Rationality in discovery

Bosch, Alexander Petrus Maria van den

Publication date:
2001

Chapter 8

Practice

8.1 Introduction

How are theory and experiments used in the practice of drug research on Parkinson's disease? Several techniques are being used to search for new drugs and explore the activity of the basal ganglia. In this chapter I report on how new drugs are investigated and how experiments are being used to explore and test new drugs and the mechanisms of the brain at the Pharmacy Department of the Groningen University.

For my case-study I interviewed researchers Dr. B. Westerink and Dr. W. Timmerman. In the following sections I will report on their views and experimental work. Section 8.2 presents an overview of my interview with Dr. B. Westerink. Sections 8.3 to 8.5 report my more extensive interviews with Dr. W. Timmerman which I partly conducted while witnessing her work in the laboratory.

The numbered paragraphs in these sections are translations of a selection of the verbatim responses to my questions, which were reviewed and approved by the interviewees. They aim to present an objective picture of the researchers' views on their work. Off course, all errors of translation and interpretation remain my responsibility. The next chapter of this thesis will present a detailed analysis of the practice that is portrayed in this chapter. The paragraphs are numbered for ease of reference.

8.2 Investigating new drugs

Dr. B. Westerink is a senior researcher, conducting his work at the Pharmacy Department of the Groningen University. The following text reports his views in response to my questions about drug discovery in the context of drug research for Parkinson in general, and more specifically at his department. The interview was conducted in December 1996.

1   Drug experiments can serve to investigate how and why a drug has a particular effect, whereas that effect is often discovered by accident. In 1960 the mechanism of neurotransmission became better understood. In 1965 it was discovered that already known kinds of compounds had a neurotransmitter function. Carlson discovered that in Parkinson's disease dopamine was deficient.

2   By an accidental observation it was discovered that chlorpromazine, while it was being administered for a different reason, improves schizophrenia. By focused experiments on rats it turned out that that this drug had an effect on the amount of dopamine. The hypothesis was proposed that chlorpromazine blocks the dopamine-receptor, which would cause the brain to compensate by increasing the synthesis of dopamine. This hypothesis is accepted today.

3   Often you see that an effect of a drug is discovered by accident, by a secondary observation. This will then initiate further research to understand the specific function of a drug. Later it was discovered that chlorpromazine causes parkinsonism as a side effect. This suggested a relation between DA and Parkinson's disease. This hypothesis [as discussed in the former chapter] is a result of further experimental investigations. This hypothesis pointed to rational strategies for therapy and novel drug design.

4   One direction that is explored in Groningen is the development of selective DA-agonists. These are chemical variants of the structure of dopamine. Those variants are experimentally tested in vivo (on live animals) and in vitro (on samples of tissue in a test tube) for their biological activity on a receptor. As a reaction to an agonist a receptor can make Cyclic-AMP. The concentration can be measured and compared with the concentration that is released after contact with dopamine.
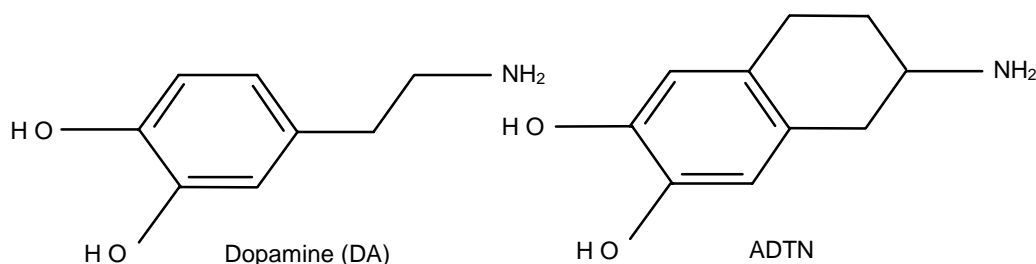
Figure 8.1: The chemical structure of dopamine and its variant ADTN

5   In 1977 a variant of DA was conceived by Prof. Horn (former professor at the Groningen University), called ADTN (Figure 8.1). This structure was the basis for further variants that were experimentally tested on four criteria:

1. The activity on the DA-receptor
2. Lipophilicity, the ability to cross the blood/brain barrier
3. Metabolism, its decomposition by enzymes
4. Selectivity, its affinity for D_1 and D_2 receptors

6   Suggestions for variants are based on experience and *fingerspitzengefühl.* It is hard to exactly predict what a receptor and enzymes will do with a compound. Yet the design of an antagonist is somewhat less difficult because it only has to obstruct a receptor, while an agonist has to fit and activate the receptor, like a key.

7   The NH_2-group of the best variant of ADTN was extended with two propyl-groups. This increased its lipophilicity so dramatically that it could even be administered by a band-aid on the skin. Removing a hydroxyl-group decreased its metabolism.

8   The experimental search also evolves the other way around. When a new receptor is discovered, its genetic expression can be used to clone it. These clones are then used by pharmaceutical companies to test all their created compounds for activity on that receptor. If one of the often more than 100.000 compounds is found to be active it can be the basis for a new drug lead.

9   Another strategy is using techniques from combinatorial chemistry to create thousands of variants of a compound at the same time and test them by rapid screening techniques. If activity is observed the compound that caused it is retrieved and will be studied to discover its structure.

10   A computational approach builds 3D models of receptors. These are used to aid the understanding of drug docking mechanisms [how a drug interacts with a receptor] by simulating and visualizing that process. Such simulations make predictions possible about how a protein folds and deforms.

11   If a new drug passes the criteria of the lab it will then be extensively tested. This is a process in three phases. The first phase tests for toxicity. It is administered to animals and later to volunteers. In the second phase the drug is given to volunteering patients to test its therapeutic strength. When it passes this barrier it goes into double blind testing and will be used in hospital trials. This is an expensive process, and yet the drug can still make victims, even when it passes all three stages. Genetically heterogeneous human beings are not the same as homogeneous mice. It is always possible that a slight genetic mutation will make a compound highly toxic for a particular group. Sometimes serious side-effects occur within isolated groups, *e.g.* in Finland or in Jewish families.

12   Newly created or discovered drugs are also used to explore biochemical mechanisms in the brain, both in normal as well as pathological conditions. This is another area of neuropharmacology. For Parkinson's disease the basal ganglia are of great interest.

8.3 Exploring the basal ganglia

In Groningen the nuclei called the basal ganglia were being studied by Dr. W. Timmerman and her students. The following text reports her responses to my questions about Parkinson's disease in general, and her experimental work on the basal ganglia in particular. I conducted these interviews in January and February 1997, and in September 1998.

In this section Dr. Timmerman talks about how the basal ganglia are involved in Parkinson's disease, how they are explored experimentally, and how knowledge about them can be used to treat Parkinson's disease. In Section 8.4 Dr. Timmerman talks about a specific experiment that was conducted during the interview. Section 8.5 reports her thoughts on interpreting data from experiments in general, and the conclusion of her experiments on the role of dopamine in the basal ganglia in particular.

13   The basal ganglia present the nuclei in the brain where the neural activity is abnormal in Parkinson's disease. When activity changes in the basal ganglia, all kinds of adjustments take place. Via the substantia nigra information is processed to other structures, to the thalamus, and then back to the pre-motor cortex. But we

do not know how, precisely. We also do not know exactly how information is processed from the basal ganglia to the periphery.

14   We know that the striatum processes information via a direct and indirect pathway to the SNR. From the nigra connections go further to the brain stem, and from there to the spinal cord. This can constitute a direct control of certain muscles. But there are also pathways going back via the thalamus to the cortex. So it is also possible that for example a change in activity of the corticospinal pathway is necessary for the deviation in behavior and motor control. I think it is a combined action. It is not just the basal ganglia and neocortex. The thalamus is involved, just like the cerebellum, which in turn also projects to the thalamus and the spinal cord. If the activity changes in the thalamus and the neocortex by input from the basal ganglia, then these changes can spread through the brain, making adjustments elsewhere.

15   Much is known about the anatomy of the basal ganglia. It is much more refined than is depicted in the model [see, Figures 3.1 and 7.3]. For example, it now seems that there are also dopaminergic neurons projecting to the Globus Pallidus (GP) [see Figure 8.2]. It also seems that the direct pathway has branches to the GP [see dotted lines in Figure 7.1].
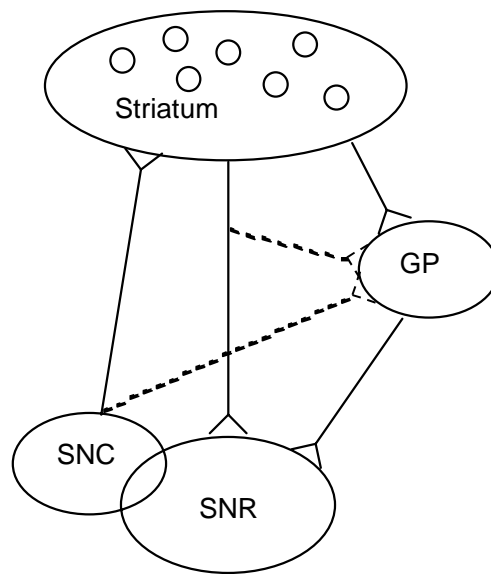
Figure 8.2: Schematic illustration of the basal ganglia by W. Timmerman

16   These pathways are discovered by means of tracing methods, *e.g.* by color coding and mRNA detection. Even so, it was discovered that the striatum is not a homogeneous structure. It is now known that it contains limbic patches in a matrix [see circles in Figure 8.2]. These areas specifically receive information from limbic areas in the cortex. From those patches specific information is processed onto the dopaminergic cells in the SNC. This is just the anatomy. These patches are chemically different, but not electrophysiologically.

17   In my study of the basal ganglia I specifically look at postsynaptic processes. I ignore anything that happens in the dopaminergic cell. I apply dopamine-agonists locally. In doing so I overrule the dopaminergic cell by activating post synaptic receptors, the receptors on *e.g.* the GABA-ergic and cholinergic cells. It is not my

problem how the endogenous dopamine is released by the cell, or how it originates from L-dopa or is broken down by MAO-B. Other people look at those specific processes. That is a research preference. Of course in the end it all has to fit together.

18   Anatomically you can look at one cell, one synapse, and you can identify projections and pathways. But for function you can learn from behavioral studies. These are often used as a measure for activity in the striatum. By local infusion of GABA-like and dopaminergic compounds in a certain part of the basal ganglia you can induce prototypical motor behaviors. By increasing and decreasing these compounds in different parts you can develop a concept of the role of GABA and DA on this level.

19   If you want to know what dopamine does in the brain then you can for instance give amphetamine, a compound that will induce the release of dopamine. If you administer it to a rat, it will show stereotypical behavior. It is a simple test that shows that dopamine is related to behavior.

20   But the question is: where is this dopaminergic effect mediated? Dopamine is not located in just one brain area. There is the nigro-striatal dopaminergic pathway, but there are also dopaminergic pathways that lead to the cortex and the accumbens. So you can specifically inject amphetamine in the cortex, striatum or accumbens. You will only see that specific stereotypic behavior if you inject amphetamine in the striatum. If you apply it in the accumbens you will mainly see locomotor activity, not stereotypical but an increase in locomotor behavior.

21   Behavior is a very accessible measure in experimental research. You have a cage, you have a rat, and you can start your research. So I start with that. In this way you can get new ideas about what a certain transmitter or pathway does in the brain, even though you are doing very little in the animal. You just look at simple behavior, is it running, is it stereotypical or not? You can also do very complicated experiments with learning models, then you explore different pathways.

22   In behavioral experiments you look at the end product of your injection. Another way is to measure the response directly in the brain in specific areas by inserting a microdialysis probe into the brain. In that way we can measure the direct effect of amphetamine, it releases an enormous amount of dopamine. The explanation for that effect is that the vesicles that contain dopamine fuse with the cell membrane, resulting in the release of dopamine in the synaptic cleft.

23   The research that I am doing aims to bridge the gap between our knowledge about the anatomy of the basal ganglia and pharmacology/physiology. From the anatomy we know that there is a connection between dopaminergic pathways and the striatum. But what is the effect of a dopamine transmitter on the striatal cells? That is still not entirely clear. It is an essential question.

24   In Parkinson's disease dopamine depletes in the striatum. That appears to be a major problem. To solve that problem you can administer L-dopa or dopaminergic agonists. But these turn out not to be ideal therapeutics because after a while side effects appear and they gradually loose their therapeutic effect. What you rather want to know is the function of dopamine in the striatum. If you have a better idea of its function it may be possible to use other, more specific compounds. So, it is essential to know what dopamine does in the striatum.

25   We have learned that there are two main subtypes of dopamine receptors in the basal ganglia, D_1 and D_2. So I work with compounds that are specific for those subtypes. But what is the function of those subtypes? In the literature this question has been asked many times. Should we just use a compound specific for D_2 or one that acts on both types, such as *e.g.* apomorphine? This compound has not been used for a while because of its many side effects. But it now seems to be a reasonable alternative.

26   Also L-dopa has D_1/D_2 affinity, simply because it results in more dopamine that acts on both subtypes. It is an ongoing discussion, what is ideal? You want to replace dopamine, you cannot use dopamine itself, so what do you need? Do you need to activate only one subtype so that you restore function, but not induce side effects? Is activation of D_2 receptors enough, or do you need a little bit of D_1 receptor activation too, and what is the ratio?

Searching a treatment

27   The problem of all dopamine-agonists is that they also have side effects in the periphery, in other places of the body like the heart and veins. You can counteract that by administering peripheral dopamine antagonists, like domperidome, together with a dopamine agonist. That will relieve side effects like nausea, but has its own side effects.

28   Another difficulty is that you have to find a proper dose that may differ per person. Too much DA-stimulation will lead to an over-activation that can induce *e.g.* spontaneous dystonias. [Dystonias are movement disorders in which sustained muscle contractions cause twisting and repetitive movements or abnormal postures.] Usually after about five years patients will be increasingly in an off-period. In an on-period a patient reacts positively to medicine. In an off period the reactions are either poor or hyper.

29   In theory you try to maintain a level of dopamine receptor stimulation by administering dopamine agonists. But when you apply a compound the sensitivity of the receptors changes. In Parkinson patients the dopamine receptors become hypersensitive as result of the dopamine depletion. By a process of up-regulation the number of receptors on cells increases. This changes, for instance, also the uptake of dopamine. There are all kinds of mechanisms that act as soon as something changes, to compensate for the change.

30   By administering an agonist you try to reestablish the situation that was normal before the degeneration of the dopaminergic cells. But you do not know what that situation was. In the clinical practice, different doses are tried until the patient's motor behavior returns to normal. But that dose might not be comparable to the amount of dopamine that was normally released before the degeneration. When you start medication the receptors are still hypersensitive. But that will change, and the induced effect will eventually decrease, so the dose should be adjusted.

31   There are methods to establish sensitivity. But there are also all kinds of compensation mechanisms on other levels than the dopamine system. Changes in dopamine induces changes in acetylcholine in the striatum, and also changes in GABA and glutamate. So, how to solve that problem, how to chart that system and how to restore it to normal?

32   The clinical studies are a kind of trial and error. The therapy is thought to be adequate when the patient responds well. Parkinson patients respond well to dopaminergic agonists, but also to cholinergic antagonists. There has been long discussion about an explanation for that effect. It is thought that there is a DA-acetylcholine balance in the striatum. When dopamine increases, acetylcholine decrease, and vice versa. That would explain why dopamine agonists and acetylcholine antagonists have a similar effect.

33   By doing basic experimental work it now appears that stimulation of D_2 receptors on cholinergic neurons does indeed inhibit the cell, explaining the balance. But, via D_1 receptors and via the cortex dopamine can also stimulate acetylcholine. So there is a delicate balance between an inhibitory and excitatory effect of DA on acetylcholine functioning. It can only be discovered by basic experimental research. How to incorporate such specific knowledge into the practice of treating Parkinson's disease is another problem.

34   Another approach is to study the effect of using NMDA antagonists. NMDA is a glutamate receptor subtype. Maybe we should use such a compound in combination with a dopaminergic therapeutic to create the optimal effect. However, the problem is that glutamatergic activation will influence the whole brain. You immediately interact with all kinds of other areas, so that will not be my best bet. Yet if you would understand how glutamate interacts with dopamine then you could judge this better. But given our current knowledge it is still a long way to go before we can easily infer what to do.

35   For my own research I want to know what the effects are of D_1 and D_2 receptor stimulation in a healthy situation. If you got a good idea of that, you can look at a lesioned model to verify if the effect is the same in the pathological situation. Is the interaction between glutamate and dopamine and dopamine receptor subtypes still traceable in the same way? If that is not the case then you must better establish what kind of compensation is involved after a dopamine cell lesion.

Using the model

36   At the moment the role of dopamine in the striatum is still a matter of debate. We have a model which claims that there are excitatory D_1 receptors on the direct pathway, being separated from the inhibitory D_2 receptors on the indirect pathway. But if you look at the literature, all kinds of gaps emerge in this story. It is pleasantly simple, but it completely lacks nuance.

37   For example, it is dubious whether D_1 receptors are located only on the direct path and D_2 receptors only on the indirect pathway. This claim alone is subject of enormous debate. There is a group of well known anatomists that claim that there is a division, based on studies of rats and monkeys with a dopaminergic cell-lesion. A way to discover the presence of receptors in pathways is by looking at messenger RNA. But with the same methods another group claims that D_1 and D_2 receptors are present on both pathways, with no absolute segregation.

38   My own data also do not fit the model. The model explains many findings but also leaves a lot of questions. But in the literature many authors appear to just treat the model as given, apparently without questioning it. This is something that intrigues me. Even though it does not fit the data well, it has gained enormous popularity. Why? I think that it is because the model fits the way you think that it

will work. It provides a prediction that you can easily understand. It is simple and it is beautiful to work with. That is why I think so many people just take it for granted without questioning it.

39   The reason that it is beautiful is the following. Upon activation of the cortex you get a glutamate activation in the striatum. Now if glutamate acts in a similar manner on both the direct and indirect pathway, you get a net reciprocal effect in the SNR, they counteract each other. The model shows that dopamine acts synergistically with glutamate stimulation via the D_1 receptor to increase the amount of GABA in the SNR, inducing an inhibition of the nigral activity. At the same time dopamine inhibits the indirect path that would increase nigral activity via the D_2 receptors, therefore diminishing the excitation of the nigral cells. So, dopamine let the activities of both pathways point in the same direction. It stimulates the direct pathway and inhibits the inhibition via the indirect pathway. The net result is a decrease of activation of the SNR. This is associated with behavioral activation. It increases the activation of the thalamus and brainstem, which coincides with all kinds of activity.

40   That is why it is beautiful, dopamine is a compound that facilitates activation. For example, with amphetamine you see stereotypical locomotion activity. You can understand that behavior using the model that says that an increase of dopamine results in SNR inhibition, which enables behavior activity.

8.4 Testing the model

In this section I report on how Dr. Timmerman used the basal ganglia model to conduct her own experiments in the laboratory. Part of the interview was conducted in the laboratory.

41   The model is subjected to heavy criticism. The first thing I did was to check whether a change in activity of striatal cells caused a change in the SNR. I infused glutamate agonists of several receptor subtypes and an immediate decrease of activity could be observed in the SNR. That means that apparently the direct route is stronger than the indirect route, as otherwise activation of the latter pathway would induce an increase in activity, given the model. So I tried several glutamate agonists to confirm the model.

42   After that we did a test with a D_1-agonist. The result was a very slight decrease. Although the effect was very limited, it would be in accordance with the model. However, application of a D_2-agonist induced a gradual but again very minor increase. If any, it does not fit the model. So is there a real segregation between the two pathways? The effects are hardly noticeable.

43   But is activation of the D_1 receptor always stimulating? In vitro studies never show a stimulation by D_1 receptor activation. If you prepare slices of the striatum and you apply a D_1 agonist you will not see a stimulation but an inhibition. That does not fit the model. So for me it is more like a model you work with, knowing that there is a lot more nuance to it. Also people that perform those in vitro studies never talk about this model. It does not fit their data, so why would they accept it.

44   What we know from other electrophysiological studies is that GABA-ergic neurons in the striatum are hardly active, under basal conditions. You can easily activate them with glutamate. We assume that dopamine modulates the glutamate-GABA interaction in the striatum. But if there is very limited activity in the striatum, a modulator will hardly be effective. So I thought, let's give a slight activation of the striatum by glutamate, and then let's see if we can make the modulating role of D_1 and D_2 agonists more apparent. The literature also implies that the role of dopamine depends on the influence of glutamate.

45   My presumption is, D_1 probably excitates, D_2 probably inhibits, possibly on different pathways. Can I confirm this, or cannot I? Well, I cannot confirm everything. Under basal conditions, without activation by glutamate, you can not speak of dopamine as a modulator, because there is nothing to modulate. That was my former study. Having finished that, I am now searching for a better start situation. That means I have to induce a slight glutamatergic activation locally in the striatum. I tried that, but it was difficult. You cannot have a nice constant activation because all kinds of other systems immediately try to compensate the increase in activity.

46   What I tried together with a student of mine, is to stimulate at the level of the cortex with a glutamate agonist, and look if this activation is noticeable in the SNR. You expect that the activation of the cortex will release glutamate in the striatum, that will consequently result in activation of GABA-ergic neurons. Depending on what pathway is the strongest, this should decrease or increase the activation of the SNR. So first we want to know which pathway dominates upon activation, but only to search a situation to again test the role of dopamine in the striatum.

47   After performing these studies it seemed that the cortex is not the best place to start the activation. So now we try to start with activating the thalamus. The thalamus projects both via the cortex and directly on to the striatum. That would create a general activation in the striatum. We have seen that if you infuse a glutamate agonist in the thalamus, just for ten minutes, then you will see a slight reaction. Yet we could not confirm this in later studies.

48   The suggestions to change experimental conditions are based on both the model and our former experiences. According to the model glutamate with D_1 receptor activation will increase the activation of the SNR, they amplify each other.

49   The test we are running now [Feb. 26, 1997] is to first infuse a D_1 agonist into the striatum. Secondly we will give a glutamatergic stimulation of the striatum to find out if D_1 cooperates with glutamate to induce an increase of GABA and hence an inhibition of the SNR. We want to find out if the presence of a D_1 agonist makes a difference. We have done this D_1 agonist infusion three times already. We have seen some reaction, but very little.

Performing microdialysis

50   In the laboratory we use brain dialysis probes. Such probes consists of a small glass tube with at the bottom a semi permeable membrane and at the top two extensions, an inlet and an outlet. If a fluid is infused via the inlet, diffusion into the surrounding tissue at the tip of the probe occurs. You can infuse compounds in this way, but you can also sample from this area. Depending on where the area of

interest is located in the brain, and what the dimensions are of this brain area you can make longer or shorter dialysis probes.

51   You can implant the probe in the brain in such a way that the tip is at a specific location. For our experiments we use Wistar rats. You can find a location in its brain using the atlas of the rat brain. Ours is falling apart because of its extensive use. The atlas portrays the whole brain from back to front in slices. We want to put our probe in the striatum. This area is relatively large, and both rats and human beings have two striata. It is a stretched out area that runs through a large part of the brain. To put in a probe you look at the coordinates of the map. These are standardized for a Wistar rat of 300 gram, and you look for particular blood vessels. The bregma at the center on top of the skull is a reference point. All brain slices portrayed in the map have a known distance from the bregma. For the striatum you look at the map that is just behind the bregma, the probe should be located 3.5 mm to the side, and 7 mm deep.
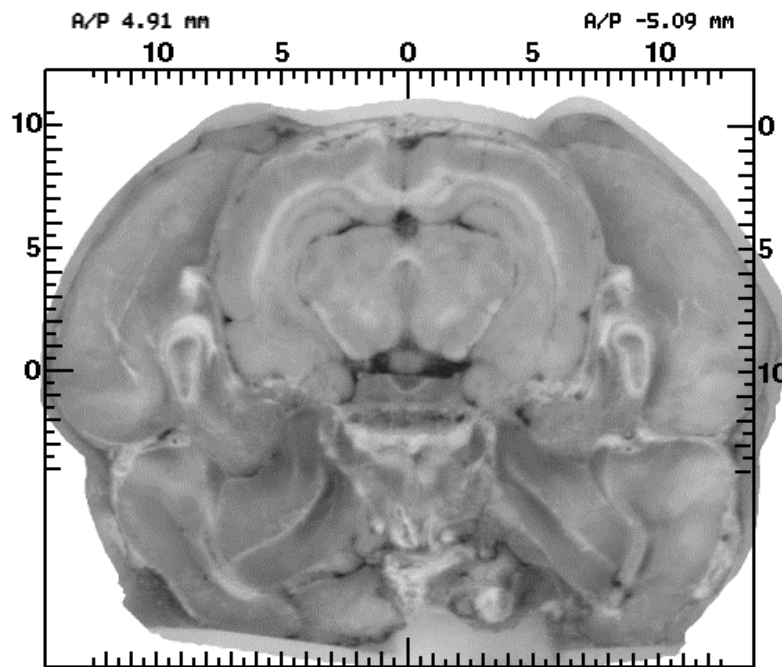

Figure 8.3: Example slice from the rat brain atlas

52   We place an anesthetized rat in a stereotactic apparatus, that clamps its scull by the ears and at the nose. Using this apparatus you can exactly determine a given location, using the bregma as a reference. When we find the given location and drill a small hole in the scull of the rat. After that we slowly lower a probe inside. We seal the probe with a screw and some cement. You prepare a rat a day in advance. When we add an electrode, to measure electrical activity, we always do this just before the actual experiment starts.

53   The inlet of the dialysis probe is connected to an infusion pump. Very slowly an ionic fluid is infused through the brain area, and it leaves the brain via the outlet tube. The fluid that comes out reflects the compounds that are present in that location at that moment. That fluid is guided through a system that analyzes a sample every given time-interval. That system can be set up to measure particular compounds, such as amino acids, dopamine, noradrenaline, etc. The compounds are separated in a column from where they are guided to a detector. For example, for amino acids we use a fluorimeter that registers the degree of fluorescence that is detected and plots those values against time.

54   In the case of our current experiment we have put in two microdialyis probes, one in the thalamus and one in the striatum. Additionally, in the SNR we place an electrode, which is an isolated wire with a small uncovered tip. This tip can measure electric activity outside the cell, it is still to large to measure intracellularly. In this way we can make an extracellular recording of action potentials. You pick up those signals from one or two nearby cells. You can determine what kind of cell you are measuring by looking at characteristics of the action potential. When we lower the electrode we actually try to find a particular cell type by looking at the kinds of signals, given descriptions in the literature. The SNR neurons are described as being tonically active, displaying a high firing frequency, and exhibiting a nice thin action potential. We have to find the correct type since other cell types may also be present in the same area. In this experiment it is easy because the SNR mostly contains the same type of cells.

55   To diagnose the cell signal type we use a computer program that records templates of signals we are interested in. You record an example of a signal and tell the program to start looking out for those types. It distills the signals from the noise. We know that an SNR action-potential lasts about 0.7 milliseconds. Any signal that takes longer will be ignored. Depending on the location of a cell and its connections, it displays a particular electrical activity. The activity depends on incoming signals from other cells or it can fire spontaneously. In our experiment we know that the cell fires about 20 to 40 times a second. An extra condition for this experiment is that the firing frequency remains stable in time. If the activity we monitor deviates from those conditions we start looking for another cell by moving the electrode again. To find good cell activity one has to learn; it will take time, patience and frustration.

56   When all conditions are met we start the experiment. We need a good base line, a good firing frequency, the activity needs to be tonic, the rat should be well anesthetized. All conditions should remain stable for half an hour. Then we record a base line of the activation for ten minutes, and start the fluid infusion. That is all still part of the preparation. When all goes well up to that point we decide to start the actual experiment or wait or look for another cell. If everything looks good we do not touch the rat anymore and start the experiment. The only thing that remains is to change the syringe from the one containing an isotonic fluid to the one that contains the isotonic fluid with the drug to be applied and start the drug infusion. The compound will enter the brain and now all we need to do is see what will happen.

57   We have done a test with a D_1 agonist. After half an hour stability we started the infusion. From that moment we knew that the compound was inside the rat's brain and an effect can appear, and than you gradually see an effect. Most of the times we hardly saw anything, but a few times we saw a slight decrease. So the D_1 agonist has little effect, it hardly deviates from the start condition, but you have the feeling that it has a slight inhibiting effect. Under these conditions amphetamine has a similar slight effect. You also have the feeling that it suppresses, but only very little. So if any, it seems to work in the same way as a D_1-agonist.

58   By trying a D2-agonist we saw that it did something different, it gave a gradual increase of activity. Therefore D_1 and D_2 agonists seem to act differently. But the effects are hardly noticeable. That is why we induced a situation where the striatum was activated. If you infuse a glutamate agonist in the striatum, you see an immediate and very strong effect, that only lasts for a limited amount of time. Hence, what I then looked for was a relatively low dose to create a more stable activation to use as an activated condition.

59   When the experiment is finished we apply a small amount of current on the electrode to burn a little hole, which will mark the location. Then we sacrifice the rat, and remove its brain. You end up with a whole series of jars with brains in them. Then you plan a day when you will slice up all those brains. With the help of the brain atlas and the marked position of the electrode tip you determine the exact location of your measurements.

8.5 Interpreting the data

In this final section I report on my questions to Dr. W. Timmerman about the interpretation of laboratory data in general, and the published conclusion about her investigation in particular.

60   Sometimes the data you obtain deviates from what you expected, or the outcome of one experiment is very different from the rest. In the latter case it is possible that the probe location was wrong. This would give you a reason to remove these results from your sample. However, if that is not the case you will have an anomaly.

61   If I find an anomaly I check the experiments just before and after in the same series to see if something can be traced from that. Also the experiment has to feel right. For instance, sometimes a signal is hardly noticeable in the noise, and then it already casts some doubt.

62   But if the template was good, the stability was in order, and you still see a deviating response, and it was one deviation in *e.g.* five others, then I just mention it in the results section of an article. One rat was an exception for an unknown reason, so be it. As an average we always repeat an experiment five to six times. You cannot base anything on one observation. Sometimes we follow one experiment, but often it turns out that it is still different. You cannot publish anything based on one experiment.

63   Another influence on your data is the anesthetic. For instance, ketamine is an anesthetic that acts on the glutamate receptor. You do not want that. There are all kinds of arguments to use anesthetic. It is less stressful for the animal. You have more stabile activity compared to animals that are awake. But because the striatum is involved in motor behavior you never can be sure that it does not influence your data. You do not know until you also check it with awake animals.

64   Yet another factor is that in Parkinson research animal models are used that are lesioned with for instance MPTP [see Section 7.2], but that may not reflect the entire or precise pathological situation. So conclusions about the model may not be true for the disease.

65   Another issue is that many effects in experiments with systemic dopaminergic injections are ascribed to the striatum. This is indeed one of the areas where effects can be mediated. But an effect can also be directly induced in the accumbens or the SNR. You could be wrong by claiming that it was the striatum. DA released from dendrites can also be involved. That is another complication. So it is not all that easy to establish the functional role of dopamine.

66   For the manipulations in our research we focused on glutamate and dopamine interactions. But in the back of your mind you know that there are also dopamine-acetylcholine interactions, and all kinds of peptides, and the influence of GABA-ergic neurons. So you leave out a great many to keep a grasp on what you are doing. So if you find things that you can not easily understand, there are many explanations possible. You know you cannot explain everything by just measuring dopamine, glutamate and GABA, there is much more to it.

This concludes my interviews with Dr. Timmerman about her work and experiments up to February 1997. In later tests Dr. Timmerman further experimented with different setups, such as beginning with a glutamate agonist infusion, followed with a glutamate agonist infusion in combination with DA-agonist. About this work she and her coworkers published the following conclusion:

67   "To gain insight into the role of striatal dopamine in basal ganglia functioning, dopaminergic drugs alone, and in combination with the glutamate receptor agonist kainic acid were infused in the lateral striatum via a microdialysis probe, while single-unit recordings of substantia nigra reticulata neurons were made in chloral hydrate-anaesthetized rats. Striatal infusion of dopaminergic drugs did not significantly affect the firing rate of substantia nigra reticulata neurons, which was related to the low activity of striatal cells under basal conditions, illustrated by the lack of effect of striatal infusion of TTX on substantia nigra reticulata activity. Under glutamate-stimulated conditions, striatal infusion of *d*-amphetamine potentiated the inhibition of substantia nigra reticulata neurons induced by striatal kainic acid. Thus, under stimulated but not basal conditions, the modulatory role of dopamine in the striatum could be demonstrated. Dopamine potentiated the inhibitory effect of striatal kainic acid on the firing rate of the basal ganglia output neurons." (W. Timmerman, F. Westerhof, T. van der Wal and B. Westerink, 1998)

8.6 Conclusion

The specific question for this chapter was: how are theory and experiments used in drug research for Parkinson's disease, in practice? I tried to present an image of a practice in neuropharmacology by interviewing two scientists about their specific work in investigating new drugs, exploring the functions of part of the brain, testing a model of those functions and interpreting the data.

Overall, neuropharmacologic research can be characterized as searching, understanding and testing a way to make the characteristics of a pathological systems resemble a healthy situation. Experiments are used to chart both situations, and to try to bring one situation closer to the other by drug manipulations. In the next chapter I will analyze the specific problems addressed in the practice, as described in this chapter, in detail.

* *