

# Expression of the immediate early gene *Arc* in ventral tegmental neurons during aging. 391.21

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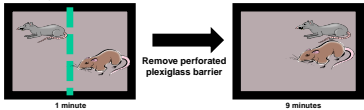
## ABSTRACT

Accumulating evidence suggests that neurons of the ventral tegmental area (VTA) of the midbrain are highly correlated with reward (e.g., Schoenbaum et al., 2002; Balfour et al., 2004). Rewards are thought to play an important role in making predictions about the outcome of future events which can guide behavior. Possible changes of the reward system during aging might lead to impairments in cognitive or behavioral flexibility. Recently, anatomical methods that monitor the expression of neural-activity-dependent, immediate-early genes (IEGs) have been developed that can map the distribution of VTA neurons during specific behavioral (Guzowski et al., 2000). IEG expression is thought to be dynamically regulated by specific forms of patterned synaptic activity that are believed to underlie information storage. To determine whether the IEG *Arc* is expressed in the VTA of young and old rats, we exposed two young and two aged male rats to a sexually receptive female, a manipulation known to activate the VTA. There was no significant difference in the behavioral responses of the young and the aged animals. Namely, both young and aged animals had similar amounts of physical contact with the female rat. Utilizing *in situ* hybridization for *Arc* mRNA as well as the confISH cellular imaging technique, we confirm *Arc* expression in VTA neurons of young and aged animals. Under caged control and maximum electro-convulsive shock conditions, similar proportions of VTA neurons in both young and aged animals express *Arc*. However, after the exposure to a sexually receptive female rat, aged animals exhibited significantly smaller proportions of *Arc*-expressing neurons in the VTA compared to young animals. These results demonstrate age-related changes in VTA neural activity which may affect the functionality and efficacy of the VTA and its projection sites. These alterations may also contribute to the deficits in learning observed in aged animals.

## METHODS

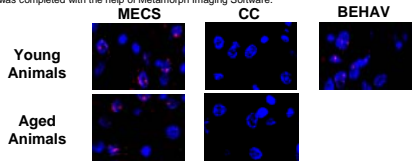
**Experimental Design:** Young (9 mo.) and aged (24 mo.) Fischer-344 rats were divided into three groups: 1) 4 yo. and 4 aged animals were used as caged controls (CC) and remained undisturbed in home cages until sacrifice 2) 4 yo. and 4 aged animals underwent maximal electro-convulsive shock (MECS) 3) 6 yo. and 6 aged animals were allowed to interact with a sexually receptive female rat in a 3' x 3' box for 10 minutes prior to sacrifice (BEHAV).

There were no notable behavioral differences observed between young and aged animals although the detailed behavioral scoring is not complete.



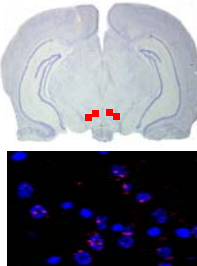
**Tissue Harvesting:** Brains were rapidly extracted and flash frozen intact in 2-methylbutane for *in situ* hybridization. **Fluorescence *in situ* Hybridization (FISH):** Twenty micron thick coronal sections were cut and arranged on a slide so that tissues from all groups were represented on a single slide. FISH was performed as previously described (Guzowski et al., 1999).

**Confocal Microscopy & Image Analysis:** Images were collected using a Zeiss 510 Metaseries laser scanning confocal microscope with a 40x oil objective. Rostral VTA was defined as -5.00 ( $\pm$  0.2) mm Bregma. Middle VTA was defined as -5.60 ( $\pm$  0.2) mm Bregma, and caudal VTA was defined as -6.30 ( $\pm$  0.2) mm Bregma. For each region of the VTA and each experimental condition,  $n=9$  sections with 4 images from each section (36 images per rat). Image analysis was completed with the help of Metamorph Imaging Software.

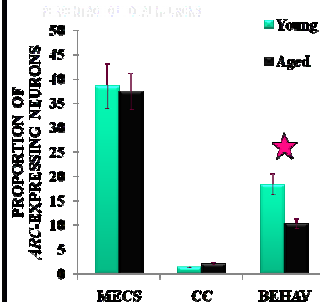


## Results

### 1. *Arc* Expression in Rostral VTA

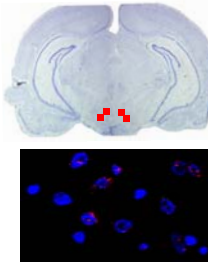


Top: Representative Nissl stain of coronal section at the level of -5.00 mm Bregma. Red squares indicate location of confocal images. Bottom: Representative image of *Arc* expression for MECS.

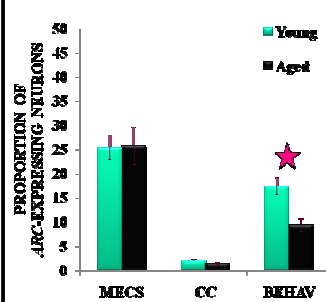


*Arc* expression in the rostral VTA of young and aged animals. Animals were individually exposed to a female rat in estrous (BEHAV). Aged animals showed significantly lower proportions of cells expressing *Arc* following behavior (\*  $p < 0.001$ ; T-test). There were no age differences in MECS or CC conditions.

### 2. *Arc* Expression in Middle VTA

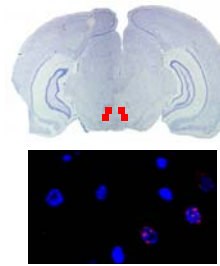


Top: Representative Nissl stain of coronal section at the level of -5.60 mm Bregma. Red squares indicate location of confocal images. Bottom: Representative image of *Arc* expression for MECS.

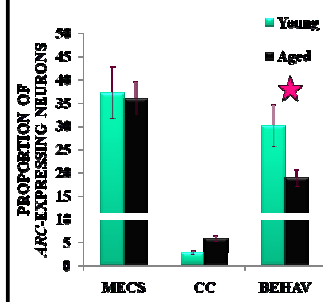


*Arc* expression in the middle VTA of young and aged animals. Aged animals showed significantly lower proportions of cells expressing *Arc* following behavior (\*  $p < 0.001$ ; T-test). There were no age differences in MECS or CC conditions.

### 3. *Arc* Expression in Caudal VTA



Top: Representative Nissl stain of coronal section at the level of -6.30 mm Bregma. Red squares indicate location of confocal images. Bottom: Representative image of *Arc* expression for MECS.



*Arc* expression in the caudal VTA of young and aged animals. Aged animals showed significantly lower proportions of cells expressing *Arc* following behavior (\*  $p < 0.001$ ; T-test). There were no age differences in MECS or CC conditions.

## CONCLUSIONS

- Arc* expression in VTA neurons was shown for the first time in both young and aged animals.
- Furthermore, sexual behavior induced *Arc* expression along the entire rostro-caudal axis of the VTA in both age groups. The proportions of *Arc*-expressing cells after behavior, however, appear to be greatest in the caudal VTA.
- This axial difference may reflect important differences in VTA afferents as well as projection sites for reward.
- Importantly, *Arc* expression in VTA neurons is significantly attenuated during aging for naturally rewarding stimuli, possibly reflecting age-associated alterations in VTA functionality and efficacy.

## REFERENCES

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## ACKNOWLEDGEMENTS

The authors would like to thank Dr. Marsha Penner and Ellen Wann for assistance with the *in situ* hybridizations and the laboratory of Dr. Patricia Hoyer for providing female animals.

This work is supported by: McKnight Brain Research Foundation; state of Arizona and ADHS

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