Physiology and Behaviour

Michael DeWitt

Downloaded 8:40, 13 December

End 15:09, 13 December

# 2. Long term potentiation

## Summary of the model

Long term potentiation is proposed mechanism of learning through which synapses are changed (e.g., more neurotransmitters, less neurotransmitters, more receptors, more synapses) through gene expression pathways. It is considered long term precisely because gene expression is altered leading to longer term differences in response to stimuli. The long term potentiation framework provides a mechanistic explanation for the existence of memory and longer lasting signal transmission between neurons. The mechanistic approach provides a framework where particular neural pathways become strong (or conversely weaker) over time, leading to preferred pathways of connections. These gene expression changes effect “store” information to different stimuli, creating preferred pathways over time and allowing for memory formation. A useful statistical proxy is the concept of neural networks in which there are entry nodes and “hidden nodes” with output nodes. In an untrained neural network, all pathways have equal weight within the network, with each weight having some activation function. As the neural network is training, pathways between nodes that are more successful with get higher weights meaning more contribution to the model outputs—these weights represent learning and a statistical proxy for “long term potentiation.”

Mechanistically, an external receptor is activated leading to increases in cyclic AMP (cAMP). The abundance of cAMP then activates protein kinase A (PKA), which phosphorylates cyclic AMP response element binding (CREB) proteins within the nucleus, which in turn binds to genes with cycle AMP response elements thereby inducing genes with CRE upstream of their promoters. This pathway results in CRE mediated genes. The genes can then induce the synthesis and migration of additional receptors (or conversely a reduction), increasing in neurotransmitters, and/or the creation of additional synapses.

## Experiment

Given the Drosophila model and the well-established operant-conditioning protocol, I would design an experiment to verify that CREB is part of the molecular signalling pathway for long term potentiation. As described above, CREB must be phosphorylated by protein kinase A which leads to binding to CRE and the associated changes in gene expression. By disrupting the availability CREB, we should break the proposed mechanistic pathway for long term potentiation, meaning that our hypothesis is that despite the amount of training/conditioning the Drosophila will not negatively respond to playing of “Sweet Caroline.”

In the first part of the experiment, we will create a mutant Drosophila that will not produce CREB and that the Drosophila can service into adulthood (i.e., assume this mutation is not so deleterious that they are nonviable). We will then use a reporter to ensure that CREB is not available (e.g., GFP tagged CREB in wild-type and the knockout mutant) so that we can confirm that we have eliminated or substantially reduced the about of CREB available in our adult drosophila.

With the wild-type and CREB knockout Drosophila, we will subject them to the well-established anti- Deal Diamond “Sweet Caroline” operant-conditioning protocol. We would then build an apparatus in with two soundproof chambers connected by “lobby”. In the center of each chamber, we would have nectar (unconditioned stimulus). In one chamber we would have “Sweet Caroline” playing while in the other chamber we would have “Country Roads” playing, as we want to ensure that sound is playing played in both chambers rather than only one. We could then release a group of drosophila (both the wild type and knockout) and count the number per group that enter each chamber. If long term potentiation is at play, we would suspect that the wild-type drosophila would preferentially go to “Country Roads” and were less likely to go to the “Sweet Caroline” chamber. Conversely, we would expect the knockout drosophila to enter both chambers at roughly equal rates as we hypothesize that due to a lack of CREB long term potentiation could not occur. In order to ensure that there isn’t a “side” effect we would run this experiment multiple times rotating the songs that were played in each chamber. Additionally, we would ensure that our study was sufficiently powered to detect the smallest meaningful difference.

If long term potentiation as described was the method of learning, we would expect the knockout drosophila to go to either chamber at roughly similar rates regardless of the music played, while the wildtype flies would go to the chamber without “Sweet Caroline”.

# 3. Research flaws

## Grosenick et al. (fish doing transitive inference)

Grosenick and collogues sought to determine if male *Astatotilapia burtoni* fish can exhibit transitive inference of male hierarchy through observation. Transitive inference is a type of logical inference where the relationship between two parameters is *inferred* though the use of other *observed* relationships (e.g., A > B; C < B, therefore by transitive inference A > C). In their experiment they allowed an observer *A. burtoni* to observe “fixed” from behind a separator matches between five fish from labeled A to E with the associated hierarchy A > B > C > D > E. The winners of each match were predetermine through the use of approaches to shock the fish lower in the hierarchy. The observer was then allowed to react to novel groupings (i.e., A and E; B and D) where evidence of transitive learning was defined as if the observed spent more time nearer the fish lower in the hierarchy (as *A. burtoni* prefer to be around less dominant fish of their species).

Design Flaw 1—The experimental design, fails to include a negative control, i.e., an experimental arm which did not observe the fights and the associated induced hierarchy. If this negative control were to exhibit similar preference for the weaker fish in the absence of training, it would indicate that there is some other signal taking place for the observer *A. burtoni* to infer a hierarchy. This design could have also been performed by using the same bystander (i.e., each fish serving as their own control). The induced hierarchy could have taken place in the absence of the bystander, and then the naïve bystander’s (i.e., the fish prior to observing the staged fights) preferences could be measured (both through the continuous measure of time and the binary preference). The experiment could be repeated as described in the paper with the bystander in position to watch the matches. If there is no change in preference after the observation of the staged fights (e.g., if both before and after observation the bystander prefers the weaker fish in the hierarchy) then we cannot conclude transitive inference took place, at least not through the design described in the paper. Perhaps a pheromone or some other physiological change took place allowing the observer to make a *direct comparison* rather than perform *transitive inference* (e.g., I see one fish is more brightly colored than the other indicating past success than the other, the hierarchy is clear and not inferred).

Design Flaw 2—The experimental design and published results failed to show that the induced hierarchy worked as anticipated. While the authors mention that males prefer to spend more time around male *A. burtoni* who are less dominant (i.e., lost a match), the authors failed to describe experiments where this was actually the case. In mechanistic experiments it is important to show that your model works before you try to induce a change (e.g., create a mutant to show that some feature exists or doesn’t before you proceed with your experiment). In this case the authors could have staged several fights and confirmed that the bystander typically chose the observed hierarch (e.g., B wins over C, does bystander spend more time with C). This serves as a kind of positive control in the experimental design demonstrating that we should expect the fish to prefer the less dominant in this experimental set-up more broadly (i.e., the artificial lab conditions of lighting, pH, habitat). Experimentally it should be confirmed that a hierarchy is induced in the first place which then leads credence to the experimental design.

Outside of the two design flaws, a more general comment that N = 8 is incredibly small and likely no sufficient to capture the variation potentially present in the model. This is somewhat confirmed by the PCA analysis in which they find that even among the small number of fish the first principal component only explains 60% of the variance in the fish behaviour. It is reasonable to assume that these heterogeneities may translate to transitive inference (or other properties important in assessing dominance). Additionally, examining some power analysis for proportions with an alpha of 0.05 and a power of 80% with N = 8 would indicate a maximum effect size of .99 (arc-sine transformation). This means that with a baseline probability of 50% the only effect we were powered to down to 8% or 92% (e.g., nearly all or nothing). Effectively the authors powered this study to detect all or nothing results (e.g., all bystanders make the first move to one of the shown pair).

## Fuxjager et al. (effects of testosterone on manikin muscles)

The experiments described by Fuxjager and collogues include the addition of testosterone pellets to an experimental arm of golden collared manakins compared to those without the addition of these pellets. In a second group, flutamide, an androgen receptor antagonist is added to a completely different species of bird, the zebra finch in an experimental group and withheld in the control group. A bioinformatic approach is then performed looking at differential mRNA abundance between those with and without the treatment. Gene expression networks are also created from the differentially abundant proteins for each species.

Experimental design flaw 1— A major missing component of this entire research is the lack of any mechanistic approaches or reasoning. For example, the authors write: “Little is known about either the way in which muscle-wide gene expression relates to extreme physical movements or how such gene expression patterns are modulated to fine-tune these behaviors”1 proposing that the research will help to shed light on gene expression. Additionally, the authors write “In our studies, we identify numerous genes that are transcriptionally regulated by androgen action within the main forelimb musculature”1 further indicating that genes have been identified which regulate these activities. Despite these comments, Fuxjager and collogues provided no real direct evidence that the genes describe led to the protein abundances detected through the RNA-Seq analysis. RNA-Seq only quantified the protein (mRNA) abundances, so while we can make comments regarding the detected differential abundance between the pooled sampled from different tissues of the golden collared manakins who received testosterone versus those who did not receive testosterone, we cannot directly describe a gene expression pathway or mechanism—only that abundances changed (e.g., we see the result without seeing any pathway). To understand the pathway and to make stronger claims about gene expression, one option could be to induce mutations in some of the androgen receptors in the different tissues. This might require several experimental groups, i.e., a control group with no mutation, a group with a mutation (or maybe an antagonist like flutamide or aromatase), and a group with the mutation and the testosterone pellet. Performing RNA-Seq amongst these groups would then allow us to observe and quantify effects are induced directly through mutations in the androgen receptor amongst these different tissues. Similarly, we could attempt to have additional downstream “reporter” mutations to confirm that gene regulatory pathways are taking place (e.g., GFP tagged proteins in genes responsible for myosin or important metabolic pathways). With these report genes we could confirm that the mutation interferes with the pathway vs the wild type, and more importantly, how testosterone impacts it. Without more mechanistically oriented experimental designs, we can only describe the outcome but not the mechanism.

Experimental design flaw 2—It is unclear why zebra finches were used as an indirect comparison group to golden collared manakins. Zebra finches occupy a different geography with vastly different ecologies, sexual mating behaviors, sharing only the order *Passeriformes*. Any kind of comparison made between the protein abundances in the two different species reflects more than just the addition of testosterone in the golden collared manakin and the introduction of an androgen receptor antagonist in the case of the zebra finch (another design flaw in the case of elevating testosterone in one species and suppressing its receptor in another experimental arm). Rather, the differential expression of proteins cannot be disentangled from completely different evolutionary histories. To use some hyperbole it is similar to comparing differentially expressed proteins between domestic chickens and albatrosses. If the authors wanted a different species to compare the wing-snapping sexual selection, another species with9n the same genus would likely have been possible such as the white-bearded manakin which does not exhibit the characteristic wing-snap of interest. Having these species more closely related on the phylogenic tree would then allow more insight into the evolutionary and physiological changes that led to the wing-snap behaviour we see in the golden collared manakins.

# Biologists describe explanations for behavioral traits as either proximal or ultimate. Define those terms, and give a real-life example of each (i.e., real organisms and actual traits).

The proximate cause of a behavioral trait relates to the biological mechanism underlying the observed behavioral trait at typically thought about at the level of the individual. This could mean the expression of some hormone which causing differential behaviour and or other physiologic/phenotypic differentiation. For example, golden-collared manakins have increased quantities of androgen receptors allowing for wing-snaps created by fast-twitch muscles. Wood frogs and painted turtles have compounds to prevent ice crystals from forming within their cells during periods of extreme cold. Additionally, *A. burtoni* dorsal fin polychromy and other cichlids changing color based on winning a fight with another competitor.2,3 In another example, male chimpanzees have lower levels of testosterone when facing an immune challenge and are thus less likely to be as aggressive4, despite general increases in testosterone (and thus aggression) in males when tumescent females are present.5

The ultimate cause relates to the purpose of the behavioral trait on an evolutionary timescale (e.g., within the fitness landscape for the species what is the *reason* for this trait, while the proximate cause is the *how* this trait is manifested). Taking the above examples further—the golden-collared manakins exhibit these wing snap likely plays into sexual selection with stronger, more fit males exhibiting louder wing-snaps (or more frequent) as these males demonstrate a reduced parasite load and higher likelihood of producing viable offspring (and will begat more offspring). This type of sexual selection is likely the ultimate cause of the heterochromia observed in *A. burtoni* and other cichlids with bright red dorsal fins or other color changes after being in competition with other males likely demonstrating higher fitness and likelihood of producing progeny likely to reproduce. Again, in an example of sexual selection, the ultimate cause for the increase in testosterone is for more aggressive males to be more successful in mating during mate selection (with infected males less likely to be successful in mating and thus passing on their genes). Rather than sexual selection, the ultimate cause of the behavioural traits observed in wood frogs and painted turtles is likely due to the resource landscape of the environment with long, cold winters and lack of resources (and predators) these behavioural traits represent the ultimate cause for these behaviours (e.g., these traits resulted in a higher fitness phenotype embodied by this behavioural trait).

# References

1. Fuxjager, M. J. *et al.* Research Resource: Hormones, Genes, and Athleticism: Effect of Androgens on the Avian Muscular Transcriptome. *Mol. Endocrinol.* **30**, 254–271 (2016).

2. Dijkstra, P. D., Seehausen, O. & Groothuis, T. G. G. Direct male-male competition can facilitate invasion of new colour types in Lake Victoria cichlids. *Behav. Ecol. Sociobiol.* **58**, 136–143 (2005).

3. Korzan, W. J., Robison, R. R., Zhao, S. & Fernald, R. D. Color change as a potential behavioral strategy. *Horm. Behav.* **54**, 463–470 (2008).

4. Sonnweber, R., Stevens, J. M. G., Hohmann, G., Deschner, T. & Behringer, V. Blood testosterone levels in sickness and in health: Male chimpanzee testosterone levels decrease in face of an immune challenge. *Am. J. Primatol.* **84**, e23334 (2022).

5. Muller, M. N. & Wrangham, R. W. Dominance, aggression and testosterone in wild chimpanzees: a test of the ‘challenge hypothesis’. *Anim. Behav.* **67**, 113–123 (2004).