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Research report

Impaired two-tone processing at rapid rates in male rats with induced microgyria

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Abstract

We previously reported that adult male rats with bilateral induced microgyria exhibit deficits in rapid auditory processing, which appear similar to auditory processing deficits seen in individuals with developmental language disabilities. The current study was designed to further elaborate that finding using an improved paradigm in which stimulus duration was uncoupled from testing experience and learning effects. Specifically, two-tone stimuli with durations of 540, 390, 332 and 249 ms were all presented within a single test session in a modified operant conditioning paradigm. Subjects were tested over a period of 12 days using this variable-stimulus format. Results confirmed microgyric male rats were impaired only in processing two-tone stimuli presented at rapid rates (i.e., 249 ms duration). Thus the current results support the previously observed link between focal malformations and deficits in rapid auditory processing.

Theme: Development and regeneration

Topic: Developmental genetics

Keywords: Language impairment; Dyslexia; Cortex; Auditory discrimination; Operant conditioning

1. Introduction

Evidence suggests that humans with developmental language disabilities, including specific language impairment (SLI) and developmental dyslexia, may exhibit a fundamental dysfunction in the ability to process brief auditory stimuli followed in rapid succession by other acoustic information (i.e., auditory temporal processing [14,18,19]). Moreover, postmortem analysis of dyslexic brains has revealed neocortical malformations, including molecular layer ectopias and microgyria [6,8,11]. Malformations similar in appearance to microgyria can be induced in rats via focal freezing lesions of the cortical plate on the first day (P1) of life [12,17]. This animal model allows for assessment of a posited relationship between neocortical malformations and defects in rapid auditory processing. Specifically, Fitch and colleagues [4,5,10] trained adult male rats with bilateral microgyric lesions and sham rats to perform a go/no-go auditory discrimination task in a modified operant conditioning apparatus. The rats were to perform a target identification of two-tone stimuli with total stimulus durations reduced from 540 to 249 ms over a period of 24 days of testing (6 days at each of 4 stimulus durations). Results demonstrated that all adult male rats were able to discriminate at the longer stimulus durations, but at the 'short' 249 ms condition, the microgyric subjects were significantly impaired compared to sham subjects. This deficit is strikingly similar to those observed in some individuals with developmental language disabilities. Hence our results may provide a critical bridge for relating developmental cortical anomalies (e.g., cerebrocortical microgyria) and impaired rapid auditory processing.

Our original procedure [4,5,10], however, contained a potential confound between stimulus duration and learning. Specifically, by beginning with the longest stimulus dura-

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tion and continuing to the shortest, stimulus-duration-specific group differences could have been affected by (or interact with) experiential learning. In the current study, therefore, we assessed auditory processing of two-tone stimuli with the same 4 stimulus durations (540, 390, 300 and 249 ms), but two-tone stimuli of different durations were randomly presented *within* each day of testing.

2. Materials and methods

2.1. Subjects

Twenty-four male Wistar rats, (12 sham/12 lesion) from 6 litters were used in the current experiment. On P1, male pups were randomly designated to receive either sham or bilateral freezing lesion surgery balanced within each litter. Focal necrotic lesions were induced based on a modification of the technique employed by Dvorák and associates [1,2] and explained in detail elsewhere [12,17]. Pups were anesthetized and a midline incision was made over the skull. A 2 mm diameter, stainless steel probe cooled to −70°C was placed on the skull approximately 2 mm lateral of the sagittal suture and 2 mm caudal to bregma for 5 s corresponding to the region of the presumptive parietal cortex. An identical lesion was placed in the opposite hemisphere with a second, cooled, probe. Sham subjects were treated identically with the exception that the steel probes were maintained at room temperature. The rats were weaned, individually housed, and maintained on a 12 h light/dark cycle. Food and water were available ad lib until testing had begun.

2.2. Behavioral testing

The behavioral testing procedures utilized are adapted from Fitch and colleagues [3–5,10]. Following several days of water restriction (15 min of water ad lib per day), subjects were introduced to an operant conditioning apparatus that utilizes water as a reward. Subjects were trained for 30 min daily through a series of phases that culminated in the requirement that the rat place its head in a Plexiglas tube and hold still for a period of 750 ms during the presentation of a white noise stimulus. This behavior was followed by the opportunity for the subject to respond by nose press for water reinforcement. Following successful completion of a full session of 48 trials, subjects were introduced to the auditory discrimination paradigm.

2.3. Two-tone auditory discrimination testing

The paradigm consisted of a go/no-go target identification task. In place of white noise, subjects were presented with one of four possible two-tone auditory sequences on each trial. The sequence was either the subjects 'target' (high-low or low-high, randomly assigned across groups), or one of three possible 'non-targets' (high-high, low-low, or the opposite mixed pair of the target). A press following the target stimulus presentation (i.e., a 'Hit') was rewarded with water. The high tone was 2300 Hz and the low tone was 1100 Hz, presented at 75 dB SPL. A press following a non-target (i.e., a 'False alarm') produced a 45 s timeout with lights extinguished. All responses and response latencies were recorded by computer.

Testing began with 3 days of testing at a long stimulus duration condition (a 540 ms stimulus composed of a 20 ms tone, 500 ms ISI, and 20 ms tone). Data were analyzed to confirm that subjects showed discrimination of their targets, based on significant differences in response latencies to targets and non-targets. Subjects were then tested for 12 days on the variable stimulus duration procedure. Stimuli were composed of two variable duration tones separated by a variable duration interstimulus interval (ISI) (i.e., 20/500/20 ms; 20/350/20 ms; 16/300/16 ms; 12/225/12 ms; as described in Fitch et al. [5]). Rise-fall times for tones were 1 ms. During any given test session, the presentation of target and non-target sequences was random with the constraints that: (1) half the presentations be the target; (2) no more than 3 target or non-target sequences occur in succession (to maintain motivation); and (3) for variable stimulus testing, all possible stimulus permutations (4 durations×4 tone-sequences) be represented in a balanced manner.

3. Results

Histological verification of post mortem data confirmed that all subjects classified as microgyric exhibited microgyria located bilaterally in SM-I cortex. No cortical anomalies were found in sham subjects.

A multi-variate ANOVA was used to analyze response latencies for the 24 subjects. Treatment was a between-subject variable with 2 levels (sham, lesion); Response Type was a within-subject variable with 2 levels (hit, false alarm); Stimulus Duration was a within-subject variable with 4 levels (540 ms, 390 ms, 332 ms, and 249 ms); and Day was a within-subject variable with 12 levels. Since all effects examined were replications of previously observed findings, one-tailed tests were used. Results showed a significant main effect of Response Type ($F_{1,22}=13.36$, $P<0.001$), indicating that response latencies for false alarms (non-targets) were significantly longer than for hits (targets). This means that, in general, subjects were able to discriminate target from non-target stimuli. However, there was a significant interaction of Treatment×Stimulus Duration×Response Type ($F_{3,66}=3.33$, $P<0.05$). This interaction reflected the fact that the treatment groups were performing differentially across stimulus durations. Separate analyses performed for each Stimulus Duration revealed a main effect of Response Type at all conditions (indicating overall discrimination). However, there was

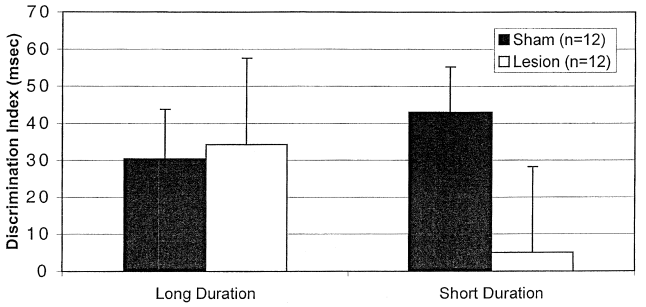

Fig. 1. Discrimination Index of microgyric lesioned and sham subjects for short (i.e., 249 ms) and long stimulus duration two-tone stimuli (i.e., 332 ms, 390 ms, and 540 ms, combined).

also a significant Treatment×Response Type interaction at the shortest (i.e., 249 ms) stimulus duration ($F_{1,22}=4.23$, $P<0.05$). Simple effects revealed a significant Response Type effect at this duration for shams only ($F_{1,22}=10.8$, $P<0.001$), while the Response Type effect for lesions was not significant ($F_{1,22}<1$). These results indicate that subjects showed significant discrimination of the target at all stimulus durations, with the exception of microgyric subjects at the shortest condition.

To examine this group difference another way, latency scores were converted to Discrimination Indices (DI) by subtracting mean hit latencies from mean false alarm latencies for each subject, at each stimulus duration, for each day. Moreover, the discrimination indices for the three 'long' stimulus durations (i.e., 540 ms, 390 ms, and 332 ms) were pooled for each subject. These DI were analyzed by a multivariate ANOVA using Treatment as a between-subjects variable with 2 levels (lesion, sham), Stimulus Duration as a within-subjects variable with 2 levels (long, short) and Day as a within-subjects variable (12 levels). This analysis confirmed the prior results by showing a significant interaction of Treatment and Stimulus Duration ($F_{1,22}=4.18$, $P<0.05$), and a group difference between shams and lesions only at the short stimulus duration (i.e., 249 ms; $F_{1,22}=4.23$, $P<0.05$). This interaction is illustrated in Fig. 1.

4. Discussion

In the current study we found that microgyric and sham male rats are equally able to process two-tone stimuli with 'long' stimulus durations of 540 ms to 332 ms, while microgyric (but not sham) rats, are impaired at processing stimuli presented at the 'short' stimulus duration of 249 ms.

Importantly, these results expand upon the previously reported findings obtained from microgyric and sham adult male rats tested in our modified operant conditioning apparatus [4,5,10]. The current results indicate that even when potentially confounding effects of experience and/or learning are removed, adult male rats with microgyric lesions exhibit impairments in rapid auditory processing. These results further support the assertion by Fitch and colleagues [5] that ''induced anomalies in neocortical development, as found in the postmortem pathology of diagnosed dyslexics, may be a significant causal factor in this temporal dysfunction [of rapid auditory processing].''

While not addressed directly by this research, prior research has addressed the question of how impairments in rapid auditory processing could be brought about by induced focal malformations displaced from areas traditionally associated with auditory processing. In particular, Herman and colleagues [10] demonstrated adult male rats were impaired in rapid auditory processing regardless of bilateral lesion location (e.g., primary somatosensory, frontal or occipital cortices). Moreover, they reported that adult male rats with bilaterally induced cerebrocortical microgyria in frontal, occipital and parietal cortices had more small and fewer large neurons in the medial geniculate nucleus (MGN) of the thalamus, again irrespective of lesion location. The relationship of developmental focal cortical anomalies and differences in cell size distribution are not restricted to rats with induced microgyria. For example, more small and fewer large neurons were evident in the left MGN of the brains of developmental dyslexics (all of whom exhibited focal cortical malformations) when compared to control human brains [7].

Connectional changes resulting from early focal damage to the developing cerebral cortex have been suggested as potentially causal to changes in the distribution of thalamic cell sizes [10]. It is thus reasonable to suggest that induced focal freezing lesions may disrupt afferent and efferent connectivity to the damaged region [9], as well as allowing for maintenance of otherwise transient connections in the developing brain [13]. Moreover, evidence suggests there are anomalous cortico-cortical connections created that relate to microgyria, and there is a marked disruption of the reciprocal connections between the affected cortex and the thalamus [15,16]. In total, the relevant evidence suggests that induced focal malformation (e.g., microgyria) may lead to anatomical changes in the thalamus, which may in turn be related to auditory processing deficits. Ongoing and future research will continue to address this hypothesis.

Prior research has demonstrated adult male rats with induced microgyria are impaired in rapid auditory processing of two-tone stimuli presented with total stimulus durations of 249 ms relative to sham rats [4,5,10]. These results provide a critical link between focal cortical malformations (e.g., microgyria) and impaired rapid auditory processing. Since our prior protocol [4,5,10] contained a potential confound of experiential learning by presenting 6 days at each of the test stimuli from longest duration (i.e., 540 ms) to shortest duration (i.e., 249 ms) over a total of 24 days, the current study examined processing of the same two-tone stimuli but presented within each test session, over 12 days. We still find that adult male

microgyric rats are significantly impaired at processing short-duration (249 ms) stimuli relative to sham rats. The results support and extend our previous research, and further substantiate the existence of a relationship between impaired focal cortical development and deficient rapid auditory processing.

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