**Waking Up Anna**

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**Preparation:**

As homework, prior to the case discussion in class, get acquainted to the case. The following resources are linked in this document as well as on the course Canvas module.

* Watch a video entitled [Waking Sleeping Beauty](https://www.youtube.com/watch?v=V9gnvWtta4M) to hear Anna’s personal account and how doctors at the Emory Sleep Center in Atlanta, GA found a treatment that finally helped her feel awake.
* Read the Emory news article, “[An antidote for hypersomnia](https://news.emory.edu/stories/2012/11/antidote_for_hypersomnia/)”, by Baker and Eastman, published on Nov. 21, 2012 describing this treatment.
* Read the abstract and introduction of the peer reviewed scientific article describing this work by [Rye *et al*., 2012](https://stm.sciencemag.org/content/4/161/161ra151).

These articles and video set the stage for the case. In order to understand how and why Anna woke up following the specific treatment given to her it will be helpful to learn a little about cellular communication in the brain, neurotransmitters, and their receptors (especially the ones involved in sleep). At the same time learning the vocabulary and key concepts of related to neurochemistry of sleep and sedatives will help in understanding the molecular basis for the cause and treatment of Anna’s sleep crises.

* Review these sub-sections and answer the questions below.

*A. Understanding sources*

Based on the video you watched and articles you have read about this case answer the following questions.

Q1 (2 pts). Describe in 1-2 sentences the main difference between the popular news report and the peer-reviewed scientific article. (*You can be more general about your answer regarding primary scientific literature and news sources*.)

Ans: The peer-reviewed articles are primary sources of information. However, since they have real data and report experimental evidences the language used in them may be full of technical jargon and understandable by those in the field.

The news report, video, and other articles in the popular press are written for non-specialists so only summarize the key findings without providing detailed technical descriptions and data.

Q2 (2 pts). For the three sources of information that you viewed/read for this case list one benefit and one drawback in the table below.

|  |  |  |
| --- | --- | --- |
| Source | Benefit | Drawback |
| Video | Real people and stories, so relatable and engaging | Personal opinions and may have bias |
| News report | Easy to read | Somewhat oversimplified |
| Journal article | Real data and evidences | Technical and complex |

Q3 (1 pt) . Define the prefixes Hypo- and Hyper- used in the description of this case in the peer-reviewed manuscript. Find at least one example where this meaning is used

Ans: The term “Hyper” refers to increased or more and “Hypo” refers to reduced or less. In the context of this case the words have been used in the peer-reviewed manuscript to describe increased sleeping (Hypersomnia) and reduced alertness (hypovigilance).

*B. Cellular Communication in the Brain*

Anna’s doctors guessed that there was something off balance between the excitation and inhibition systems in her brain, which might be causing her extreme sleepiness. To understand her condition, we must first understand normal cellular communication works in the brain and how it is inhibited in sleep.

**Neurons** are cells in the nervous system that transmit information to other nerve cells, muscle, or glands in the body to changes its physiology and elicit a response. Communication between neurons and other cells is carried out by small chemicals called **neurotransmitters**. The space between the 2 cells is called the **Synapse**. (see Figure 1). When a suitable electrical/chemical signal reaches end of the pre-synaptic cell it causes vesicles full of the neurotransmitter to be released into the synapse. Specific receptors on the surface of second (post-synaptic) cell binds it and sends a signal.



Figure 1: Neuronal synapse showing a presynaptic cell, post synaptic cell, neurotransmitters, synaptic vesicles, and receptors

Q4 (2 pts). Match the names listed in column A with the Label numbers of Fig 1 (listed in column B).

|  |  |
| --- | --- |
| **Column A** | **Column B** |
| Neurotransmitters | 4 |
| Synaptic vesicle | 2 |
| Presynaptic cell | 1 |
| Postsynaptic cell | 3 |
| Receptors | 5 |

* Review the vocabulary in Box 1 to learn more about signaling in the brain and answer the following questions

*Box 1: Vocab*

**Neurotransmitters** are small molecules derived from basic building blocks of life, e.g., amino acids. For example, gamma amino butyric acid (or GABA) is derived from the amino acid Glutamate by a decarboxylation reaction. They bind to proteins (such as receptors) and affect their functions. They are also referred to as ligands and may include naturally occurring molecules or man-made drugs.

**Receptors** are large transmembrane proteins that change shape when they bind to small molecules. Many clinically important drugs also bind to receptors in the brain to change our behavior, to treat diseases, and in special situations like inducing anesthesia for surgery.

There are many different combinations of neurotransmitters and receptors in the nervous system that add together to control the types of physiological and behavioral responses that we can see in ourselves. Two main types of neurotransmitter receptors are listed here:

1. **Metabotropic** receptors control metabolic pathways inside the cell, which can change the activity and expression of proteins.
2. **Ionotropic** receptors open pores, or channels, to allow specific ions to cross the cell membrane. These “ion channels” alter the electrical properties of the membrane to increase or decrease the likelihood that the cell will continue the chain of communication by firing an action potential.

Some neurotransmitters (e.g. glutamate) are excitatory (i.e., make it easier to fire action potentials while others (e.g., GABA) are inhibitory (i.e., make it harder to fire action potentials).

Q5 (1 pt). Review Figure 1 and examine what happens to the receptors when the neurotransmitter binds. Does it open or close? What can you say about the type of neurotransmitter receptor shown in figure 1?

Ans: Neurotransmitter binding opens the closed receptor channels. The receptor shown here is an ion channel.

There are additional 2-min videos on the Canvas site if you wish to review the concepts of membrane potential, action potential and the GABA A receptor.

*C. Neurochemistry of Sleep*

Normal brain function relies on a balance between inhibitory signaling mediated by the molecule GABA and excitatory signaling driven by Glutamate. Key players involved in inducing sleep are discussed here.

* Neurotransmitter: The chemical **GABA** (gamma-amino butyric acid) is one of the main **inhibitory** chemicals of the nervous system. It dampens/slows down brain activity and promotes sleep.
* Receptor: There are two main types of GABA receptors. GABA type A (GABA-A) receptors are ionotropic and GABA type B (GABA-B) receptors are metabotropic, but the overall response of both types of receptors is to inhibit the activity of the cell.
* Structure and Function of GABA-A receptors:
  + Structure: These receptors are pentameric, i.e., they are composed of 5 transmembrane protein subunits that together form a chloride ion-selective channel. Although there are many different isoforms of the protein, the most common type of GABA-A receptors found in the human brain are composed of 2 alpha (**α**), 2 beta (**β**), and 1 gamma (**γ**) subunit.
  + Function: Binding of neurotransmitters triggers ion channels to open. When GABA binds to the GABA-A receptor, the ion channel is open and chloride ions can cross the cell membrane. Other ligands, including clinically important drugs, *e.g.* benzodiazepines (a sedative), anesthetics and depressants can also bind to the GABA-A receptor to alter its function by opening or closing the Cl− channel. A simple cartoon of the GABA-A receptor is shown in Figure 2 along with the binding sites of various ligands.

A close up of text on a white background

Description automatically generated

Figure 2: GABA-A receptor, chloride (Cl−) ionophore complex. The cut-away view shows binding sites for a variety of compounds that influence the function of the receptor complex. (Adapted from Olsen, R.W. and DeLorey, T.M., (1996), GABA Receptor Physiology and Pharmacology, Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th edition.)

* Key vocabulary often used in describing the binding of drugs and small molecules to their target receptors is included in Box 2.

*Box 2: Vocab*

**Agonists**: These are drugs or molecules that turn on the function of a protein. The natural ligand usually acts as an agonist.

**Antagonists**: These are drugs or molecules that decrease or inhibit the function of a protein.

**Allosteric modulators**: The word root “allo” means “other”. These are drugs or molecules that bind at a location other than the natural ligand’s binding site. Modulators could be positive (increase activity of the protein) or negative (decrease its activity).

* Based on what you have learned here answer the following questions.

Q6 (1 pt). The GABA-A receptor is a transmembrane protein. Where would you expect a chemical signal (GABA, Benzodiazepine etc.) to bind this receptor? Circle ONE and explain your choice.

1. extracellular side
2. transmembrane domain
3. intracellular side
4. in the central channel

Q7 (1 pt). Explain why you circled the answer in Q6 above.

Ans: Explanation - Receptors receive signals from outside the cell, therefore you would expect them to bind neurotransmitters on the extracellular side of the protein.

Advanced students may also discuss the nature of the ligand - Most neurotransmitters are hydrophilic molecules (derived from amino acids, etc.). These molecules are impermeable to the membrane and therefore bind to the extracellular domains of cell surface receptors to transmit a signal within the cell.

Q8 (4 pt). Complete the following table describing the effect of ligand/ drug binding to the GABA-A receptor.

|  |  |  |  |
| --- | --- | --- | --- |
| # | Ligand/Drug bound | Effect on Chloride channel | Explain your answer |
| 1 | Agonist | Open | An agonist would directly activate the GABA-A receptor. It would open the chloride channel and slow down brain activity to induce sleepiness. As the natural ligand, GABA is an agonist for the GABA-A receptor. |
| 2 | Positive Allosteric Modulator | Open | A positive allosteric modulator (PAM) would increase the inhibitory effects of GABA. It would increase the likelihood that the chloride channel is open and slow down brain activity to induce sleepiness. Examples of GABA-A receptor PAMs include Anesthetic drugs, Alcohol, barbiturates, neurosteroids, and benzodiazepines – they all enhance the effects of GABA and lead to sedation/drowsiness/sleep. |
| 3 | Antagonist | Close | An antagonist would decrease or block the inhibitory effects of GABA, so it would decrease the likelihood of channel opening and the channel would remain closed. When the antagonist binds to the GABA binding site it competes with it for the same space, so this is called a competitive antagonist. |
| 4 | Antagonist as above + high concentration of GABA | Possibly open | Increased concentration will increase the likelihood that GABA is bound to the channel and the channel is open. GABA can “outcompete” the antagonist. |