Polysaccharide Modulation of AMPA-type glutamate receptors:

Importance to synaptic function and neuronal maintenance

Linda Chicoine, Ph.D.
Cognetix, Inc.

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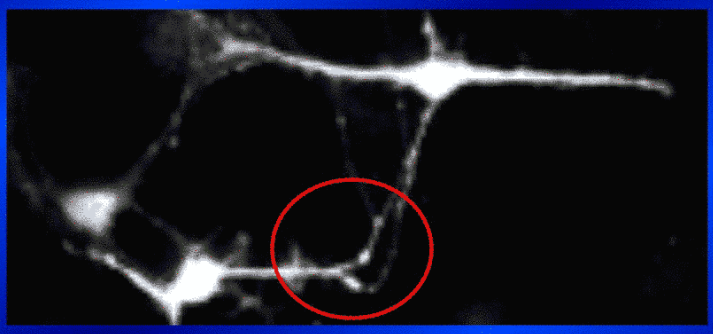

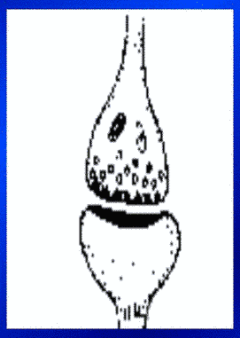

- ♣ Regulation of synapse functionality (AMPA receptors)
- ♣ Synaptic activity promotes cell survival

- ¬ Extracellular matrix (ECM) is involved in synaptic plasticity and neuronal maintenance
  - interactions between intra-/extracellular matrices

(Bahr et al, 1997, J. Neurosci)

  - polysaccharide component

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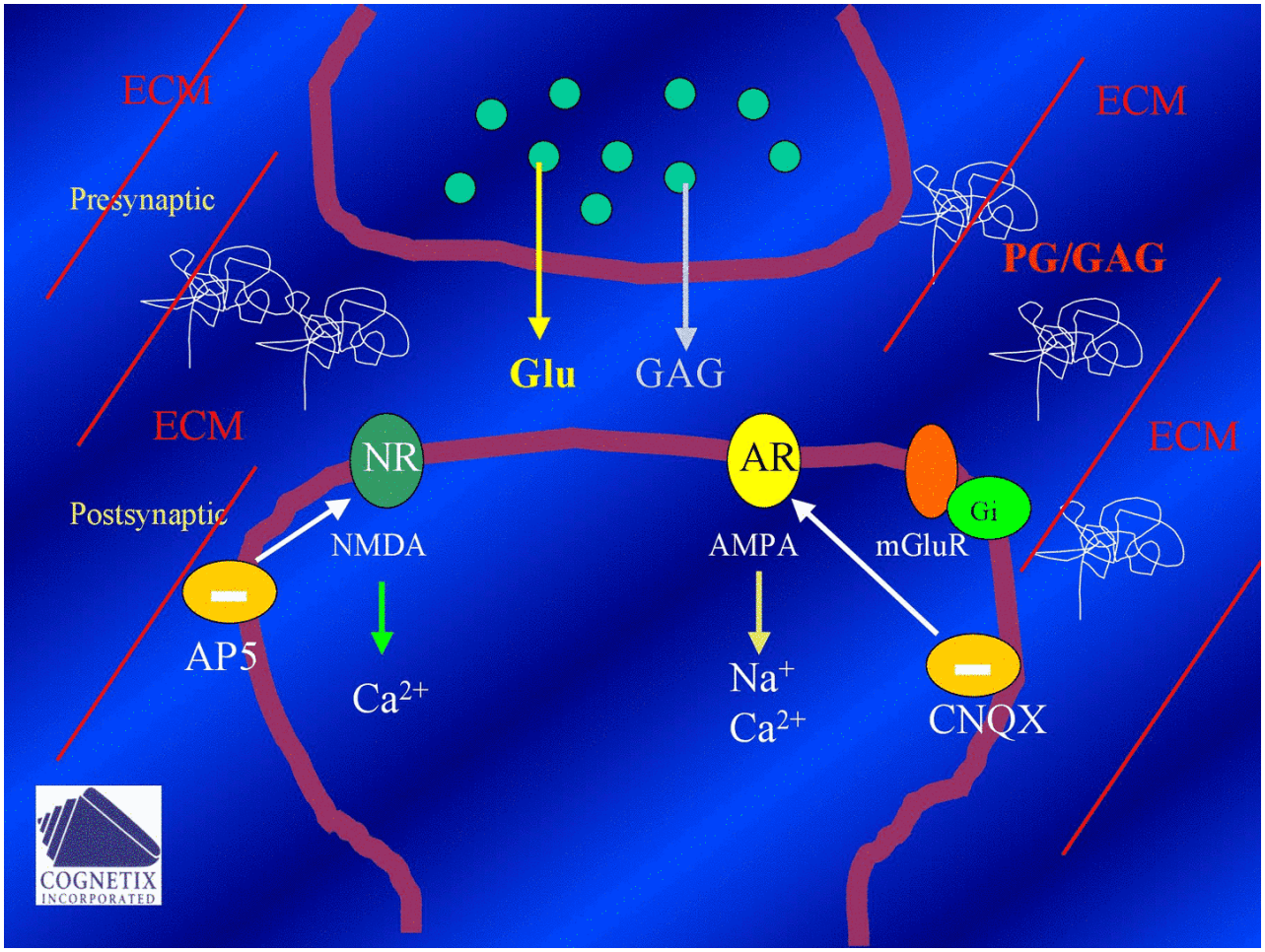
ECM
Presynaptic
ECM
PG/GAG
Glu
GAG
ECM
ECM
NR
AR
Postsynaptic
Gi
NMDA
AMPA
mGluR
AP5
Ca2+
Na+
Ca2+
CNQX
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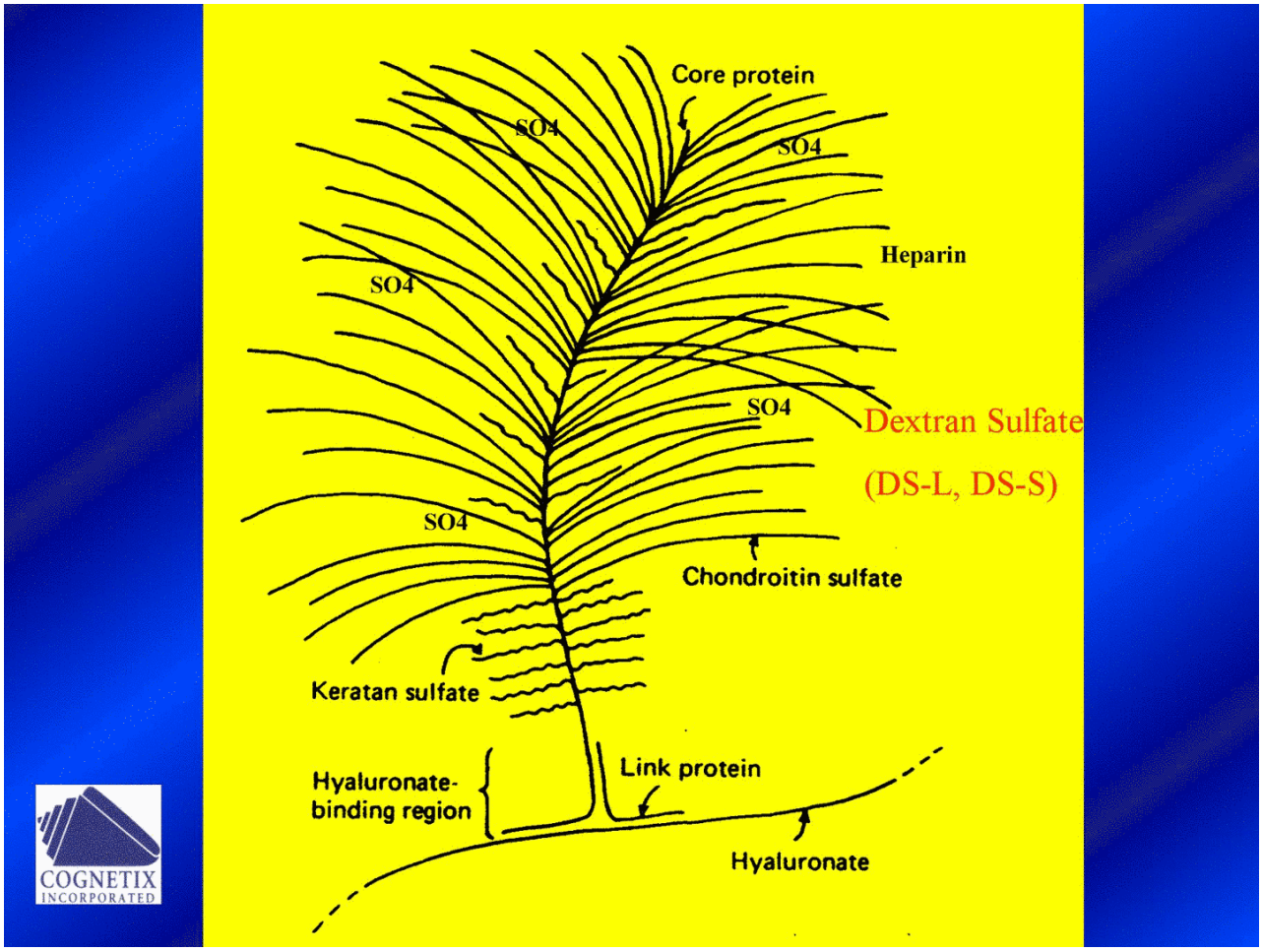
Core protein
SO4
SO4
Heparin
SO4
SO4
Dextran Sulfate
(DS-L, DS-S)
SO4
Chondroitin sulfate
Keratan sulfate
Hyaluronate-
binding region
Link protein
Hyaluronate
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Part I: Receptor Regulation

- Channel kinetics
- Binding affinity
- Receptor regulation

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Channel Kinetics

- Sulfate dependence
- Size dependence

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A
control
B
AMPA
C
AMPA + dextran
D
AMPA + DS-S
E
AMPA + DS-L
F
AMPA + DS-L + CNQX
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Open probability, % Po
0
20
40
60
80
NT
AMPA
A + Dextran
A + DS-S
A + DS-L
ANOVA p<0.001
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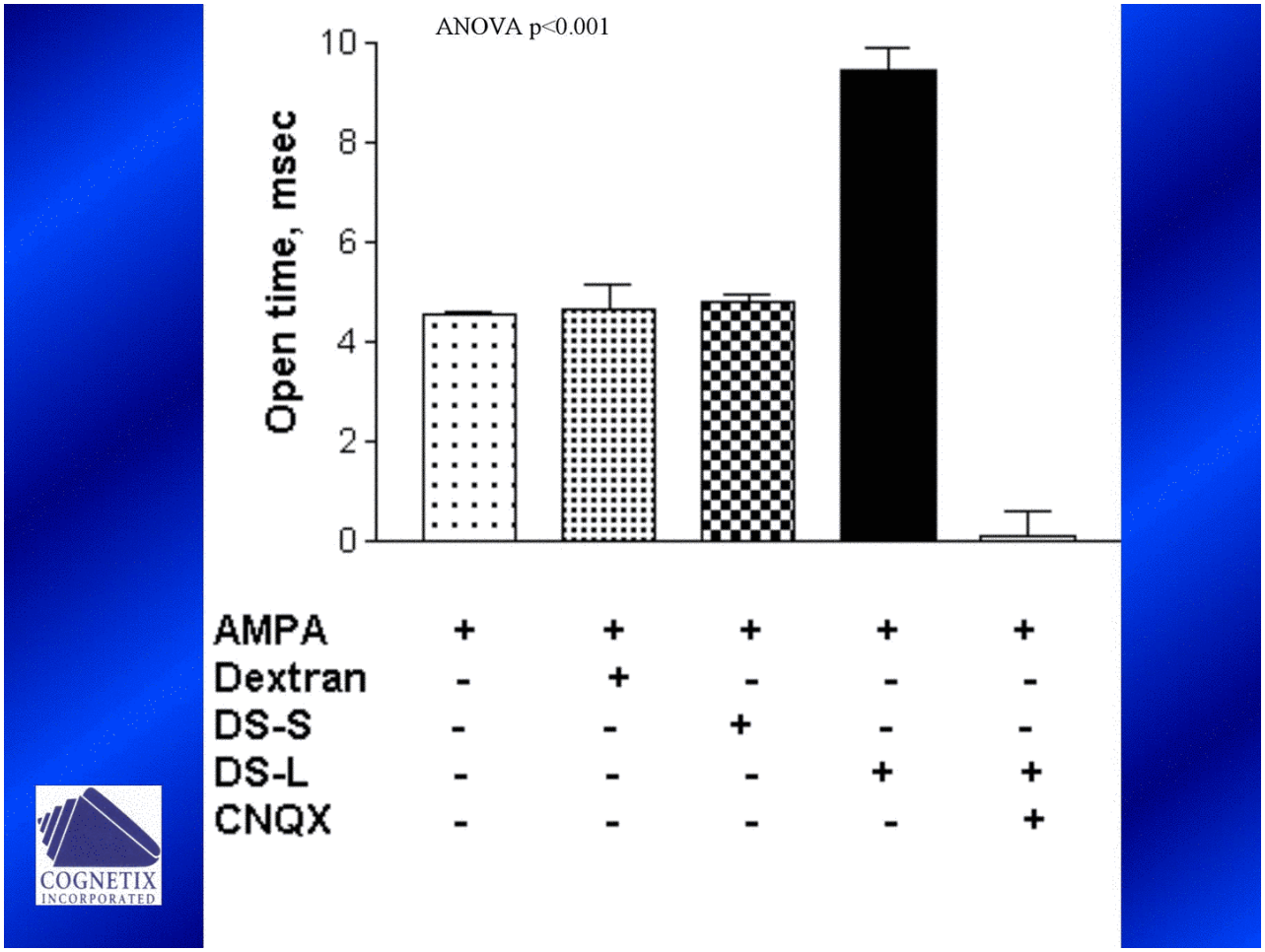

Binding Affinity

- Sulfate dependence
- Size dependence

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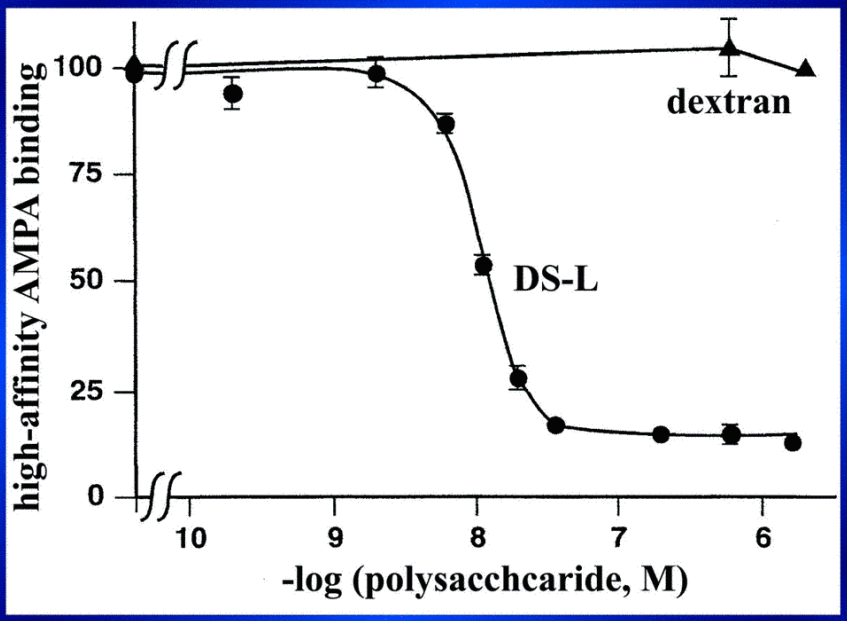
DS-L Further Studied for Changes to Binding Affinity

IC_{50} for decrease in binding affinity

Dextran >>30 _M

DS-S 800 nM

DS-L 10 nM



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Receptor Regulation

- Ca^{2+} influx

- Sulfate dependence
- Size dependence

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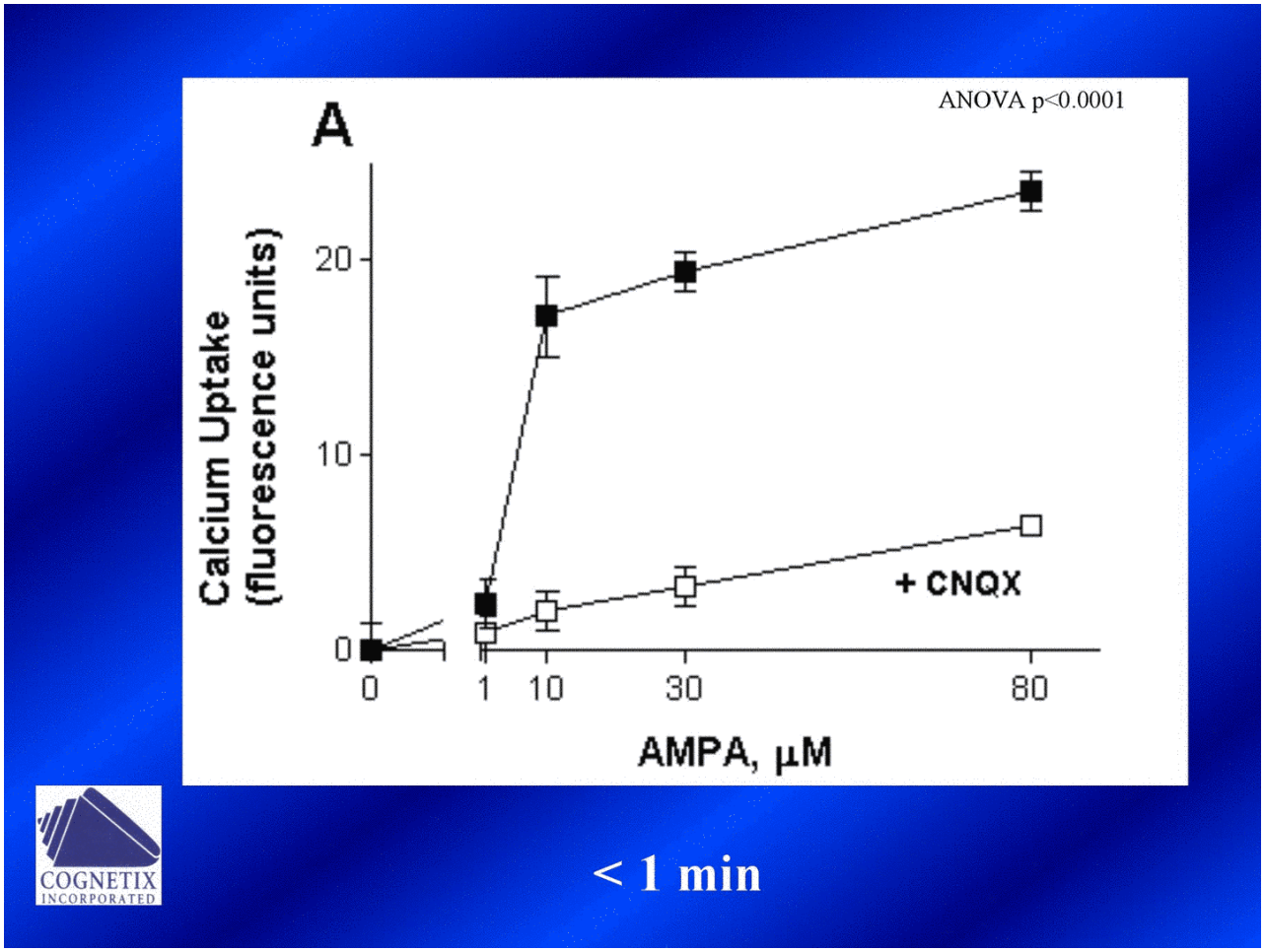

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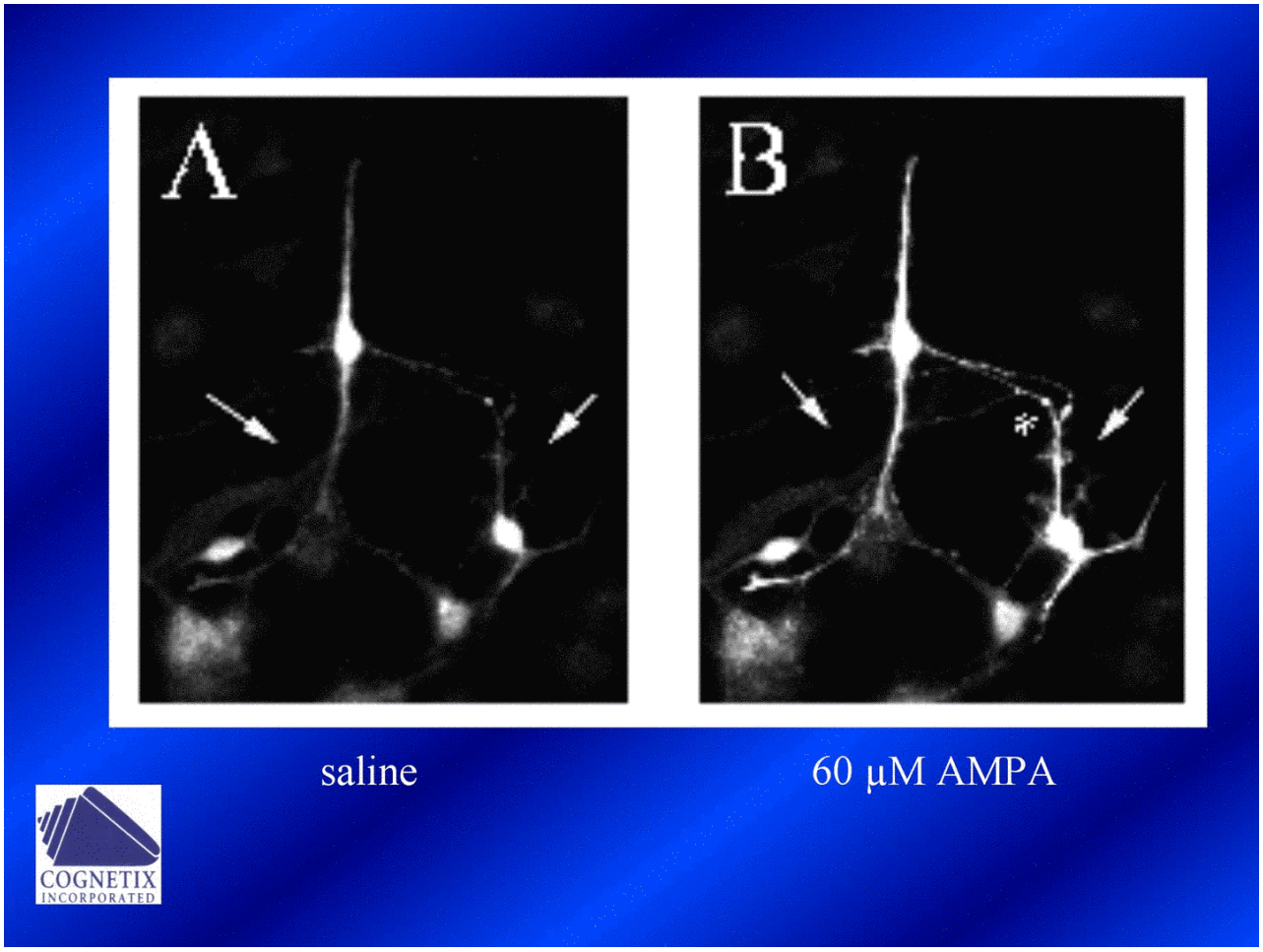
< 1 min



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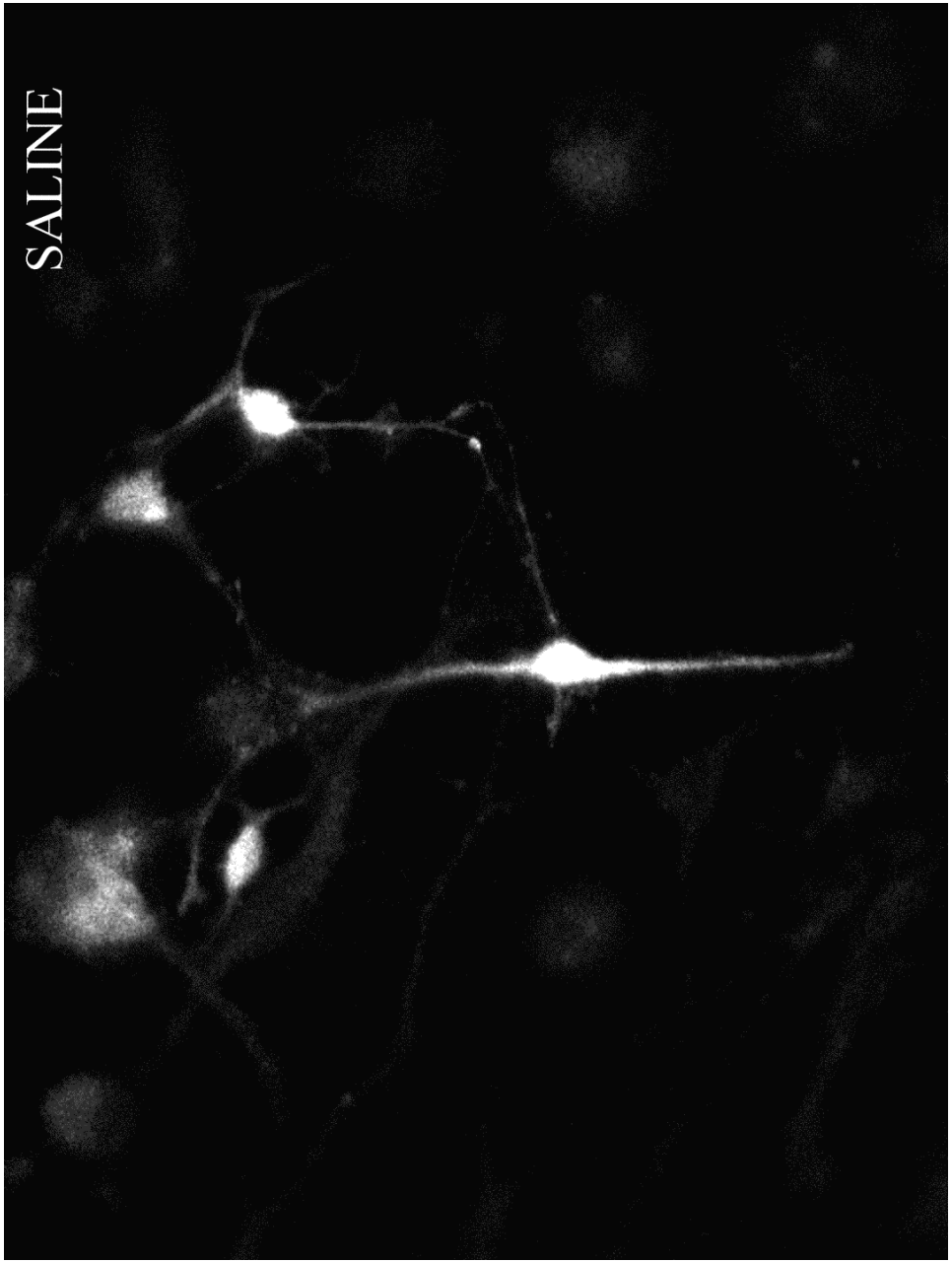
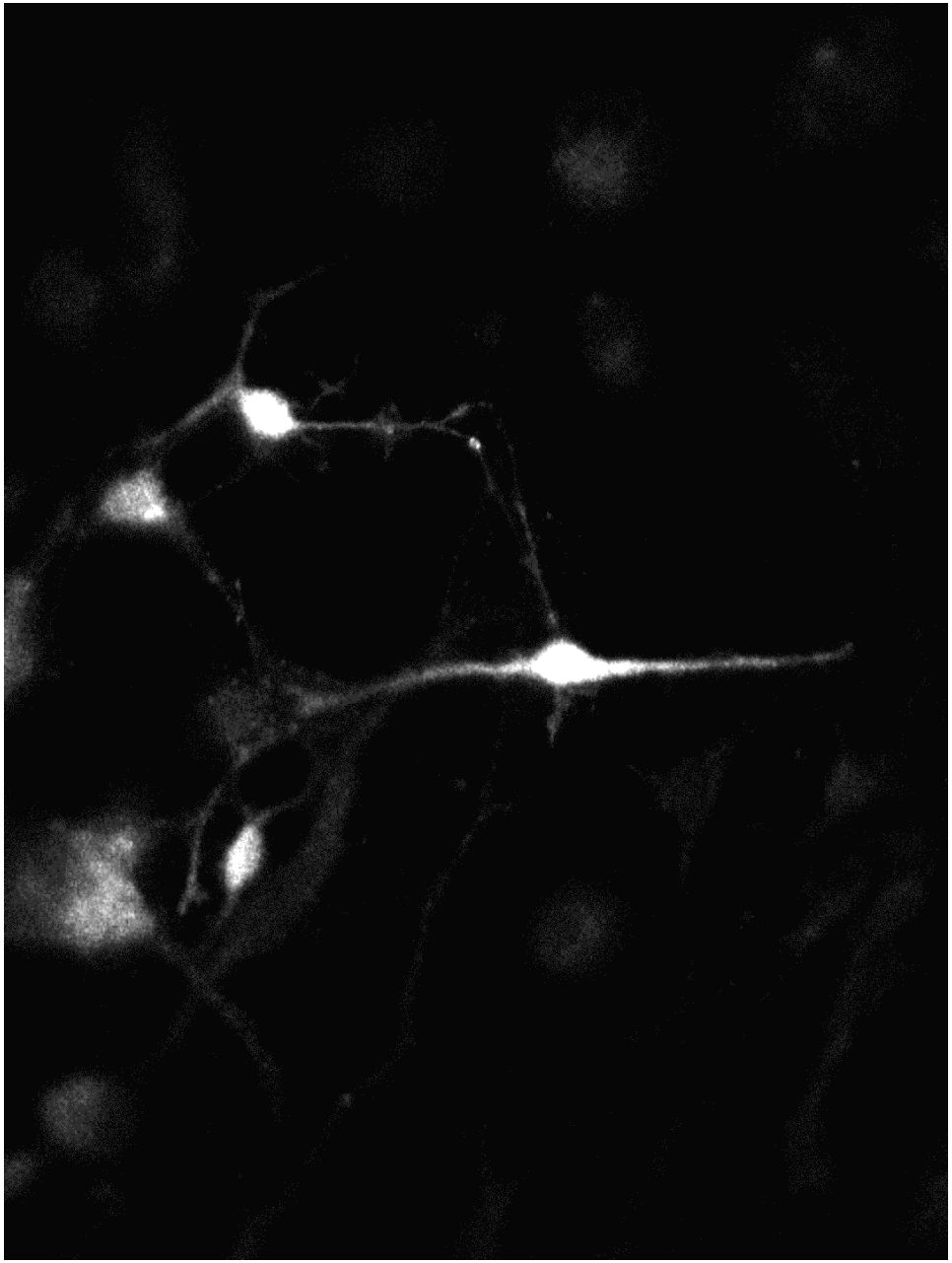
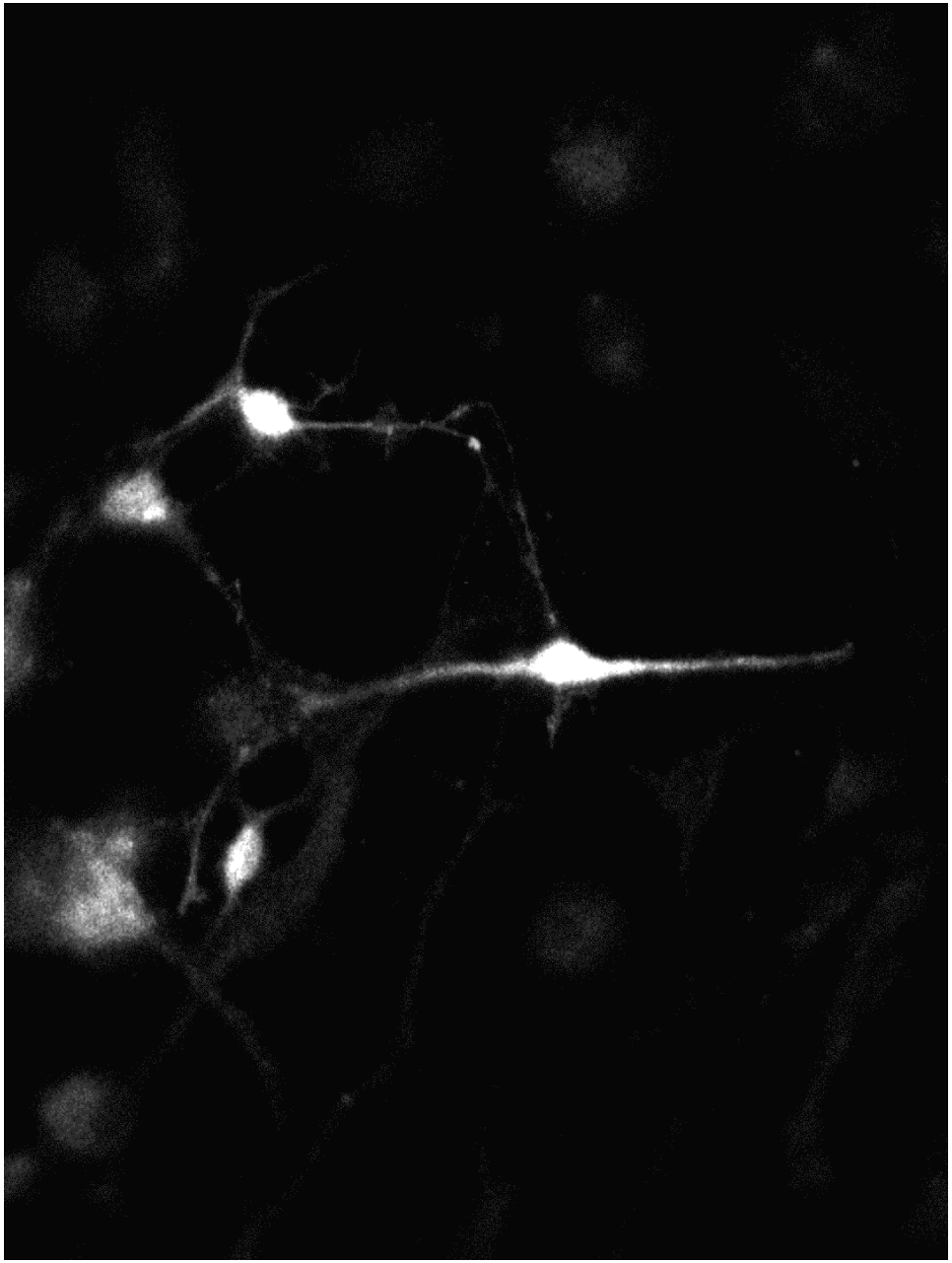
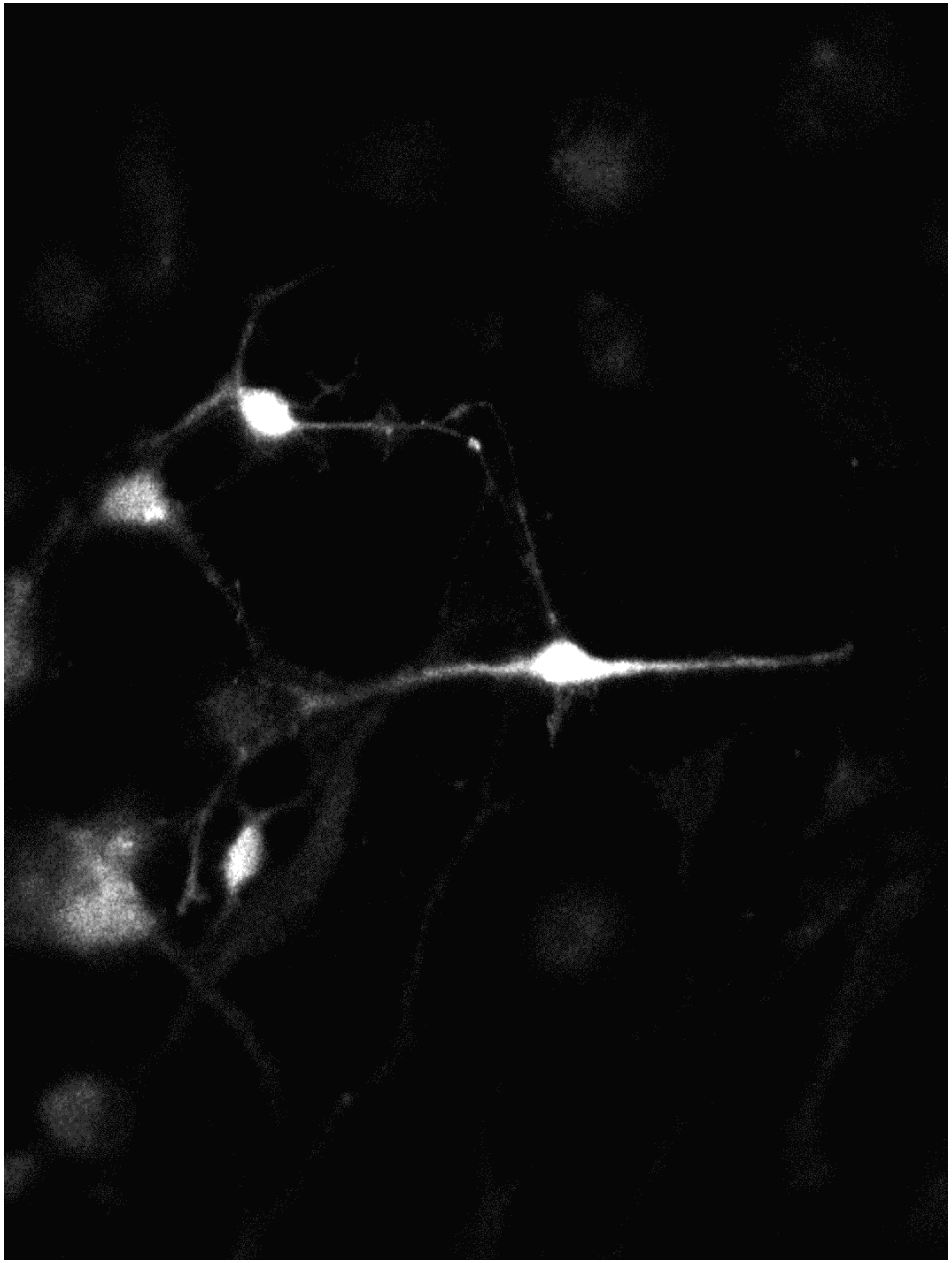
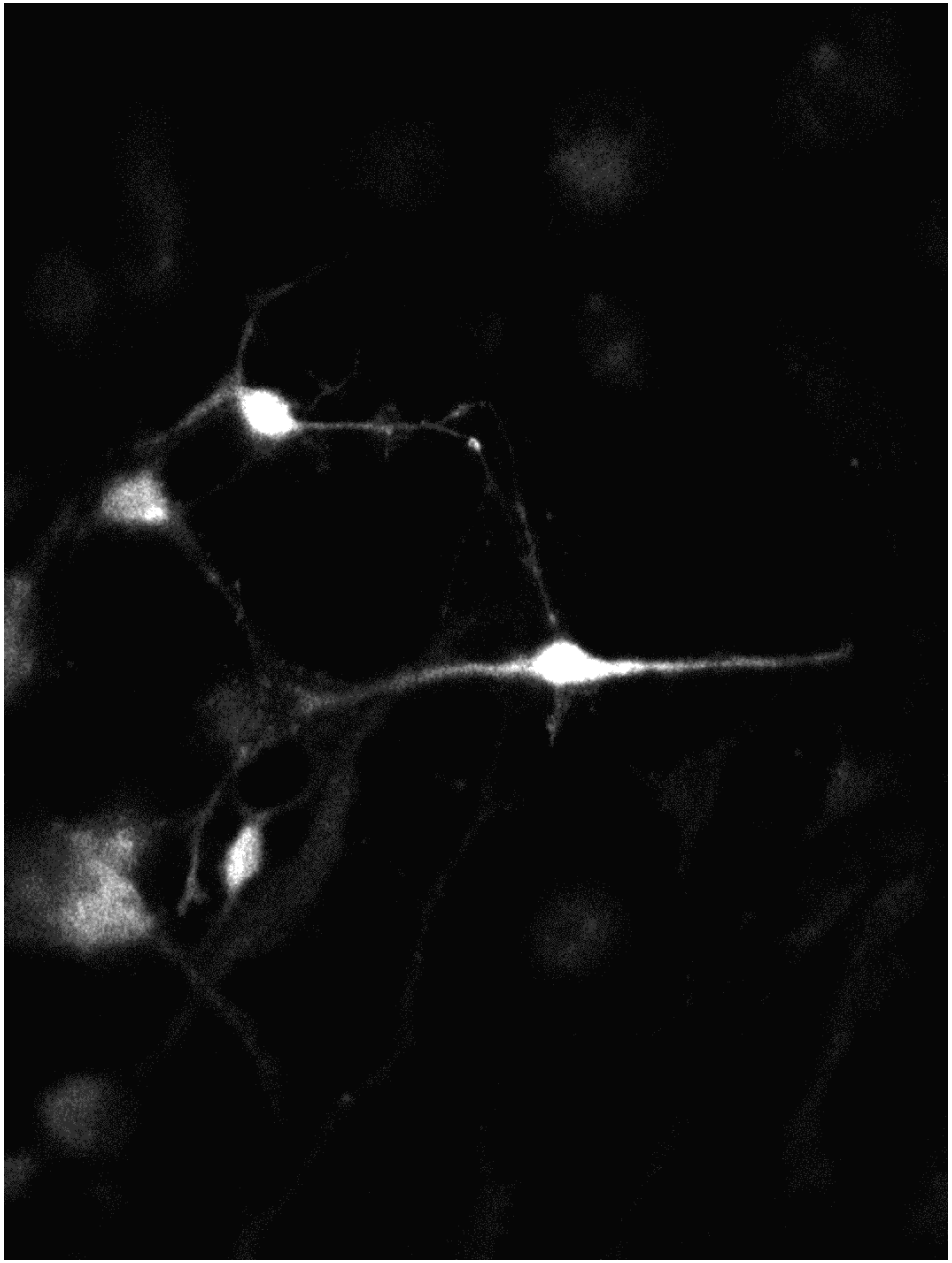
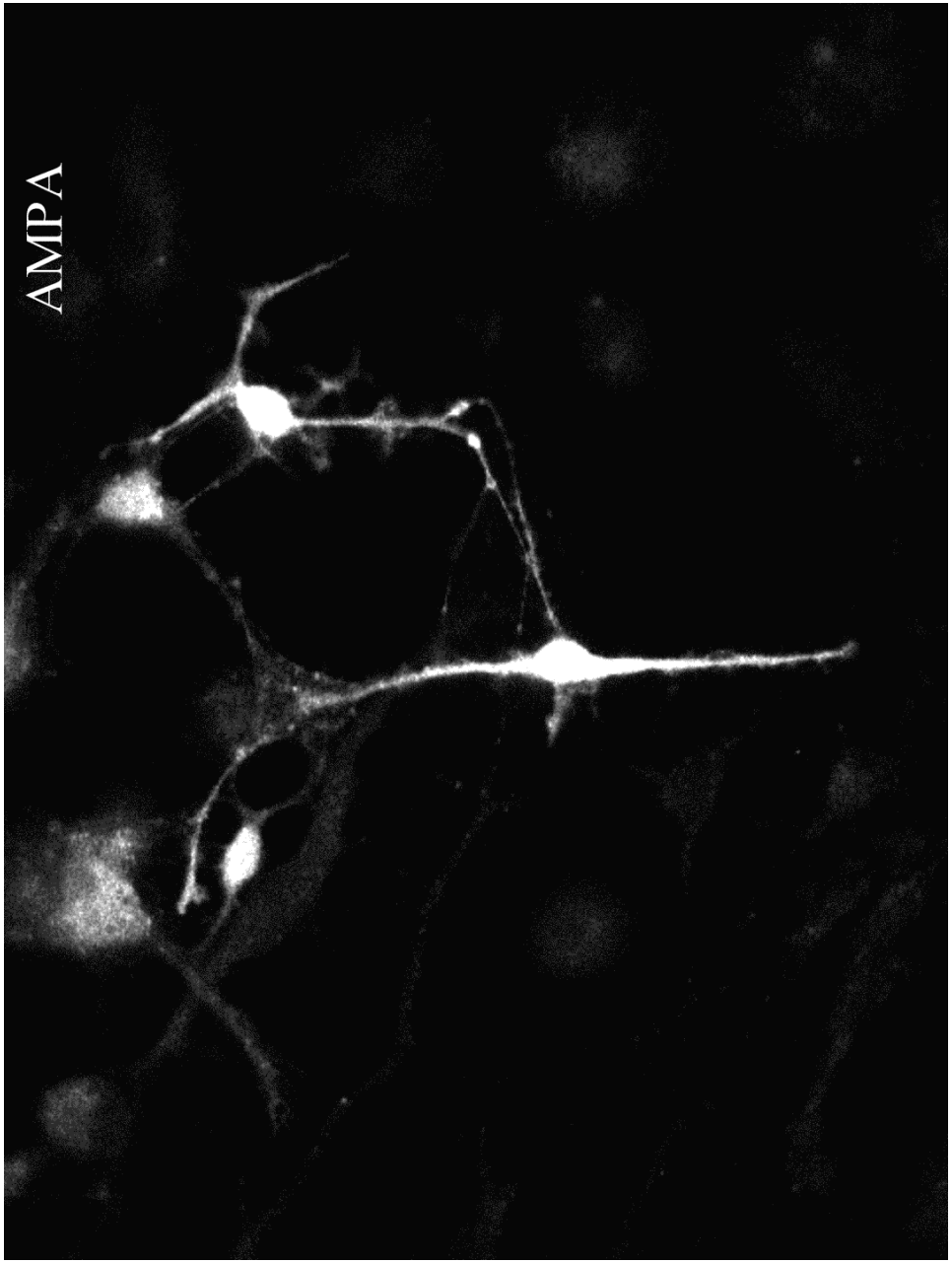

SALINE

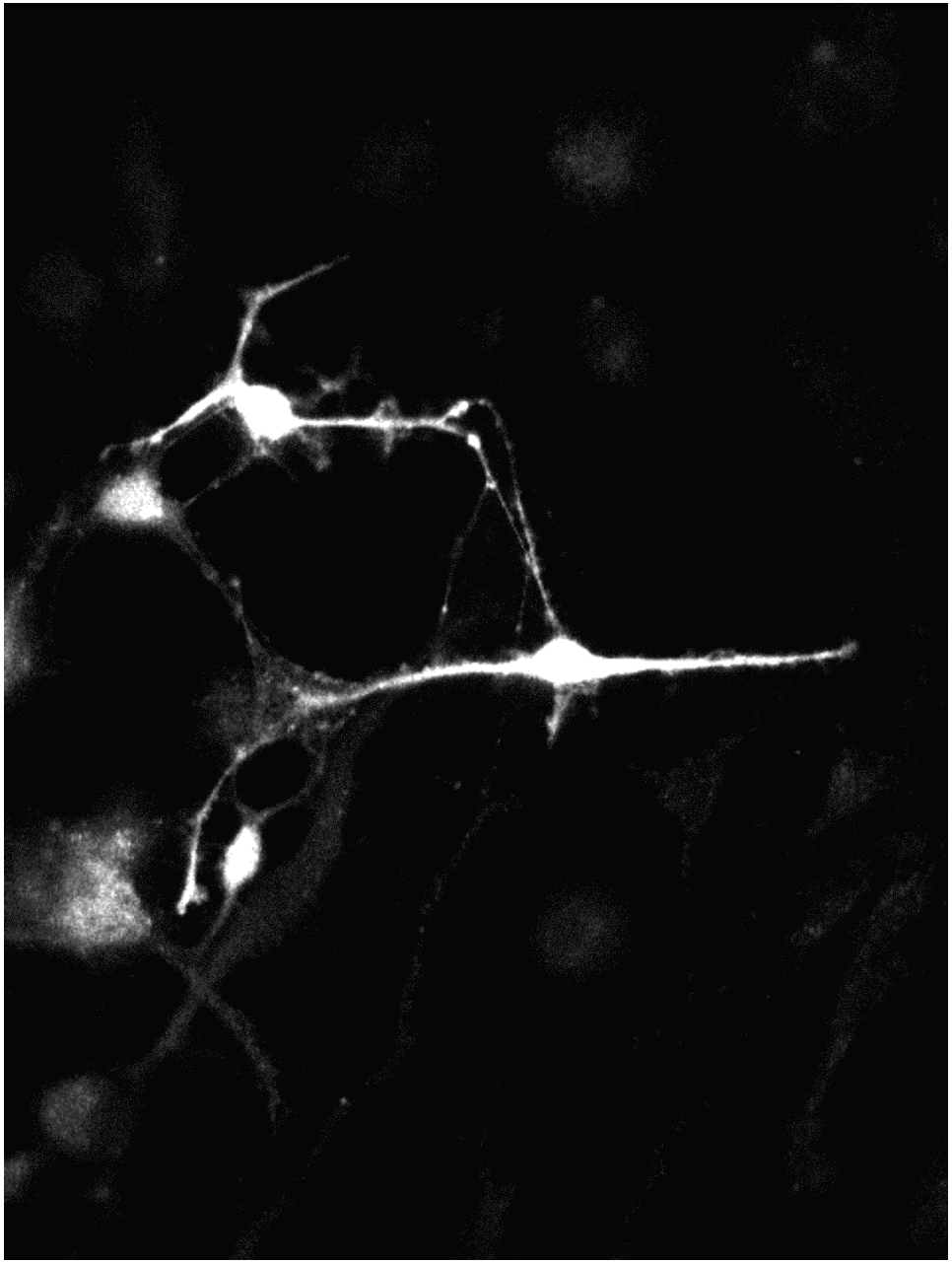


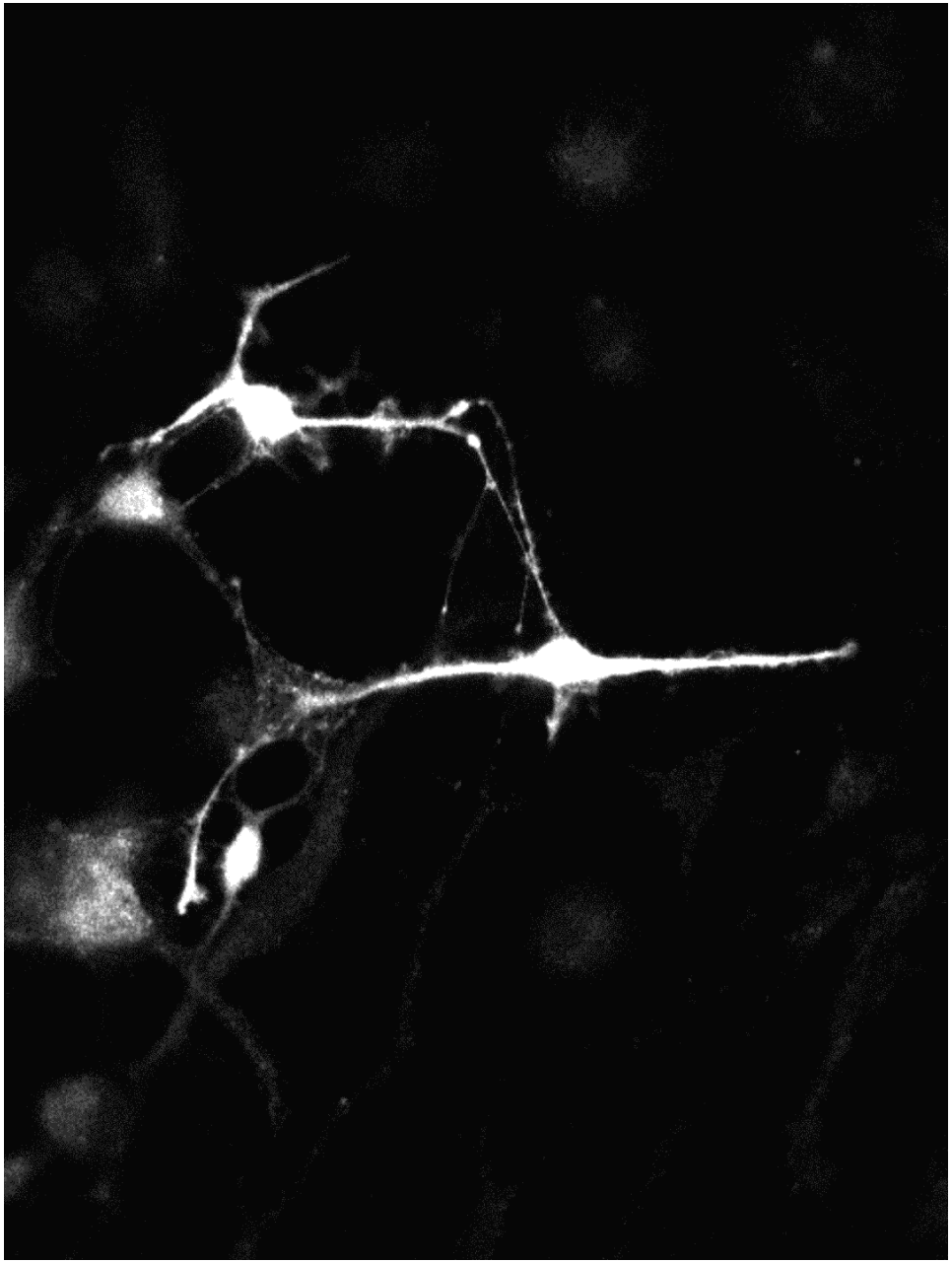


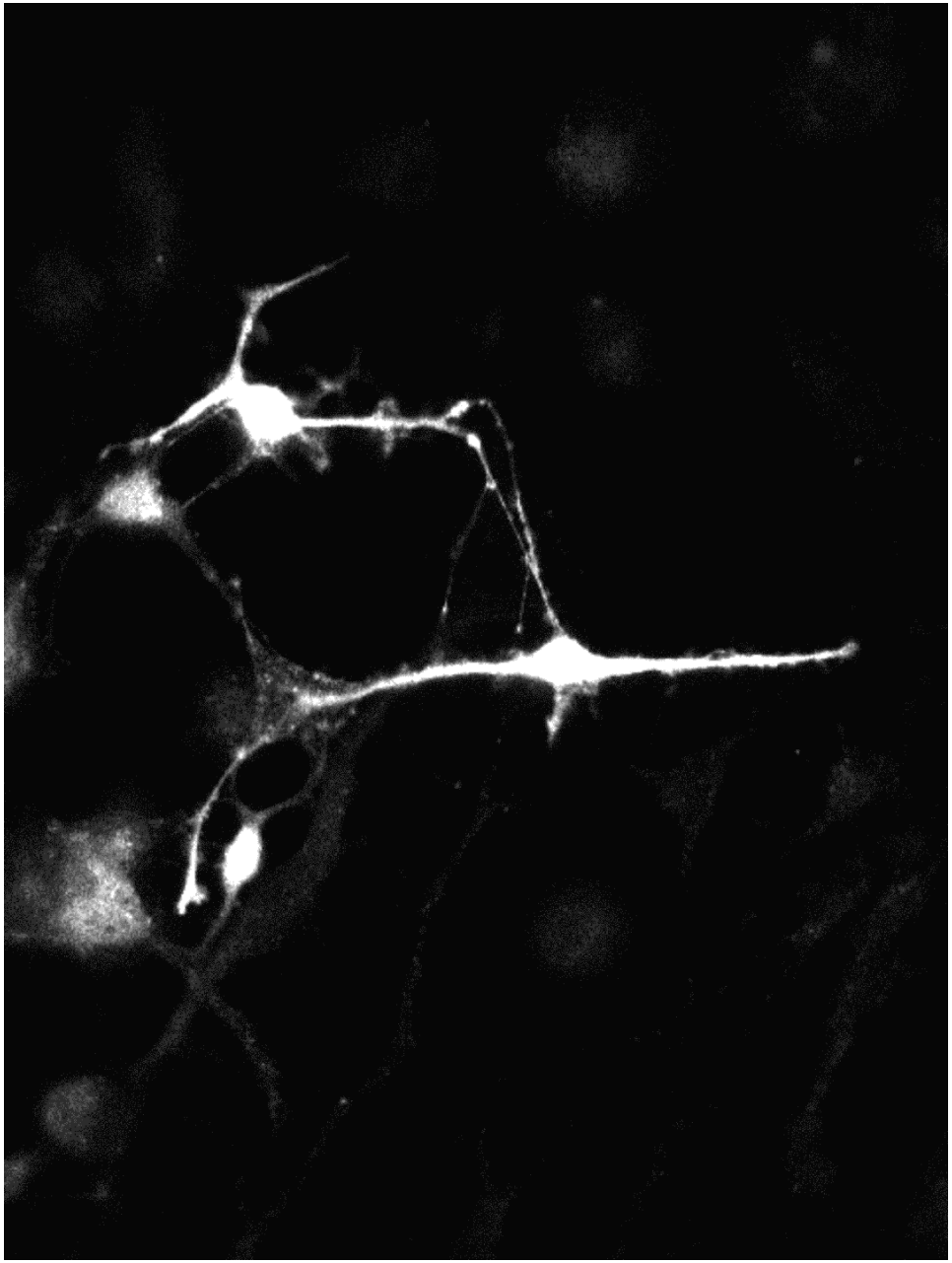


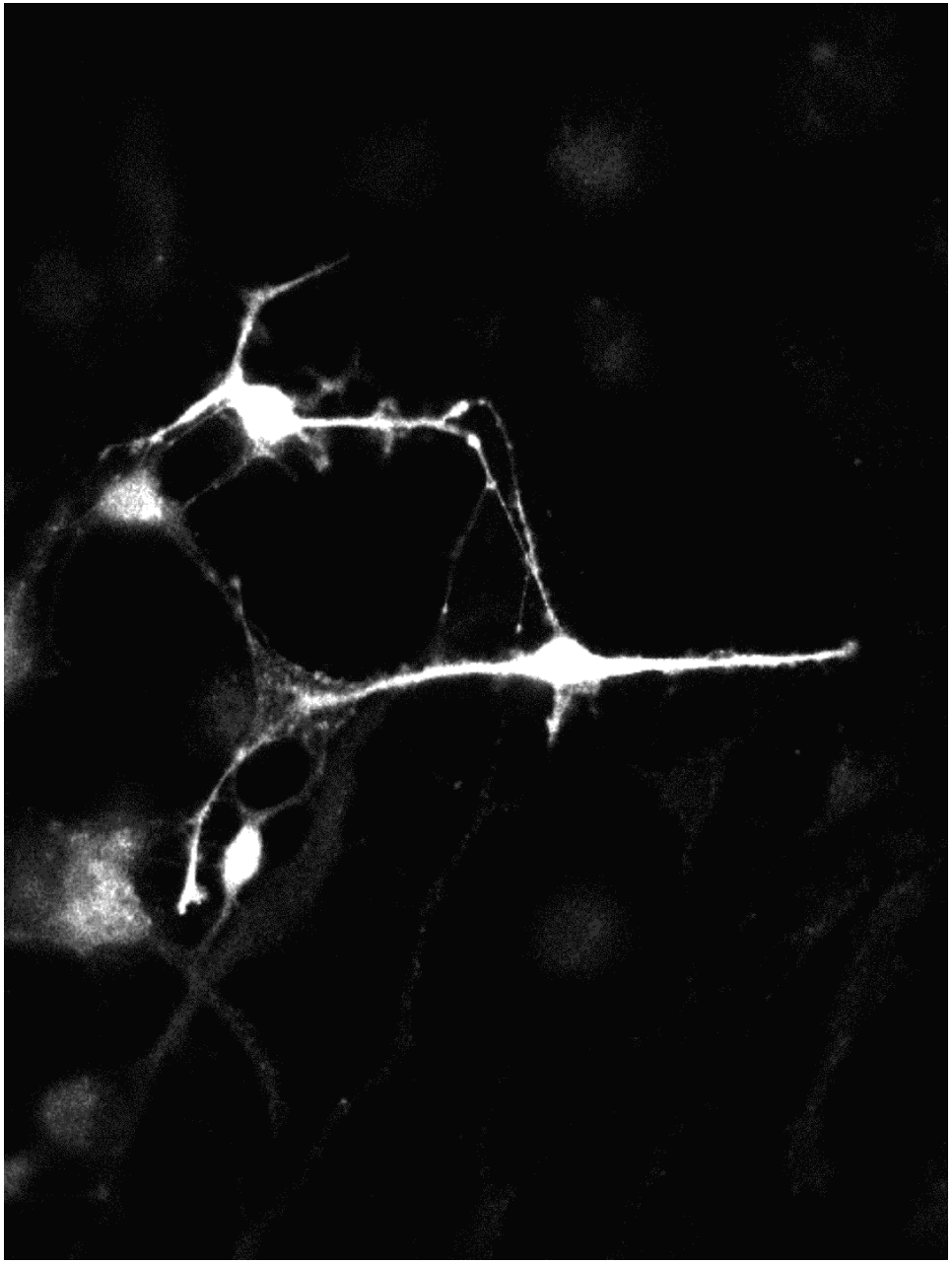


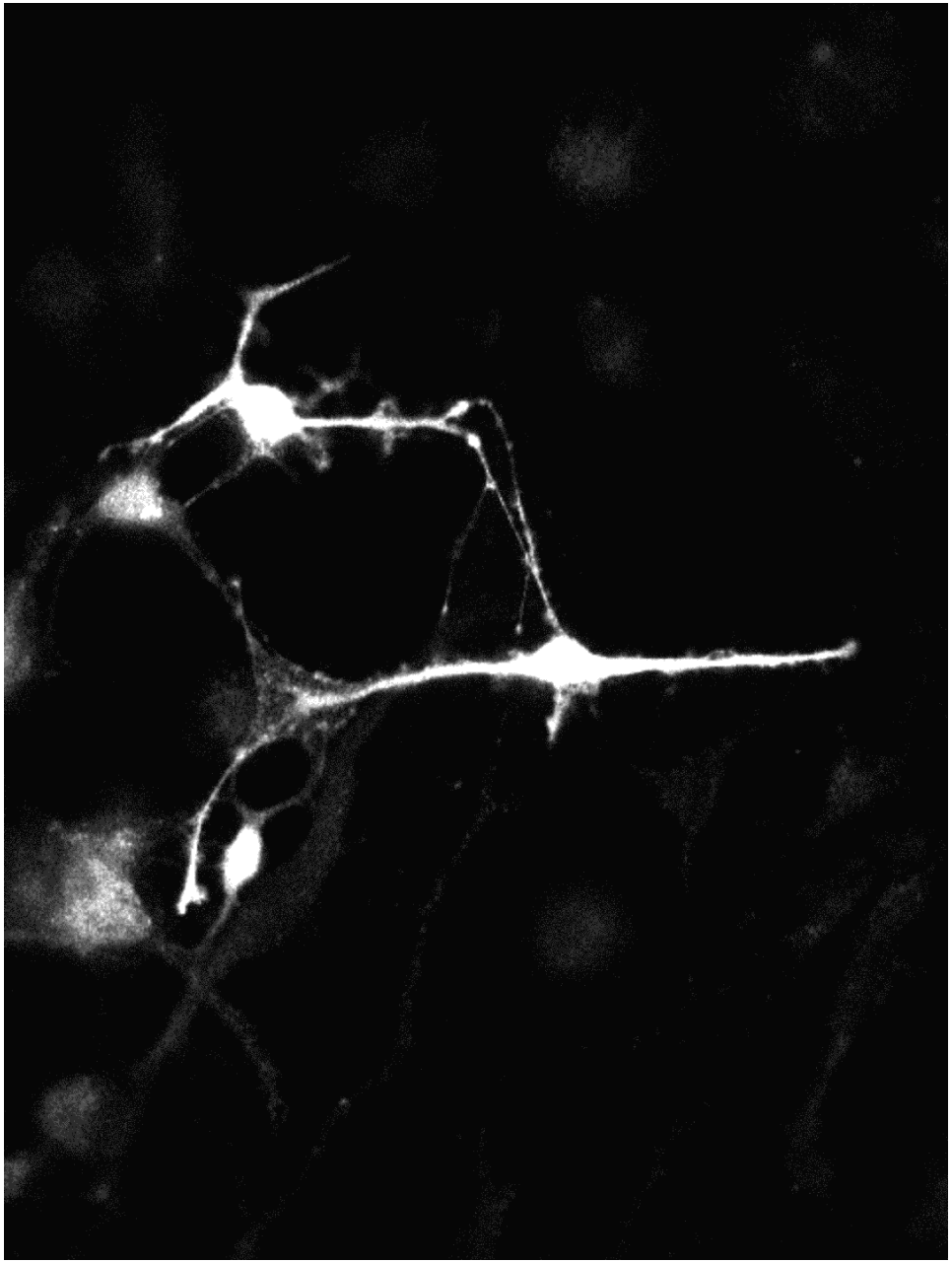

AMPA

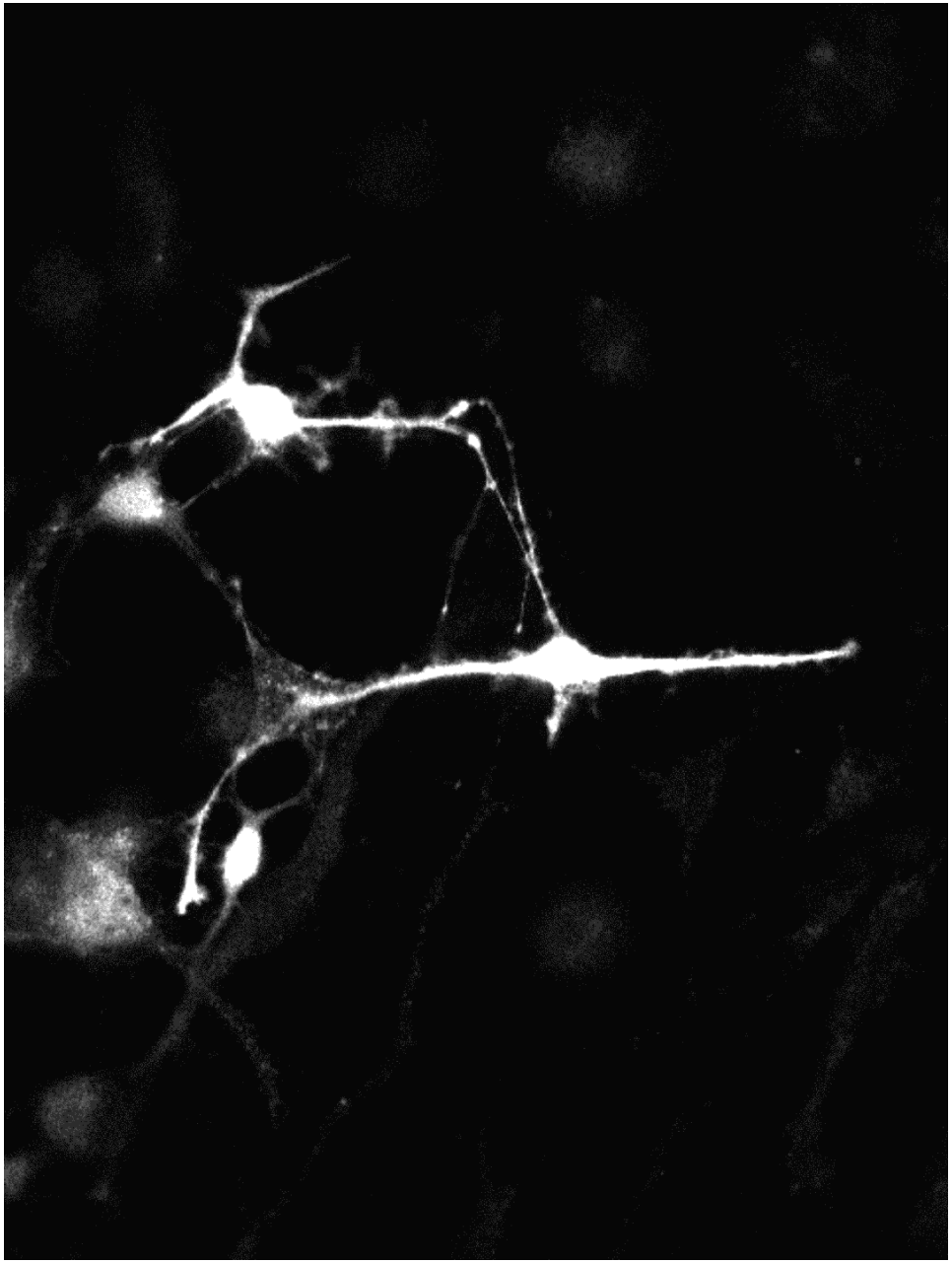


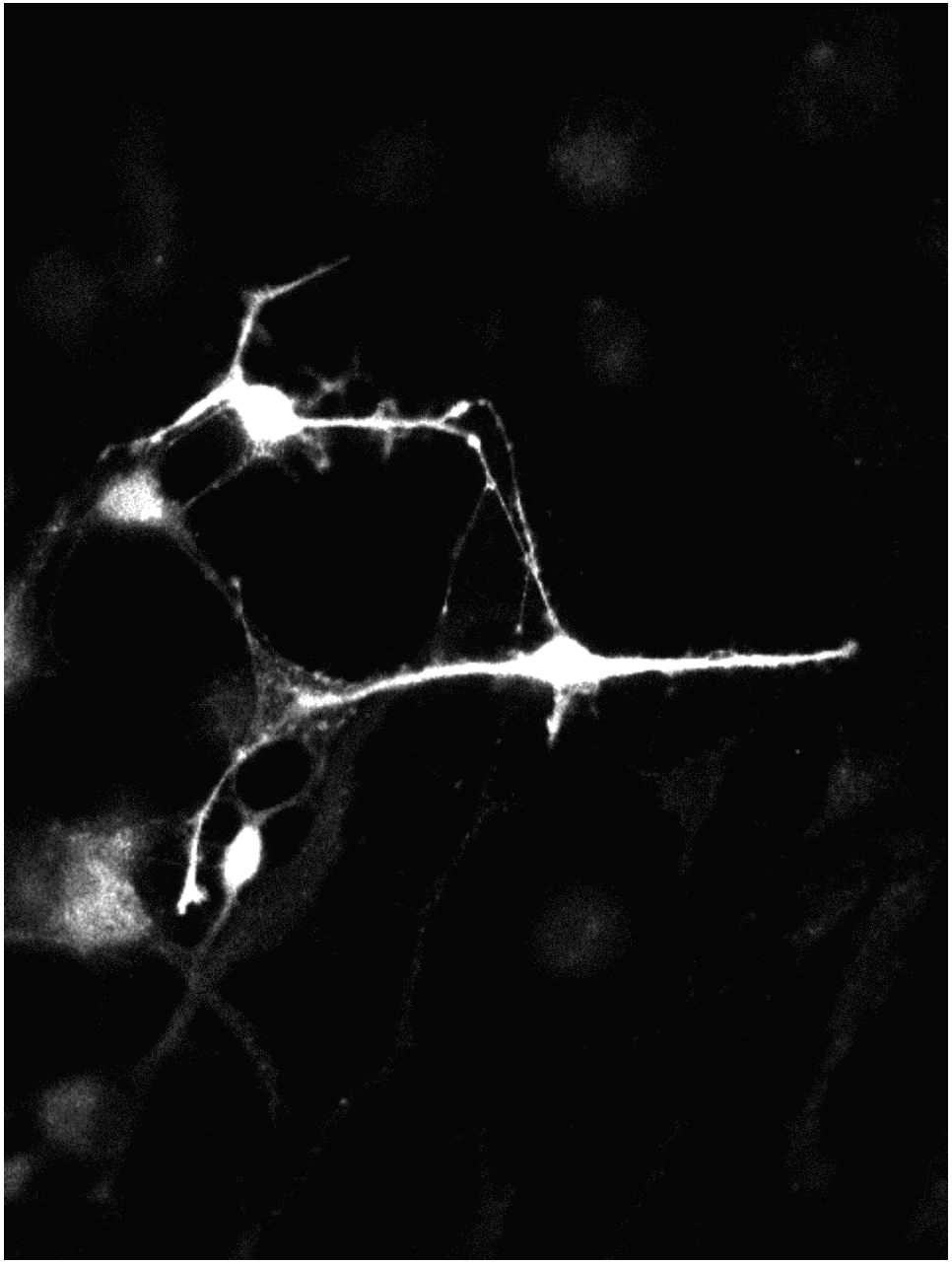


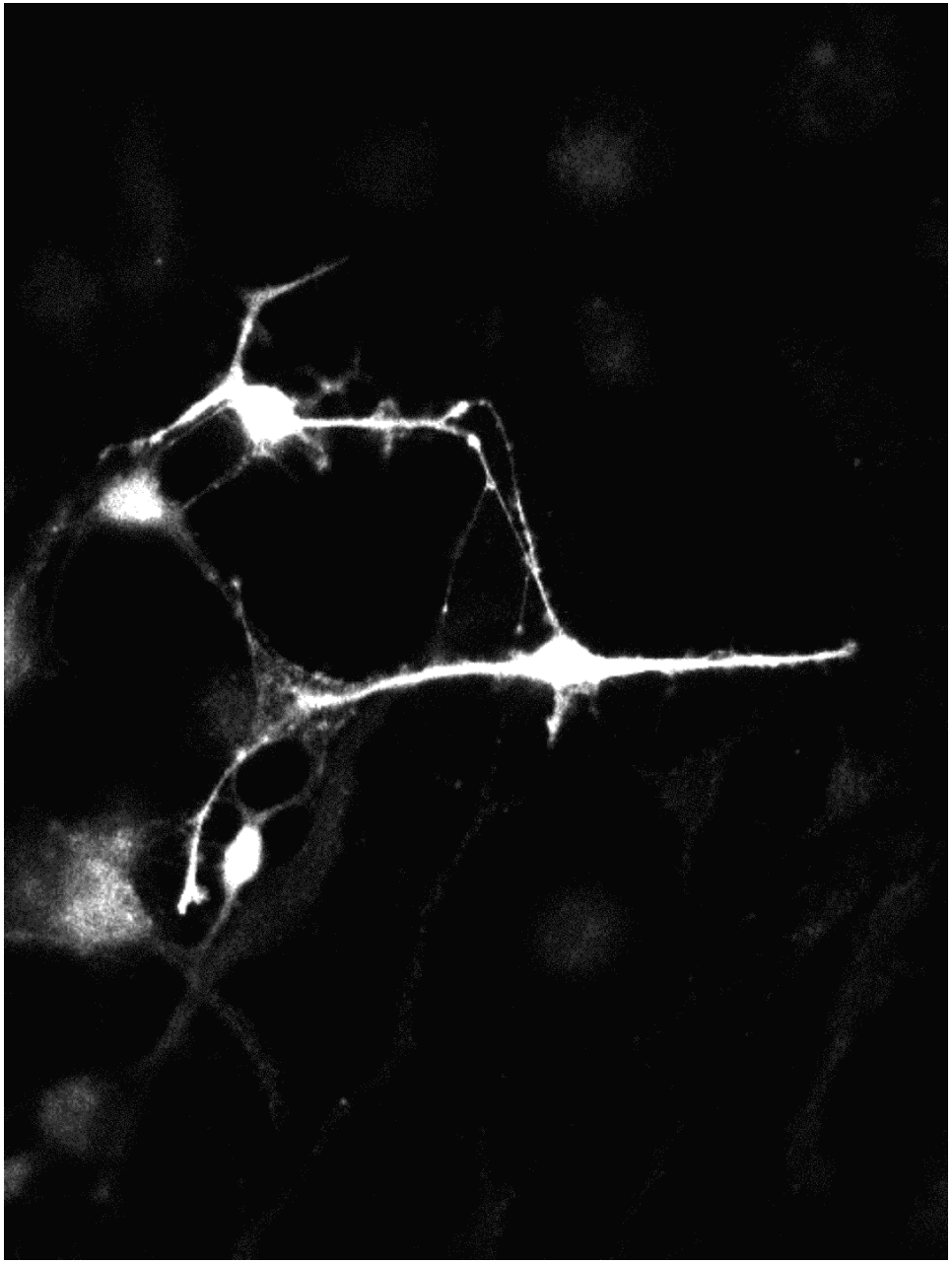


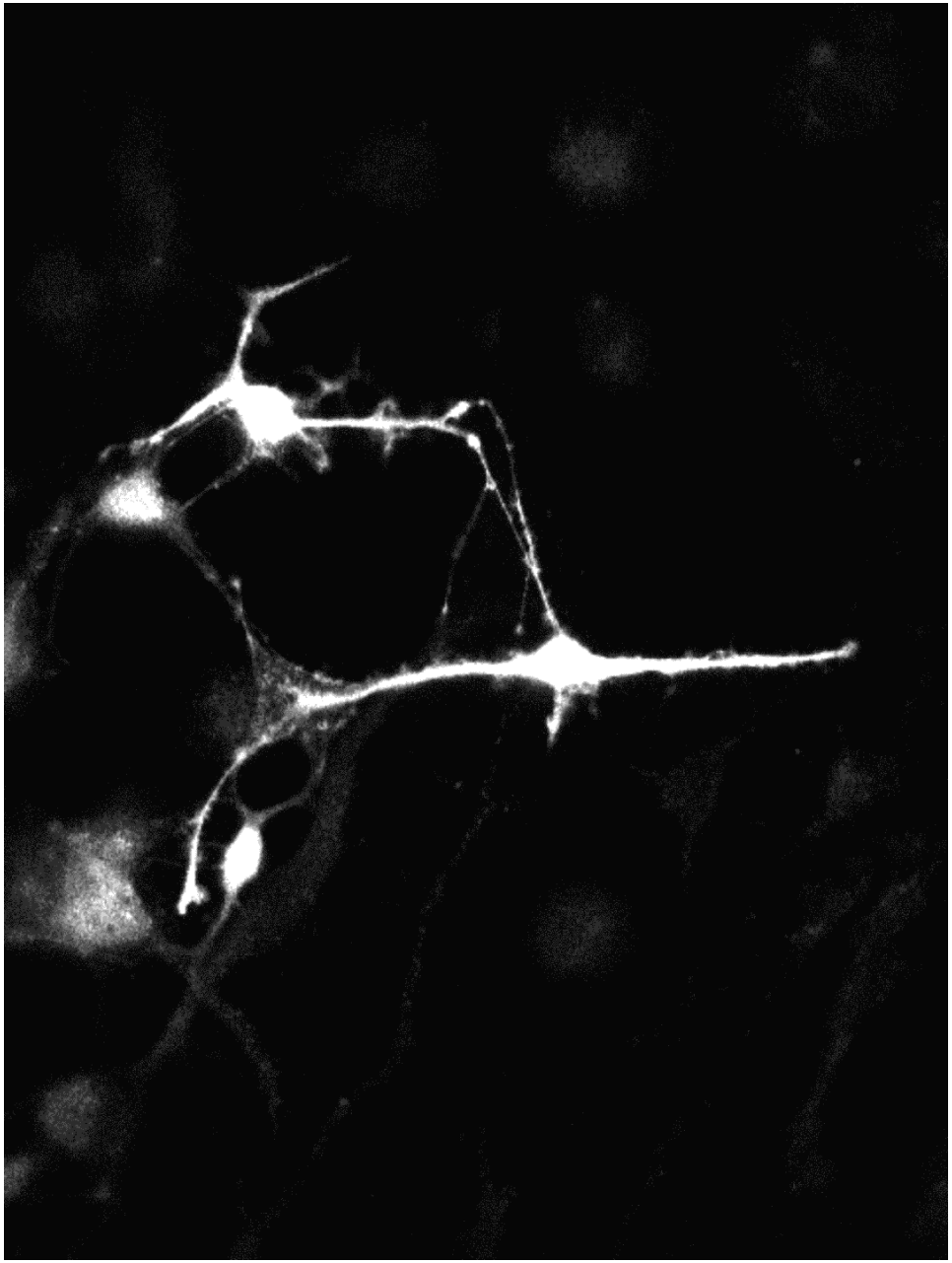


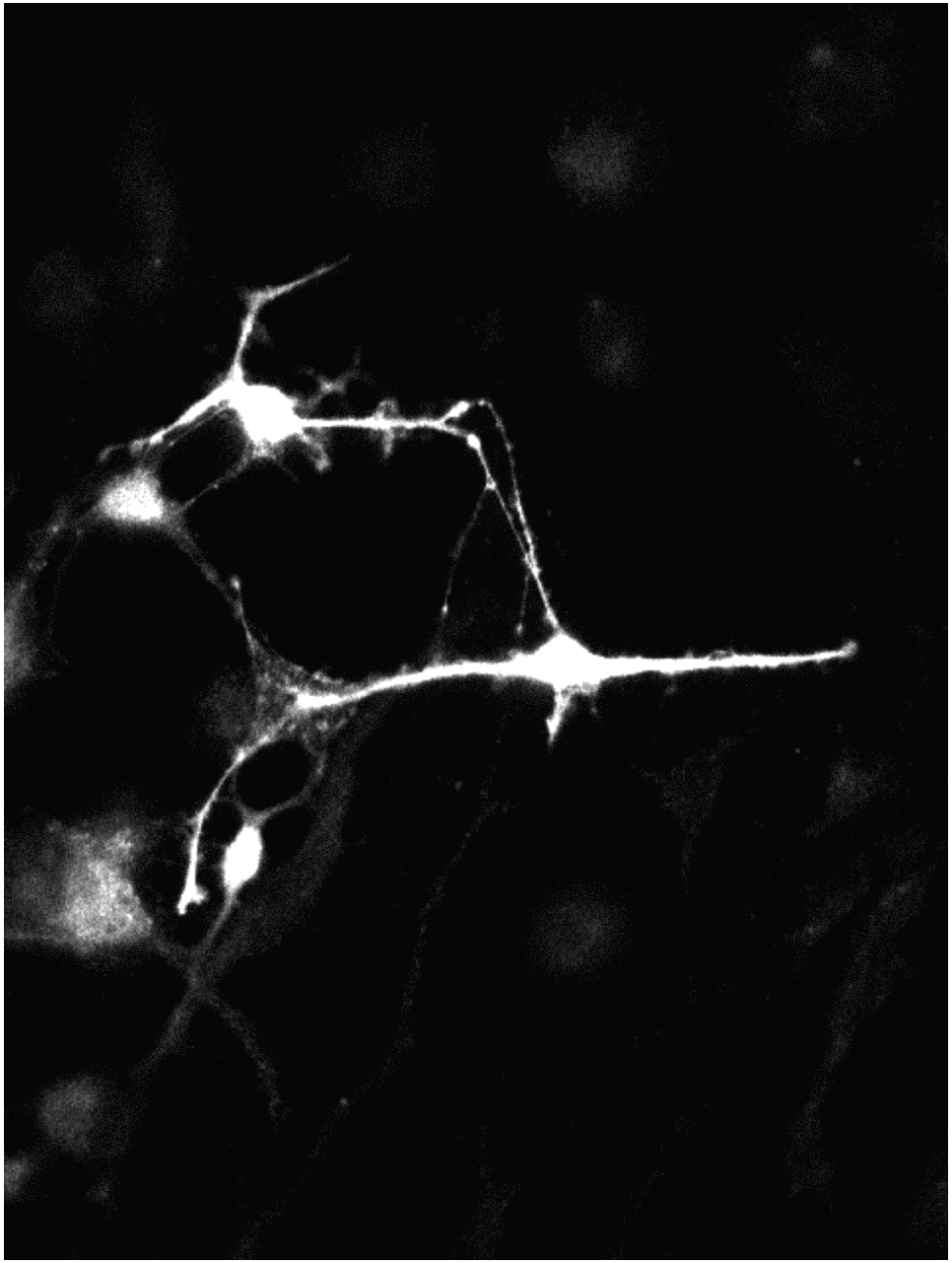












60 μM AMPA
AMPA +
10 nM DS-L
AMPA + DS-L
+ 30 μM CNQX
A
B
C
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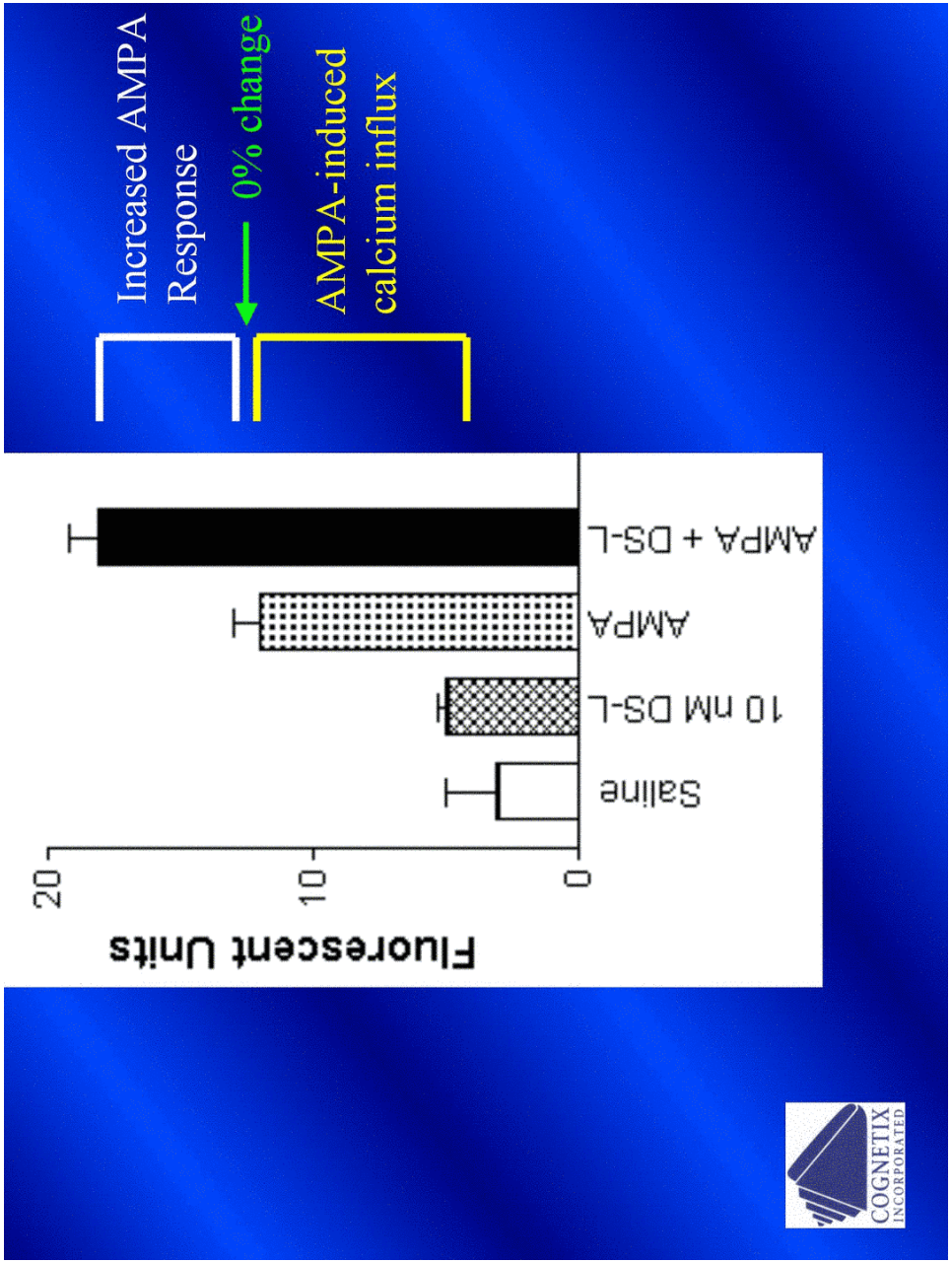
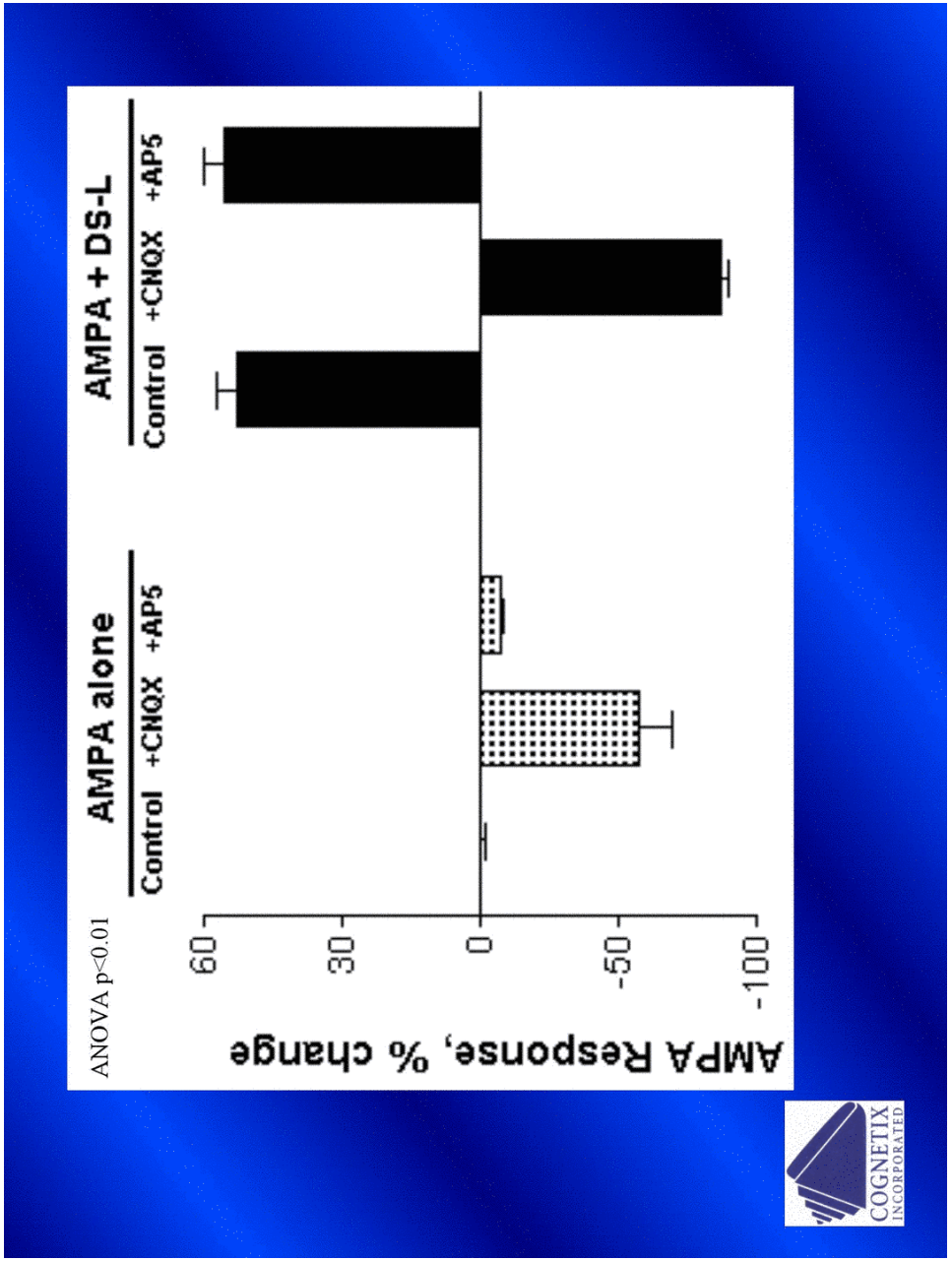
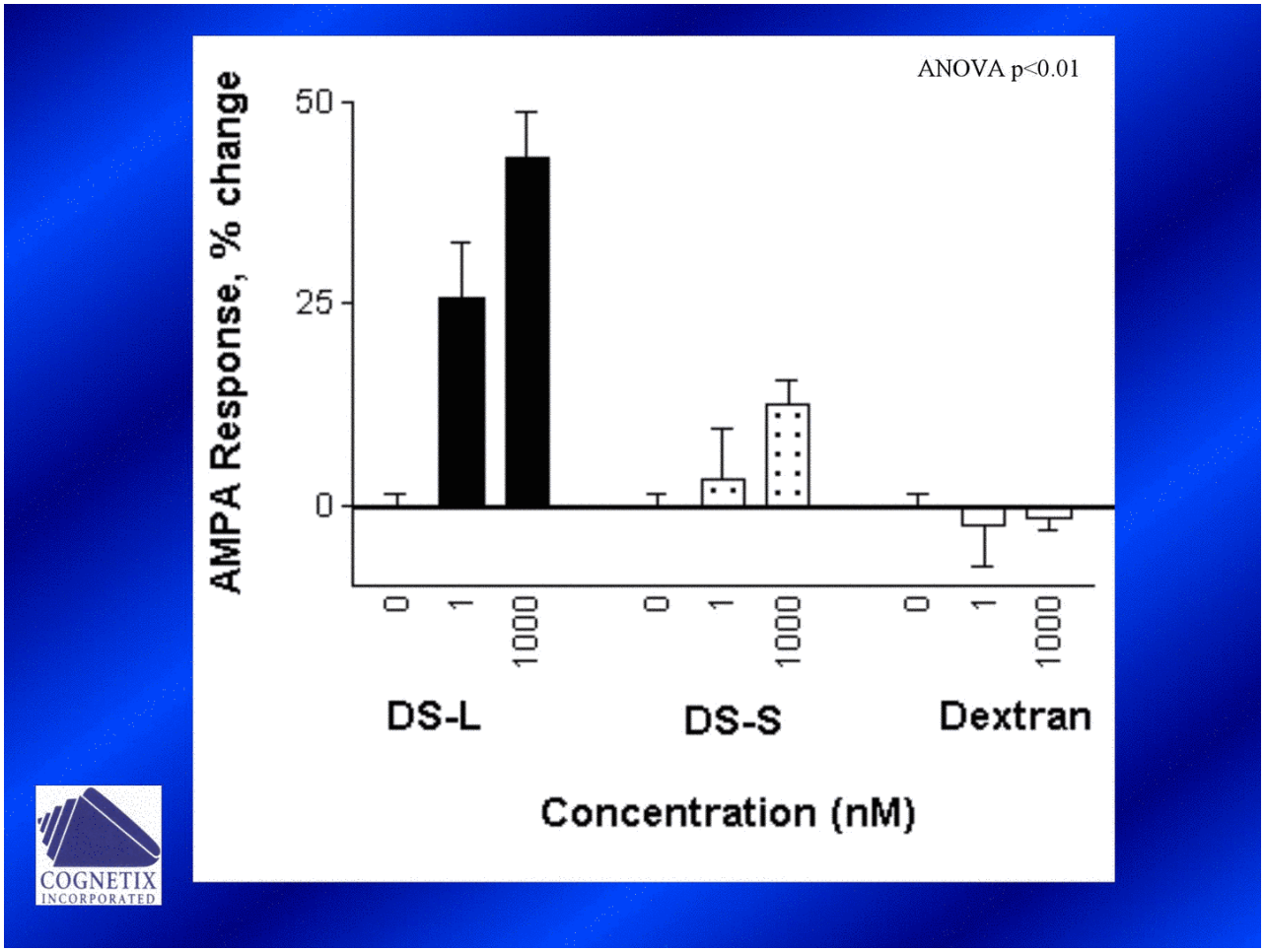
Fluorescent Units
20
10
0
Saline
10 nM DS-L
AMPA
AMPA + DS-L
+ calcium
- calcium
ANOVA p<0.05
COGNETIX
INCORPORATED


Increased AMPA Response
0% change
AMPA-induced calcium influx
Fluorescent Units
0
10
20
Saline
10 nM DS-L
AMPA
AMPA + DS-L
COGNETIX INCORPORATED


AMPA + DS-L
AMPA alone
Control +CNQX +AP5
Control +CNQX +AP5
AMPA Response, % change
60
30
0
-50
-100
ANOVA p<0.01
COGNETIX INCORPORATED


ANOVA p<0.01
AMPA Response, % change
50
25
0
0
1
1000
0
1
1000
0
1
1000
DS-L
DS-S
Dextran
Concentration (nM)
COGNETIX
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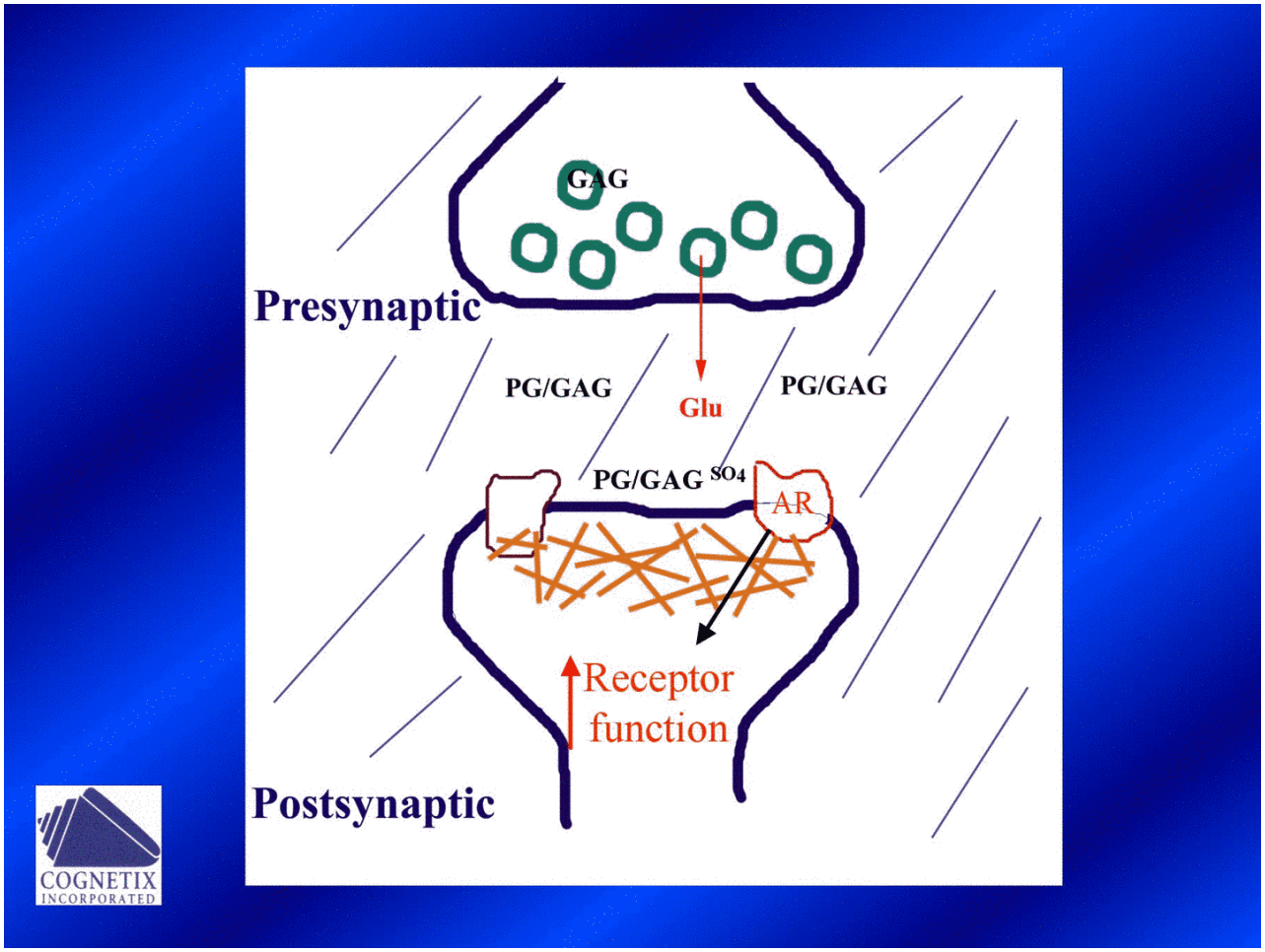
GAG
Presynaptic
PG/GAG
Glu
PG/GAG
PG/GAG SO4
AR
Receptor
function
Postsynaptic
COGNETIX
INCORPORATED

Part II: Neuroprotection

- **Dissociated hippocampal cell system**
  - DS-L-mediated protection
  - Correlation between receptor modulation and neuroprotection
- **Hippocampal slice culture system**
  - Maintenance of synaptic integrity
  - MAPK link

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Dissociated Hippocampal Cell System

- **DS-L-mediated protection**
  - Sulfate dependence
  - Size dependence

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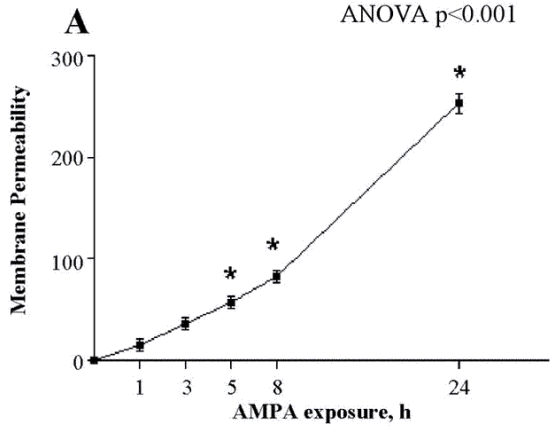
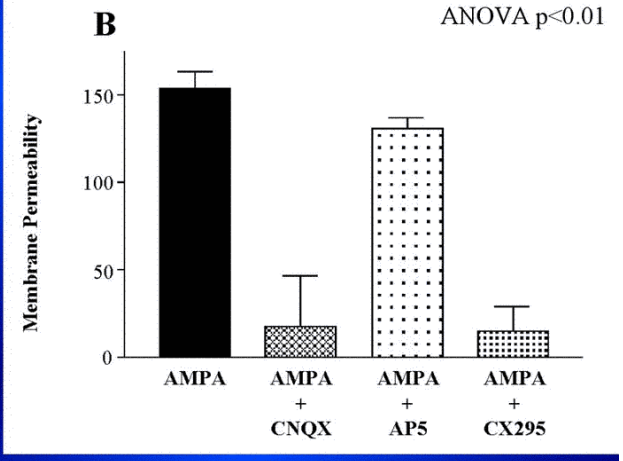
24 hr AMPA





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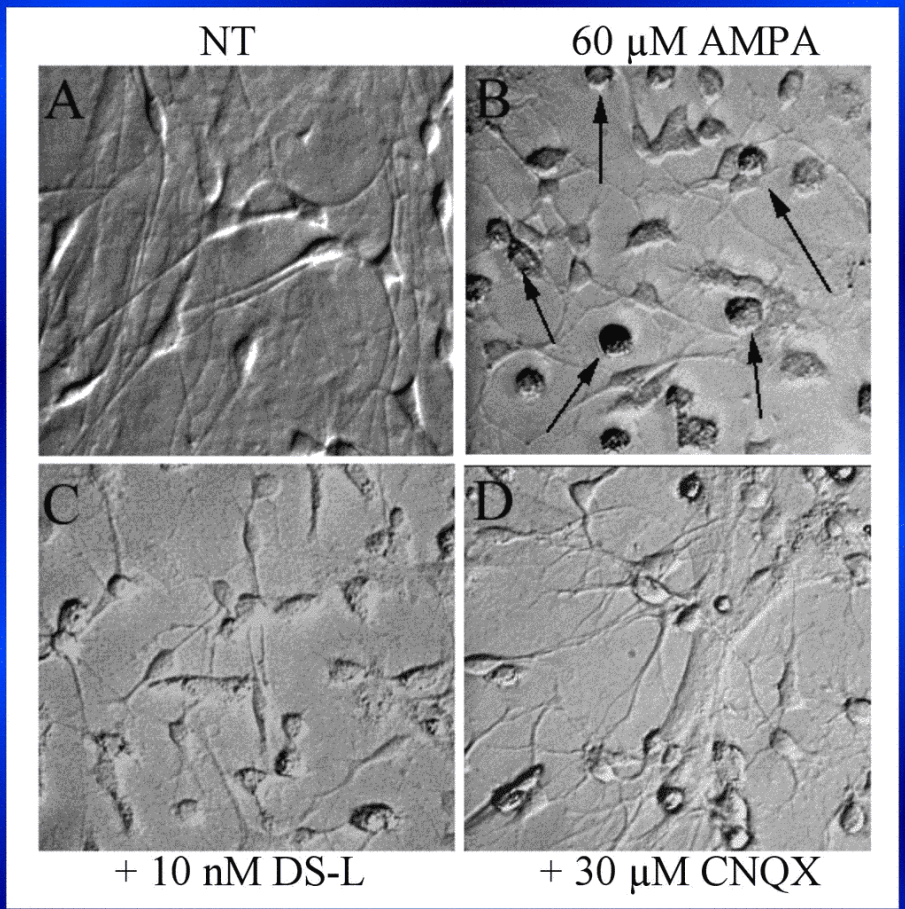

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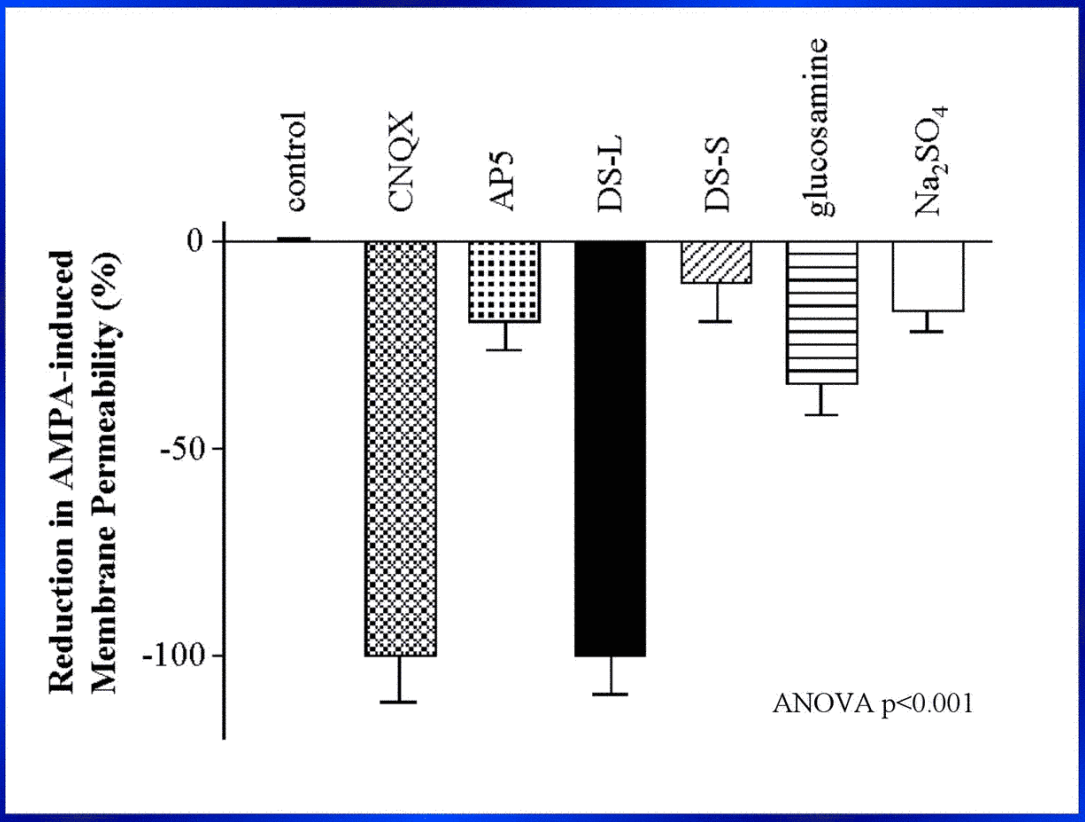
Antagonist and Polysaccharide-mediated protection



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Links to Receptor Regulation

- Correlation
  - Sulfate dependence
  - Size dependence

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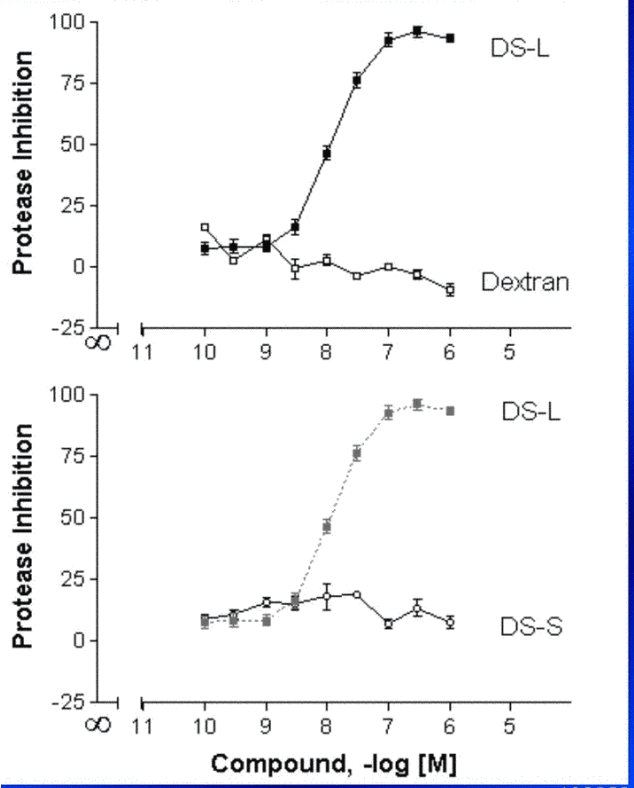
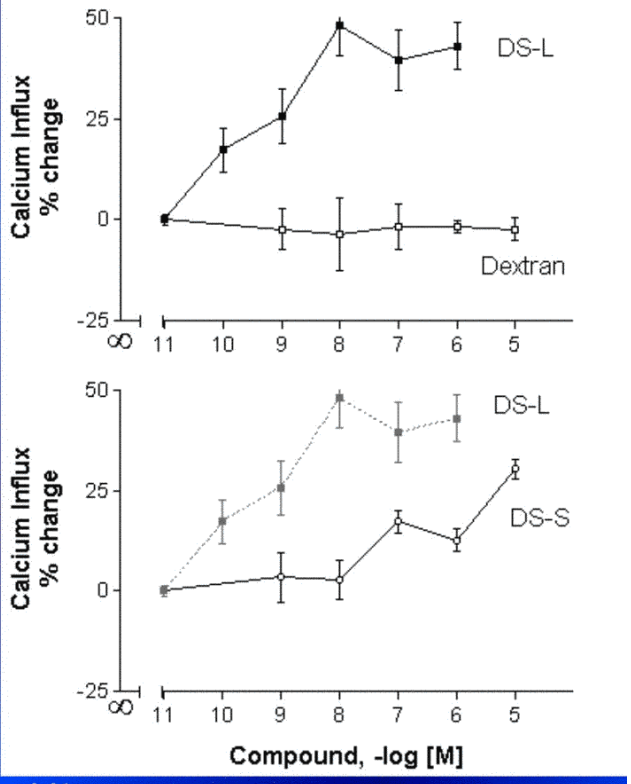
Block Neuronal Damage



AR Modulation



ANOVA $p<0.01$

Links to Synaptic Integrity

- Pre-synaptic synapsin
- Post-synaptic GluR1, spectrin breakdown

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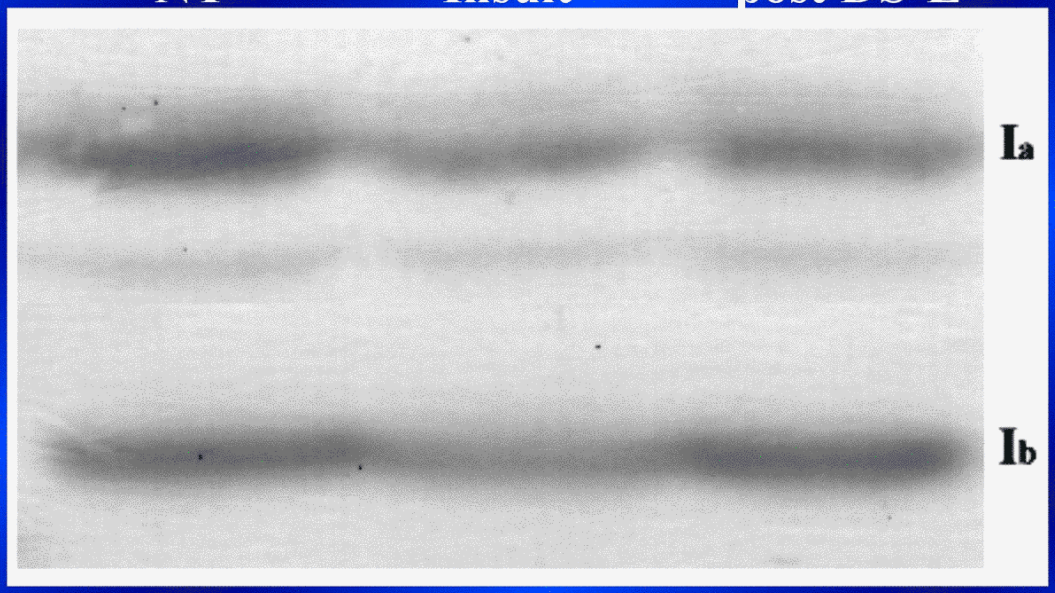
Pre-synaptic Synapsin



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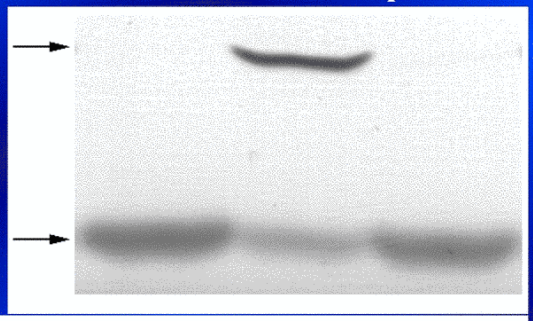
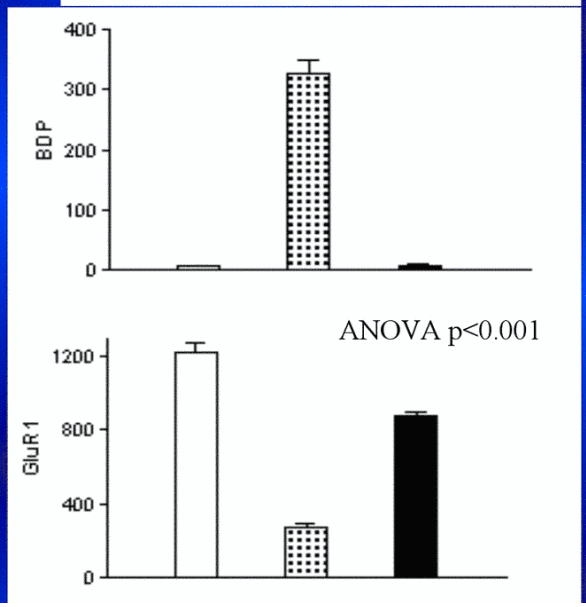
Spectrin breakdown

GluR 1





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Links to Survival Signaling

- MAPK (mitogen-activated protein kinase)
  - ERK 1 and ERK 2 (extracellular related kinase)

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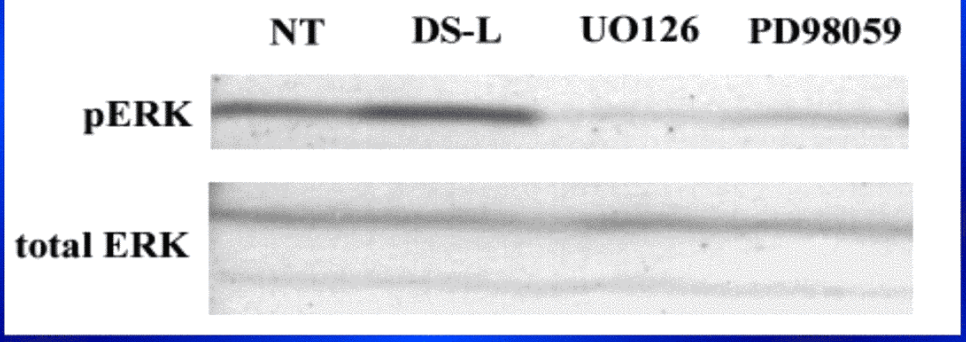

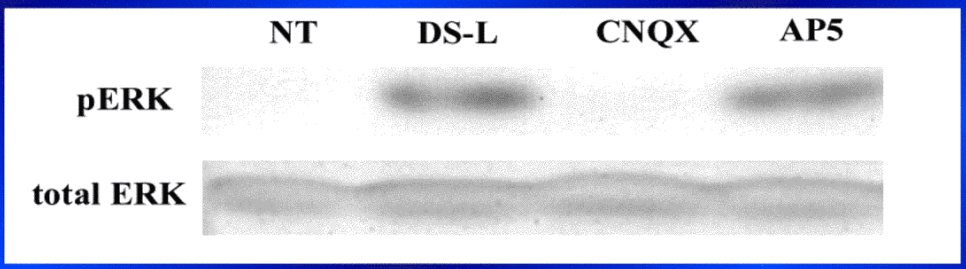

AR → MEK → MAPK (anti-pERK)

UO126 (−) MEK

PD98059 (−) MAPK (anti-pERK)

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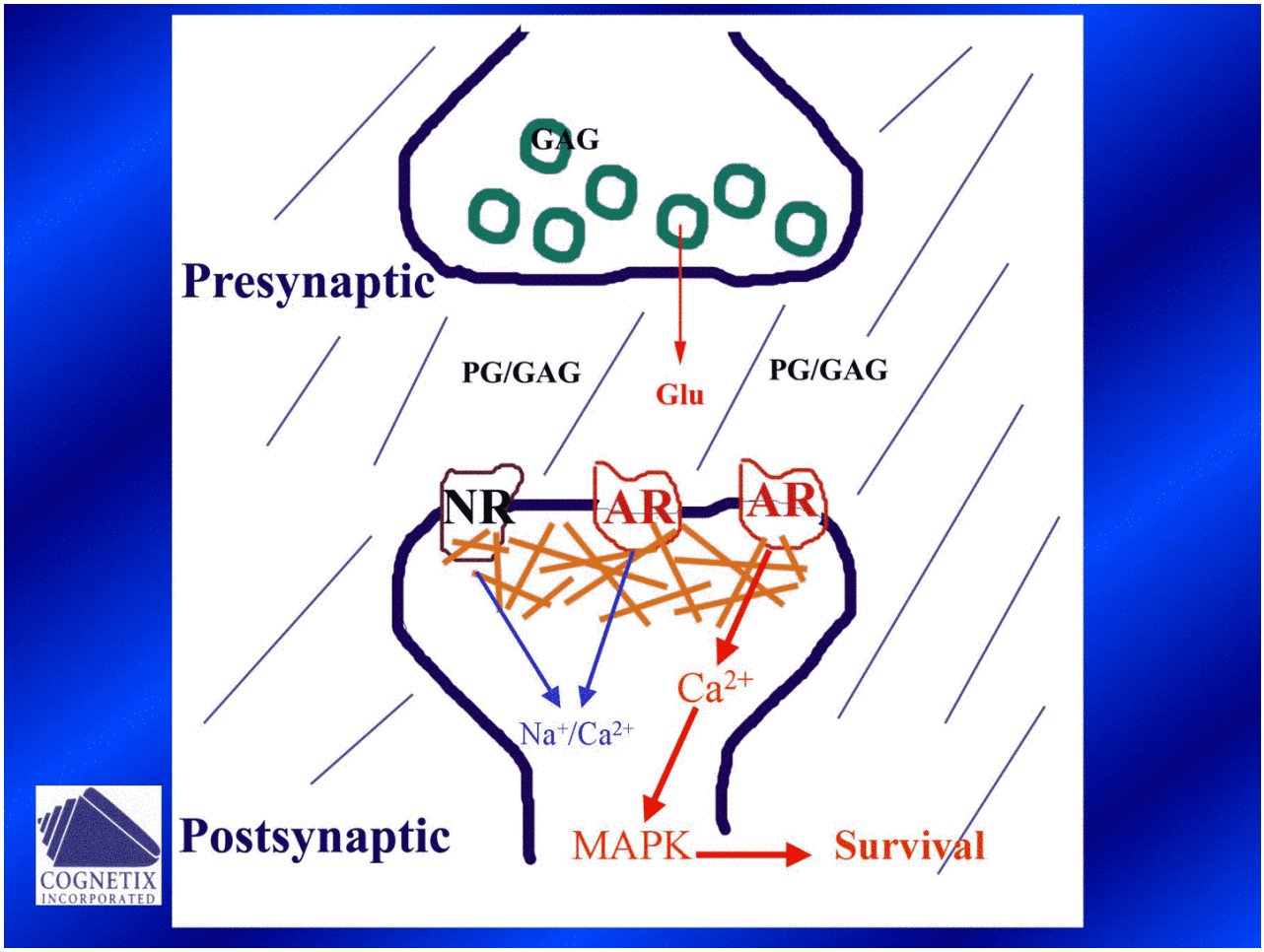

Conclusions

- Dextran sulfate positively modulates AMPA receptor activity to regulate channel kinetics and binding affinity
- Dextran sulfate enhances the AMPA-mediated calcium response
- AMPA receptor modulation mediated by dextran sulfate may be linked to the maintenance of synaptic integrity and the ERK/MAPK survival pathway

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- Kehinde Ajayi