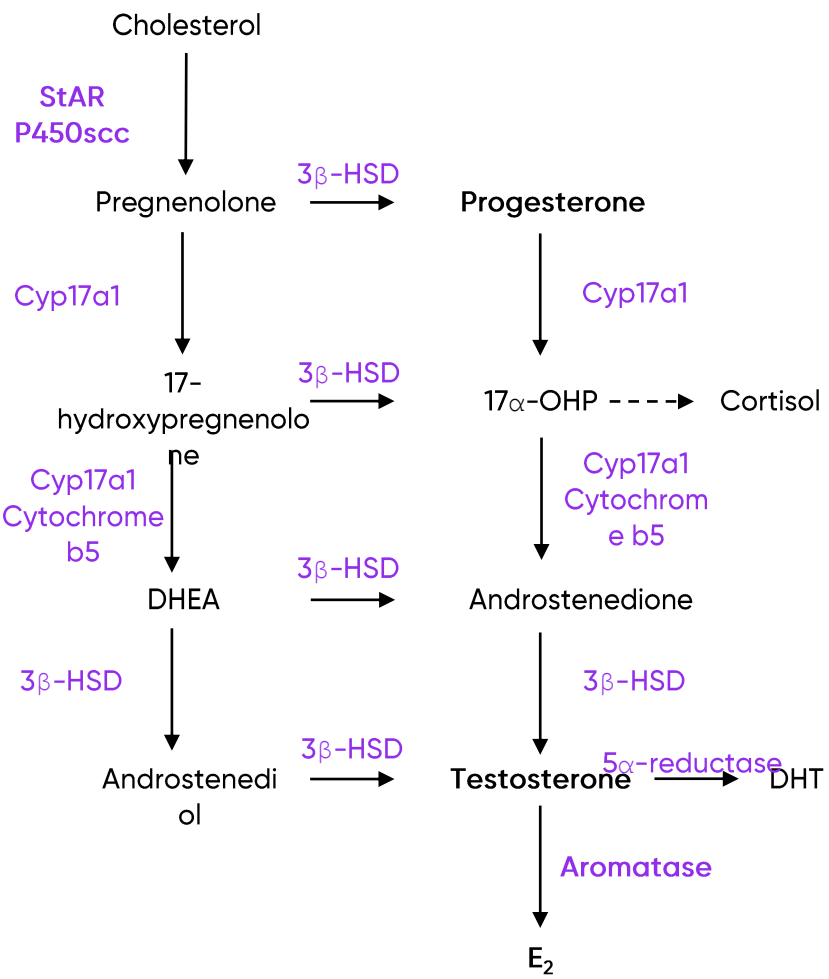
Section B

Synthesis of sex hormones

testosterone to an experimental model, we can't be sure that 100% of that testosterone is causing the effects we're seeing – what if 40% of it was aromatised to estrogen, and that was responsible for the effects instead? However, if we add DHT, we know that it can't be aromatised, so any effect we see is completely down to androgen action.

Figure 1

The steroidogenic pathway of steroid hormone synthesis. Purple text is used to indicate enzymes and bold text indicates key hormones/enzymes.



Resource One
Data Source

Section B

Synthesis of sex hormones

Many of the steps in steroid synthesis involve the same enzymes. This means that a deficiency in just one enzyme can result in decreased synthesis of many hormones! The functions of each of the enzymes in figure 1 are given below:

StAR (Steroid Acute Regulatory Protein) is the rate limiting step in steroid synthesis. It transports cholesterol to the inner membrane of mitochondria. This isn't really an enzyme since it doesn't catalyse a reaction, but it is absolutely required for steroidogenesis.

P450scc (cytochrome P450 side chain cleavage enzyme) is found in the inner mitochondrial membrane. It cleaves the cholesterol side chain to convert cholesterol to pregnenolone.

Cyp17a1 is part of the cytochrome family of enzymes. It has both an hydroxylase activity (adds an –OH group) and a lyase activity (breaking molecular bonds).

Extra information

Cytochrome b5 transports electrons for cytochrome enzyme reactions.

3β-HSD is the only enzyme in the steroidogenic pathway that isn't part of the cytochrome family of enzymes. Different isoforms are involved in each step of steroidogenesis – but this is more information than we need for this coursebook!

Resource One
Data Source

Section B

Synthesis of sex hormones

5α-reducatase is a reducing factor – it adds electrons to molecules. Deficiencies in this enzyme affect male sexual development before birth and during puberty. Men with this condition cannot synthesise DHT, one of the androgens required for normal development of the male reproductive organs. This leaves them with "ambiguous", or "intersex" genitalia, that resemble a mixture between a penis and vagina.

Aromatase converts testosterone to estrogens, the main one being 17β-estradiol (E2).

Section C

The hormone regulatory centre of the brain

Sex hormones are made in the adrenal cortex and the gonads, but the signal for their synthesis actually comes from the brain. A schematic of this process, which is further described below, is shown in figure 2.

The first signal comes from the hypothalamus; a small region at the base of the brain that controls the release of hormones from the pituitary gland. It does this by itself releasing hormones that either stimulate or inhibit the actions of the pituitary gland.

The pituitary gland is a small, bean-sized gland at the base of the brain, just below the hypothalamus. It is split into two parts: the anterior and posterior pituitary. The anterior pituitary responds to hormones released from the hypothalamus by synthesising and secreting 7 different hormones. The posterior pituitary is an extension of the hypothalamus. It does not secrete its own hormones, rather, it stores hypothalamic hormones in specialised neurones called neurosecretory cells and secretes them when needed.

Resource One
Data Source

Section C

The hormone regulatory centre of the brain

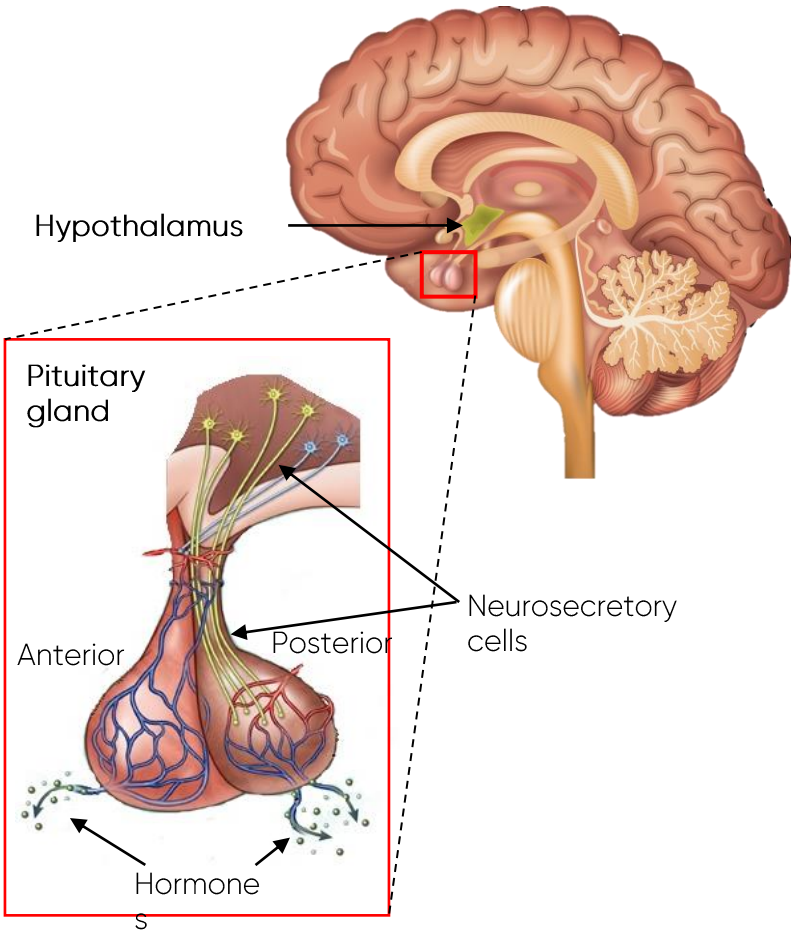


Figure 2

The hypothalamus and pituitary gland.

Sagittal brain section image taken from thescienceofpsychotherapy.com (author unknown), modified by J. L. Dovey

Pituitary image created by Bricelyn H. Strauch, Staywell©, modified by J. L. Dovey.

Resource One
Data Source

Section D

The HPG axis

The *hypothalamic-pituitary-gonadal (HPG)* axis is the main control mechanism for regulating steroid hormone synthesis and is shown in figure 3. It begins with the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus. GnRH acts on the anterior pituitary, causing the release of luteinising hormone (LH) and follicle-stimulating hormone (FSH) into the bloodstream, where they travel until they reach the gonads.

In males, LH and FSH stimulate the release of testosterone from the testes, which feeds back to the brain to tell it to stop releasing LH and FSH. This is called negative feedback. LH and FSH also stimulate spermatogenesis (the production and development of sperm).
In females, LH and FSH stimulate the release of estrogen and progesterone from the ovaries, which provide negative feedback to the brain to stop any more LH and FSH being released. LH and FSH also stimulate the growth and maturation of endometrial tissues leading to the release of an egg in ovulation.

The brain can also stimulate estrogen production in other 'extra-gonadal' tissues, such as bone, fat and even the brain itself! Estrogens that are synthesised in the brain are referred to as neuroestrogens.

Resource One
Data Source

Section D

The HPG axis

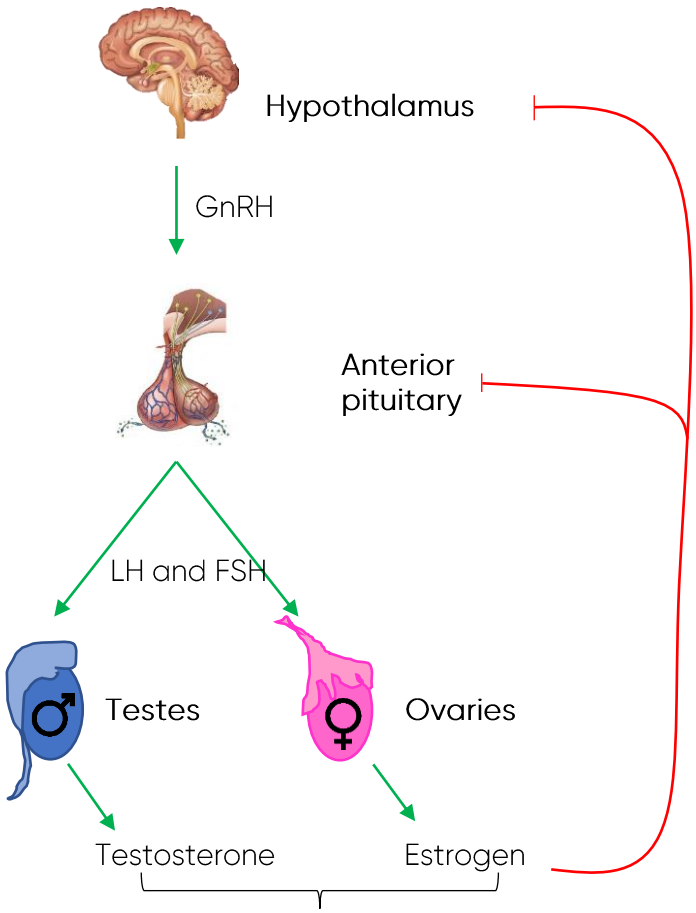


Figure 3

The HPG axis in action. Green arrows represent positive feedback, i.e. stimulation of hormone production. Red bluted arrows represent negative feedback, i.e. inhibition of hormone production.

Resource One Activities

Activities

1. Name the enzyme that converts testosterone to estrogen.

2. Describe the events that take place that lead to cholesterol's conversion into pregnenolone.

3. Use figure 1 to list the hormones it would be possible to make with a deficiency in the 3β-HSD enzyme.

4. What approach would you use to compare the effects of androgens and estrogens in a biological model that expresses aromatase?

5. What is the relationship between the hypothalamus, pituitary gland, and the gonads? Explain how it works.

Problem solving

Resource One
Further Reading

Explore https://courses.lumenlearning.com/suny-ap2/chapter/hormones/#:~:text=Steroid%20hormones%20are%20derived%20from%20cholesterol%20and%20therefore%20can%20readily,and%20can%20enter%20the%20cell

An easy-to understand, in-depth coverage of different types of hormones and endocrinology. Read from the beginning to 'Pathways of Hormone Action'.

https://www.researchgate.net/publication/331362345_Anatomy_and_Physiology_of_the_Hypothalamic-Pituitary-Gonadal_HPG_Axis

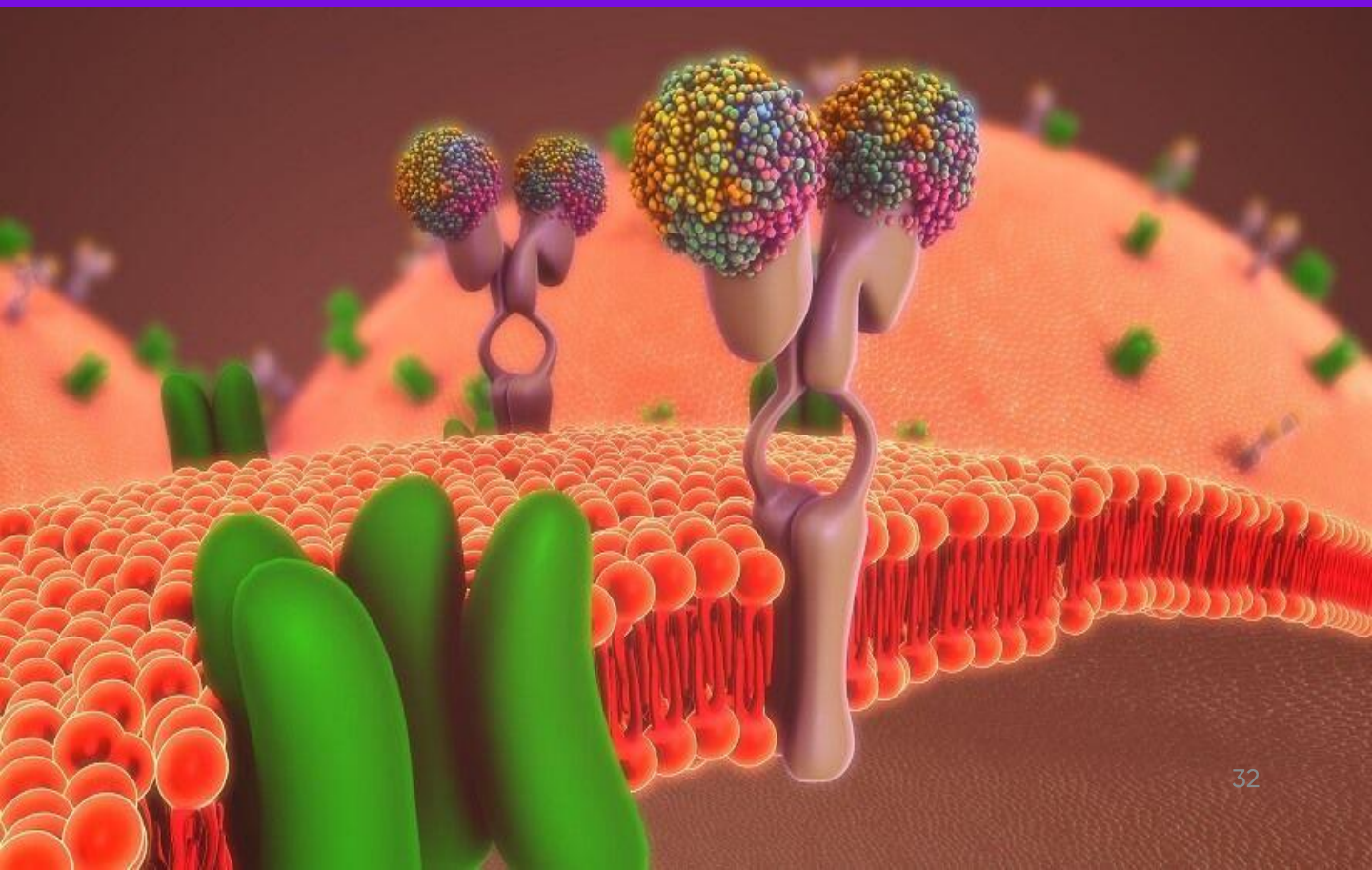
Anatomy and Physiology of the HPG axis from an online version of a book chapter. A free downloadable PDF is available by this link.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5227141/

Short review article on extra-gonadal sites of estrogen synthesis and function. This is above KS5 but covers all the areas in which estrogen acts and functions.

Resource Two Overview

Topic	Modes of signalling via estrogen receptors
A-level Modules	Responding to stimuli; Cell signalling; Cell recognition by antibodies; Gene transcription
Objectives	By the end of this resource, you will be able to: ✓ Understand and compare genomic and nongenomic estrogen signalling ✓ Describe and appreciate scientific methods for understanding estrogen receptor expression, distribution and function
Instructions	1. Read the data source 2. Complete the activities 3. Explore the further reading



Resource Two
Data Source

Section A

Estrogen receptors

Hormones travel throughout the body in the bloodstream until they reach a target cell. The target cell could be right next to the cell that made the hormone (paracrine action) or it could be very far away (endocrine action). Alternatively, the parent cell can also be the target cell (autocrine action)!

At the target cell, the hormone binds to specific protein receptor which initiates a response. Some hormones have several types of receptor they can bind to, meaning there are many possible responses.

Estrogen has several estrogen receptors which it can bind, and all are expressed at varying levels across different tissues. We can visualise where receptors and other proteins are expressed using a technique called immunohistochemistry. The technique is shown as a diagram in figure 4.

Active reading

Resource Two
Data Source

Section A

Estrogen receptors in the brain

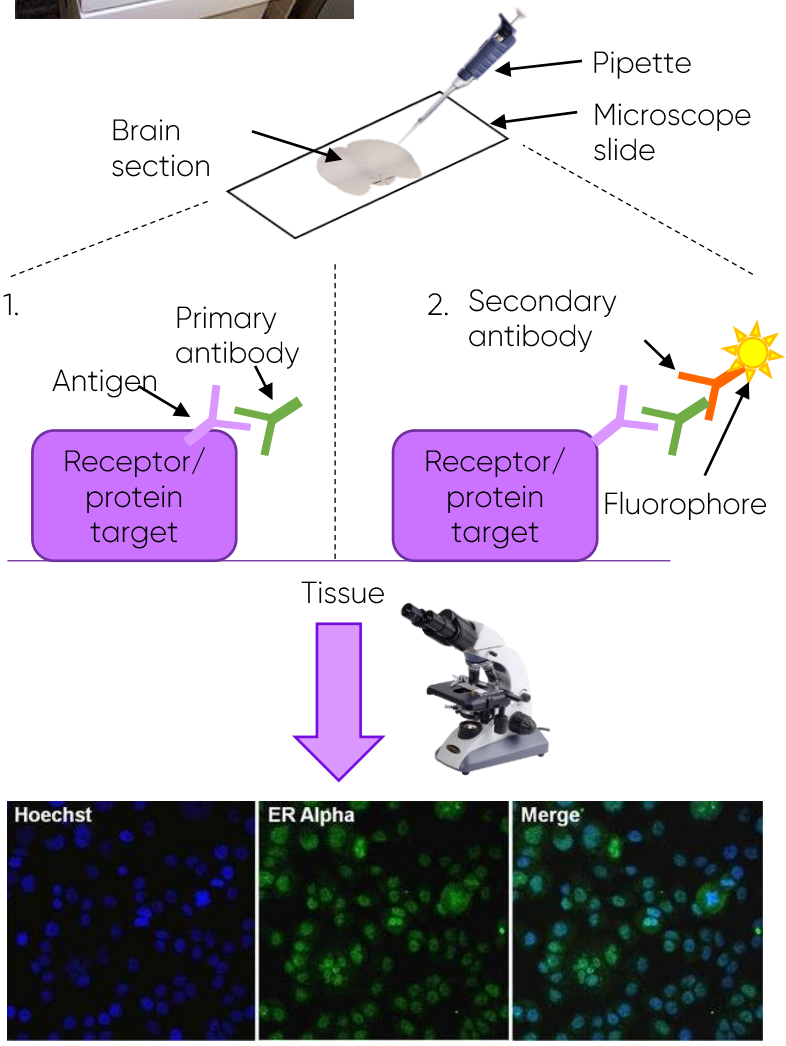


Figure 4

Immunohistochemistry technique. After the brain has been dissected and preserved in a fixative such as paraformaldehyde, it can be sectioned on the cryostat (top two images). The brain sections are mounted on microscope slides and a pipette is used to apply the antibodies, which are diluted in a solution. The first antibody is a primary antibody, which binds the antigens present on the receptor/target protein. The antibodies are left to bind for 24h. Next, a secondary antibody conjugated to a fluorescent protein (fluorophore) is added. This is visualised under a fluorescent microscope, producing images such like those seen at the bottom of this figure. You can add multiple primary and secondary antibodies to stain multiple proteins in lots of different colours!

Image taken from ThermoFisher Scientific Antibody Testing Data for their Invitrogen Estrogen Receptor alpha Monoclonal Antibody (MA1-310).

Resource Two
Data Source

Section B

Genomic signalling by classical estrogen receptors

The classical method of estrogen signalling is known as the genomic signalling pathway (shown in **figure 5**) and it involves two receptor subtypes: estrogen receptor (ER)α and ERβ. Both of these receptors belong to the nuclear receptor family. Receptors in this family have an evolutionarily conserved structure that allows them to directly bind specific DNA sequences to regulate the transcription of genes. Nuclear receptors such as ERα and ERβ are located intracellularly (inside the cell), usually found floating in the cytoplasm near the nucleus. Since estrogen is a lipophilic steroid hormone, it can easily diffuse through the cell's plasma membrane to reach the receptors inside!

When estrogen enters a cell and binds one of the nuclear estrogen receptors, a conformational change occurs. This means that the structure of the receptor changes to allow it to interact with other proteins. In the case of estrogen signalling, this interaction is with other nuclear estrogen receptors coming together to form dimers. Dimers can be made up of two of the same receptor (e.g. ERα + ERα or ERβ + ERβ); this is a homodimer. Alternatively, it can be an interaction between different receptors (ERα + ERβ); this is a heterodimer.

Once the dimer has formed, it translocates to the nucleus where it binds to a specific sequence of DNA called an estrogen response

Resource Two
Data Source

Section B

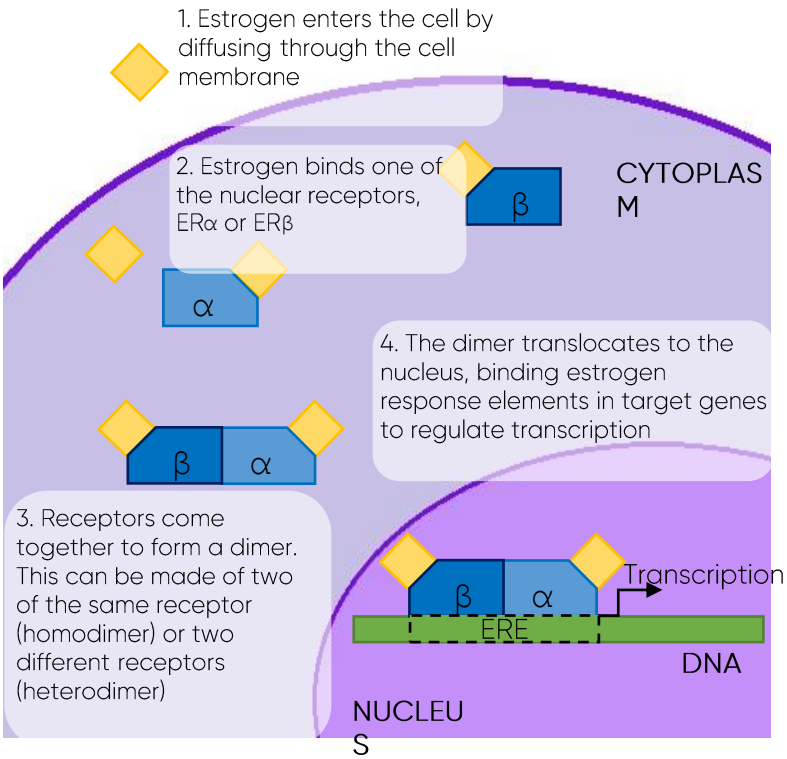
Genomic signalling by classical estrogen receptors

element (ERE). EREs are found in the promoter regions, which are the regions of DNA that initiate the transcription (reading) of a gene. The receptor dimer binds to the ERE and can then act as a transcription factor to regulate the expression of specific genes.

Genomic signalling typically takes minutes to hours to produce a cellular response

Figure 5

Genomic signalling mechanism



Resource Two
Data Source

Section C

Nongenomic signalling by membrane estrogen receptors

The two subtypes of estrogen receptor (ERα and ERβ) can be modified to make receptors with slightly different properties, called isoforms. Some of these isoforms are found in the plasma membrane of cells, meaning estrogen can bind the receptor without having to diffuse into the cell. Around 5-10% of all estrogen receptors are located in the plasma membrane, and their localisation is controlled by the relative expression of receptor transporters and anchoring proteins which attach the receptor to the membrane. One example of an ERα isoform is ERα-36. The expression of ERα-36 is a result of a process called alternative splicing, in which certain sequences of the gene encoding ERα are removed, resulting in a different amino acid sequence and thus a slightly different protein.

Active reading

Another type of membrane estrogen receptor is the G protein-coupled estrogen receptor 1 (GPER1). This receptor is not related to the classical estrogen receptors ERα and ERβ. It is found in many cell types including those found in the breast, bone and brain. The localisation of the receptor inside cells is still an issue of debate with some research groups identifying the receptor in the plasma membrane, and others identifying the receptor inside cells (in the endoplasmic reticulum), with barely any GPER1 expressed in the membrane. However, GPER1 does partake in estrogen signalling, making some scientists believe that GPER1 is trafficked to the membrane only under certain conditions, e.g. when estrogen levels are low.

Resource Two
Data Source

Section C

Nongenomic signalling by membrane estrogen receptors

The observation of rapid cellular responses to estrogen led to the development of the hypothesis that estrogen could be acting via mechanisms that do not involve gene transcription in a process known as nongenomic signalling, shown in figure 6.

Upon ligand-activation of membrane estrogen receptors, a cascade of other molecules, proteins and cell signals is activated. These are all known as second messengers. Some of these second messengers, called kinases, can activate (or deactivate) other proteins by phosphorylating them (adding a phosphate group). For example, estrogenic activation of GPER1 can increase the expression of protein kinase A. Protein kinase A then phosphorylates a transcription factor, such as CREB1, which binds DNA to rapidly regulate gene transcription.

Nongenomic signalling is an indirect mechanism since the estrogen receptors don't partake in gene transcription themselves. It is a rapid process (giving effects within seconds to minutes), which might have particular importance in the brain and changing behaviours.

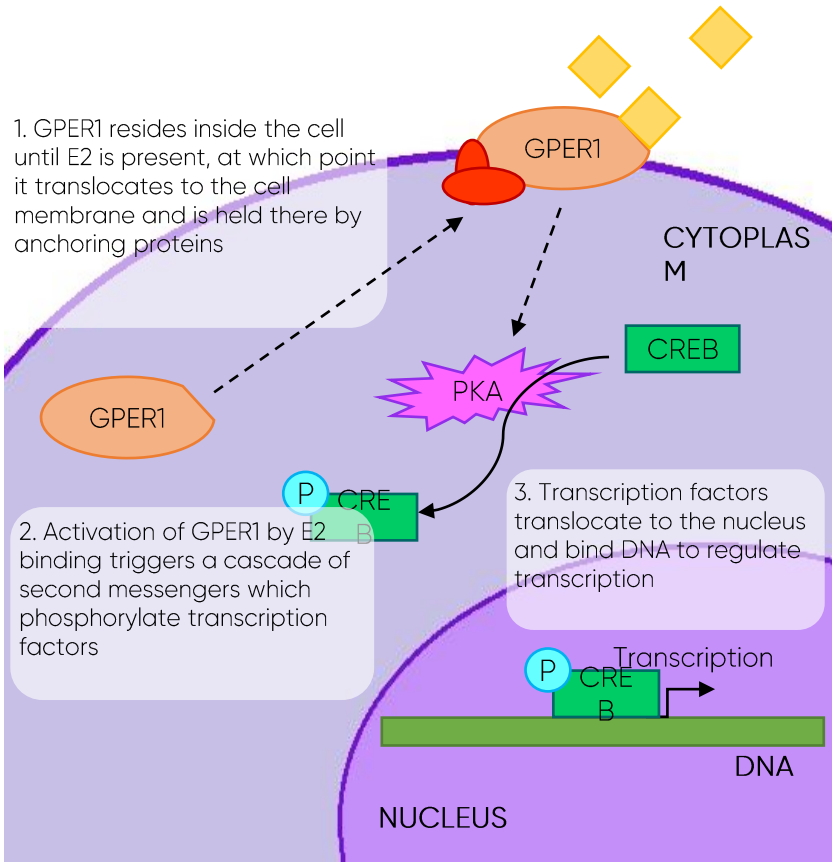
Resource Two
Data Source

Section C

Nongenomic signalling by membrane estrogen receptors

Figure 6

Nongenomic signalling mechanism



Resource Two
Data Source

Section D

Receptor agonists and antagonists

All receptors have specific agonists which stimulate the receptor, and antagonists which inhibit the receptor. Agonists and antagonists can be endogenous (found naturally in the organism) or synthetic (manufactured chemicals). Many drugs are synthetic agonists or antagonists. They can be used for treating diseases or they can be used in experiments to help us understand the function of receptors.

A summary of some of the main agonists and antagonists of the estrogen receptors is shown in the table below. All estrogen receptors can be stimulated by endogenous estrogens such as E2, so have not been included in the table. You will see that many synthetic compounds manufactured to target one receptor sometimes show weak, non-specific binding to other receptors (indicated by italics). This is due to similarities in the structure of compounds. Other compounds show clear agonistic activity towards one type of receptor with antagonistic activity towards others. These compounds are called selective estrogen receptor modulators (SERMs).

Resource Two
Data Source

Table 1

Agonists and antagonists of the estrogen receptors. All of these agonists and antagonists are either endogenous compounds conjugated to other molecules*, or synthetic compounds. *Italic* writing is used to highlight weak, non-specific binding activities.

Receptor	Receptor type	Agonists	Antagonists
ERα	Nuclear	Propylpyrazole triol (PPT), *G-15*	Tamoxifen; ICI-182,780 (commercial name Faslodex™)
ERβ	Nuclear	Diarylpropionitrile (DPN), *G-15*	Tamoxifen; ICI-182-780
ERα-36	Membrane isoform of nuclear ERα	E_2-BSA*; tamoxifen, *G-1, PPT*	
GPER1	Membrane	E_2-BSA*, G-1, tamoxifen, ICI-182,780	G-15, G-36

* E2-BSA is a conjugated form of E2. It is conjugated to bovine serum albumin (BSA) which cannot permeate the plasma membrane, preventing it from reaching nuclear estrogen receptors. Therefore, it can only target membrane estrogen receptors!

Resource Two Activities

Activities

1. Where in the cell would you expect to find ERα and ERβ? How does estrogen access them?

2. What is an estrogen response element, and what is required for it to initiate gene transcription?

3. Compare genomic and nongenomic estrogen signalling pathways, including names of the receptors involved and the timespan of the signalling mechanism.

4. Explain how we are able to see proteins using antibodies in immunohistochemistry.

5. If I wanted to conduct an experiment in which nuclear estrogen receptors are inhibited but membrane estrogen receptors aren't, which compound(s) could I use to agonise/antagonize the receptors?

Problem solving

Resource Two
Further Reading

Explore

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6533072/
Review article on the modes of estrogen signalling. Read section 6: Structural properties of estrogen receptors and section 11: Genomic and non-genomic signaling crosstalk.

https://www.creative-diagnostics.com/estrogen-signaling-pathway.htm
A shorter overview of the modes of estrogen signalling with coverage on how this is related to disease, specifically cancer.

https://bitesizebio.com/20929/getting-started-with-immunohistochemistry/
An in-depth, beginner-friendly introduction to immunohistochemistry.

Resource Three Overview

Topic	Neuronal signalling and the synaptocrine signalling hypothesis of estrogens
A-level Modules	Nervous coordination; Synaptic transmission; Neurotransmitters; Biopsychology
Objectives	By the end of this resource, you will be able to: ✓ Identify the voltage changes during an action potential and explain the biological processes underlying them. ✓ Understand and appreciate electrophysiological techniques for recording action potentials in neurones. ✓ Explain the synaptocrine signalling hypothesis and the contribution of estrogen to neuronal physiology.
Instructions	1. Read the data source 2. Complete the activities 3. Explore the further reading

Resource Three
Data Source

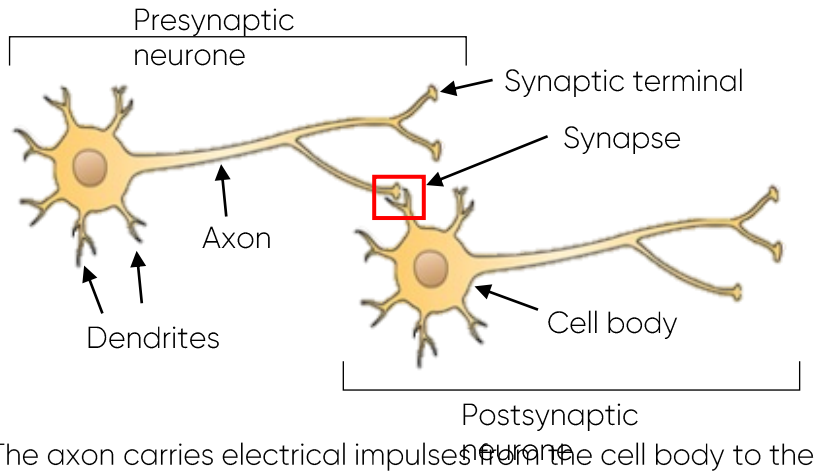
Section A

Estrogen in the nervous system

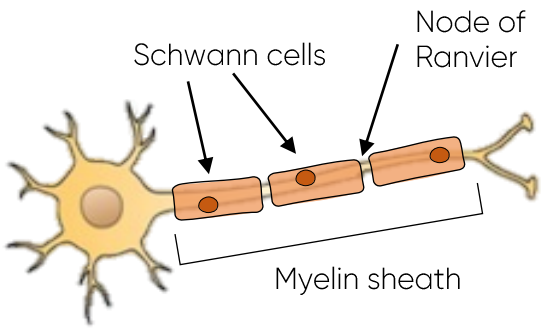
Neurones are nerve cells that are adapted to carry electrical impulses through the brain. The general anatomy of a neurone and its connections is shown in figure 7. Electrical impulses from one neurone are passed to another at the synapse.

Figure 7

General anatomy of a neurone and how it connects to other neurones.



The axon carries electrical impulses from the cell body to the synapse, triggering a response (usually another action potential) in the postsynaptic neurone. Some neurones have an insulating layer around the axon called a myelin sheath. The cells that make this sheath are called Schwann cells. Myelinated neurones carry electrical impulses much quicker than unmyelinated neurones because the impulse can jump between gaps in the cells, called nodes of Ranvier. A myelinated neurone is shown in figure 8.



Resource Three
Data Source

Section A

Estrogen in the nervous system

When a cell is at rest, the electrical charge across the membrane (resting membrane potential) is -70 millivolts (mV). Electrical impulses, or action potentials, result when there is a change in charge across the neurone membrane. During an action potential, sodium channels open to allow Na+ ions to flow inside the cell following their concentration gradient. This makes the inside of the cell less negative, and the membrane is said to be depolarised. The charge of the membrane must increase to the *threshold* potential of -55mV, otherwise the action potential does not go ahead. This is called the all-*or*-*nothing principle*. When the threshold potential has been exceeded, the membrane continues to *depolarise* up to +30mV – the peak of the action potential. Here, two things happen: 1. sodium channels close and are inactivated, preventing any more positive charge moving into the cell; 2. voltage-gated potassium channels open to *repolarise* the membrane by allowing K+ ions to flow out, making the cell more negative. Voltage-gated potassium channels are slow to close so the repolarisation phase overshoots, making the membrane even more negative than -70mV. This overshoot is called *hyperpolarisation*. This is important because it prevents the possibility of more action potentials being stimulated, which would overwhelm the neurone. A typical action potential is shown in figure 9.

Resource Three Data Source

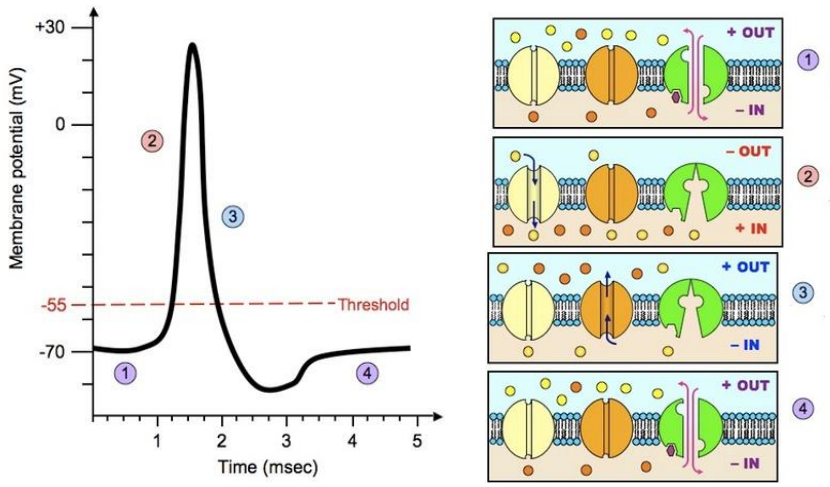


Figure 9

A typical action potential. 1. Resting membrane potential, maintained by the sodium-potassium pump (green). 2. Depolarisation, mediated by sodium channels opening (cream). 3. Repolarisation, mediated by potassium channels opening (orange) and sodium channels closing. There is a drop in membrane potential (hyperpolarisation) before returning to resting membrane potential in 4.

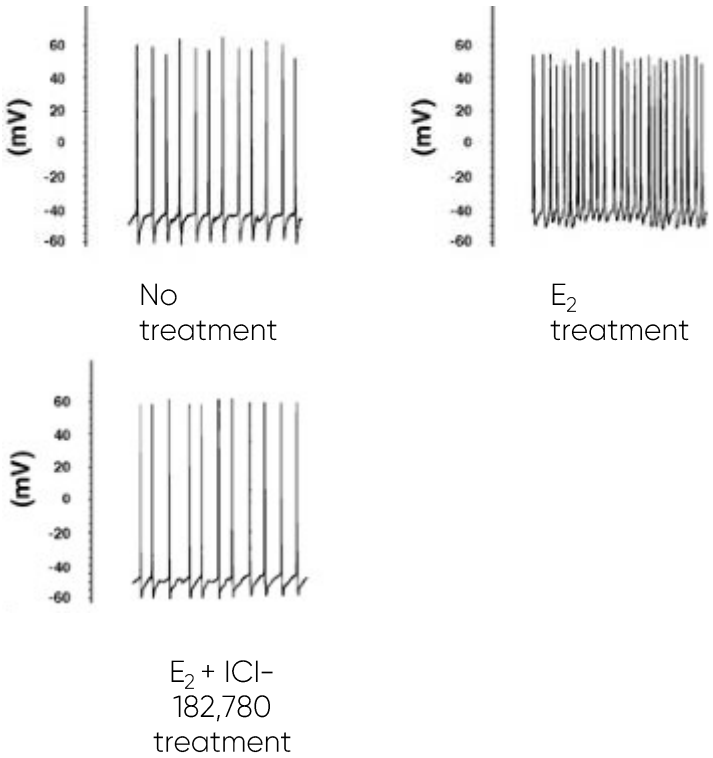
Estrogen is able to modulate the intrinsic electrical properties of neurones by increasing their excitability. This means action potentials can fire more frequently. Figure 10 shows recordings of neurone action potential in the absence of estrogen, with estrogen, and with the ERα/ERβ inhibitor ICI-182,780. In the presence of estrogen the number of action potentials – or in other words, the excitability – of the neurones is increased. Notice how this effect is reversed when the estrogen receptor antagonist is applied.

Resource Three Data Source

Figure 10

Action potentials of hypothalamic neurones in the absence or presence of E_2 and ICI-182,680.

Image taken from Fatehi and Fatehi-Hassanabad, 2007



Resource Three
Data Source

Section A

Estrogen in the nervous system

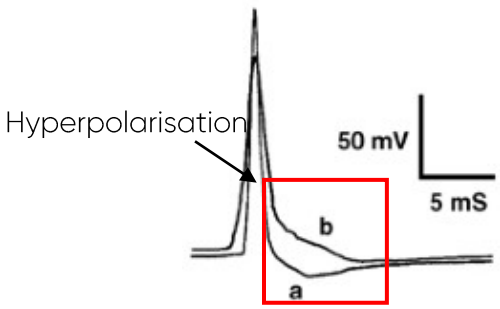
Figure 11

Superimposed action potentials of a hypothalamic neurone before (line a) and after (line b) E_2 treatment.

Image taken from Fatehi and Fatehi-Hassanabad, 2007.

We know that neurones can only fire so many action potentials within a given time because of hyperpolarisation. So how is estrogen managing to increase excitability in these neurones? Figure 11 shows a graph from the same scientific paper that shows the average action potentials of neurones with no treatment (line a) or estrogen treatment (line b). Notice how hyperpolarisation is almost completely absent when estrogen treatment is applied! This means that the ion channels will be ready for another action potential sooner than they normally would be.

Line a: action potential before E_2 treatment
Line b: action potential after E_2 treatment



Critical thinking

Resource Three
Data Source

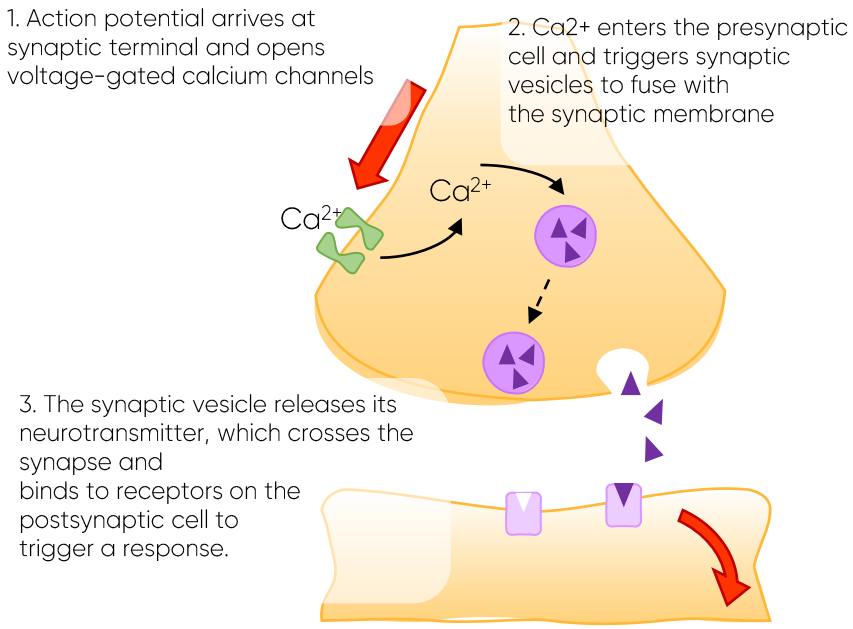
Section B

Estrogen as a neurotransmitter in "synaptocrine" signalling

When an action potential arrives at the synaptic terminal of the presynaptic neurone, voltage-gated calcium channels open and Ca2+ ions move into the presynaptic cell (i.e. the cell that the action potential has been generated in). These Ca2+ ions trigger the synaptic vesicles to fuse with the membrane of the presynaptic terminal and release their contents into the synapse. The contents of the vesicles, called neurotransmitters, cross the synapse and bind to receptors on the postsynaptic cell to trigger a response which could be hormone release, or another action potential! This is shown in **figure 12**.

Figure 12

Signal transmission across the synapse.



Resource Three
Data Source

Section B

Estrogen as a neurotransmitter in "synaptocrine" signalling

Neurotransmitters are typically small molecules of amino acids – not large, complex molecules like steroid hormones. However, there is evidence to suggest that estrogen can also act as a neurotransmitter! The synthetic estrogen enzyme *aromatase* has been found in synaptic terminals and may synthesise estrogen locally in response to electrical signals from action potentials. This novel function has been labelled as "*synaptocrine*" signalling ("-ocrine" coming from "endocrine" and "synapto" coming from "synapse"), and the hypothesis for this novel signalling mechanism was suggested by Saldanha, Remage-Healey and Schlinger in 2011.

Active reading

Neurotransmitters have a well-defined set of properties. They are: 1. synthesised and stored in neurones and 2. released into the synapse, where they 3. rapidly modulate neuronal excitability and action potential generation. Synaptocrine signalling differs from regular signalling by neurotransmitters because neurotransmitters can be stored. Estrogen, due to its lipophilic properties, cannot be stored and must be made when required. Therefore, the release of synaptocrine signals is not dependent on vesicles, but on the activity of aromatase.

The synaptocrine signalling hypothesis is shown in **figure 13**. It suggests that androgenic precursors from the circulation or adjacent neurones are converted to estrogen by aromatase, which is present in presynaptic terminals. These estrogens may then have an

Resource Three
Data Source

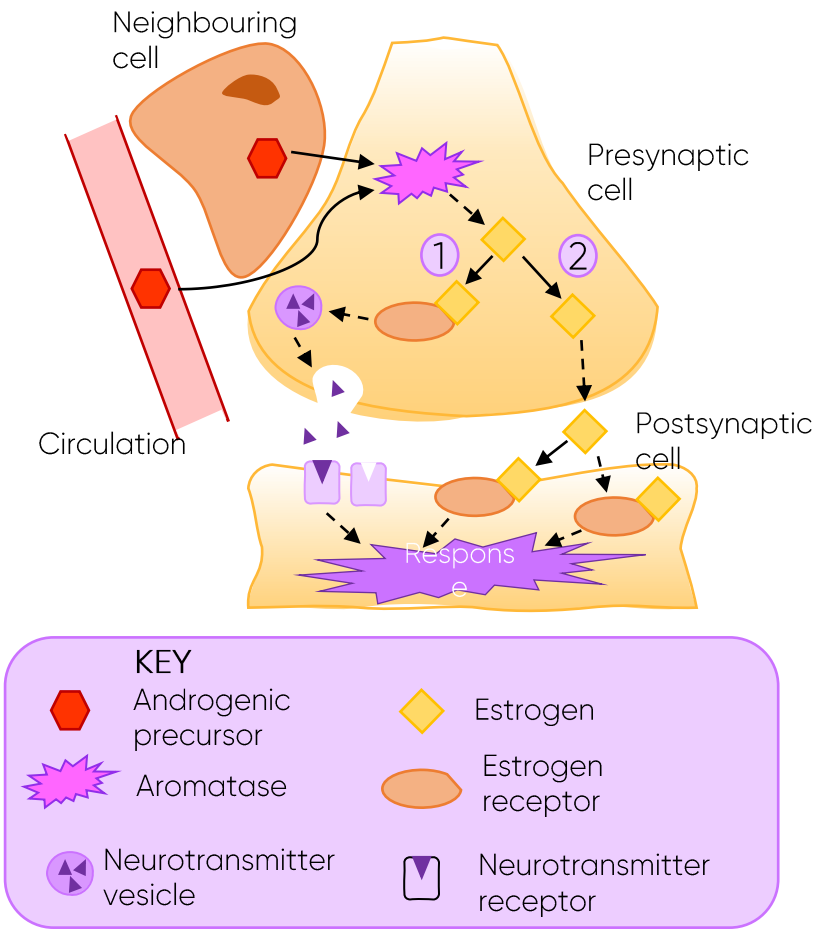
Section B

Estrogen as a neurotransmitter in "synaptocrine" signalling

autocrine action, binding to estrogen receptors within the presynaptic cell from which they were synthesised. This could then modulate neurotransmitter release - similar to how action potentials activate voltage-gated calcium channels which promote neurotransmitter release from vesicles. Alternatively, estrogen synthesised from the presynaptic cell could diffuse across the synapse like a neurotransmitter, binding to estrogen receptors on the postsynaptic neurone. This would then induce genomic/ nongenomic signalling to produce a response from the postsynaptic cell.

Figure 13

The synaptocrine signalling hypothesis.



Resource Three
Data Source

Section B

Estrogen as a neurotransmitter in "synaptocrine" signalling

Androgenic precursors from neighbouring cells or the circulation can enter the synaptic terminal of a neurone. Here they either: 1. bind estrogen receptors in the presynaptic cell to stimulate neurotransmitter release, or 2. diffuse out of the synaptic terminal and across the synapse to estrogen receptors on the postsynaptic membrane. Both possibilities trigger a response (action potential or hormone release) from the postsynaptic neurone.

Resource Three
Data Source

Section C

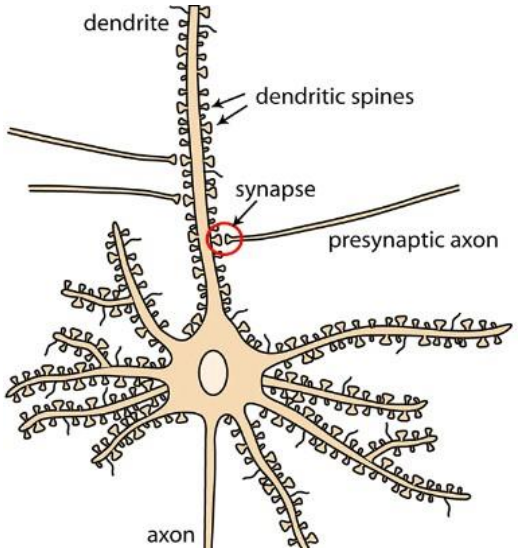
Estrogen modulates neuronal architecture

Many neuronal axons form excitatory synapses with dendritic spines. These are small protrusions from a neurone's dendrite that transmit electrical signals to the neurone's cell body. The dendrites of a single neurone can contain thousands of spines, but each one of those spines receives information from one axon only, as shown in **figure 14**.

Figure 14

Dendrites have many protrusions called dendritic spines. Each one of these spines forms a single synapse with one other axon.

Image taken from Smrt and Zhao, 2010.



Plasticity is the word given to describe the adaptability of spines that is rapidly modulated by sensory experiences, learning, and development. Plasticity involves changes in the shape and number of dendritic spines that either increases or decreases neuronal connections. The growth of new spines is called spinogenesis, although these spines take some time to mature before they stabilise and establish strong neural connections, as shown in figure 15. If new spines are not used, they are re-internalised. This really gives a biological basis to the phrase "use it or lose it"! For example, if you learn to play piano, you must continue to practice to strengthen the connections between spines and axons.

Resource Three
Data Source

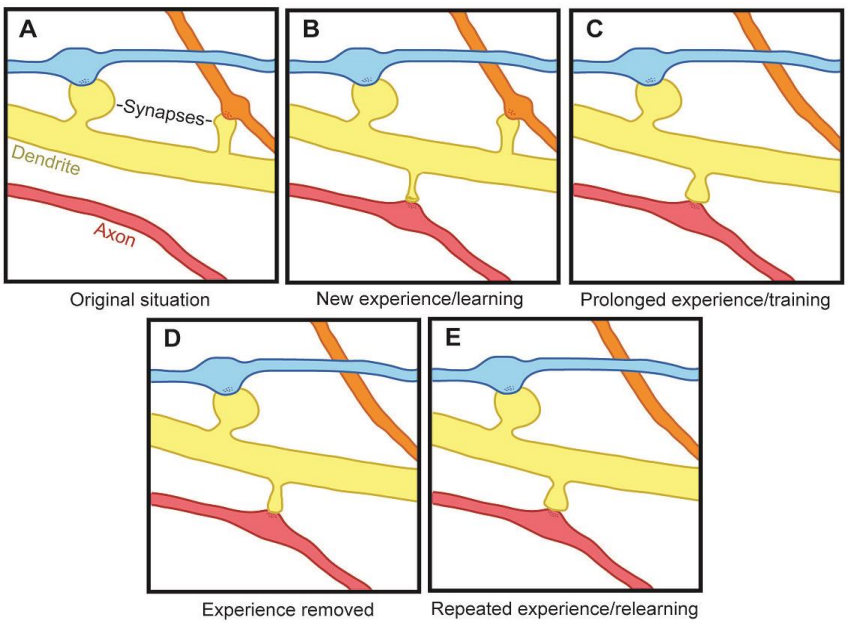
Section C

Estrogen modulates neuronal architecture

If you played for a year before giving up, you will forget how to play much quicker than someone who has been playing for 20 years. This is because the person who has played for 20 years has many more spines and neural connections, which will take longer to recede and re-internalise!

Figure 15

Spinogenesis and re-internalisation of spines depends on repeated experiences and practice which are eventually learnt.



Spinogenesis is often used as a measure for the consolidation of learning, but it also occurs during brain development and reproduction. For example, spinogenesis in the female rat hypothalamus establishes neural connections that can facilitate the expression of sexual behaviours, readying the animal for reproduction. Based on this, it's no surprise that spine plasticity can be modulated by estrogen. Figure 16 shows a representation of a female neurone during the 4-5 day estrous cycle.

Resource Three
Data Source

Section C

Estrogen modulates neuronal architecture

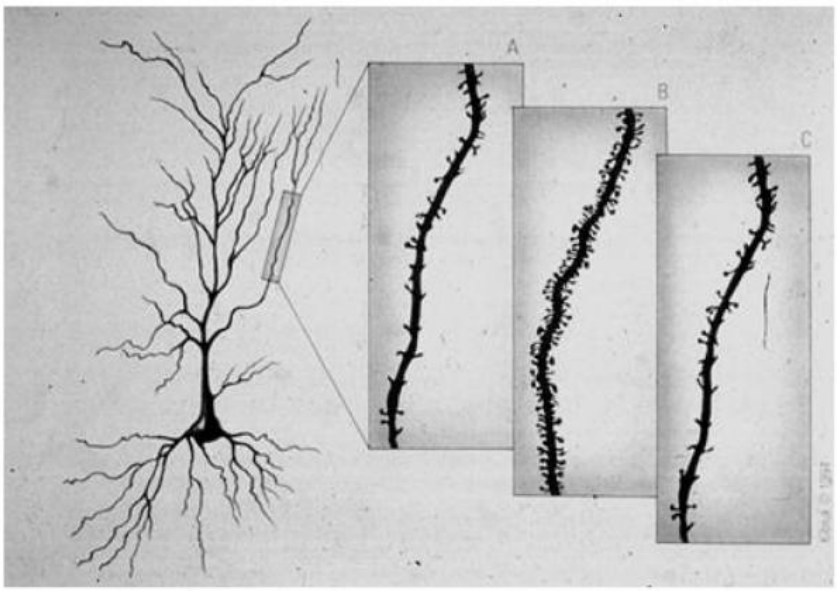


Figure 16

Representation of a female rat neurone and its dendritic spines during the 4-5 day estrous cycle. Panel A is during diestrus (E_2 levels gradually increase); panel B is during proestrus (E_2 levels continue to increase); panel C is during estrus (E_2 levels fall).

Image from McEwen and Schmeck, The Hostage Brain Rockefeller University Press, 1994. Drawing by Lidia Kibiuk.

Resource Three Activities

Activities

1. What are the membrane potentials you would expect to measure in a neurone at:
   resting membrane potential?
   threshold potential?
   peak of the action potential?
   hyperpolarisation?

2. Reorder these statements and fill in the blanks to describe an action potential:

a. When enough Na+ moves into the neurone, it surpasses its ___ ___ and the action potential goes ahead. This is the ___-___-___ principle.

b. Membrane potential restabilizes to resting potential.

c. Membrane potential continues to increase up to +30mV as more Na+ moves ___ the cell and the action potential spreads across the ___ of the neurone.

d. A change in membrane potential starts when sodium channels open and Na+ moves ___ the cell, following its concentration gradient. This is called ___.

Problem solving

e. Voltage-gated potassium channels are slow to close making the membrane even more negative than -70mV. This is ___.

f. At the peak of the action potential, sodium channels ___, preventing any more Na+ moving ___ the cell. At the same time, voltage-gated potassium channels ___ to repolarise the membrane by allowing K+ to flow ___ of the cell, following its concentration gradient.

Resource Three Activities

Activities

Problem solving

3. Based on the evidence in figure 10, which estrogen receptors would you say are responsible for altering the intrinsic excitability of neurones? (You may want to go back and look at table 1 to help with this!).

4. Explain the main differences between a typical neurotransmitter and a synaptocrine transmitter like estrogen.

5. How is the phrase 'use it or lose it' related to neurones and dendritic spines?

Resource Three
Further Reading

Explore

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3369574/
This is the original paper for the synaptocrine signalling hypothesis, put forward by Saldanha, Remage-Healey and Schlinger in 2011. It's a very lengthy read, but I would recommend looking at sections 1-4.

https://www.physiologyweb.com/lecture_notes/resting_membrane_potential/resting_membrane_potential_membrane_ionic_current_equations.html
For the more mathematically minded, this website gives a really nice explanation of the electrical properties of neurones using ionic current equations. This is also covered in most undergraduate biomedical science, physiology and neuroscience courses, so getting a grip of it now would be really beneficial if this is something you're thinking about studying!

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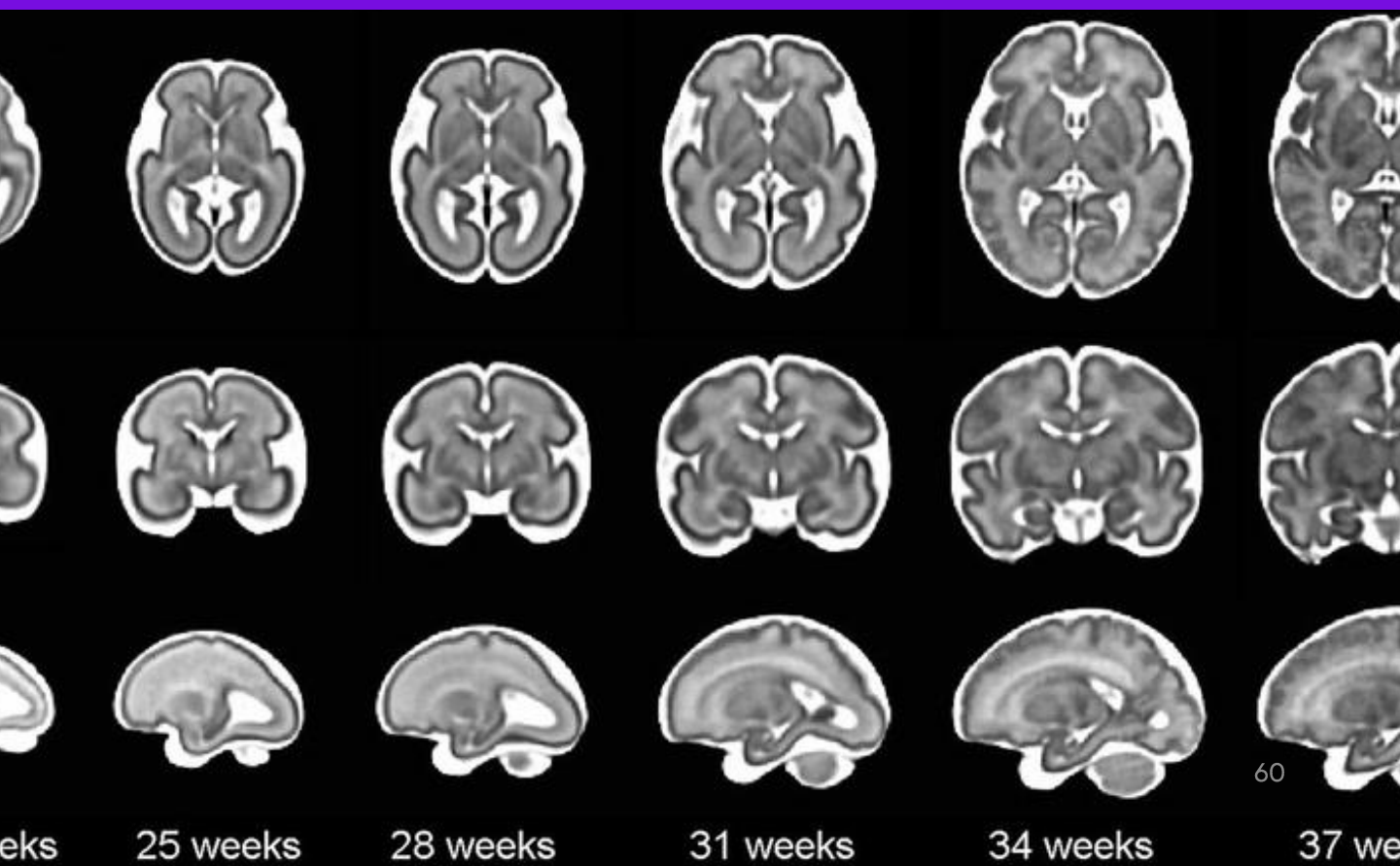
Resource Four Overview

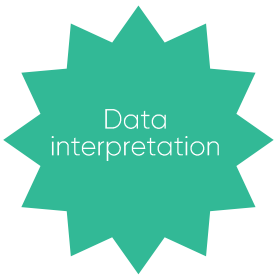
Topic	Estrogen and the sexually dimorphic brain
A-level Modules	Cognition and development; Biopsychology
Objectives	By the end of this resource, you will be able to: ✓ Understand and explain how sexual dimorphisms in the brain arise and how these dimorphisms are related to sex steroid hormones under the organisational-activational hypothesis. ✓ Critically analyse the evidence for and against estrogen in the development and function of the female brain. ✓ Begin to interpret data logically with critical thinking.
Instructions	1. Read the data source 2. Complete the activities 3. Explore the further reading



Resource Four
Data Source

Section A

Sexually dimorphic behaviour



A sexual *dimorphism* is any difference between two sexes of the same species that goes beyond differences in genitals and reproductive organs. Some examples of sexual dimorphisms are secondary sex characteristics such as hair distribution (e.g. male vs female lions), and colour/markings (e.g. male vs female ducks). Many behaviours are also sexually dimorphic and they depend on sexual dimorphisms in brain structure. Typical sexually dimorphic behaviours are: reproductive behaviours (males *mount* females; females display sexual receptivity by arching their backs – a behaviour known as *lordosis*), behaviours relating to care of offspring such as maternal behaviour and aggression, and anxiety and stress behaviours. Humans show a wider range of identifiable sexually dimorphic behaviours, such as play behaviours in children and use of language. However, human behaviours tend to be more fluid, existing more on a scale of sex typicalness.

Section B

Estrogen and sexually dimorphic behaviour

In 1959, a study by CH Phoenix administered testosterone to a pregnant guinea pig, then observed the behaviour of the offspring when they grew to adulthood. The group were interested in whether the offspring would behave like males or females due to the testosterone they received prenatally. The results were as follows:

Females treated prenatally with testosterone showed less *lordosis* (female behaviour) and more mounting (male behaviour) than control females in adulthood.

The behavioural effects of prenatal treatment with testosterone were permanent, being present throughout the animal's life.

It was later shown that it was not testosterone exerting these effects, but estrogen that had formed as a result of aromatisation! Therefore, testosterone is aromatised to estrogen, which "masculinises" the brain to produce male behaviours in adulthood.

Resource Four
Data Source

Section C

The organisational-activational hypothesis

The observation that *perinatal* exposure to estrogen permanently masculinises adult behaviours led to the proposal of the organisational-activational hypothesis.

The organisational-activational hypothesis states that exposure to steroid hormones during early development permanently **organises** neural circuits and brain structures. These circuits and structures are then **activated** by steroid hormones in adulthood.

During perinatal development, the testes begin to secrete testosterone. This travels in the bloodstream to the brain, where it is aromatised to estrogen. Estrogen then "organises" the structure of the brain, making it more "male". These alterations could involve inducing cell death (*apoptosis*), promoting cell survival (anti-apoptosis), or inducing spinogenesis. In contrast, the ovaries don't actively begin secreting estrogen until puberty. Therefore, the female brain is believed to develop as a result of a lack of estrogen.

In adulthood, sex steroid hormones function to "activate" the neural circuits that were organised perinatally. Removal of the gonads, and hence removal of the main source of sex steroid hormones, does not drastically alter brain structure, indicating that the organisation of the brain during perinatal development is permanent. However, some groups have identified that adult *gonadectomy* does induce some small changes in cell numbers within discrete areas of the hypothalamus. Thus, hormones may not only activate the neural circuitry, but also play a part in maintaining it. However, the "maintenance concept" is a matter of debate.

Resource Four
Data Source

Section C

The organisational-activational hypothesis

Table 2 summarises these developmental changes in the brain.

Table 2

Critical periods of development in males and females.

	Perinatal (includes prenatal and early postnatal)	Pubertal	Adulthood
	Testes become active and secrete testosterone just before birth. This is aromatised to estrogen which masculinises many neural structures permanently.	Testes become active again due to HPG axis activation. Estrogen acts to further masculinise some brain structures.	HPG axis is fully matured and testes are active. Secreted hormones activate neural circuitry.
	Ovaries are quiescent. Some discrete areas of the brain *may* make their own estrogen. For the most part, the perinatal female brain develops in the absence of estrogen.	Ovaries become active for the first time due to HPG axis activation. Here, estrogen may have a *feminising* effect, although how this happens is not understood.	HPG axis is fully matured and ovaries are cyclically active. Secreted hormones activate neural circuitry.

Resource Four
Data Source

Section D

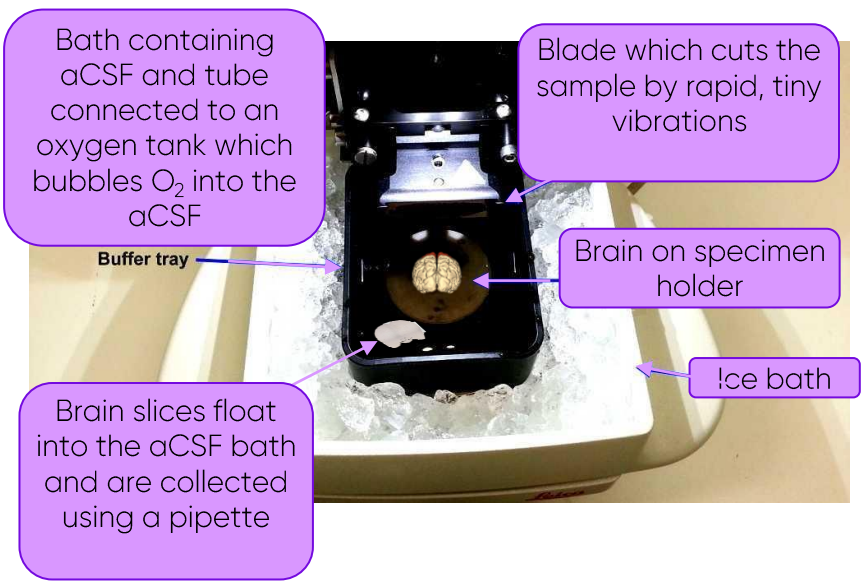
Measuring estrogen in the female mouse brain

Since other scientists have shown low levels of aromatase expression in the female brain, I have been trying to measure the amount of estrogen that is actually being produced using an enzyme-linked immunosorbent assay (ELISA). The steps I have taken to do this are:

1. I have dissected an adult female mouse brain and sliced it into 200μm thick slices using a vibratome, shown in figure 17. The brain slice is kept alive in artificial cerebrospinal fluid (aCSF) that has oxygen bubbling through it. The dissection process can be quite stressful for the brain cells, inducing the expression of apoptotic proteins and release of stress hormones that can interfere with my results. Therefore, after collecting the brain slices I let the brain "recover" in a tube of oxygenated aCSF.

Figure 17

Vibratome set up.



Resource Four
Data Source

Section D

Measuring estrogen in the female mouse brain

Research techniques

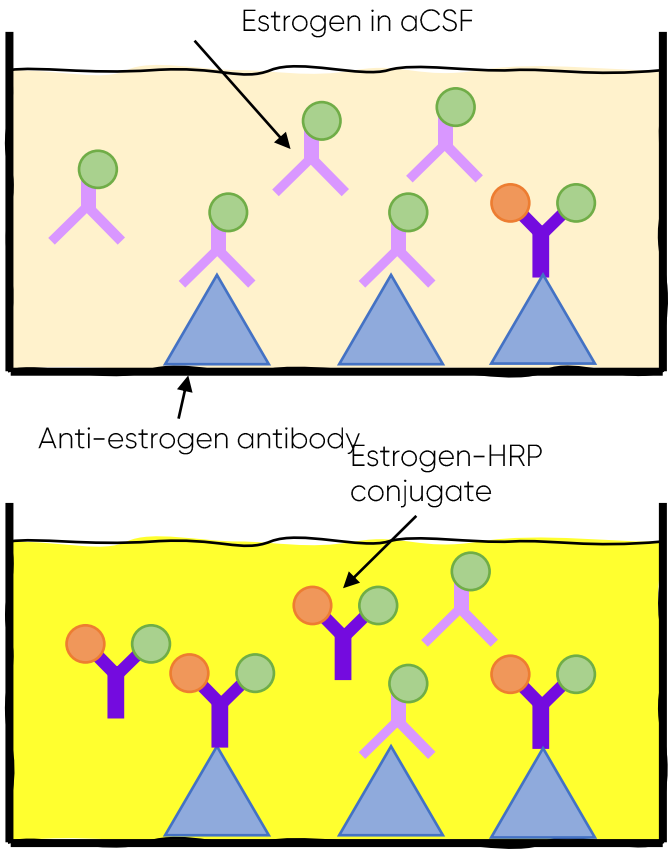
2. After the slice has recovered, I trap oxygen in the tube by bubbling oxygen into the aCSF and putting a lid on it. The oxygen will stay in solution to keep the brain slice alive. I then put the tube in an incubator and collect the aCSF at different timepoints. If the brain is making its own estrogen, the estrogen should be released from the brain slice into the surrounding aCSF in the tube!

3. At each timepoint, I collect the aCSF and use it in a competitive estrogen ELISA. For this, I coat a 96-well plate with an anti-estrogen antibody, which sticks to the surface of the wells. I then add my estrogen standards. These are samples with a known concentration of estrogen. I use these to generate a standard curve which I compare to my samples to measure their estrogen content. After the standards, I add my samples – the aCSF I collected from the tube – to each of the 96 wells. Then I add an estrogen-HRP conjugate. HRP is an enzyme, which is chemically linked to estrogen. This will compete with the estrogen in my sample in binding the anti-estrogen antibody. Finally, I add a substrate for the HRP. If the estrogen-HRP conjugate binds the anti-estrogen antibody coating the plate, it turns the substrate yellow. The more estrogen-HRP binding the anti-estrogen coating, the more yellow the sample. The more estrogen there is in my aCSF, the less estrogen-HRP binding, and so the clearer the sample. This is shown in **figure 18**.

Resource Four
Data Source

Figure 18

Competitive ELISA for estrogen. In the top well, the aCSF sample has a high amount of estrogen so it outcompetes estrogen-HRP in binding to the anti-estrogen antibody. Because less estrogen-HRP conjugate is bound, there is less of a colour change when the substrate is added. In the bottom well, the aCSF sample does not have much estrogen and is out-competed by estrogen-HRP. This means there is a greater degree of colour change when the substrate is added.



Resource Four
Data Source

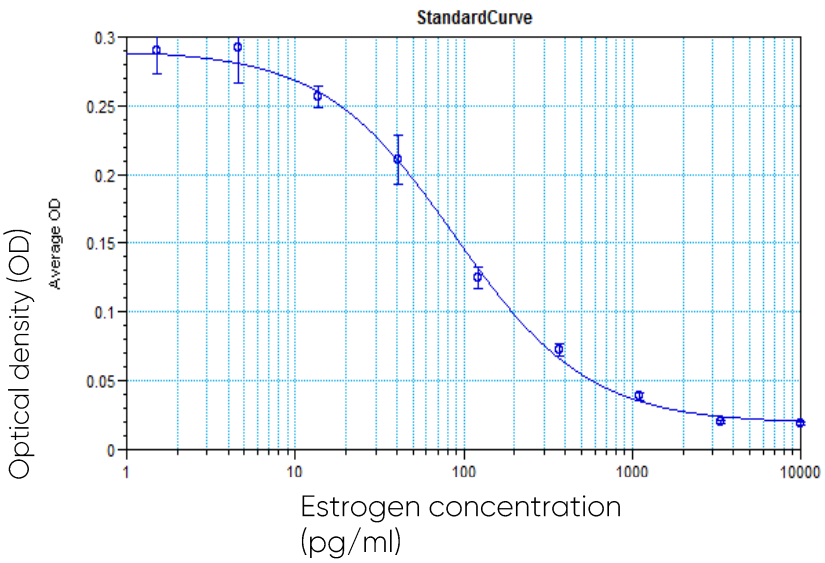
Section D

Measuring estrogen in the female mouse brain

4. After adding the samples and reagents, I put the 96-well plate in a spectrophotometer which measures the degree of colour change. Less estrogen = more yellow sample = high optical density. I then use the standard curve shown in **figure 19** to work out the amount of estrogen in my samples based on their optical density. I use a computer to do this, but you can do it manually! First, look at the optical density of the sample and find this on the y axis of the standard curve. Next, draw across from the y axis until you hit the standard curve, then draw down to the x axis to see the concentration.

Figure 19

A standard curve from one of my ELISA plates. The known concentrations of estrogen range between 0 and 10,000 pg/ml. The optical density is the arbitrary measure of colour change quantified by the spectrophotometer. Because the OD is arbitrary, you have to run a standard curve on every single ELISA plate you do!



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Resource Four
Data Source

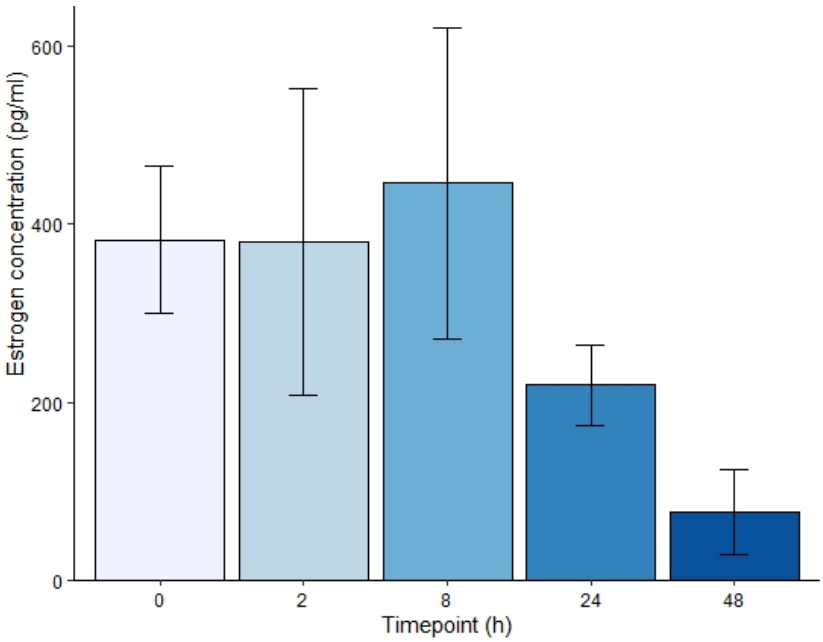
Section D

Measuring estrogen in the female mouse brain

5. Finally, I graph my results, shown in **figure 20.**

Figure 20

Some of my own results quantifying the amount of estrogen being made by the adult female mouse brain at different timepoints.



Research techniques

Resource Four Activities

Activities

1. Use your own words to define the organisational-activational hypothesis.

2. Which idea(s) and evidence justify the idea that the female brain develops in the absence of estrogen?

3. Can you make an argument against the hypothesis that the female brain develops in the absence of estrogen?

4. Based on what you know about estrogen from the ovaries being able to travel to the brain, what could you say about my ELISA results shown in figure 20?

Problem solving

5. Use the standard curve in figure 19 to estimate how much estrogen you would have in a sample with an optical density of 0.15.

6. Why do you think the estrogen concentration in my samples (shown in figure 20) increases at 8h but then begins to decrease over time?

Resource Four
Further Reading

Explore https://en.wikipedia.org/wiki/Neuroscience_of_sex_differences

Wikipedia might not be the preferred resource of your teachers or university lecturers, but it makes a great starting point! This article gives more examples of sex differences observed in the brain.

Resource Five Overview

Topic	Estrogen in sexually dimorphic reproductive behaviours
A-level Modules	Reproductive behaviour and partner preference; The role of hormones in sex behaviours; Research methods
Objectives	By the end of this resource, you will be able to: ✓ Critically analyse and interpret data from published scientific papers. ✓ Design an experiment to investigate the role of estrogen in reproductive behaviours. ✓ Understand and debate ethical issues relating to science and sexuality.
Instructions	1. Read the data source 2. Complete the activities 3. Explore the further reading

Resource Five
Data Source

Section A

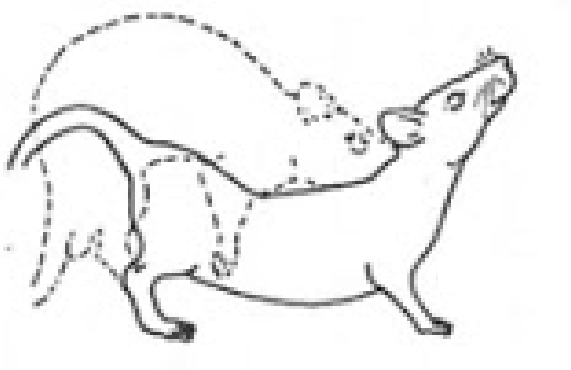
Female sexual behaviour: lordosis

Lordosis, shown in **figure 21**, is a typical female sexual behaviour observed in mammals that involves arching the back as a display of sexual receptivity to a male. Lordosis is a reflex action with a reflex arc that involves the hypothalamus. The expression of lordosis is hormonally regulated by the *HPG axis* in accordance with estrous stage and circulating estrogen, as shown in **figure 22**. As estrogen levels increase, the pulses of GnRH secretion from the hypothalamus accelerate, leading to the LH surge. The LH surge increases sexual receptivity.

Figure 21

Lordosis posture in a sexually receptive female rodent.

Image taken from Hardy and Debold, 1971.



Resource Five
Data Source

Section A

Female sexual behaviour: lordosis

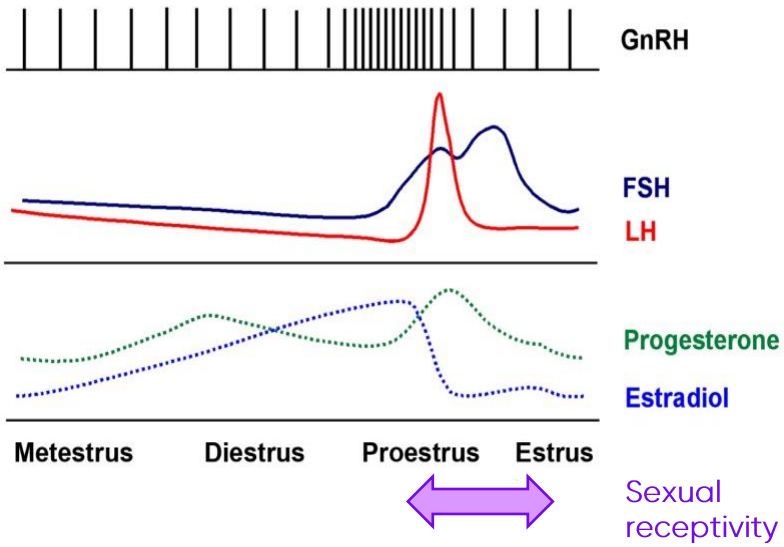


Figure 22

Rodent estrous cycle and period of sexual receptivity. In mice, the estrous cycle lasts 4-5 days, with each phase lasting only a matter of hours! Metestrus and diestrus are characterised by low but increasing levels of ovarian estrogens. Ovarian estrogen levels peak at proestrus and quickly decrease in estrus, allowing for ovulation.

Image taken from Miller and Takahashi, 2013.

To investigate the contribution of different steroid receptors and sex hormones to behaviour, many experimental female animals have their ovaries removed (a procedure called ovariectomy, abbreviated to OVX). This removes any confusion that might be caused by ovarian hormone release.

OVX females do not show typical lordosis behaviour as the major source of estrogen (which activates the neural circuitry underlying the behaviour) has been removed. However, when the experimental estrogen, estradiol benzoate (EB), is administered, OVX females are able to express lordosis.

Resource Five
Data Source

Section A

Female sexual behaviour: lordosis

Which receptor are estrogens acting through to facilitate this behaviour? The ERα agonist PPT facilitates lordosis behaviour, but the ERβ agonist DPN does not. What about GPER1?

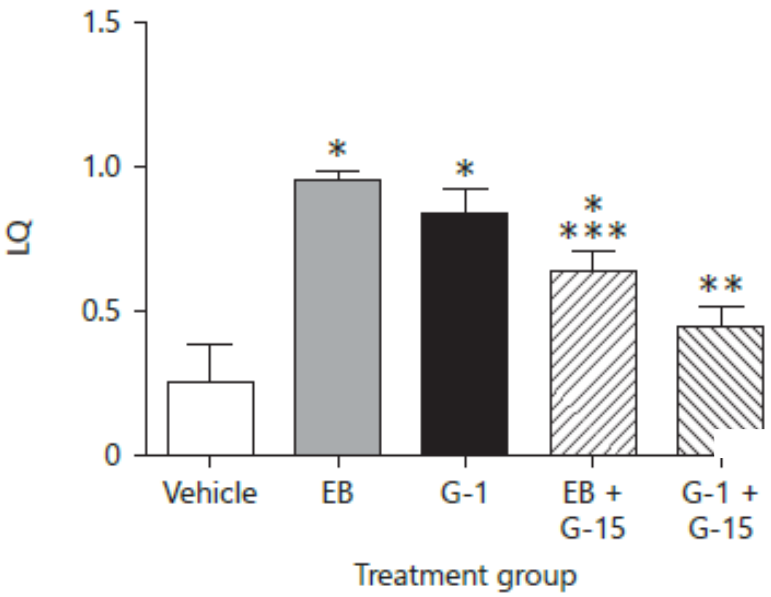
Data interpretation

Figure 23 shows a graph from a published scientific research paper where different agonists and antagonists have been administered to OVX female mice. They quantified the amount of times animals showed lordosis behaviour (termed as lordosis quotient, LQ) in response to males. We will interpret this data logically on the next page.

Figure 23

Lordosis quotient (LQ) scores in OVX female mice administered with a vehicle (control), the estrogen estradiol benzoate (EB), the GPER1 agonist G-1, the GPER1 antagonist G-15, or a combination of these. * indicates a significant difference compared to vehicle.

Graph taken from Anchan et al, 2014. Lordosis image taken from Hardy and DeBold, 1971.



Resource Five
Data Source

Section A

Female sexual behaviour: lordosis

Control animals are OVX and are treated with a *vehicle* (control injection), therefore none of their estrogen receptors are being stimulated. This demonstrates what we would expect to see if there was no estrogen at all, and we can compare all experimental groups to this one.

As expected, the data shows that estrogen facilitates the highest levels of lordosis. But we don't know how much of this behaviour is due to the contribution of GPER1.

Critical thinking

To understand the role of GPER1, the specific GPER1 agonist G-1 is administered. Here, we can see that GPER1 also facilitates lordosis, but not to the same extent as estrogen. This indicates that GPER1 is plays a role in female sex behaviour but isn't the only estrogen receptor involved.

Estrogen administered with G-15 creates a scenario where ERα is functional but GPER1 is inhibited. In this situation, lordosis behaviours are decreased but not absent.

G-1 administered with G-15 creates a scenario where GPER1 is first activated, then inhibited. No other estrogen receptor is active, since G-1 only stimulates GPER1. This allows us to isolate GPER1 from the other estrogen receptors and inhibit it. Here, we see the greatest reduction in lordosis behaviours, although these animals still express lordosis more than the controls.

Resource Five
Data Source

Section A

Female sexual behaviour: lordosis

Altogether, these data suggest that GPER1 contributes to lordosis, but other estrogen receptors are activated to facilitate maximum potential expression of sexual behaviours in female mice.

Aggression is a typical male sexual behaviour, for example the display of aggression between two males over a potential female mate. However, aggression can be circumstantial, so our understanding of the steroidogenic control of this behaviour is much more limited.

Data interpretation

In experimental animals, scientists can measure aggression in the resident-intruder test, shown in figure 24. Before the test, a male and female mouse are caged together for at least one week. During this time, the bedding of the cage is not cleaned to allow the resident male mouse to establish its own territory. One the day of the test, the female mouse is removed from the cage and an unfamiliar "intruder" male is placed into the cage instead. The behaviour of the resident male is recorded using a video camera for a test duration of 10 minutes. After completion of the test, the intruder is removed and the resident male is reunited with its female companion.

Resource Five
Data Source

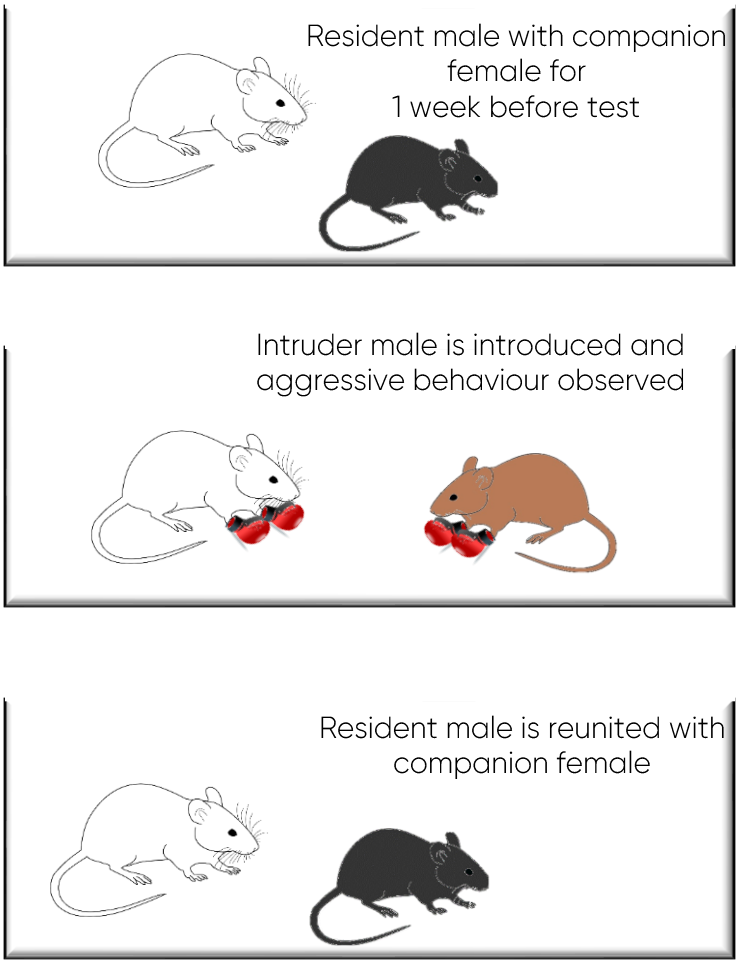
Section A

Female sexual behaviour: lordosis

Figure 24

Resident-intruder behavioural test for aggression in male mice.

Images are licensed under CC BY-SA and were further modified by J. L. Dovey.



Resource Five
Data Source

Section A

Female sexual behaviour: lordosis

Data interpretation

Which sex steroid hormones are contributing to male aggression? We commonly hear the term "roid rage" in relation to feelings of anger in anabolic steroid users. It is commonly believed that testosterone acting through androgen receptors causes this. However, aggressive behaviour appears to be the result of estrogen, aromatised from testosterone, acting through estrogen receptors. Evidence for this is shown in **figure 25**.

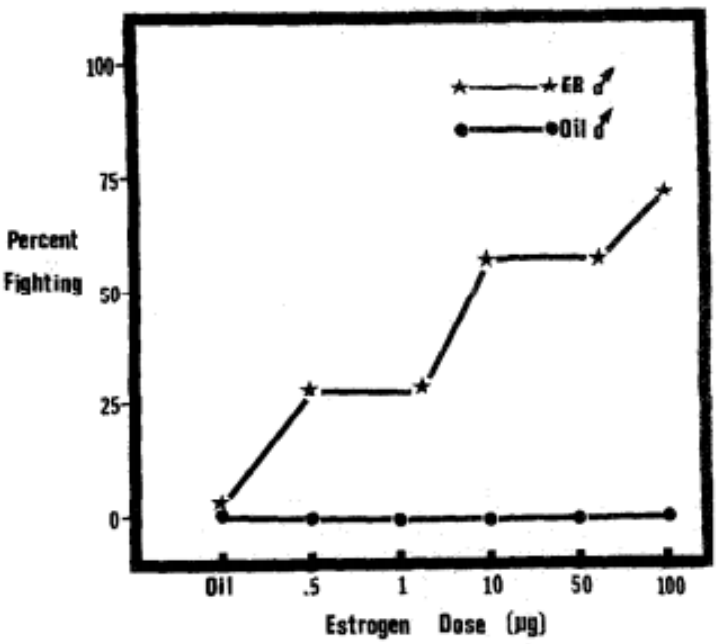


Figure 25

Effect of castration on aggressive behaviour in two groups of male mice treated with either oil (control) or the estrogen estradiol benzoate (EB). The number of mice showing aggression increases with estrogen concentration, highlighting a dose-response effect.

Image taken from Edwards and Burge, 1971.

Resource Five
Data Source

Section B

Male social behaviour: aggression

Genetically modified mice have been used to understand the effects of the estrogen receptors on male aggression. In these mice, the gene encoding the estrogen receptor(s) has been silenced, or "*knocked out*", so the receptors are not expressed. The advantage of this is that we can be sure of no non-specific actions of drugs. For example, PPT not only stimulates ERα, but can stimulate the membrane isoform ERα-36, too. The disadvantage of genetic *knockout* is that the receptors are knocked out from birth, meaning that some important functions of the receptor during critical developmental periods are missed. Thus, we can't be 100% sure that our observations are authentic and due to the receptor not being there at the time of investigation, or if they're due to the underlying effect of not expressing that receptor during neural organisation in development.

Data interpretation

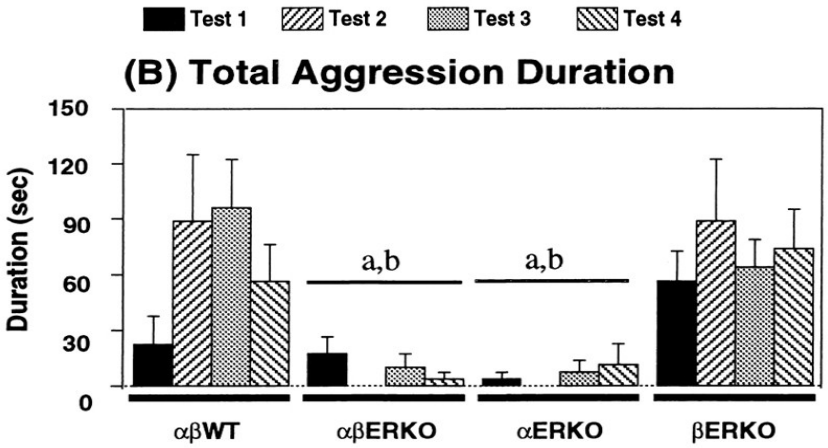
Figure 26 shows data from a published scientific research paper on the contributions of ERα and ERβ to male aggression using genetic knockout models. They have carried out the resident intruder test several times using the same resident and intruder mice. Generally, we would expect aggressive behaviour to increase over the first 2-3 tests and decrease with concurrent tests as the mice get used to each other and establish territorial boundaries. We will interpret this data logically on the next page.

Resource Five
Data Source

Figure 26

Duration of aggressive behaviours in genetically normal, or *wildtype* (WT) male mice, male mice with both ERα and ERβ genetically knocked out (αβERKO), and male mice with either ERα or ERβ knocked out (αERKO or βERKO).

Image taken from Ogawa et al, 2000.



a = significant difference compared to αβWT
b = significant difference compared to βERKO

In these experiments, *wildtype (WT)* mice are genetically normal and are used as the control group. Knockout of both receptors (αβERKO) severely reduces aggression compared to WT mice, giving us a strong indication that estrogen receptors are involved in aggressive behaviours in male mice.

Knockout of ERβ (βERKO) does not affect the duration of aggressive behaviours compared to WT control mice. However, aggression during the first test is much higher in βERKO mice than in WT control mice. The fact that genetic silencing of ERβ **increases** aggression suggests that ERβ normally **inhibits** aggressive behaviours in genetically normal mice.

Critical thinking

Resource Five
Data Source

Section B

Male social behaviour: aggression

Knockout of ERα (αERKO) severely reduces the duration of aggressive behaviours. Unlike other groups, αERKO mice tend to show an increase in aggressive behaviours over concurrent tests. This could be because ERβ is still expressed and may be compensating for the lack of behavioural output from ERα. Therefore, ERα **facilitates** aggression, because silencing of it results in **reduced** aggressive behaviour.

It's not uncommon for receptors to have opposite effects and it actually makes good sense. ERα and ERβ are typically expressed together in the brain as well as in other cell types and tissues and form heterodimers. The interaction between the receptors may provide an important regulatory action on ERα-mediated male aggression.

The contribution of GPER1 to male aggressive behaviours has not been investigated.

Resource Five
Data Source

Section C
Human reproductive behaviour: sexual orientation

Critical thinking

Since the organisational effects of sex steroid hormones can influence reproductive behaviour in animals, it has been suggested that differential exposure to these hormones during human development in the womb can alter sexual orientation.

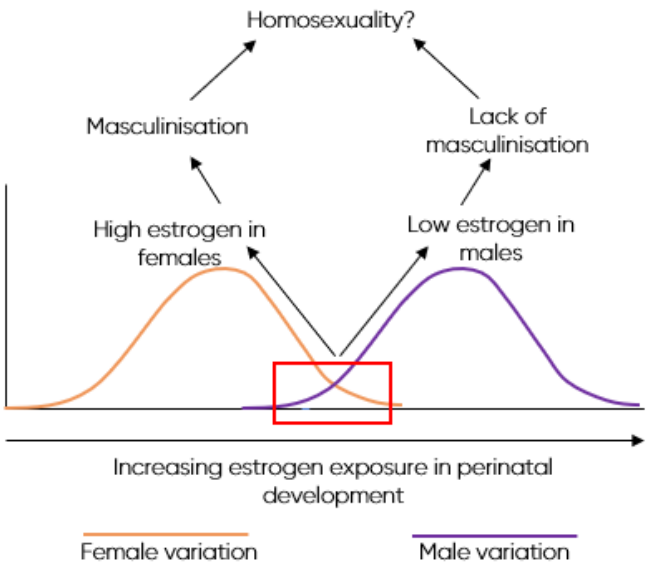
Male embryos are exposed to higher concentrations of testosterone (and through aromatisation, estrogen) than female embryos. This is because of the prenatal activity of the testes. Female embryos may also be exposed to estrogen because of the natural presence of estrogen in the womb and the mother's circulation. This estrogen does not normally pass to the foetal circulation, but if it did then it opens an opportunity for masculinisation of the female's brain.

The concentration of hormones that foetuses are exposed to can vary. This could mean that male embryos exposed to concentrations of estrogen at the lower end of the spectrum could express an interest towards other males in adulthood. Likewise, females exposed to estrogen concentrations at the higher end of the spectrum could express a sexual interest towards females in adulthood. This is depicted in figure 27.

Resource Five Data Source

Figure 27

Proposed hormonal mechanism for homosexuality. There is a great degree of variability in what is considered "normal" hormone exposure. The idea suggests that females exposed to high perinatal estrogens, and males exposed to low perinatal estrogens, undergo different organisational effects on the brain which can alter sexual behaviours and orientation.



Such a theory may well be impossible, since embryonic exposure to sex steroid hormones also determines genital structure. If the above theory were true, we would expect to see males with feminised genitalia and females with masculinised genitalia, which is clearly not the case in homosexual individuals.

In addition, organisational effects of sex steroid hormones are permanent, but sexuality is fluid and can change depending on a person's age and experiences.

Critical thinking

Investigating the biological basis of homosexuality is no doubt a socially difficult topic. On one hand, attributing homosexuality to biology could help to reduce stigma. On the other hand, finding a biological basis for homosexuality could give non-supporters a reason for finding an unneeded "cure".

Resource Five
Data Source

Activities

Problem solving

1. Describe how ovarian estrogen secretion fluctuates across the estrous cycle.

2. In **figure 23**, why do you think EB + G-15 treatment results in higher lordosis quotients (LQ) than G-1 + G-15 treatment?

3. What is the importance of removing the gonads (gonadectomy) of animals before treating them with estrogen receptor agonists/antagonists in behavioural tests?

4. Design an experiment to test the contribution of GPER1 to male aggressive behaviours.

5. Give your opinion on finding a biological basis for homosexuality. Is it something we should explore scientifically?

Resource Five
Further Reading

Explore

- https://en.wikipedia.org/wiki/Lordosis_behavior
  This article explains the reflex arc and neural circuitry that mediates the expression of lordosis.
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3826214/
  In this section, we have focused on male aggression as a sexually dimorphic behaviour. However, females also show sexually dimorphic aggression when they become mothers. You can read about maternal aggression in this review article.

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Resource Six Overview

Topic	Estrogen and mood
A-level Modules	Psychopathology and depression; Biological approach to drug therapy; Research methods
Objectives	By the end of this resource, you will be able to: ✓ Understand and explain the importance of investigating sexual dimorphisms in the brain. ✓ Design an experiment to investigate the role of estrogen in anxious behaviours. ✓ Recall the 3Rs and explain how they align with animal welfare and ethics.
Instructions	1. Read the data source 2. Complete the activities 3. Explore the further reading

Resource Six
Data Source

Section A

Importance of understanding sex differences in the brain

Why is it important for us to understand the development and function of sexual dimorphisms in the brain?

Many neuropathological and psychopathological conditions don't affect men and women equally. For example:

- Parkinson's disease, a neurodegenerative disease that affects movement. It is more commonly diagnosed in men.
- Autism spectrum disorder and attention deficit hyperactivity disorder (ADHD) are conditions that affect behaviour and social interactions. They are more commonly diagnosed in men.
- Major depressive disorder and generalised anxiety disorder are psychological conditions affecting mood and behaviour that are more commonly diagnosed in women*. Studies have shown that changes in estrogen levels after the menopause are responsible for feelings of depression and anxiety in older women.

Contextual learning

Studying sexual dimorphisms can help us understand the reason for unequal sex ratios in brain disease and disorder. Importantly, it could open avenues for us to explore personalised treatments and therapy options. This could improve a patient's life in many ways, from seeing faster treatment effects (many people spend years finding the right treatment for them), to having less side effects. Estrogen treatments and/or specifically targeting receptors may be a way we can achieve personalised medicine.

- *Many men can also experience depression, and perhaps one of the reasons we see a sexual dimorphism in depression diagnoses is because of the stigma surrounding men's mental health that stops many men reaching out for help. Depression in men may also be harder to diagnose because men are more likely to behave aggressively as a result of depression as opposed to behaving 'depressed'. Interestingly, men are much more likely to commit suicide than women, most likely because they go for so long without a diagnosis or appropriate treatment.*

Resource Six
Data Source

Section B

Behavioural paradigms for anxiety and depression

Psychological behaviours are a complex response to a stimulus which can take on many different modalities. When conducting behavioural experiments in laboratory rodents, the behaviours must be objectively measurable and bear some similarity to what we would observe in human disease and disorder. All tests must be conducted twice: the training procedure (to acclimate the rodent to a novel situation) and the test itself (the session where results are recorded).

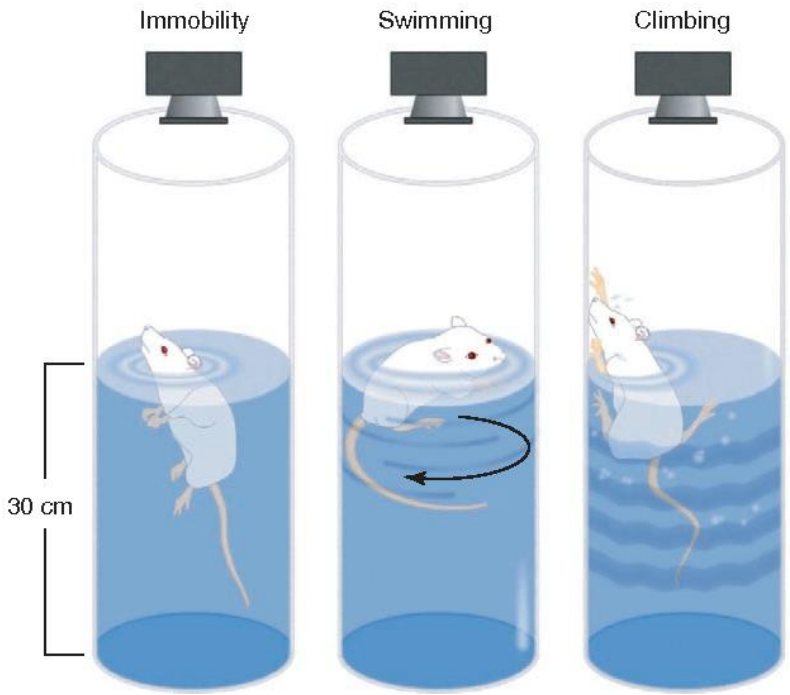
Forced swim test for depressive behaviours

The forced swim test, shown in **figure 28**, allows us to measure "behavioural despair". The rodent is dropped into a cylinder of water too deep for it to stand in. The assumption is that the rodent will make efforts to escape by swimming or trying to climb the side of the container before giving up and being immobile. The time an animal spends moving (swimming or climbing) and the time it spends immobile is recorded. Immobility, or behavioural despair, is the objective measurement for depression.

Figure 28

Forced swim test for assessing depressive behaviours in laboratory rodents. The time spent in each of the three states (immobility, swimming, climbing) in response to different drugs (e.g. antidepressants) is recorded. The test is 6–15 minutes long and animals are removed if they show any signs of potential drowning.

Image taken from Cryan et al, 2002.



Resource Six
Data Source

Section B

Behavioural paradigms for anxiety and depression

Elevated plus maze for anxious behaviours

The elevated plus maze is a plus-shaped (+) maze that is elevated off the ground. Two of the arms are "closed" with walls and two of the arms are "open" with no walls. As rodents are foragers in the wild, they have a natural instinct to explore novel areas. However, they are also prone to predation and have a tendency to avoid open, brightly lit areas. The elevated plus maze, shown in figure 29, measures anxiety by timing how long a rodent spends in the open arms of the maze as opposed to the closed arms. Reduced anxiety is indicated by a greater amount of time spent in the open arms of the maze as well as a greater number of head dips (looking over the edge of the maze).

Figure 29

Elevated plus maze for assessing anxious behaviours in laboratory rodents. The time spent in the open arms and the closed arms is recorded. The test is 5 minutes long.

Resource Six
Data Source

Section C

Estrogen receptors in anxiety

We can assess the contributions of each estrogen receptor to behaviour by using either specific agonists/antagonists or receptor knockout models.

In this study, ERα knockout and ERβ knockout male and female mice were generated and placed in the elevated plus maze. The results are shown in **figure 30**.

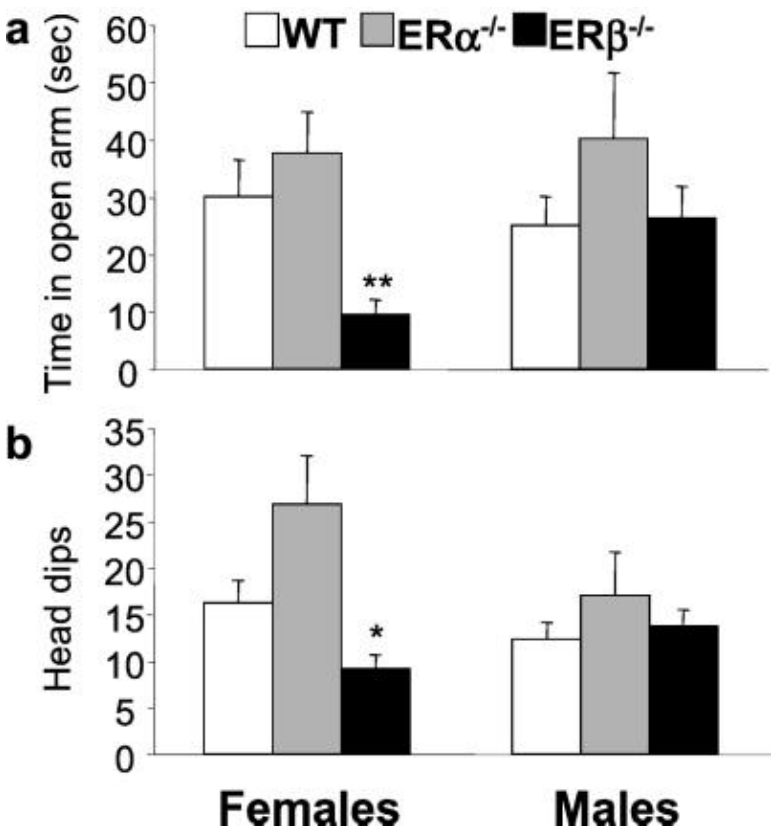


Figure 30

Effects of estrogen receptor knockout on anxiety in the elevated plus maze. The top panel shows the time spent in the open arms of the maze, where more time spent indicates less anxiety. The bottom panel shows the number of head dips where the mouse looked over the edge of the open arm. More head dips is indicative of less anxiety.

Image taken from Krezel et al, 2001.

Both male and female ERα knockout mice show a higher tendency to enter the open arms with more head dips. Thus, in the absence of ERα, the mice are less anxious.

Resource Six
Data Source

Section C

Estrogen receptors in anxiety

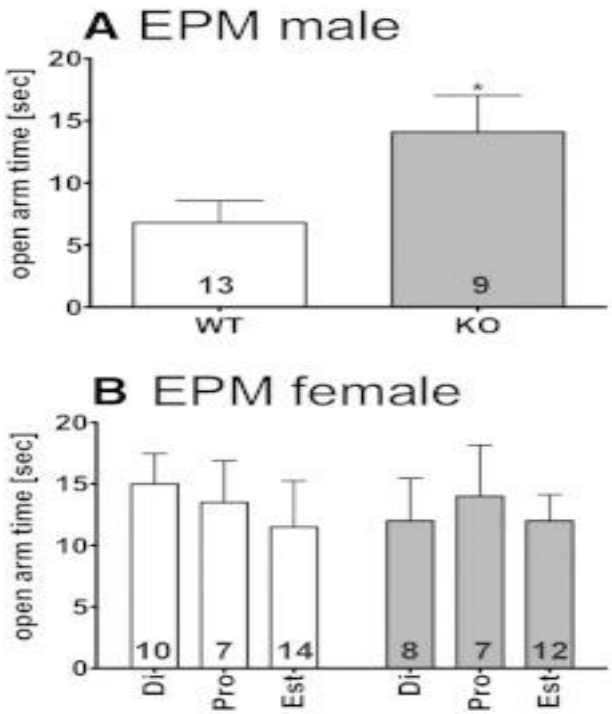
Data interpretation

In the absence of ERβ, mice are more anxious, showing less time in the open arms of the maze and less head dipping. However, this effect is only statistically significant in female mice. This highlights yet another sexual dimorphism – ERβ reduces anxiety to a greater extent in females than males! The reason for this is not clear and it still under investigation.

Opposite results have been shown with GPER1 knockout, as seen in **figure 31**. Male GPER1 knockout mice show a greater reduction in anxiety (measured as a greater amount of time spent in the open arms of the maze) than GPER1 knockout females. This suggests that GPER1 increases anxiety to a greater in extent in males than females. The reason for this sexual dimorphism is also unclear.

Figure 31

Effects of GPER1 knockout on anxiety in the elevated plus maze. *Image taken from Kastenberger and Schwarzer, 2014.*



Resource Six
Data Source

Section C

Estrogen receptors in anxiety

Top panel shows the time spent in the open arms of the elevated plus maze in male wildtype and GPER1 knockout mice. Bottom panel shows time spent in the open arms in wildtype and GPER1 knockout female mice at different points in their 4-5 day estrous cycle
(Di = diestrus, estrogen levels rising;
Pro = proestrus, estrogen levels peaking;
Est = estrus, estrogen levels low).

Section D

Ethics of using animals in research and the 3Rs

The use of animals in research is a tricky topic. No responsible scientist wants to use animals in research unless it is absolutely necessary and their use can be ethically and morally justified. Furthermore, pain and suffering can alter an animal's physiology and behaviour, leading to variation in experimental results that impairs the reliability of the study.

Any scientist working with animals has to be properly trained in how to handle, care for, and kill animals to minimise their suffering. There are also trained Animal Technicians who check on animals every day, make sure they are fed, and are trained to identify signs of physical and/or psychological illness. Then, experiments must be approved by an Animal Welfare and Ethical Review Body, who advise the scientific investigator on the regulations enforced by the Animals (Scientific Procedures) Act 1986 (ASPA). ASPA adopts the principles of the *3Rs*: Replacement, Reduction, and Refinement.

Active reading

Replacement refers to methods, technologies and approaches that directly replace the use of animals in experiments. These methods could involve the use of cell lines or mathematical models.

N.B. animals can also be **partially replaced**. Partial replacement involves the use of animals that, based on current scientific thinking, are not considered capable of experiencing suffering. These animals include, for example, *Drosophila* flies and embryonic forms of mammals, birds and reptiles that are earlier than the last third of their gestation.

Resource Six
Data Source

Section D

Ethics of using animals in research and the 3Rs

Reduction refers to limiting the numbers of animals that are used in animal experiments. Scientists must use the minimum number of animals possible (6-10 per group is a good number) whilst also ensuring the sample size is enough to make results robust, reproducible, and reliable.

Refinement refers to minimising the pain and suffering felt by experimental animals to improve their welfare. This applies not only to experimental procedures but also their housing and care.

More information on this topic can be found under the Further Reading section.

Active reading

Resource Six Activities

Activities

Problem solving

1. Can you assess the value of personalized treatments? What are the factors currently holding us back from achieving them?

2. Usually patients are treated and monitored over the course of weeks. How and why does this differ from the behavioural studies we do on animals?

3. How else could you analyse the contribution of ERα and ERβ to anxious behaviours, rather than using genetic knockout models?

4. Explain how you would set up an elevated plus maze test, and what behaviours you are looking for that may denote anxiety.

5. What is your opinion of the use of animals in research and the 3Rs? Can you justify your views?

Resource Six
Further Reading

Explore

- https://www.frontiersin.org/articles/10.3389/fendo.2020.595895/full
  This is a Mini Review article I wrote and had published this year. It's quite complex, but I recommend reading the sections 'Discussion: Therapeutic Potential for GPER1' and 'Future Perspectives' to get a better understanding of how we can use estrogen receptors in mood therapies.

For information of animal welfare in science:

- https://www.nc3rs.org.uk/the-3rs
- https://www.understandinganimalresearch.org.uk/
- https://www.gov.uk/guidance/guidance-on-the-operation-of-the-animals-scientific-procedures-act-1986

References in this resource

Cryan, J. F. et al. (2002). Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci.* 23: 238-245.

Kastenberger, I. and Schwarzer, C. (2014). GPER1 (GPR30) knockout mice display reduced anxiety and altered stress response in a sex and paradigm dependent manner. *Horm Behav.* 66: 628-636.

Krezel, W. et al. (2001). Increased anxiety and synaptic plasticity in estrogen receptor β-deficient mice. *Proc Natl Acad Sci USA.* 98: 12278-12282.

Final Reflection Activity

Deep Dive

BROUGHT TO YOU BY RBC ACCESS ED

Imagine you have conducted a forced swim test with GPER1 knockout male mice to test the effects of GPER1 on depressive symptoms in males. The test lasted a total of 15 minutes and the results have been split into the early part of the test (1st – 6th minute) and late part of the test (11th–15th minute). The results you obtained are shown below:

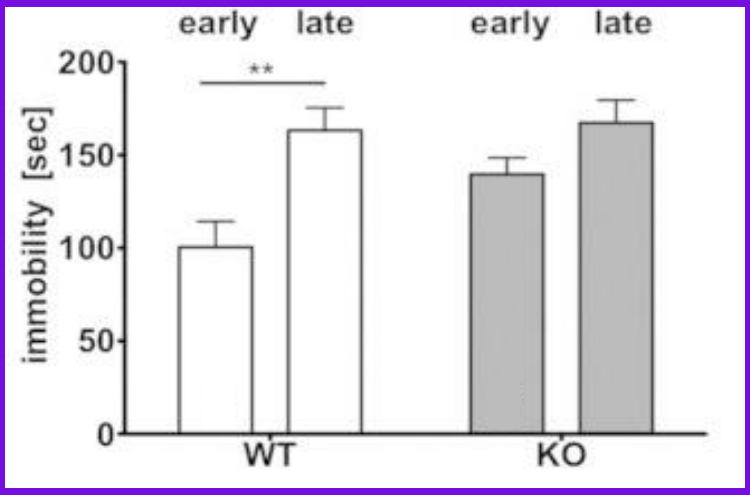


Image from Kastenberger and Schwarzer, 2014, modified by J L Dovey.

The results show a statistically significant difference in immobility time of wildtype (WT) mice between the early and late parts of the test, i.e. WT mice spent more time swimming in the early part of the test than they did in the late part.

Using the critical thinking skills you have developed throughout this coursebook, write a lab report based on these results. You may also include an additional behavioural experiment with the results you would expect to see. The lab report should contain the following sections:

- Introduction – a brief background to the topic and why you are doing this experiment.
- Methods – details of the number of animals used and how the experiment(s) were set up and conducted.
- Results – an explanation of the above results (state what you see, not what it means), and a hypothetical graph or representation of the results for any additional experiments.
- Discussion – explain what the results mean and a scientific justification for why you may have seen these results.
- Conclusion – contextualise your results – what do they mean? Based on the results above and your hypothetical results, is there a therapeutic potential for GPER1 in male depression?

Part 3 – Study Skills, Tips & Guidance

This section includes helpful tips to help you complete this pack, as well as improve your study skills for any courses you take next year.

It also includes a few fantastic easy-to-use resources to know what to do next if you are hoping to go to university in the next few years, like UCAS advice and web links to more academic opportunities.

In this section:

University Study Skills:
- ✓ Cornell Notes
- ✓ Key Instruction Words
- ✓ Academic Writing
- ✓ Referencing
- ✓ Evaluating Your Sources

University Guidance:
- ✓ What next?

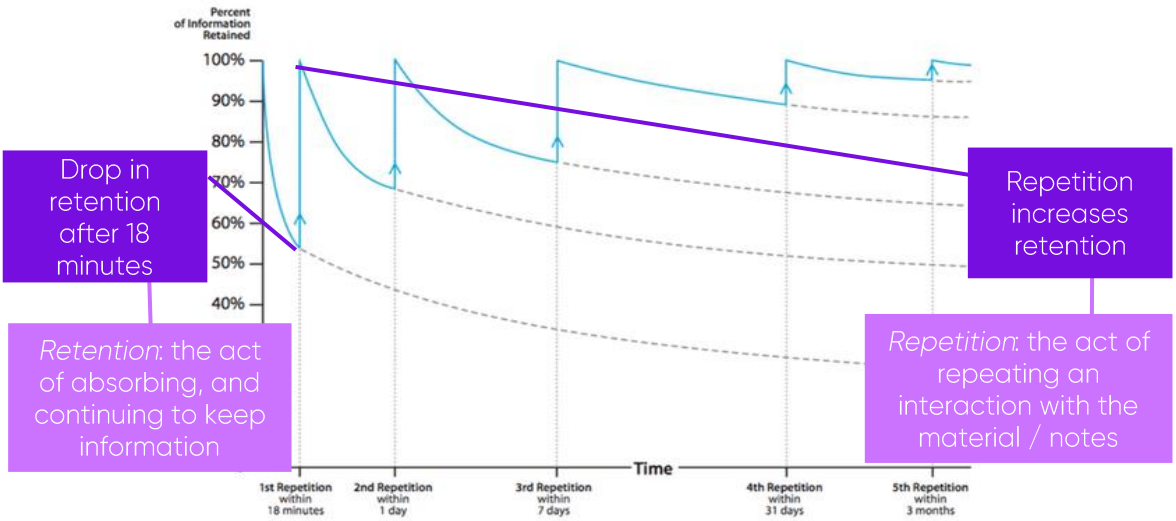
Subject Guidance:
- ✓ More on studying your subject

University Study Skills
Cornell Notes

Why is good note taking important?

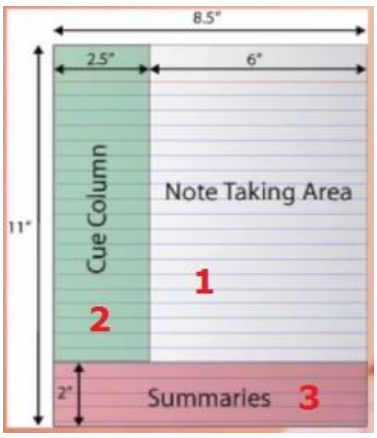
If it feels like you forget new information almost as quickly as you hear it, even if you write it down, that's because we tend to lose almost 40% of new information within the first 24 hours of first reading or hearing it.

If we take notes effectively, however, we can retain and retrieve almost 100% of the information we receive. Consider this graph on the rate of forgetting with study/repetition:



Learning a new system

The Cornell Note System was developed in the 1950s at the University of Cornell in the USA. The system includes interacting with your notes and is suitable for all subjects. There are three steps to the Cornell Note System.



Step 1: Note-Taking

1. Create Format: Notes are set up in the Cornell Way. This means creating 3 boxes like the ones on the left. You should put your name, date, and topic at the top of the page.

2. Write and Organise: You then take your notes in the 'note taking' area on the right side of the page. You should organise these notes by keeping a line or a space between 'chunks' /main ideas of information. You can also use bullet points for lists of information to help organise your notes.

University Study Skills
Cornell Notes

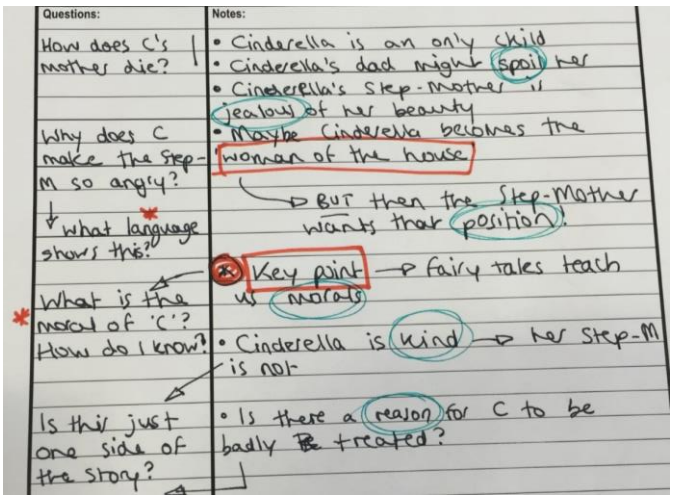
Step 2 Note-Making

1. Revise and Edit Notes: Go back to box 1, the note taking area and spend some time revising and editing. You can do this by: highlighting 'chunks' of information with a number or a colour; circling all key words in a different colour; highlighting main ideas; adding new information in another colour

2. Note Key Idea: Go to box 2 on the left hand side of the page and develop some questions about the main ideas in your notes. The questions should be 'high level'. This means they should encourage you to think deeper about the ideas. Example 'high level' questions would be:

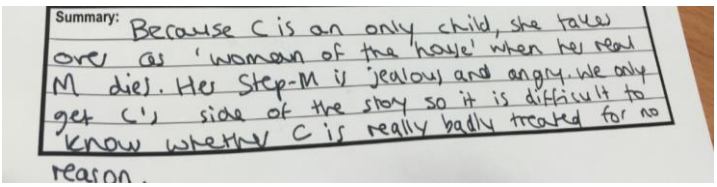
- Which is most important / significant reason for...
- To what extent...
- How does the (data / text / ideas) support the viewpoint?
- How do we know that...

Here is an example of step 1 and step 2 for notes on the story of Cinderella:



Step 3 Note-Interacting

1. Summary: Go to box 3 at the bottom of the page and summarise the main ideas in box 1 and answer the essential questions in box 2.



Give the Cornell Note Taking System a try and see if it works for you!

University Study Skills
Key Instruction Words

These words will often be used when university tutors set you essay questions- it is a good idea to carefully read instruction words before attempting to answer the question.

Analyse – When you analyse something you consider it carefully and in detail in order to understand and explain it. To analyse, identify the main parts or ideas of a subject and examine or interpret the connections between them.

Comment on – When you comment on a subject or the ideas in a subject, you say something that gives your opinion about it or an explanation for it.

Compare – To compare things means to point out the differences or similarities between them. A comparison essay would involve examining qualities/characteristics of a subject and emphasising the similarities and differences.

Contrast – When you contrast two subjects you show how they differ when compared with each other. A contrast essay should emphasise striking differences between two elements.

Compare and contrast – To write a compare and contrast essay you would examine the similarities and differences of two subjects.

Criticise – When you criticise you make judgments about a subject after thinking about it carefully and deeply. Express your judgement with respect to the correctness or merit of the factors under consideration. Give the results of your own analysis and discuss the limitations and contributions of the factors in question. Support your judgement with evidence.

Define – When you define something you show, describe, or state clearly what it is and what it is like, you can also say what its limits are. Do not include details but do include what distinguishes it from the other related things, sometimes by giving examples.

Describe – To describe in an essay requires you to give a detailed account of characteristics, properties or qualities of a subject.

Discuss – To discuss in an essay consider your subject from different points of view. Examine, analyse and present considerations for and against the problem or statement.

University Study Skills
Key Instruction Words

Con't

Evaluate – When you evaluate in an essay, decide on your subject's significance, value, or quality after carefully studying its good and bad features. Use authoritative (e.g. from established authors or theorists in the field) and, to some extent, personal appraisal of both contributions and limitations of the subject. Similar to **assess**.

Illustrate – If asked to illustrate in an essay, explain the points that you are making clearly by using examples, diagrams, statistics etc.

Interpret – In an essay that requires you to interpret, you should translate, solve, give examples, or comment upon the subject and evaluate it in terms of your judgement or reaction. Basically, give an explanation of what your subject means. Similar to **explain**.

Justify – When asked to justify a statement in an essay you should provide the reasons and grounds for the conclusions you draw from the statement. Present your evidence in a form that will convince your reader.

Outline – Outlining requires that you explain ideas, plans, or theories in a general way, without giving all the details. Organise and systematically describe the main points or general principles. Use essential supplementary material, but omit minor details.

Prove – When proving a statement, experiment or theory in an essay, you must confirm or verify it. You are expected to evaluate the material and present experimental evidence and/or logical argument.

Relate – To relate two things, you should state or claim the connection or link between them. Show the relationship by emphasising these connections and associations.

Review – When you review, critically examine, analyse and comment on the major points of a subject in an organised manner

University Study Skills
Academic Writing

What is academic writing?

'Academic writing' is a specific way of writing when communicating research or discussing an argument/point of view. It has a logical structure, and it uses formal language. There is a particular tone, 'voice' and style to the language. Unlike creative or narrative writing, academic writing will also use different sources of information to support what is being said.

The language of academic writing: do's and don'ts

- Do use words you know the meaning of and are confident using, it doesn't have to be complicated to be clear!
- Do not use contractions; don't, can't, doesn't, it'd. Do write out fully; do not, cannot, does not, it would.
- Do not use colloquialisms- this is 'writing as you speak'. Examples include misuse of the words 'literally' or 'basically', common phrases, such 'like chalk and cheese'.
- Do not use slang or jargon. For example, 'awks', 'lit', 'woke'.

Expressing your opinion in academic writing

In academic writing, it is best practice to express an opinion without writing in the first person, which can often be challenging. Always bear in mind that your work should read like a voice that is guided by the evidence and not basic personal intuition.

Therefore, rather than saying 'In my opinion, this proves that', you can express the outcome of your reasoning in other ways:

- 'This indicates that…';
- 'The aforementioned problems in Smith's argument reveal that…';
- 'Such weaknesses ultimately mean that…', and so on.

Signposting

Signposting guides your reader through different sections of your writing. It lets those who read your writing know what is being discussed and why, and when your piece is shifting from one part to another. This is crucial to for clear communication with your audience.

Signposting stems for a paragraph which expands upon a previous idea	Signposting stems for a paragraph which offers a contrasting view
Building on from the idea that … (mention previous idea), this section illustrates that … (introduce your new idea).	However, another angle on this debate suggests that … (introduce your contrasting idea)
To further understand the role of …(your topic or your previous idea) this section explores the idea that … (introduce your new idea)	In contrast to evidence which presents the view that … (mention your previous idea) an alternative perspective illustrates that …
Another line of thought on … (your topic or your previous idea) demonstrates that …	However, not all research shows that … (mention your previous idea). Some evidence agrees that …

University Study Skills
Referencing

What is a reference or referencing?

A reference is just a note in your assignment that tells your reader where particular ideas, information or opinions that you have used from another source has come from. It can be done through 'citations' or a 'bibliography'.

When you get to university, you will need to include references in the assignments that you write. As well as being academic good practice, referencing is very important, because it will help you to avoid plagiarism.

Plagiarism is when you take someone else's work or ideas and pass them off as your own. **Whether plagiarism is deliberate or accidental, the consequences can be severe.** You must be careful to reference your sources correctly.

Why should I reference?

Referencing is important in your work for the following reasons:

- It gives credit to the authors of any sources you have referred to or been influenced by.
- It supports the arguments you make in your assignments.
- It demonstrates the variety of sources you have used.
- It helps to prevent you losing marks, or failing, due to plagiarism.

When should I use a reference?

You should use a reference when you:

- Quote directly from another source.
- Summarise or rephrase another piece of work.
- Include a specific statistic or fact from a source.

University Study Skills
Referencing

Is it a source worth citing?

Question your sources before referencing using these tips:

Currency: the timelines of the information

- When was it published or posted? Has it been revised or updated? Does your topic require current information, or will older sources work as well?

Relevancy: the importance of the information for your needs

- Does the information relate to your topic or answer your question? Who is the intended audience? Have you looked at a variety of sources?

Authority: the source of the information

- Who is the author/publisher/source/sponsor? What are the author's credentials? Is the author qualified to write on the topic?

Accuracy: the reliability and correctness of the source

- Is the information supported by evidence? Has the information been reviewed or refereed? Can you verify whether it is a personal or professional source? Are there errors?

Purpose: the reason the information exists

- Does the author make the intensions/ purpose clear? Is the information fact opinion or propaganda? Are there are biases? Does the viewpoint appear objective?

University Study Skills
Referencing

How do I reference?

- There are a number of different ways of referencing, but most universities use what is called the Harvard Referencing Style. Speak with your tutor about which style they want you to use, because the most important thing is you remain consistent!

- The two main aspects of referencing you need to be aware of are:

1. In-text citations

- These are used when directly quoting a source. They are located in the body of the work, after you have referred to your source in your writing. They contain the surname of the author of the source and the year it was published in brackets.
    - E.g. *Daisy describes her hopes for her infant daughter, stating "I hope she'll be a fool—that's the best thing a girl can be in this world, a beautiful little fool."* ***(Fitzgerald, 2004)****.*

2. Bibliography

- This is a list of all the sources you have referenced in your assignment. In the bibliography, you list your references by the numbers you have used and include as much information as you have about the reference. The list below gives what should be included for different sources.

- Websites – Author (if possible), *title of the web page*, 'Available at:' website address, [Accessed: date you accessed it].
    - E.g. *'How did so many soldiers survive the trenches?'*, Available at: http://www.bbc.co.uk/guides/z3kgjxs#zg2dtfr [Accessed: 11 July 2019].

- Books – Author surname, author first initial, (year published), *title of book*, publisher
    - E.g. Dubner S. and Levitt, S., (2007) *Freakonomics: A Rogue Economist Explores the Hidden Side of Everything,* Penguin Books

- Articles – Author, *'title of the article'*, where the article comes from (newspaper, journal etc.), date of the article.
    - E.g. Maev Kennedy, *'The lights to go out across the UK to mark First World War's centenary'*, The Guardian Newspaper, 10 July 2014.

University Study Skills
Evaluating your sources

Knowing about the different types of sources and what makes them worth using is important for academic work.

When doing research you will come across a lot of information from different types of sources. How do you decide which source to use? From newspaper articles to books to tweets, this provides a brief description of each type of source, and breaks down the factors to consider when selecting a source.

A platform for millions of very short messages on a variety of topics.

Blogs (e.g. Tumbler) are an avenue for sharing both developed and unpublished ideas and interests with a niche community.

A collection of millions of educational, inspirational, eye-opening and entertaining videos.

A reporting and recording of cultural and political happenings that keeps the general public informed. Opinions and public commentaries can also be included.

A collection of analytics reports that outline the objectives, background, methods, results and limitations of new research written for and by scholars in a niche field.

The information presented is supported by clearly identified sources. Sometimes each chapter has a different author.

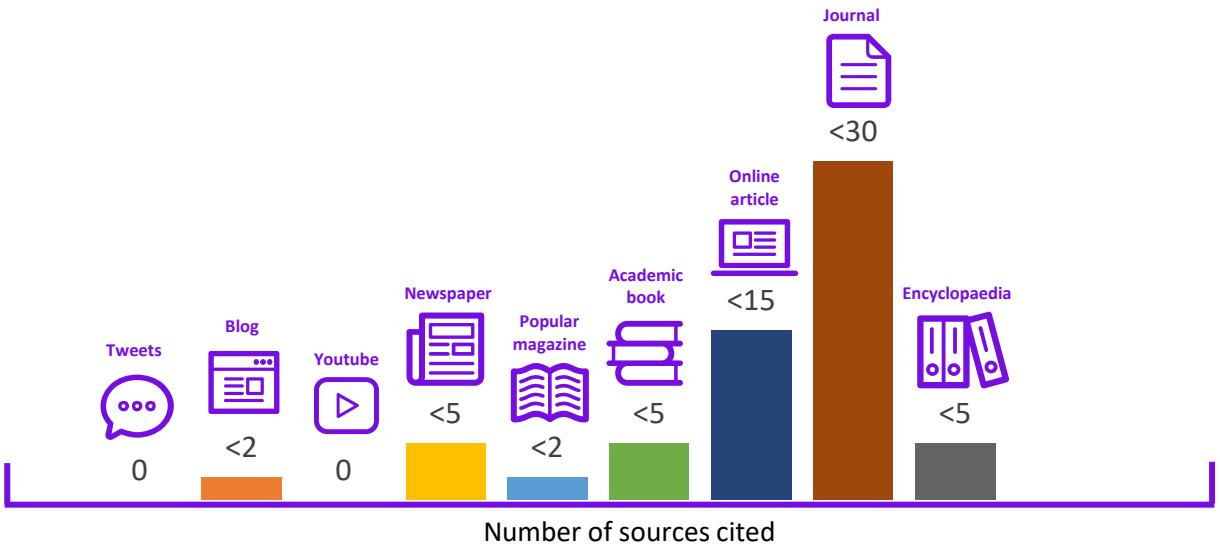
Books or online – giving information on many different subjects. Some are intended as an entry point into research, some provide detailed information and onwards references.

A glossy compilation of stories with unique themes intended for specific interests.

University Study Skills
Evaluating your sources

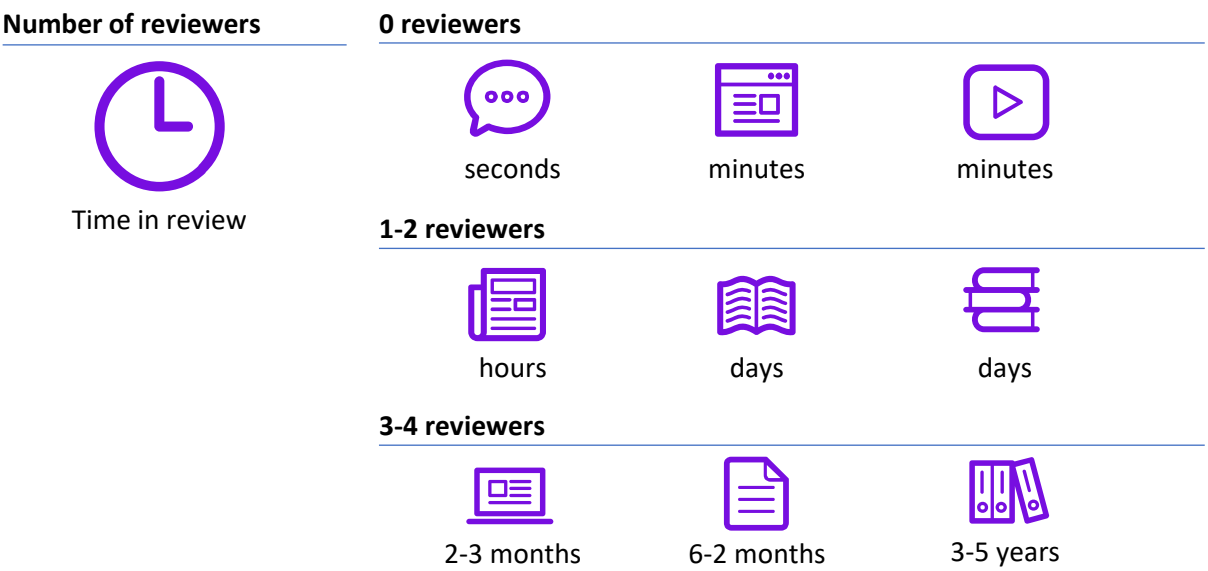
Number of outside sources

When an author used many outside sources into their writing, they demonstrate familiarity with ideas beyond their own. As more unique viewpoints are pulled into a source, it becomes more comprehensive and reliable. This shows the typical number of outside sources used in each publication.



Degree of review before a source is published

Two factors contribute to the amount of inspection that a source receives before it might be published: the number of reviewers fact-checking the written ideas, and the total time spent by reviewers as they fact-check. The more people involved in the review process and the longer the review process takes, the more credible the source is likely to be.



University Guidance
What next?

University Guidance

Different people go to university for different reasons. You might have a particular job in mind or just want to study a subject you are passionate about. Whatever your motivations, going to university can help improve your career prospects, as well as develop your confidence, independence and academic skills.

Choosing a course and university

Choosing the right course to study is an important decision so make sure you take time to research the different options available to you. Here are some top tips:

- ✓ You don't have to choose a course which you have already studied, there are lots of courses which don't require prior knowledge of the subject. You can apply skills gained from school studies to a new field.
- ✓ The same subject can be taught very differently depending on the course and university you choose. Take a look at university websites to find out more about the course content, teaching styles and assessment types.
- ✓ When choosing a university, think about what other factors are important to you. Do you want to study at a campus university or be based in a city centre? What accommodation options are there? Does the university have facilities for any extracurricular activities you're involved in?
- ✓ To research your options, have a look at university prospectuses and websites, as well as seeing if there are opportunities to speak to current students who can give you a real insight in to what life is like there.

Insight into: University of Reading

The author of this coursebook attends the University of Reading.

The University of Reading runs a large number of sessions to help find out more about the process of applying to university as well as taster sessions and Open Online Courses in a number of different subjects. To find out more, visit: www.reading.ac.uk/virtual-events.

Chat to current University of Reading students via Unibuddy and get their views on what university life is like!

University Guidance
What next?

Exploring Careers and Subject Options

- ✓ Find job descriptions, salaries and hours, routes into different careers, and more at https://www.startprofile.com/
- ✓ Research career and study choices, and see videos of those who have pursued various routes at http://www.careerpilot.org.uk/
- ✓ See videos about what it's like to work in different jobs and for different organisations at https://www.careersbox.co.uk/
- ✓ Find out what different degrees could lead to, how to choose the right course for you, and how to apply for courses and student finance at https://www.prospects.ac.uk/
- ✓ Explore job descriptions and career options, and contact careers advisers at https://nationalcareersservice.direct.gov.uk/
- ✓ Discover which subjects and qualifications (not just A levels) lead to different degrees, and what careers these degrees can lead to, at http://www.russellgroup.ac.uk/media/5457/informed-choices-2016.pdf

Comparing Universities

Use our platform ThinkUni.org to take a short quiz about your preferences and interests to find out which universities might be a great fit for you.

Other popular resources:

- ✓ https://www.ucas.com/
- ✓ https://www.whatuni.com/
- ✓ http://unistats.direct.gov.uk/
- ✓ https://www.thecompleteuniversityguide.co.uk/
- ✓ https://www.opendays.com/

University Guidance
What next?

UCAS and the university application process

All applications for UK degree programmes are made through UCAS. There is lots of information on the UCAS website to guide you through the process and what you need to do at each stage.

Apply

- Applications **open in September** the year before you plan to start university.
- You can apply for up to **five courses.**
- The deadline for most courses is **15 January**, though there is an earlier deadline of **15 October** for Oxford and Cambridge, medicine, veterinary medicine/science and dentistry.

Decisions

- Some courses may require an interview, portfolio or admissions test in addition to UCAS application. Check individual university websites details.
- Check UCAS Track which will be updated with decisions from the universities you have applied for and to see your deadline for replying to any offers.
- You should choose a firm (or first) choice university and an insurance choice. If you already have your exam results or a university thinks your application is particularly strong, you might receive an **unconditional offer**.

Results

- If you're holding a conditional offer then you will need to wait until you receive your exam results to have your place confirmed.
- Clearing & Adjustment allows you to apply to courses which still have vacancies if you didn't meet the conditions of your offer, have changed your mind about what or where you want to study, or have met and exceeded the conditions of your offer and would like to look at alternate options.

Personal statements

A really important part of your application is the personal statement. The personal statement gives you the opportunity to tell universities why they should offer you a place.

Here a few top tips for making your personal statement stand out:

- You can only submit one personal statement so it's important that you are consistent in your course choices. Make sure you have done your research to show your understanding of the subject area and passion for it.
- Start by brainstorming all your skills, experience and attributes. Once you have everything written down, you can begin to be selective – you only have 47 lines so won't be able to include everything.
- The ABC method: action, benefit and course can be a useful way to help demonstrate your relevant experience and how it applies to the course you're applying for.

University Guidance What next?

Personal Statement do's and don'ts

Read the tips below from real life professors and admissions staff in university Biology and Psychology departments, on the 'do's' and 'don'ts' of what to include in your personal statement:

Biology

- Tell us why you want to study Biology
- What area of Biology fascinates you? I.e. ecosystems
- Demonstrate your interest by telling us what you have recently read, watched or listened to and how they helped your understanding of Biology
- What activities or practical work have you completed which helped to develop your lab-based skills?
- Describe how your school or individual work has equipped you with the necessary knowledge and ability to be a successful Biology student.

Further useful resources

Be sure you know what you'll need to do to apply to university in the UK:

- ✓ Key dates and deadlines: www.access-ed.ngo/timelines-for-applying-to-university
- ✓ Get tutor advice on writing a UCAS personal statement at www.access-ed.ngo/writing-your-ucas-personal-statement
- ✓ An easy template to start practising your personal statement: https://www.ucas.com/sites/default/files/ucas-personal-statement-worksheet.pdf
- ✓ Untangle UCAS terminology at https://www.ucas.com/corporate/about-us/who-we-are/ucas-terms-explained
- ✓ Discover more about the application process including when to apply and how to fill in your application on the UCAS website.
- ✓ Read more useful advice about what to include in your personal statement on UCAS, the Complete University Guide and The Student Room.
- ✓ Attend one of our virtual sessions to find out more about applying and personal statements.

More on studying this subject

A Deeper Look Into Biology and Psychology

- ✓ **Read:** Sex differences, neural and behavioural functions affected by estrogens. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5286723/

- ✓ **Watch:** A must watch! The differences between the male and female brain and how research has contributed to our knowledge. https://www.youtube.com/watch?v=z5c7ubF0u-U
  The connection between estrogen and autism, with a nice description of how hormone exposure varies during gestation. https://www.youtube.com/watch?v=MQQwhnCVkXY

- ✓ **Listen**: A TED talk on how estrogen affects female physiology and the brain. https://www.youtube.com/watch?v=ryNjSP5VVI8

- ✓ **Do:** Have a look at the British Neuroscience Association and consider registering. They offer considerably reduced membership fees for 16+ students, which will give you the chance to attend conferences, meet other scientists, and access tailored career guidance. https://www.bna.org.uk/about/membership/undergraduate/

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Kemp House, 160 City Road
London, EC1V 2NX

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