What key experiments using amphibian embryos led to the neural default model of nervous system induction? Include a discussion of the resulting model and its potential weaknesses.

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The neural default model of nervous system induction has been built on numerous important experiments using amphibian embryos. These experiments I would argue start with Spemann and Mangold's 1924 dorsal lip transplantation experiment, which won a Nobel prize and has been described as the most influential paper in developmental biology. I would also argue that Wolpert's line about gastrulation being the most important point in your life, applies completely to nervous system induction because without it we would not have a central nervous system and would not exist, therefore it is essential to the field of developmental biology to get an accurate understanding of nervous system induction, and identify potential weaknesses with the current model to progress. This essay will discuss amphibian embryos, early development, early developmental biology experiments leading to the neural default model, the neural default model, and its potential weaknesses.

Xenopus is a good model organism as the eggs develop very quickly, becoming a neurula within 24 hours, egg can be up to 1,500 per brood and can be visualised under the microscope, it can also easily manipulate, and the effects can be seen with a simple light microscope. But we always must question its relevance to humans with these being amphibians. These factors were large reasons for them being valuable organisms to learn about early development and was why they were being used in the 1920s, equalling the Northern crested newt (*Tritus cristantus*) shares many of these properties (which interestingly during earlier experiments two species of newt were mixed *cristantus* – non pigmented, emphtaeniatus – pigmented to allow for tracing of cells). This meant that through many traditional developmental biology "cut and paste" experiments, fate maps were well established. Gastrulation involves the migration of cells form the inside to the outside causing the formation of the three germ layers; mesoderm, endoderm and ectoderm. The ectoderm eventually forms the central nervous system, peripheral nervous system and skin. Neurulation is where the ectoderm thickens to form the neural plate, which then bends by apical constriction through Rho, to fold and form the neural tube. Neurula cells are formed by convergent extension of ectodermal cells and is induced by the notochord which produces long and short range inducive signals.

Spemann and Mangold's classic experiment of grafting the dorsal lip of the blastopore onto the ventral side creates 2 heads and 2 nervous systems, and if it was taken later but still grafted into an early host the inducing signal is weaker, varying with time. Despite these only working 1/100 times it was still enough evidence to show that it was an organiser which induces ectoderm to become the nervous system. This organiser was thought to perform vertical induction and Holtfreter through the use of high salt conditions developed a gastrula, which had cells that would stay outside, but found no neural tissue as it prevented vertical induction and only allowed for planar induction. Mangold took anterior neural plate and implanted it into the cavity of the early gastrula which induced an extra head, but without a brain and transplanting the posterior gave an extra tail and spinal cord thus showing that vertical induction was needed. However, when hox genes came about, we looked at genetic markers for neural tissue Holtfreter gastrulas and found them where only planar induction was possible. So planar induction signals from the mesoderm had the potential to induce ectoderm to express neural markers. A "Keller sandwich" is where the blastopore tissue forms two embryos sandwiched under glass, they show that nervous system was still induces with homeobox marker genes (e.g. krox20 in 3rd and 5th rhombomeres) they were in the right sequence even with only planar induction. But Keller Sandwiches and exogastrula didn't have a floor plate and Keller sandwich embryos had no anterior CNS structures. Therefore, it is clear that we need both planar and vertical induction.

We wrongfully assumed for over 70 years that a single molecule was responsible for the transition of ectoderm into neural tissue, and that neural tissue is the default state for ectoderm not epidermis. The theory came from disassociating the cells of the animal cap and if reaggregation was delayed by ¿5hs there was only neural tissue, while reaggregation after 1hour showed some neural markers, and immediate reaggregation causes epidermis. This meant that it is in fact the absence of intercellular signalling is required for differentiation, so with no signal they become neural, therefore they are neural by default, hence neural default model. So, it was hypothesised that the Spemann-Mangold organiser emits an antagonist into the dorsal ectoderm which blocks inductive signals and causes dorsal ectoderm to default to this neural state.

More recent experiments have been focused on discovering the molecules and receptors causing this, which with the building evidence reinforces that the neural default model is correct. Activin inhibition was shown to be present and to cause neural tissue through mRNA blocking, truncating its receptor. So, in the search for activin inhibitors began and showed that knockout noggin, chordin and follistatin together gives disrupted neural plate formation, they all block the BMP4 receptor, a receptor that is part of the TGFBeta family that binds to activin receptors to induce epidermis. Various experiments including ; injection of BMP4 mRNA gives ventralised embryo, grafting the BMP source onto neural plate causes a thin neural plate, adding BMP4 to disassociated animal cap giving epidermal tissue, and adding BMP4 inhibitor or mutant BMP4 receptor to animal cap cells induce neural tissue implicates them with the neural default model. But BMP antagonism alone does not induce neural tissue as FGF signalling is needed so there may be even more induces required. This unknown points to a potential weakness with the model, along with the pathways found in amphibians not working exactly in mouse/chick/zebrafish experiments, meaning that it probably does not exactly translate (as expected) to mammals. The previous weaknesses of it not being easily replicate I would argue has been overcome by modern techniques replicating it and edging this theory forward over time to the point where it has triumphed over the previously assumed epidermal default model, even if the neural default model is not fully characterised.

In conclusion the experiments using amphibian embryos leading to the neural default model of nervous system induction has been long, complex, but eventually reinforcing. Although not directly applicable to mammals, it is clear these experiments are ground-breaking and the animals have been a fantastic model organism for exploring this early vital stage of development.