NATURE VS NURTURE: LEARNING AND MEMORY (Reverse Genetics)

"FORWARD" GENETICS
(mutational analysis, functional analysis)

FUNCTION
(learning)
mutation
GENE
(loss-of-function)
clone
PROTEIN
(stupidity)

LEARNING TEST
IDENTIFY MUTANT STRAIN
MAP GENE CLONE GENE
POSITIONAL CLONING
PROTEIN SEQUENCE

NEURONAL EXCITABILITY

dCREB2
dunce
rutabaga
PACAP (amnesiac)

fosB

1) Transcription factor
2) Immediate Early Gene
3) Normally activated in some learning responses

Q. Is fosB activated by CREB in long term memory?
(i.e. in a fosB mutant, is long term memory blocked?)

"REVERSE" GENETICS

FUNCTION
(learning?)
mutation
GENE
(loss-of-function)
(fosB)
clone
PROTEIN
(stupidity?)

MUTANT STRAIN
LEARNING TEST
REPLACE NORMAL GENE w/ MUTATED GENE
PROTEIN SEQUENCE
CLONE GENE MUTATE GENE w/ IN CLONE (knockout)

GENE REPLACEMENT
(targeted gene exchange in mouse)

cloned knockout
gene introduced by electroporation
ES cell (in tissue culture)
(Embryonic Stem cell)
normal fosB gene
(in the ES genome)

1. Pairing of mutant & normal fosB genes
2. Double cross-over event occurs
3. Mutant & normal fosB genes exchanged
4. Rare event (ca. 10^7)
STRAIN OF KNOCKOUT MICE

MOUSE KNOCKOUT MUTATIONS SHOWN TO AFFECT LEARNING AND MEMORY BY REVERSE GENETICS

CaMKII  Ca/calmodulin-dependent protein kinase
fyn      tyrosine kinase
PKC      protein kinase C
CREB     cAMP-responsive transcription factor

THE fosB KNOCKOUT MOUSE

LEARNING - NORMAL - no learning defect
  high performance in Morris Water Maze test

MOTHERING - BEHAVIORAL DEFECT -
  Mutant fosB mothers cannot take care of their pups
  Mutant females let their pups die

fosB MUTANT MOTHERS DO NOT FEED THEIR PUPS

- time spent nursing (20min observation)
- note: mutant mothers have a normal milk supply; mainly do not keep pups warm or spend much time nursing
fosB- MUTANT MOTHERS DO NOT RETRIEVE LOST PUPS

- fosB mutant mothers retrieve the 1st pup
- normal mothers retrieve the 1st pup
- normal mothers retrieve the 3rd pup

Note: No mutant mother retrieved 3 pups; mutant males are also poor at nurturing behavior.

fosB- MUTANT MOTHERS LET THEIR PUPS DIE

- normal mothers (n = 65)
- fosB mutant mothers (n = 27)

Time taken to retrieve 3 pups moved to corners of the cage (20 min observation time)

Graph showing the number of surviving pups per pregnancy:
- Normal mothers: 8 pups
- fosB mutant mothers: 2 pups