

Decreased Somal Size of Deep Layer 3 Pyramidal Neurons in the Prefrontal Cortex of Subjects With Schizophrenia

Joseph N. Pierri, MS, MD; Christine L. E. Volk, BS; Sungyoung Auh, MS; Allan Sampson, PhD; David A. Lewis, MD

Background: Schizophrenia is associated with deficits in working memory, a cognitive function that depends on the connections of the prefrontal cortex (PFC) with the thalamus and other cortical regions. Pyramidal neurons in PFC deep layer 3 play a central role in both thalamocortical and corticocortical circuitry. Given that somal size tends to be associated with both the dendritic and axonal architecture of a neuron, abnormalities in these circuits in schizophrenia may be associated with a change in the somal size of deep layer 3 pyramidal neurons.

Methods: We used design-based stereology to estimate the somal volume of pyramidal neurons in deep layer 3 of PFC area 9 in 28 subjects with schizophrenia, each of whom was matched to 1 normal comparison subject for sex, age, and postmortem interval.

Results: The geometric mean of the somal volume estimates in the subjects with schizophrenia was significantly ($P=.02$) decreased by 9.2%. This decrease was associated with a shift in the distribution of somal volumes toward smaller sizes. Neither antipsychotic medication treatment history nor duration of illness was associated with somal size.

Conclusions: These findings independently replicate previous reports of decreased somal size in the PFC in schizophrenia. The reduction in size of deep layer 3 pyramidal neurons is consistent with abnormalities in thalamocortical and corticocortical circuitry, suggesting that disruption of these circuits may contribute to cognitive abnormalities in schizophrenia.

Arch Gen Psychiatry. 2001;58:466-473

WORKING memory is impaired in persons with schizophrenia,^{1,2} and these abnormalities are associated with altered function of the dorsal prefrontal cortex (dPFC).^{3,4} In addition, studies in nonhuman primates indicate that working memory requires the integrity of dPFC connections with other cortical regions and the thalamus.^{5,6}

Recent studies have reported alterations in layer 3 of the dPFC in schizophrenia. Rajkowska et al⁷ described a decrease in the mean somal size of all layer 3 neurons and a decrease in the density of the largest neurons in deep layer 3. The authors interpreted this finding to suggest that large pyramidal neurons in deep layer 3 may be most affected in schizophrenia. Interestingly, the density of dendritic spines, a marker of the number of excitatory inputs to pyramidal neurons,⁸ has been found to be significantly decreased on layer 3 pyramidal neurons in subjects with schizophrenia,⁹ and this abnormality was reported to be most prominent on pyramidal neurons in deep layer 3 of the dPFC.¹⁰

Pyramidal neurons in deep layer 3 of the dPFC play a key role in both corticocortical and thalamocortical circuitry. The principal axons of these neurons project to cortical association areas, such as the superior temporal gyrus and the inferior parietal cortex.¹¹⁻¹³ In addition, intrinsic axon collaterals of layer 3 pyramidal neurons furnish wide-spreading, horizontal excitatory connections within the dPFC.¹⁴⁻¹⁷ Furthermore, deep layer 3 pyramidal neurons are located in the termination zone of axon projections from the mediodorsal thalamic nucleus,¹⁸ and thus likely receive excitatory input from this nucleus.

Because neuronal size is correlated with the extent of a neuron's dendritic^{19,20} and axonal arbor,^{21,22} a decrease in somal size may reflect decreased afferent and/or efferent connectivity of these neurons in schizophrenia. Consequently, we tested the hypothesis that the somal size of deep layer 3 pyramidal neurons in dPFC area 9 is decreased in individuals with schizophrenia.

RESULTS

The primary MANCOVA model revealed a significant ($F_{1,26}=5.76$; $P=.02$) 9.2% de-

From the Departments of Psychiatry (Drs Pierri and Lewis and Ms Volk), Statistics (Ms Auh and Dr Sampson), and Neuroscience (Dr Lewis), University of Pittsburgh, Pittsburgh, Pa.

SUBJECTS AND METHODS

SUBJECTS

With the consent of the next of kin and the approval of the Health Sciences Institutional Review Board of the University of Pittsburgh, we obtained brain specimens from 56 subjects during autopsies conducted at the Allegheny County (Pittsburgh) Coroner's Office (**Table**). Neuropathological examinations revealed no abnormalities in any of the subjects except for the following: thioflavin-S staining revealed a few neuritic plaques in 3 normal comparison subjects (pairs 3, 4, and 24) and in 1 subject with schizophrenia (pair 6), but they did not meet either clinical or neuropathological criteria for Alzheimer disease.²³ Two subjects with schizophrenia died of cerebrovascular events (pair 6, left parietal subdural hematoma; pair 12, intracerebral hemorrhage in the right temporal lobe), but the left dPFC was not affected. An independent panel of experienced clinicians made consensus *DSM-III-R* diagnoses as described previously.²⁴

Twenty-eight subjects diagnosed as having schizophrenia or schizoaffective disorder (**Table**) were each matched to 1 comparison subject for sex, age, and post-mortem interval (PMI). Individual pairs were completely matched for sex, and the mean \pm SD differences in age and PMI within pairs were 3.8 ± 3.0 years and 2.6 ± 2.1 hours, respectively. Mean values for these variables (**Table**) and percentage of out-of-hospital deaths (comparison group, 96.4%; schizophrenia group, 89.3%) did not differ between the 2 groups. For subjects with schizophrenia, the mean \pm SD age of onset was 27.3 ± 9.0 years and the duration of illness was 25.8 ± 11.8 years. Five of these subjects died by suicide, and 13 had a history of an alcohol or substance use-related disorder; these diagnoses were current at the time of death for 8 subjects. Toxicology examinations were positive for plasma alcohol in 3 subjects with schizophrenia (pairs 4 [0.13%], 7 [0.12%], and 27 [0.09%]) and in 2 comparison subjects (pairs 11 [0.03%] and 20 [0.01%]). Six subjects with schizophrenia had not been taking antipsychotic medication for at least 1 month prior to death, and 1 (pair 16) never received treatment (**Table**).

TISSUE PREPARATION

The left hemisphere of each brain was cut into 1.0-cm-thick coronal blocks, immersed in ice-cold 4% paraformaldehyde in phosphate buffer for 48 hours, washed in a graded series of sucrose solutions, and stored in an antifreeze solution at -30°C . Tissue storage time did not differ between the subject groups (**Table**). From blocks located 2 to 4 cm from the frontal pole, 40- μm coronal sections were cut on a cryostat. Every tenth section was mounted on slides and stained for Nissl substance with thionin. From a series of sections determined by cytoarchitectonic criteria^{25,26} to contain dPFC area 9 (**Figure 1**), we selected 4 sections, each separated by 400 μm , for somal size estimation. These slides were placed in random order and coded for blinded quantification.

MEASUREMENT OF SOMAL SIZE

Quantification was performed without knowledge of diagnosis by one rater (C.L.E.V.). Using a Zeiss Axioplan microscope equipped with Stereo Investigator software²⁷ and a Microvid Monitor (MicroBrightField, Inc, Colchester, Vt), area 9 was identified at low magnification ($\times 50$), the border between layers 3 and 4 was located (**Figure 1**), and a contour outlining the lower third (determined by measuring the width of layer 3) of layer 3 was drawn (**Figure 2A**). The mean \pm SD contour area per section was $1.93 \pm 0.33 \times 10^6 \mu\text{m}^2$ for the comparison subjects and $1.85 \pm 0.36 \times 10^6 \mu\text{m}^2$ for the subjects with schizophrenia. Magnification was then changed to $\times 1000$, using a 1.4 numerical aperture, $\times 100$ oil immersion objective, for cell measurements. To randomly sample cells, we used the optical fractionator²⁸ probe of the Stereo Investigator software, which systematically and randomly placed 18 to 22 sampling boxes throughout the region of interest (**Figure 2B**). Each box was $110 \times 75 \times 8 \mu\text{m}$ in the x, y, and z directions, respectively (**Figure 2C**). At each sampling site, sampling was begun 2 μm below the section surface. Sections had a mean \pm SD thickness of $14.0 \pm 1.0 \mu\text{m}$ for both the comparison and schizophrenia groups. To estimate somal volume, we used the nucleator probe²⁹ of the Stereo

Continued on next page

crease in the geometric mean of the somal volume of the subjects with schizophrenia ($2084.3 \mu\text{m}^3$; 25% quartile, $1795.4 \mu\text{m}^3$; 75% quartile, $2383.2 \mu\text{m}^3$) compared with that of the matched comparison subjects ($2295.7 \mu\text{m}^3$; 25% quartile, $1980.3 \mu\text{m}^3$; 75% quartile, $2600.3 \mu\text{m}^3$) (**Figure 4**). In 18 of 28 of the pairs, the subject with schizophrenia had a geometric mean somal volume estimate that was lower than the matched comparison subject. Somal volume was decreased in subjects with "pure" schizophrenia and in those with schizoaffective disorder relative to their matched controls, although in separate exploratory analyses neither difference was significant ($F_{1,39} = 1.81$; $P < .19$ and $F_{1,11} = 3.55$; $P < .09$, respectively).

The distributions of somal sizes (**Figure 5**) revealed that the subjects with schizophrenia had higher percentages of neurons in the smaller cell size categories and lower percentages in the larger 2 categories compared with the comparison subjects. The MANCOVA model used to com-

pare the cell category percentages between comparison subjects and subjects with schizophrenia showed a significant diagnosis \times somal size category interaction ($F_{2,108} = 4.9$; $P = .009$). To determine the differences in the distributions of somal size that accounted for this interaction, we used paired *t* tests, adjusted to have a simultaneous .05 significance level, to examine the differences in changes in percentages of neurons across size categories for the 2 diagnostic groups. This analysis showed that between the "1001-3000 μm^3 " and the "3001-5000 μm^3 " categories, the subjects with schizophrenia exhibited a decrease in the percentage of neurons, whereas the comparison subjects exhibited an increase. The difference in these changes was significant ($t_{108} = 2.8$, simultaneous $P = .02$), and consistent with a shift in the somal size distribution toward smaller cell sizes for the subjects with schizophrenia.

The mean \pm SD width of layer 3 did not differ ($F_{1,27} = 0.18$; $P > .68$) between the comparison subjects

Investigator software, in a local vertical section design.³⁰ That is, we only measured neurons in regions of area 9 where the long axis of the neurons was judged to be parallel to the plane of the section and perpendicular to the pial surface (Figures 1 and 2D). This design assumes that pyramidal neurons in deep layer 3 are isotropically oriented about the vertical axis of the tissue.

For each neuron, the nucleolus was used as the cell's uniquely associated reference point. To estimate somal size, the operator clicked on the nucleolus, which brought up a set of 5 two-dimensional isotropic random rays (Figure 2D). The operator clicked on each ray where it intersected the boundaries of the soma. The formula used to calculate volume was as follows: $\text{volume} = 4/3 \pi \bar{l}_n^3$, where \bar{l} equals the mean segment length from the nucleolus to the cell boundary, and n equals the number of segments, in this case, 5.

Only cells within the 3-dimensional sampling box meeting the following criteria for pyramidal neurons were measured: (1) a clearly identifiable nucleolus, (2) an abundance of Nissl-stained cytoplasm, (3) a clearly visible vertical apical dendrite, and (4) a triangular shape. The mean \pm SD number of neurons measured for each subject did not differ between the control subjects (265 ± 44) and the subjects with schizophrenia (261 ± 45). The mean \pm SD coefficients of error for average somal volume were $4.5\% \pm 0.6\%$ for comparison subjects and $4.6\% \pm 0.6\%$ for subjects with schizophrenia.

STATISTICAL METHODS

Individual and group somal volume distributions for all measured neurons in each diagnostic group showed a skew toward the larger somal sizes (Figure 3A). To normalize the data, all single neuron volume estimates were transformed using a natural log function³¹ (Figure 3B). For each subject, estimates of somal volume were averaged over the neurons within each of the 4 sections. These averages were treated as 4 correlated observations. Exploratory regression analyses done to assess the effects of sex, age, PMI, and tissue storage time on somal volume indicated a potential effect of PMI on somal volume, which was confirmed in formal modeling. To examine a

main effect of diagnosis, a multivariate analysis of covariance (MANCOVA) model assuming a compound symmetric covariance structure³² was used. To test for a main effect of diagnosis, pair was used as a blocking factor and tissue storage time as a covariate. In this model, the pair factor accounts for the effect of PMI, as subjects were matched, on a pairwise basis, for sex, age, and PMI. This model was validated by using the MANCOVA procedure to assess both diagnosis and pair factors, with tissue storage time and PMI as covariates, as well as by including all pairing factors (age, sex, and PMI) and tissue storage time as covariates in the MANCOVA model. As all 3 models yielded similar results, only the results of the primary model are reported.

Analyses were implemented in SAS PROC Mixed.³³ All statistical tests were performed on the log-transformed estimates of somal volume, and summary descriptions of somal volume were obtained by back transformation of the summary statistics of log-transformed somal volume. Back transformation yields geometric means that are estimates of median somal volume.³⁴ The distributions of somal size for the 2 diagnostic groups are described using 25% and 75% quartiles, obtained through back transformation.

To examine changes in the distribution of somal volumes between diagnostic groups, arbitrary cutoff values were used to create 4 somal size categories. The mean percentages of neurons for each subject in the 3 smallest size categories were compared across diagnostic groups using a MANCOVA model that assumed an intraclass covariance matrix with pair as a blocking variable.

Within the schizophrenia group, we also used an intraclass covariance matrix MANCOVA model to evaluate the effects of age of onset and duration of illness on somal volume. Because age at the time of death was correlated with both age of onset and duration of illness, it was included as a covariate for these analyses. In exploratory analyses, we used an analysis of covariance model with PMI as the covariate to evaluate the effects of suicide, substance abuse history, and medication status at the time of death on differences in log-transformed somal volumes between subjects with schizophrenia and comparison subjects.

All statistical tests were conducted with $\alpha = .05$.

($993.1 \pm 81.7 \mu\text{m}$) and the subjects with schizophrenia ($1003.7 \pm 86.3 \mu\text{m}$).

We found no significant associations between age of onset ($F_{1,24} = 0.11$; $P = .75$) or duration of illness ($F_{1,24} = 0.09$; $P = .77$) and somal size. In addition, differences in log-transformed somal volumes between the subjects with schizophrenia and their matched comparison controls did not vary ($F_{1,25} < 0.10$; $P > .75$) as a function of sex, suicide, or history of alcohol and/or substance abuse (Figure 6A-C). Finally, differences in somal size between subjects with schizophrenia and their matched controls were not affected by antipsychotic medication treatment status at the time of death ($F_{1,25} = 0.25$; $P = .62$) (Figure 6D).

COMMENT

In this study, subjects with schizophrenia showed a significant 9.2% decrease in the somal volume of pyrami-

dal neurons in deep layer 3 of PFC area 9, with a shift in the somal volume distribution toward smaller cell sizes. Decreased somal size was not related to duration or age of onset of illness, sex, death by suicide, history of alcohol or substance use, or antipsychotic medication treatment at the time of death.

The strengths of this study include (1) the sample size; (2) the use of an optical fractionator design, which enabled systematic random sampling within area 9; and (3) the use of the nucleator, which permitted estimates of somal volume.²⁹ However, several potential confounds must be considered in interpreting the pathophysiological significance of our observations.

First, the stereological design of this study has limitations. Although a relatively large volume of area 9 was sampled, we did not sample throughout the region of interest. Consequently, caution must be used when generalizing findings from our sampling scheme to all deep layer 3 pyramidal neurons in area 9. Also, our method

Subjects Examined in This Study*

Pair	Comparison Subjects				Subjects With Schizophrenia						
	Sex/ Age, y	PMI, h	Storage Time, mo	Cause of Death	Diagnosis†	Sex/ Age, y	PMI, h	Storage Time, mo	Duration of Illness	Cause of Death	
1	F/47	5.3	42.2	ASCVD	SA	F/41	10.3	0.4	20	Pulmonary embolism	
2	M/62	3.3	20.3	ASCVD	CUS ^d	M/62	3.9	0.4	30	Pneumonia	
3	M/74	17.5	0.8	Trauma	RS	M/69	17.0	25.9	49	Thermal burns	
4	M/60	11.0	0.3	ASCVD	CUS ^{a,+}	M/64	8.6	33.6	45	ASCVD	
5	M/48	12.0	15.4	ASCVD	CUS	M/48	8.3	13.6	31	Bronchopneumonia	
6	M/77	6.2	40.0	ASCVD	CUS ⁺	M/72	3.8	33.5	30	Subdural hematoma	
7	M/48	7.8	19.6	ASCVD	CUS ^a	M/52	10.0	16.0	29	Gastrointestinal bleeding	
8	F/40	14.3	15.4	ASCVD	CUS ^c	F/47	14.5	22.1	27	Suicide by chlorpromazine overdose	
9	F/67	19.5	5.9	Accidental CO poisoning	CUS	F/66	17.9	23.0	38	ASCVD	
10	M/41	17.5	4.7	ASCVD	CPS	M/46	19.8	21.4	26	ASCVD	
11	M/50	6.8	44.8	ASCVD	CPS	M/54	11.0	16.8	15	ASCVD	
12	F/47	4.3	17.7	Accidental CO poisoning	CDS ^a	F/48	3.7	3.0	20	Intracerebral hemorrhage	
13	M/42	14.2	31.9	Aortic stenosis	CUS	M/48	19.0	4.2	?	ASCVD	
14	F/72	11.0	35.9	ASCVD	SA ^d	F/67	9.0	14.9	30	COPD	
15	M/41	22.1	14.7	ASCVD	CUS ^{b,g,+}	M/48	22.0	6.1	24	Suicide by jumping	
16	M/51	11.6	2.3	Hypertrophic cardiomyopathy	CPS ⁺⁺	M/51	12.8	49.6	34	Cardiomyopathy	
17	M/42	12.3	33.6	Pericardial tamponade	CUS	M/40	8.5	36.2	4	Suicide by combined drug overdose	
18	F/46	15.0	11.5	Mitral valve prolapse	SA ⁺	F/37	14.5	17.6	8	Suicide by hanging	
19	M/26	16.0	3.0	Trauma	SA	M/27	16.5	9.4	9	Heat stroke	
20	F/60	9.5	19.0	ASCVD	SA ^a	F/61	16.8	20.5	20	ASCVD	
21	M/64	17.3	10.2	Accidental drowning	CUS ^d	M/63	18.3	6.2	20	ASCVD	
22	F/27	16.5	7.5	Trauma	CUS ^d	F/38	17.8	2.6	18	Myocardial hypertrophy	
23	F/55	11.3	17.4	ASCVD	SA	F/46	10.1	13.8	25	Pneumonia	
24	M/83	19.0	88.1	Tuberculosis	CUS ⁺	M/83	16.0	9.6	55	Accidental asphyxiation	
25	M/61	16.4	8.6	Cardiac tamponade	CUS ⁺	M/58	18.9	10.0	16	Right MCA infarction	
26	M/52	16.2	8.7	ASCVD	CDS ^c	M/49	23.5	9.8	14	ASCVD	
27	F/49	13.4	31.1	Hypertensive CVD	SA ^a	F/47	20.1	6.5	30	Suicide by gunshot	
28	M/50	24.0	7.8	ASCVD	CPS ^{a,f}	M/46	28.1	17.7	30	Accidental combined drug overdose	
Mean	52.9	13.3	19.9			52.8	14.3	15.9			
SD	13.8	5.3	18.7			12.4	6.2	11.9			

*PMI indicates postmortem interval; ASCVD, atherosclerotic coronary vascular disease; CD, carbon monoxide; SA, schizoaffective disorder; CUS, chronic undifferentiated schizophrenia; RS, residual schizophrenia; CPS, chronic paranoid schizophrenia; CDS, chronic disorganized schizophrenia; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; and MCA, middle cerebral artery.

†Superscripts are as follows: a, alcohol dependence, current at time of death; b, alcohol dependence, in remission at time of death; c, alcohol abuse, current at time of death; d, alcohol abuse, in remission at time of death; f, other substance dependence, current at time of death; g, other substance abuse, in remission at time of death; +, schizophrenic subjects not taking medications at time of death; and ++, schizophrenic subject who never received antipsychotic medication.

for estimating somal volume did not take into account the possibility that alterations in the shape or orientation of pyramidal neurons in schizophrenia could lead to underestimates or overestimates of somal volume. However, despite these caveats, the fact that a decrease in somal size of deep layer 3 pyramidal neurons has been reported by another independent group,⁷ using a different sampling scheme and estimator of cell size, strongly suggests that decreased somal size of deep layer 3 pyramidal neurons in schizophrenia is unlikely to be the result of biased measurement methods.

Second, terminal conditions associated with changes in brain volume, such as prolonged hypoxemia, could result in altered somal size. However, in this study, 92% of the subjects died suddenly, out of hospital, consistent with a limited agonal state.

Third, neuronal size may change as a function of PMI,^{19,35} as was observed in this study. However, subject pairs were closely matched for PMI, and inclusion of PMI

as a covariate revealed that subjects with schizophrenia still showed a significant decrease in somal volume.

Fourth, the duration of brain tissue fixation represents a potential confound since tissue volume shrinks as much as 14% after prolonged fixation.³⁶ In the present study, each specimen was processed following a standard protocol, with a brief fixation time (48 hours), that was identical for both diagnostic groups.

Finally, long-term exposure to antipsychotic medications might confound estimates of somal volume, as dose of antipsychotic medication has been found to be negatively associated with frontal lobe volume in schizophrenia.³⁷ In the present study, the somal volumes of subjects with schizophrenia who were not taking antipsychotic medications at the time of death did not differ from subjects who were (Figure 6D). Moreover, the somal volume of the subject with schizophrenia who had never been treated with antipsychotic medications was less than that of the matched comparison subject.

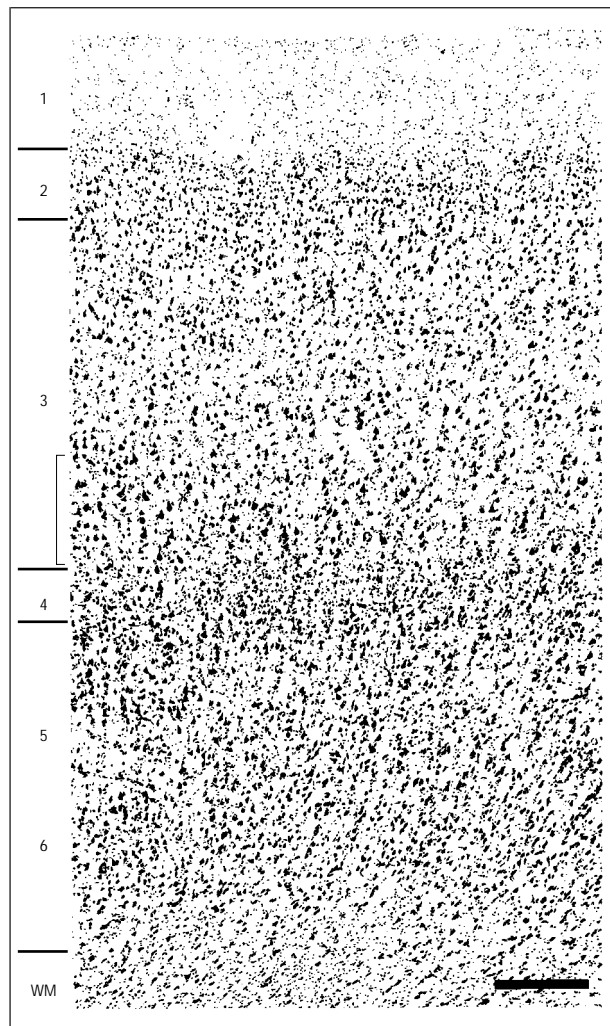


Figure 1. Brightfield photomicrograph of Nissl-stained neurons in prefrontal cortex area 9. Note that pyramidal neurons in deep layer 3 are oriented parallel to the plane of section and perpendicular to the pial surface, consistent with the concept of a local vertical design. The 6 cortical layers and white matter (WM) are identified. The bracket indicates the lower one third of layer 3, where pyramidal neurons were sampled and measured. Scale bar = 300 μ m.

Decreased somal size is consistent with and possibly related to other reported abnormalities of dPFC layer 3 pyramidal neurons in schizophrenia, such as decreased dendritic spine density.^{9,10} Specifically, a decrease in the density of basilar dendritic spines on deep layer 3 pyramidal neurons was associated with a decrease in the total length of these dendrites, as well as a nonsignificant decrease in somal size.¹⁰

Similar to previous studies of PFC pyramidal neurons,^{19,20} we also found no relationship between somal size and age at time of death in our subjects, confirming that somal size is stable across adulthood. Furthermore, somal volume in schizophrenia was not related to age of onset or duration of illness, suggesting that reductions in somal volume may not progress with time, and that the events leading to decreased somal size may have occurred during development before illness onset or during a limited progressive phase of the illness.³⁸

Alterations in deep layer 3 pyramidal neurons may be related to changes in chandelier cells, a specific sub-

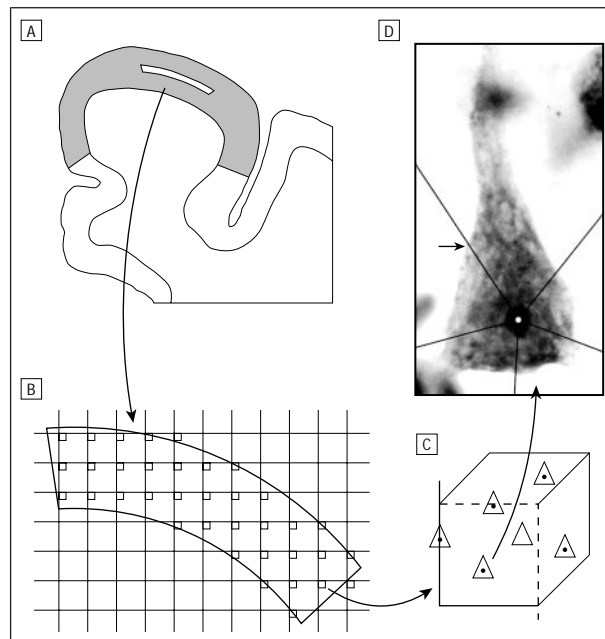
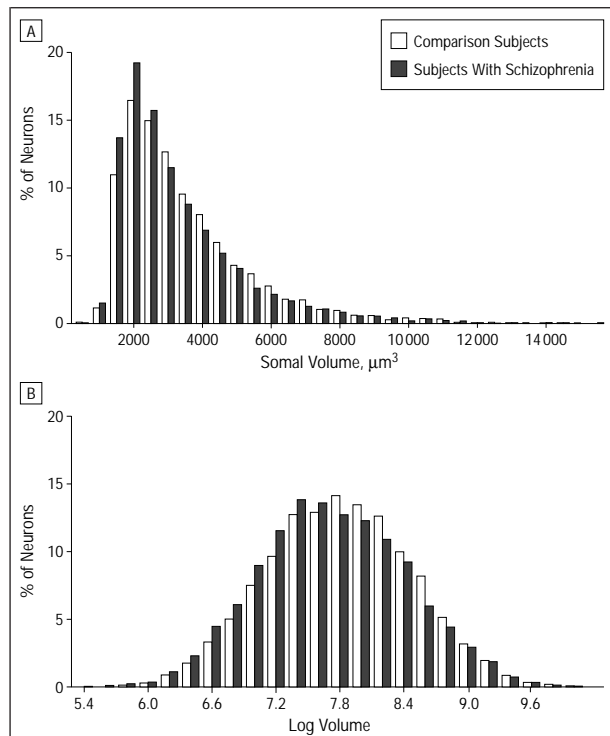


Figure 2. Schematic of the sampling and somal volume estimation procedure. Panel A shows a camera lucida drawing of the superior frontal gyrus in area 9. The gray area indicates the approximate medial and lateral boundaries of area 9. Within this area, where pyramidal neurons met criteria for a local vertical design, a contour was drawn outlining the lower one third of layer 3. B, A sampling grid was randomly superimposed over this area to designate sampling sites. C, At each sampling site, a 3-dimensional sampling frame was used to identify neurons for measurement according to unbiased inclusion and exclusion rules (broken and solid lines indicate inclusion and exclusion boundaries, respectively). Each neuron was measured at a final magnification of $\times 1000$ using the nucleator principle (D), which involves identifying the nucleolus of each pyramidal neuron by clicking on it with the cursor, and marking (arrow) where each of a set of 5 equidistant (separated by 72°) rays, which randomly overlay the neuron, intersect the boundary of the soma. The photomicrograph depicts a pyramidal neuron undergoing this procedure.

set of cortical inhibitory neurons. Chandelier neuron axon terminals (termed “cartridges”) synapse on pyramidal neuron axon initial segments, providing powerful regulation of pyramidal neuron output.³⁹ In the dPFC, subjects with schizophrenia exhibit a decrease in the density of cartridges immunoreactive for the γ -aminobutyric acid transporter (GAT-1)^{40,41}; interestingly, this decrease appeared to be greatest in deep layers 3 and 4 of the same subjects studied herein.⁴¹

Our findings also suggest that a reduction in somal volume may contribute to the subtle reductions in dPFC volume found in neuroimaging studies of schizophrenia.⁴²⁻⁴⁷ In addition, our findings may account for the reports of decreased concentrations of dPFC N-acetylaspartate⁴⁸⁻⁵¹ in schizophrenia, since pyramidal cells make up the majority of neurons in the cortex,⁸ and since the concentration of N-acetylaspartate may be greatest in these neurons.⁵² Interestingly, in subjects with schizophrenia, N-acetylaspartate levels in the dPFC were positively correlated with changes in cortical activation, as measured by regional blood flow, in the prefrontal, temporal, and parietal association cortices during performance of a working memory task.⁵³ This relationship, found only for the dPFC, suggests that activation of the working memory network may be determined by the integrity of cortico-cortically projecting dPFC pyramidal neurons.^{13,53}



Figures 3. Percentage of neurons across somal size bins for both the comparison subjects and the subjects with schizophrenia, both before (A) and after (B) the natural log transformation of individual somal volume estimates.

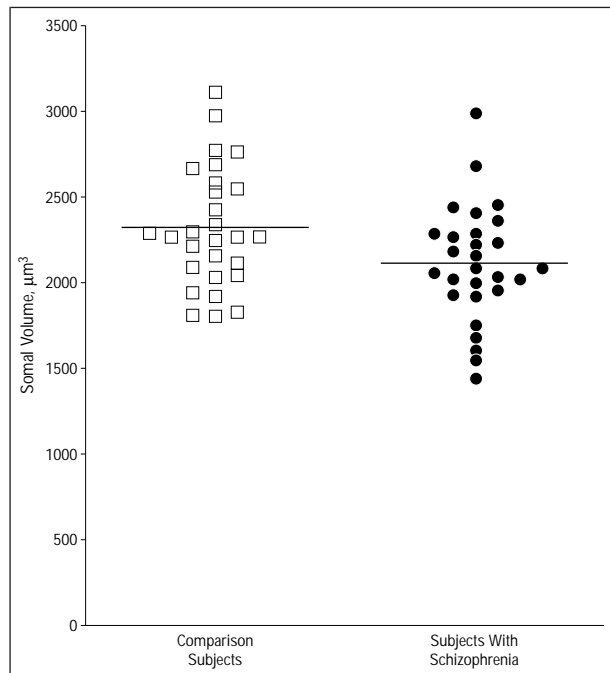


Figure 4. Geometric means of the somal volumes for the comparison subjects and the subjects with schizophrenia. Cross bars indicate the geometric means for each diagnostic group.

Understanding the pathophysiological significance of a decrease in the somal size of pyramidal neurons in deep layer 3 in persons with schizophrenia depends on whether this abnormality reflects a defect intrinsic to deep layer 3 pyramidal neurons or an extrinsic defect in

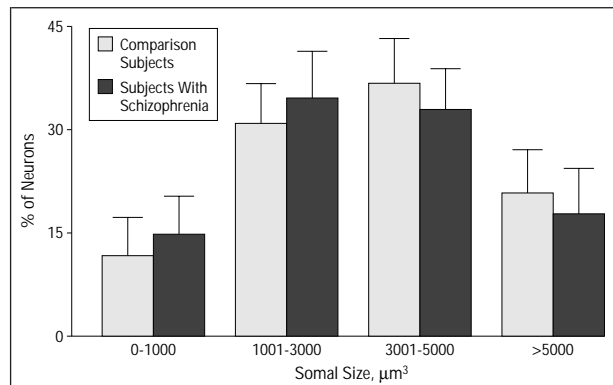


Figure 5. Mean \pm SD percentages of deep layer 3 pyramidal neurons in arbitrarily defined somal volume categories for the comparison subjects and the subjects with schizophrenia. Note that the subjects with schizophrenia appear to have fewer large neurons and more small neurons than the comparison subjects.

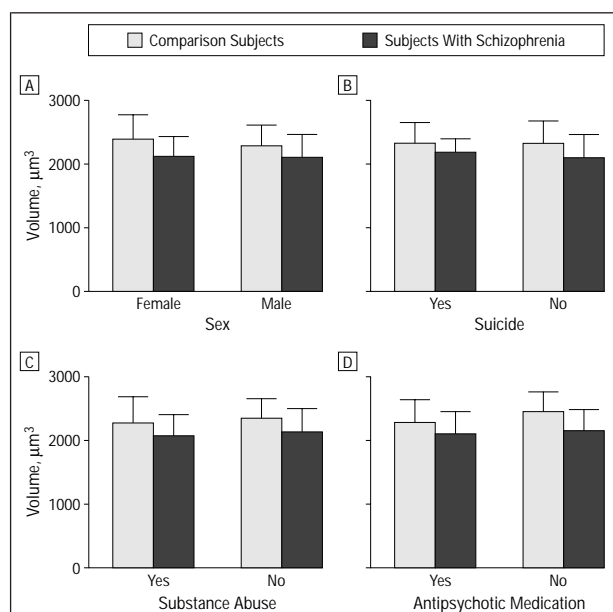


Figure 6. Mean \pm SD of the geometric means of somal volume estimates for the comparison subjects and the subjects with schizophrenia grouped by sex (A) (female, $n=10$; male, $n=18$), cause of death (B) (suicide, $n=5$; other, $n=23$), history of substance abuse (C) (no, $n=15$; yes, $n=13$), and antipsychotic medications at the time of death (D) (no, $n=7$; yes, $n=21$). Groupings were based on the status of the subjects with schizophrenia for each of these variables.

the inputs they receive. An intrinsic defect may be manifest in a decrease in the capacity of these neurons to form and maintain appropriate afferent and efferent connections. For example, in certain neuronal populations, somal size correlates with the extent of a neuron's dendritic^{19,20} and axonal arbor.^{21,22} If these correlations apply to the findings of the present study, we would expect evidence for decreases in these components of pyramidal neuron architecture. Indeed, this possibility is suggested by reported abnormalities in spine density and total dendritic length. Interestingly, for 12 subjects with schizophrenia in the present study, who were also examined in a previous study of spine density,¹⁰ average total dendritic length, measured in Golgi-stained deep layer 3 pyramidal neurons, and average somal volumes, esti-

mated herein, are significantly correlated ($r=0.64$, $P=.02$). In contrast to measures of a neuron's dendritic arbor, the axonal arbor of a neuron is much more difficult to measure in the postmortem state. However, given that layer 3 pyramidal neurons participate in reciprocal short- and long-range circuits intrinsic to the dPFC,¹⁶ a proportion of the decrease in pyramidal neuron spine density in schizophrenia may be due to a decrease in axonal arbor.

On the other hand, a decrease in somal size could result from a loss of input from other brain areas, such as the thalamus. Recent studies⁵⁴⁻⁵⁷ of the mediodorsal thalamic nucleus, which projects to the dPFC, have reported a decrease in the number of neurons in subjects with schizophrenia. These findings suggest that, in schizophrenia, deep layer 3 pyramidal neurons may receive less excitatory drive from the thalamus, and consequently, they are less active and hypotrophic. This possibility of "denervation atrophy" is supported by experiments in which lesioning a subset of afferent inputs to the PFC induced decreased somal size of layer 3 pyramidal neurons and decreased performance on frontal lobe mediated tasks.⁵⁸

Further studies are needed to determine whether abnormalities in dPFC deep layer 3 pyramidal neurons in schizophrenia reflect an intrinsic defect or are the result of altered inputs from other brain regions. Either possibility would support the hypothesis that abnormal thalamocortical and corticocortical circuitry underlie dPFC dysfunction in schizophrenia.

Accepted for publication January 22, 2001.

This work was supported by US Public Health Service grants MH45156, MH00519, MH18951, and MH60473 from the National Institute of Mental Health, Rockville, Md, and a grant from the Stanley Foundation, Bethesda, Md.

We thank Mary Brady for assistance with the figures and Bente Pakkenberg, MD, for advice on the experimental design.

Corresponding author and reprints: David A. Lewis, MD, University of Pittsburgh, 3811 O'Hara St, W1650 BST, Pittsburgh PA 15213 (e-mail: Lewisda@msx.upmc.edu).

REFERENCE

- Park S, Holzman PS. Schizophrenics show spatial working memory deficits. *Arch Gen Psychiatry*. 1992;49:975-982.
- Goldman-Rakic PS. Working memory dysfunction in schizophrenia. *J Neuropsychiatry*. 1994;6:348-357.
- Weinberger DR, Berman KF, Zec RF. Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia, I: regional cerebral blood flow evidence. *Arch Gen Psychiatry*. 1986;43:114-124.
- Steinberg JL, Devous MD, Paulman RG. Wisconsin card sorting activated regional cerebral blood flow in first break and chronic schizophrenic patients and normal controls. *Schizophr Res*. 1996;19:177-187.
- Fuster JM. *The Prefrontal Cortex: Anatomy, Physiology, and Neuropsychology of the Frontal Lobe*. 3rd ed. Philadelphia, Pa: Lippincott-Raven; 1997.
- Goldman-Rakic PS. Cellular basis of working memory. *Neuron*. 1995;14:477-485.
- Rajkowska G, Selemon LD, Goldman-Rakic PS. Neuronal and glial somal size in the prefrontal cortex. *Arch Gen Psychiatry*. 1998;55:215-224.
- DeFelipe J, Farinas I. The pyramidal neuron of the cerebral cortex: morphological and chemical characteristics of the synaptic inputs. *Prog Neurobiol*. 1992;39:563-607.
- Garey LJ, Ong WY, Patel TS, Kanani M, Davis A, Mortimer AM, Barnes TRE, Hirsch SR. Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. *J Neurol Neurosurg Psychiatry*. 1998;65:446-453.
- Glantz LA, Lewis DA. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry*. 2000;57:65-73.
- Goldman-Rakic PS. Topography of cognition: parallel distributed networks in primate association cortex. *Ann Rev Neurosci*. 1988;11:137-156.
- Barbas H. Architecture and cortical connections of the prefrontal cortex in the rhesus monkey. *Adv Neurol*. 1992;57:91-115.
- Hof PR, Nimchinsky EA, Morrison JH. Neurochemical phenotype of corticocortical connections in the macaque monkey: quantitative analysis of a subset of neurofilament protein-immunoreactive projection neurons in frontal, parietal, temporal, and cingulate cortices. *J Comp Neurol*. 1995;362:109-133.
- Levitt JB, Lewis DA, Yoshioka T, Lund JS. Topography of pyramidal neuron intrinsic connections in macaque monkey prefrontal cortex (areas 9 & 46). *J Comp Neurol*. 1993;338:360-376.
- Kritzer MF, Goldman-Rakic PS. Intrinsic circuit organization of the major layers and sublayers of the dorsolateral prefrontal cortex in the rhesus monkey. *J Comp Neurol*. 1995;359:131-143.
- Pucak ML, Levitt JB, Lund JS, Lewis DA. Patterns of intrinsic and associational circuitry in monkey prefrontal cortex. *J Comp Neurol*. 1996;376:614-630.
- Melchitzky DS, Sesack SR, Pucak ML, Lewis DA. Synaptic targets of pyramidal neurons providing intrinsic horizontal connections in monkey prefrontal cortex. *J Comp Neurol*. 1998;390:211-224.
- Giguere M, Goldman-Rakic PS. Mediodorsal nucleus: areal, laminar, and tangential distribution of afferents and efferents in the frontal lobe of rhesus monkeys. *J Comp Neurol*. 1988;277:195-213.
- Hayes TL, Lewis DA. Hemispheric differences in layer III pyramidal neurons of the anterior language areas. *Arch Neurol*. 1993;50:501-505.
- Jacobs B, Driscoll L, Schall M. Life-span dendritic and spine changes in areas 10 and 18 of human cortex: a quantitative Golgi study. *J Comp Neurol*. 1997;386:661-680.
- Lund JS, Lund RD, Hendrickson AE, Bunt AH, Fuchs AF. The origin of efferent pathways from the primary visual cortex, area 17, of the macaque monkey as shown by retrograde transport of horseradish peroxidase. *J Comp Neurol*. 1975;164:287-304.
- Gilbert CD, Kelly JP. The projections of cells in different layers of the cat's visual cortex. *J Comp Neurol*. 1975;63:81-106.
- Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Bell G. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD), part II: standardization of the neuropathological assessment of Alzheimer's disease. *Neurology*. 1991;41:479-486.
- Glantz LA, Lewis DA. Reduction of synaptophysin immunoreactivity in the prefrontal cortex of subjects with schizophrenia: regional and diagnostic specificity. *Arch Gen Psychiatry*. 1997;54:943-952.
- Rajkowska G, Goldman-Rakic PS. Cytoarchitectonic definition of prefrontal areas in the normal human cortex, I: remapping of areas 9 and 46 using quantitative criteria. *Cereb Cortex*. 1995;5:307-322.
- Daviss SR, Lewis DA. Local circuit neurons of the prefrontal cortex in schizophrenia: selective increase in the density of calbindin-immunoreactive neurons. *Psychiatry Res*. 1995;59:81-96.
- Stereo Investigator Version 3.0*. Colchester, Vt: MicroBrightField Inc; 1998.
- Gundersen HJG, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, West MJ. The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *Acta Pathol Microbiol Immunol Scand*. 1988;96:857-881.
- Gundersen HJG. The nucleator. *J Microscopy*. 1988;151:3-21.
- Howard CV, Reed MG. *Unbiased Stereology: Three Dimensional Measurement in Microscopy*. New York, NY: Springer-Verlag; 1998.
- Korbo L, Andersen BB. The distributions of Purkinje cell perikaryon and nuclear volume in human and rat cerebellum with the nucleator method. *Neuroscience*. 1995;69:151-158.
- Neter J, Kutner MH, Nachtsheim CJ, Wasserman W. *Applied Linear Statistical Models*. 4th ed. Chicago, Ill: Irwin; 1996.
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD. *SAS System for Mixed Models*. Cary, NC: SAS Institute Inc; 1996.
- Johnson NL, Kotz S, Balakrishnan N. *Continuous Univariate Distributions*. New York, NY: Wiley & Sons; 1994.
- Hayes TL, Lewis DA. Anatomical specialization of the anterior motor speech area: hemispheric differences in magnopyramidal neurons. *Brain Lang*. 1995;49:289-308.
- Mai JK, Assheuer J, Paxinos G. *Atlas of the Human Brain*. San Diego, Calif: Academic Press; 1997.

37. Gur RE, Cowell P, Turetsky BI, Gallacher F, Cannon T, Bilker W, Gur RC. A follow-up magnetic resonance imaging study of schizophrenia. *Arch Gen Psychiatry*. 1998;55:145-152.
38. Lieberman JA. Is schizophrenia a neurodegenerative disorder? a clinical and neurobiological perspective. *Biol Psychiatry*. 1999;46:729-739.
39. Fairen A, Defelipe J, Regidon J. Nonpyramidal neurons, general account. In: Peters A, Jones EG, eds. *Cerebral Cortex*. Vol 1. New York, NY: Plenum; 1984: 201-245.
40. Woo T-U, Whitehead RE, Melchitzky DS, Lewis DA. A subclass of prefrontal gamma-aminobutyric acid axon terminals are selectively altered in schizophrenia. *Proc Natl Acad Sci U S A*. 1998;95:5341-5346.
41. Pierri JN, Chaudry AS, Woo T-U, Lewis DA. Alterations in chandelier neuron axon terminals in the prefrontal cortex of schizophrenic subjects. *Am J Psychiatry*. 1999;156:1709-1719.
42. Shelton RC, Karson CN, Doran AR, Pickar D, Bigelow LB, Weinberger DR. Cerebral structural pathology in schizophrenia: evidence for a selective prefrontal cortical defect. *Am J Psychiatry*. 1988;145:154-163.
43. Zipursky RB, Lim KO, Sullivan EV, Brown BW, Pfefferbaum A. Widespread cerebral gray matter volume deficits in schizophrenia. *Arch Gen Psychiatry*. 1992; 49:195-205.
44. Andreasen NC, Flashman L, Flaum M, Arndt S, Swayze V II, O'Leary DS, Ehrhardt JC, Yuh WTC. Regional brain abnormalities in schizophrenia measured with magnetic resonance imaging. *JAMA*. 1994;272:1763-1769.
45. Schlaepfer TE, Harris GJ, Tien AY, Peng LW, Lee S, Federman EB, Chase GA, Barta PE, Pearlson GD. Decreased regional cortical gray matter volume in schizophrenia. *Am J Psychiatry*. 1994;151:842-848.
46. Sullivan EV, Lim KO, Mathalon D, Marsh L, Beal DM, Harris D, Hoff AL, Faustman WO, Pfefferbaum A. A profile of cortical gray matter volume deficits characteristic of schizophrenia. *Cereb Cortex*. 1998;8:117-124.
47. Goldstein JM, Goodman JM, Seidman LJ, Kennedy DN, Makris N, Lee H, Tourville J, Caviness VS, Faraone SV, Tsuang MT. Cortical abnormalities in schizophrenia identified by structural magnetic resonance imaging. *Arch Gen Psychiatry*. 1999;56:537-547.
48. Bertolino A, Nawroz S, Mattay VS, Barnett AS, Duyn JH, Moonen CTW, Frank JA, Tedeschi G, Weinberger DR. Regionally specific pattern of neurochemical pathology in schizophrenia as assessed by multislice proton magnetic resonance spectroscopic imaging. *Am J Psychiatry*. 1996;153:1554-1563.
49. Bertolino A, Callicott JH, Elman I, Mattay VS, Tedeschi G, Frank JA, Breier A, Weinberger DR. Regionally specific neuronal pathology in untreated patients with schizophrenia: a proton magnetic resonance spectroscopic imaging study. *Biol Psychiatry*. 1998;43:641-648.
50. Buckley PF, Moore C, Long H, Larkin C, Thompson P, Mulvany F, Redmond O, Stack JP, Ennis JT, Waddington JL. ¹H-magnetic resonance spectroscopy of the left temporal and frontal lobes in schizophrenia: clinical, neurodevelopmental, and cognitive correlates. *Biol Psychiatry*. 1994;36:792-800.
51. Deicken RF, Zhou L, Corwin F, Vinogradov S, Weiner MW. Decreased left frontal lobe *N*-acetylaspartate in schizophrenia. *Am J Psychiatry*. 1997;154:688-690.
52. Moffet JR, Nambodiri MA. Differential distribution of *N*-acetylaspartylglutamate and *N*-acetylaspartate immunoreactivities in rat forebrain. *J Neurocytol*. 1995; 24:409-433.
53. Bertolino A, Esposito G, Callicott JH, Mattay VS, Van Horn JD, Frank JA, Berman KF, Weinberger DR. Specific relationship between prefrontal neuronal *N*-acetylaspartate and activation of the working memory cortical network in schizophrenia. *Am J Psychiatry*. 2000;157:26-33.
54. Pakkenberg B. Pronounced reduction of total neuron number in mediodorsal thalamic nucleus and nucleus accumbens in schizophrenics. *Arch Gen Psychiatry*. 1990;47:1023-1028.
55. Jones L, Mall N, Byne W. Localization of schizophrenia-associated thalamic volume loss. *Soc Neurosci Abstr*. 1998;24:985.
56. Popken GJ, Bunney WE Jr, Potkin SG, Jones EG. Neuron number and GABAergic and glutamatergic mRNA expression in subdivisions of the thalamic mediodorsal nucleus of schizophrenics. *Soc Neurosci Abstr*. 1998;24:991.
57. Young KA, Manaye K, Liang C-L, Hicks PB, German DC. Reduced number of mediodorsal and anterior thalamic neurons in schizophrenia. *Biol Psychiatry*. 2000; 47:944-953.
58. Wellman CL, Logue SF, Sengelau DR. Maze learning and morphology of frontal cortex in adult and aged basal forebrain-lesioned rats. *Behav Neurosci*. 1995; 109:837-850.