Development of Multisensory Processing in Ferret Parietal Cortex.

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Abstract

It is well known that the nervous system adjusts itself to its environment during development. Although a great deal of effort has been directed toward understanding the developmental processes of the individual sensory systems (e.g., vision, hearing, etc.), only one major study has examined the maturation of multisensory processing of cortical neurons. Therefore, the present investigation sought to evaluate multisensory development in a different cortical region and species. Using multiple single-unit recordings in anesthetized ferrets (n=18) of different ages (from postnatal day 80 through 300), we studied the responses of neurons from the rostral posterior parietal area (PPr) to presentations of visual, tactile and combined visual-tactile stimulation. The results showed that multisensory neurons were infrequent at the youngest ages (pre-pubertal) and progressively increased through the later ages. Significant response changes that result from multisensory stimulation (defined as multisensory integration, MSI) were observed in post-pubertal adolescent animals and the magnitude of these integrated responses also increased across this age group. Furthermore, non-significant multisensory response changes were progressively increased in adolescent animals. Collectively, at the population level, MSI was observed to shift from primarily suppressive levels in infants to increasingly higher levels in later stages. These data indicate that, like the unisensory systems from which it is derived, multisensory processing shows developmental changes the specific time course of which may be regionally and species dependent.

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Abstract

It is well known that the nervous system adjusts itself to its environment during development. Although a great deal of effort has been directed toward understanding the developmental processes of the individual sensory systems (e.g., vision, hearing, etc.), only one major study has examined the maturation of multisensory processing of cortical neurons. Therefore, the present investigation sought to evaluate multisensory development in a different cortical region and species. Using multiple single-unit recordings in anesthetized ferrets (n=18) of different ages (from postnatal day 80 through 300), we studied the responses of neurons from the rostral posterior parietal area (PPr) to presentations of visual, tactile and combined visual-tactile stimulation. The results showed that multisensory neurons were infrequent at the youngest ages (pre-pubertal) and progressively increased through the later ages. Significant response changes that result from multisensory stimulation (defined as multisensory integration, MSI) were observed in post-pubertal adolescent animals and the magnitude of these integrated responses also increased across this age group. Furthermore, non-significant multisensory response changes were progressively increased in adolescent animals. Collectively, at the population level, MSI was observed to shift from primarily suppressive levels in infants to increasingly higher levels in later stages. These data indicate that, like the unisensory systems from which it is derived, multisensory processing shows developmental changes the specific time course of which may be regionally and species dependent.

Introduction

How the cerebral cortex organizes itself to analyze and perceive the sensory world around it has been the topic of intense scrutiny for decades now (e.g., see (Crain, 1952; Grossman, 1955; Eayrs & Goodhead, 1959). In general, the course of neural sensory development has been extensively examined from a unisensory perspective by evaluating neuronal responses evoked by the presentation of stimuli from one sensory modality. For example, development of cortical sensory responses have been described for visual (Wiesel & Hubel, 1963), somatosensory (Rubel, 1971) and auditory (Kral *et al.* , 2001) areas in a variety of mammalian models from mice to non-human primates. From these and numerous other studies, the general pattern of cortical sensory development indicates that early postnatal responses are often comparatively weak, with long response latencies, higher levels of spontaneous activity and broad receptive fields regardless of the modality involved. These features were found to progressively change across postnatal development to eventually reach values consistent with neurally mature adults. However, the brain does not habitate a unisensory world and events commonly occur that are detected by more than one sensory modality. Correspondingly, multisensory neurons have been identified so frequently that it has been asserted that the neocortex is "essentially multisensory" (Ghazanfar & Schroeder, 2006).

Despite the ubiquitous presence of cortical multisensory neurons, few studies of the development of cortical neuronal multisensory properties have been published. A very early study of multisensory development observed neurons in feline association (Lateral Suprasylvian area) cortex that primarily responded unisensory stimulation early in the postnatal period (e.g., 67% at postnatal day 8), but were largely replaced by multisensory neurons (89%) by postnatal day (P) 50 (Mayers*et al.* , 1971). This general multisensory developmental sequence was reiterated for a different feline cortical region, the Anterior Ectosylvian Sulcal cortex (AES), in a study by (Wallace *et al.* , 2006). Here, the functional onset for somatosensory responsivity was present for the earliest age group (P28), auditory onset occurred near P56 and the onset of the first multisensory neurons (bimodal somatosensory-auditory) was also seen at that same time. Further, visual

response activity was first observed at P84 at which time the first multisensory neurons with visual inputs were also identified. Thus, although unisensory neurons had earlier functional onsets than did multisensory ones, it seemed that once a particular modality became active, its functional effect applied to both unisensory and multisensory neurons. Furthermore, the time course of these respective sensory onsets corresponded with the expression of specific sensory guided behaviors (Larson & Stein, 1984). The Wallace et al. (2006) study went even further to examine the development of multisensory integration (MSI), which is the effect that multisensory neurons can generate when responding to concurrent stimulation from more than one sensory modality. Even though bimodal neurons were identified by P56, neurons that exhibited MSI were not observed until P84, after which their incidence progressively increased into adulthood. However, the magnitude of MSI response change did not change across this developmental period, since the levels observed at early stages (e.g., P84) were essentially the same as those seen in adults. Thus, this study (Wallace *et al.* , 2006) revealed that multisensory features develop gradually during maturation, but when they do they immediately exhibited adult-like properties.

More recently, an additional form of multisensory neuron has been identified that is different from the traditional bimodal (and trimodal) types. This form, which responds to a single unisensory stimulus, but has that response significantly modified by the presence of a stimulus from a different modality, is termed a subthreshold multisensory neuron (Dehner *et al.* , 2004; Allman & Meredith, 2007; Allman *et al.* , 2009). Like bimodal neurons, subthreshold multisensory neurons can show multisensory response enhancement (as studied in (Wallace *et al.* , 2006)) as well as response depression (Dehner *et al.* , 2004). However, it is not known when these subthreshold multisensory neurons occur developmentally nor has the developmental time-course of multisensory response depression been reported. In addition, multisensory neurons can respond to multisensory stimulation without exhibiting MSI, but with responses that fail to achieve a statistically significant difference from their most effective unisensory response (e.g., see (Perrault Jr *et al.* , 2005)). Such non-significant response changes, however, can contribute to substantial effects at the population level (Merrikhi *et al.* , 2022a; Merrikhi *et al.* , 2022b) although their developmental presentation is not known. Furthermore, the AES area is a conglomerate region consisting of 3 different sensory representations, each with their own connectional features and functional properties (for review, see (Meredith *et al.* , 2018)). Consequently, the developmental effects for a singular multisensory cortical region, to our knowledge, has not yet been described. Therefore, the present investigation seeks to incorporate these recent details about multisensory processing into an assessment of the development of multisensory processing within a singular cortical region, the ferret Rostral Posterior Parietal cortex (PPr).

Like many higher mammals, ferrets have a gyrencephalic brain, demonstrate a high proportion of white matter (Schwerin *et al.* , 2017), and their visual cortices exhibit ocular and orientation-selectively columns (Issa *et al.* , 1999; Medina*et al.* , 2005). As a consequence, ferrets have been used to examine amblyopia (Liao *et al.* , 2004; Krahe *et al.* , 2005) or crossmodal plasticity (Roe *et al.* , 1990) as well as in neuropsychiatric and neurologic conditions such as Fetal Alcohol Spectrum Disorders (Medina *et al.* , 2003; Medina *et al.* , 2005; 2006; Paul *et al.* , 2010; Keum *et al.* , 2023), TBI (Schwerin *et al.* , 2017; Goodfellow *et al.* , 2022) and cortical dysplasia (Noctor *et al.* , 1999; Abbah & Juliano, 2014). The cortical location of the PPr is depicted on the lateral view of the ferret brain shown in Figure 1A. This region has been demonstrated to contain unisensory somatosensory and unisensory visual neurons, as well as bimodal and subthreshold multisensory neurons affected by those same sensory modalities (Manger *et al.* , 2002; Foxworthy *et al.* , 2013; Foxworthy *et al.* , 2014). The receptive fields of these neurons are comparatively large, such that a standardized stimulus set is effective in activating a large proportion of sensory neurons within a multiple single-unit recording site. In addition to ferrets, the parietal cortex is well studied in humans, monkeys and rodents and behavioral involvements have been established for the region in relation to attention, rectification of spatial maps, goal-directed behaviors and self-awareness (Calton & Taube, 2009; Nitz, 2009; Reep & Corwin, 2009; Save & Poucet, 2009; Alais *et al.* , 2010; Kaas *et al.* , 2011; Blanke, 2012). Because all eutherians studied reveal a multisensory visual–somatosensory region between visual and somatosensory cortical representations (Manger *et al.* , 2002; Kaas, 2009), the properties of bimodal and unisensory neurons observed in the ferret PPr can be generalizable to a wide number of species.

Methods

Experiments were performed in compliance with the Guide for Care and Use of Laboratory Animals (National Institutes of Health, publication 86-23), the National Research Council's Guidelines for Care and Use of Mammals in Neuroscience and Behavioral Research (2003), and with approval from the Institutional Animal Care and Use Committee at Virginia Commonwealth University. Details of these procedures are provided in a previous report (Foxworthy *et al.* , 2013). Ferrets were obtained from a licensed vendor (Marshall Farms, Inc.) and were housed in VCU Department of Animal resources until used. All ferrets were male and their age on the experiment day ranged from P80 – P300. Based on these ages, each ferret was assigned to one of four developmental groups. (1) Infancy: ferrets less than P90 are weaned but have not yet reached sexual maturity (have not yet become adolescents). Next, because onset of sexual maturity can occur as early as 4 months of age, animals aged >P120 are post-pubertal and were considered (2) early adolescent (P120-155); (3) mid-adolescent (P160-200) and (4) late adolescent (P240-P300). There is, as yet, no consensus on when ferrets become neurologically mature adults.

For the recording experiments, the animal was anesthetized (8mg/kg ketamine; 0.03mg/kg dexmedetomidine intramuscularly) and their head were secured in a stereotaxic frame. A craniotomy was made to expose the rostral posterior parietal (PPr) portion of the suprasylvian gyrus. Across this opening a recording well/head supporting device was implanted using stainless steel screws and dental acrylic to support the head without obstructing the eyes or ears. The implant was then secured to a supporting bar. The animals were intubated through the mouth, ventilated (expired CO_2: ˜4.5%) and immobilized (pancuronium bromide; 0.3 mg/kg initial dose; 0.2 mg/kg h supplement i.p.) to prevent ocular drift or spontaneous limb movements during testing. Fluids (lactated Ringer's solution) and supplemental anesthetics (4mg/kg h ketamine; 0.5 mg/kg h acepromazine i.p.) were administered continuously with an infusion pump. Heart rate was monitored continuously, and body temperature was monitored and maintained at ˜38°C with a heating pad.

Within the craniotomy the dura was opened to identify the location of the PPr (based on gyral patterns) and to insert the recording electrode array. Neuronal activity was recorded using a four shank, 32-channel silicon probe (A4×8-5mm 200–200-413 array; impedance ˜1 MΩ· NeuroNexus Technologies, Ann Arbor, MI) as described in previous reports (Allman *et al.* , 2009; Keniston *et al.* , 2009; Foxworthy *et al.* , 2013). Neuronal activity was digitized (rate>25kHz) using a TDT System III Workstation (TuckerDavis Technologies Alchua, FL) using MatLab software and archived for off-line analysis. Spike waveforms were clustered by principal component feature space analysis and sorted into individual units using an automated Bayesian sort-routine. Spikes which exhibited interspike intervals < 2ms were rejected.

Once PPr neurons were identified and templated, their responses to sensory stimulation were assessed. Quantitative sensory testing consisted of somatosensory stimuli produced by a calibrated 1-gram monofilament fiber moved by an electronically-driven, modified shaker that displaced hair or indented the skin. Visual stimulation consisted of a bar or spot of light, whose movement direction, velocity, and amplitude across the visual receptive field was computer-controlled and projected onto the translucent hemisphere. These somatosensory and visual stimuli were presented separately and in combination, and each stimulus or combination was repeated 50 times (randomly interleaved with 3-7 second presentation interval). During combined presentations, the onsets of the stimuli were offset by 40ms (visual preceded tactile) to accommodate for the difference in response latency among these sensory modalities. Attention was given to maintaining the consistency of sensory stimulation between different experiments, such that the somatosensory stimulus was always positioned on the contralateral side of the face and moved at the same velocity and amplitude; visual stimulation always consisted of a moving (150 º/sec) bar (5x20º) of light that crossed ˜45º of contralateral visual space in the nasal-to-temporal direction.

Neuronal responses to somatosensory, visual, and combined visual-somatosensory stimuli analyzed using custom software (MatLab; described in (Allman *et al.* , 2009; Keniston *et al.* , 2009). A neuronal response was defined as spiking activity which exceeded 3 standard deviations from spontaneous activity, lasted for a minimum of 15ms, and ended when activity returned to baseline for at least 15ms. Neurons showing suprathreshold activation to individual stimuli from more than one sensory modality were defined as bimodal

multisensory neurons. Neurons which showed suprathreshold activation by only one modality but exhibited responses that were significantly different in the combined stimulus condition than in the unisensory stimulus condition (determined by t-test) were classified as subthreshold multisensory neurons. However, neurons that were driven exclusively by one modality and did not show change in responses after combined stimulation were categorized as unimodal.

Multisensory (bimodal and subthreshold) neurons were further analyzed to determine if they demonstrated integrated responses to multisensory stimulation. Specifically, responses showing a significantly different (assessed by t-test) activation (mean spikes/trial) to multisensory stimuli versus that elicited by the most effective single modality stimulus were defined as exhibiting multisensory integration (Meredith & Stein, 1983). Significant response increases were termed multisensory response enhancement, while those showing a significantly reduced activation were regarded as demonstrating multisensory response depression (also called multisensory suppression (Keum *et al.* , 2023)). The magnitude of multisensory integration was calculated according to the method of (Meredith & Stein, 1986) using the formula: (CM-SM_{max})/ SM_{max} x 100 = % Integration. In this equation, SM_{max} was the neuron's response to the most effective unisensory stimulus (mean spikes/trial) and CM was the response to the multisensory stimulus. Ultimately, the neuronal response type (unisensory, bimodal, subthreshold) as well as the direction (enhancement, depression) and magnitude (% response change) of multisensory responses were tabulated in relation to the age of the experimental animal.

Once the recording session was completed, the animal was overdosed (Euthasol), perfused intracardially with saline and fixed (4% paraformaldehyde). The brain was blocked stereotaxically and the cortex containing the recording site(s) was processed for histological verification of the recording sites. Recording tracks confirmed within the grey matter of the PPr were included in this study.

Results

The sensory and multisensory properties of neurons in PPr (Fig. 1A) were electrophysiologically recorded in 18 male ferrets that ranged in age from P80 to P300. A total of 538 PPr neurons were tested with visual, tactile and combined visual-tactile stimulation presented in a random, interleaved manner. Fig. 1B depicts the experimental setup. Recorded neurons were grouped by their response patterns into the following categories: unimodal visual (V), unimodal tactile (T), bimodal (VT) and subthreshold multisensory (S). Fig. 1C shows representative examples of PPr neurons from each of these categories. Neuronal responses defined as multisensory integration (MSI) showed a statistically significant response change (either depression or enhancement) evoked by a combined visual-tactile stimulus when compared to that elicited by the most effective unimodal cue (Meredith & Stein, 1983). Examples of responses showing MSI are illustrated in the raster/histograms shown in the middle row of Fig. 1C. In addition, subthreshold multisensory responses are defined as a statistically significant response change elicited by combined visual-tactile stimulation when a neuron was activated by only one sensory modality presented alone. Examples of such subthreshold multisensory responses are shown in Fig. 1C (bottom row).

To evaluate the incidence and distribution of the different neuronal categories during postnatal development, the sampled PPr neurons were sorted by age-group. As defined in Methods, animals were divided by age in the following groups: Infancy, (P80-P90; Neurons/Animals=49/2), Early adolescence (P120-P155; 241/6), Mid adolescence (P160-P200; 179/6) and Late adolescence (P240-P300; 69/2). As shown in Fig. 2, these results show that, although each neuronal response type was observed in each age group, their relative proportions systematically changed. Chi-square tests (with Bonferroni corrections for multiple comparisons) showed that the Infant group exhibited more unimodal V but fewer bimodal VT cells than all the other groups ($P<0.008$). All other comparisons did not reach significance. Figure 2B replots this same data to emphasize the progressive change in the proportions of neurons in each response category. From this it is evident that the proportions of unimodal V cells markedly reduce from infancy to early adolescence and decrease even further through late adolescence. In contrast, the number of bimodal VT cells dramatically increase from infancy to early adolescence and these numbers remain high through late adolescence. Likewise, the incidence of the other form of multisensory neuron, the subthreshold category, also proportionally increases steadily

during development.

Next, the level of neuronal responsiveness across postnatal development was examined. The average number of spikes evoked by sensory stimulation was collated by age group, as illustrated in Fig. 3 and summarized in Table 1. In general, response activity tended to increase from infant to early adolescence but then decreased through late adolescence.

For unimodal neurons, responses were only significantly different between early and mid-adolescence stages (univariate ANOVA, $f = 4.7$, $p = 0.004$; followed by post hoc Bonferroni test, $p = 0.002$). In bimodal neurons, the early-adolescence group showed significantly higher responses than all other age groups (univariate ANOVA, $f = 7.2$, $p < 0.001$; followed by post hoc Bonferroni test; Early vs Infancy, $p = 0.03$; Early vs Mid, $p = 0.02$; Early vs Late, $p = 0.001$). No significant difference in responsiveness across ages was observed in subthreshold neurons.

Next, we evaluated the rates of spontaneous activity over the course of development. This was calculated by recording the number of spikes per second fired during the 500ms interval before the onset of the sensory stimulus for each age group. These results are illustrated in Fig. 4 and summarized in Table 2.

No significant differences in spontaneous activity were observed between age groups in unimodal and subthreshold neurons. In bimodal neurons, the Late-adolescence group showed significantly higher spontaneous activity (univariate ANOVA, $f = 4$, $p = 0.008$; followed by post hoc Bonferroni test) than Early ($p = 0.03$) and Mid-adolescence ($p = 0.004$). That spike rates are elevated in bimodal PPr neurons has been reported previously (Foxworthy et al., 2014).

Next, we quantified the proportion of bimodal neurons that exhibited significant MSI. No bimodal neurons showed integration during infancy but, as shown in Figure 5A, the proportions of bimodal neurons that exhibit MS enhancement gradually increase during development (Infancy=none, Early=23%, Mid=28%, Late 67%). Only a single example of multisensory depression was observed among the bimodal neurons sampled, which occurred in the early adolescence group. In the context of the levels of MSI, an increase in the magnitude of response change was observed from early to mid-adolescence and remained constant after that. Figure 5B shows that the magnitude of enhancement was significantly higher for bimodal neurons during mid- and late-adolescence when compared to early. Kruskall-Wallis non-parametric ANOVA showed significant differences (KW-H= 10.07, $p < 0.001$). Post Hoc Mann Whitney tests comparisons between groups showed significant differences between Early vs Mid (Z= -2.87, $p = 0.004$) and Early vs Late (Z= -4.21, $p < 0.001$) but not Mid vs Late ($Z = -0.71$, $p = 0.48$). Median response changes in these groups: Early= 32.7%; Mid= 49.9%; Late= 51.3%.

Not all bimodal neurons exhibit MSI in response to effective multisensory stimulation. Neurons presenting small changes in firing in response to combined visual-tactile stimulation may fail to achieve the statistical significance that defines MSI. Nevertheless, these non-integrative responses contribute to the population response of an area and small changes in response can collectively produce a major change at the population level. Therefore, it seems conceivable that population responses can reveal differences not observed when only neurons that meet MSI criteria are considered (Keum *et al.* , 2023). Fig 5C shows response changes in bimodal neurons that failed to show MSI. Importantly, these non-integrative response changes tended to increase during development, such that the median value of multisensory response change was: Infancy= 9.8%; Early= 6.4%; Mid= 13%; Late= 15%. Kruskall-Wallis non-parametric ANOVA showed that the differences observed were significant (KW-H= 10.07, $P < 0.001$). Post Hoc Mann Whitney tests comparisons between groups showed significant differences between Infancy Vs Mid (Z= -2, $p = 0.037$); Infancy Vs Late (Z= -2, $p = 0.036$); Early Vs Mid (Z=-2.4, $p = 0.016$); Early Vs Late (Z= -2.4, $p = 0.018$).

A similar, progressive increase in magnitude of multisensory response change is shown in Fig. 5D which displays cumulative probability curves of the multisensory response change measured for all bimodal neurons (integrative and non-integrative) from the different age groups. In this figure, a value of zero indicates that the combined visual-tactile stimulation resulted in a number of spikes that was equal to the most effective unimodal stimulus, while positive and negative values indicate increases or decreases in response

changes, respectively, regardless of statistical significance. The cumulative response curves shown in Figure 5D consistently shift upwards from infancy to late adolescence. A Kruskall-Wallis non-parametric ANOVA (KW) followed by post-hoc Kolmogorov-Smirnov tests showed that all groups were significantly different from each other ($p<0.05$ for all comparisons).

Last, we evaluated the development of subthreshold multisensory neurons (Fig. 6). Only a single subthreshold neuron was observed within the infant age-group (which showed MS enhancement), while progressively more examples of subthreshold responses were observed during the adolescent periods (Early, 3.3% of PPr neurons; Mid, 7.6%; Late 12.5%; also illustrated in Figure 2B). For all subthreshold responses to combined VT stimulation, the median magnitude of multisensory response change during the adolescent periods are shown in Figure 6A: Early (41%), Mid (64%), Late (54%) with no statistical significance observed between these groups. Overall, subthreshold multisensory responses were most frequently enhanced while only a few showed response depression (Early: 3 out of 14 neurons with an average depression of -29%±4.2; Mid: 1 out of 14 neurons that showed a depression of -22%). Figures 6B and C summarize the levels of multisensory enhancement and multisensory depression, respectively, observed in subthreshold neurons; no statistical differences in proportion or effects were observed between these age groups.

DISCUSSION

These results show that multisensory neurons are present in ferret PPr cortex at developmental stages that precede sexual maturity (<˜P120). Designated as the "infant" period, multisensory neurons at that time, however, were the minority of neurons observed and only one showed MSI (which was the singular subthreshold neuron identified in this age group). At this early developmental stage, these data from infant, pre-pubertal ferrets closely resemble findings in cat cortex (Wallace *et al.* , 2006), as well as superior colliculus (Wallace & Stein, 1997), where early multisensory neurons respond independently to stimulation from more than one sensory modality but fail to integrate responses when those cues are presented together. Thus, despite the presence of multisensory convergence, the capacity to integrate multisensory responses apparently is not yet developed in such young cats or ferrets.

For the series of older ferrets (e.g., >P120), the presence of multisensory neurons progressively increased, as was also seen in developing cats following the epoch in which the first multisensory neurons appeared (Wallace *et al.* , 2006). In the present study, this period is referred to as "adolescence" because, like post-pubertal humans, neural development continues beyond the age of sexual maturity. It is well known that neural maturity in humans is delayed for approximately a decade after sexual onset, especially for higher-order regions, such as inferior parietal and frontal cortices (Spear, 2000a; Spear, 2000b). To our knowledge such conditions have not yet been identified in ferrets (or cats) and hence, there is ambiguity in what is actually defined as being neurally adult in these animals. Indeed, the study of multisensory development in cats did not define neural adulthood, except that the cats designated as adults were older than P180, which is the age of sexual maturity for cats. As a consequence, all the developmental age groups studied in cats were pre-pubertal (Wallace *et al.* , 2006). In contrast, the ferret age groups in which MSI appeared and developed in bimodal neurons were all post-pubertal adolescents. These conflicting results may be accounted for by the species and methodological differences between these studies, but it might also be possible that PPr neurons mature more slowly than those of the AES perhaps as a reflection of cortical hierarchical differences.

Further evidence that multisensory changes occur in PPr during the ferret adolescent stages comes from the observation that multisensory integration increases in magnitude after the age of sexual maturity at ˜P120. Figure 2B summarizes this feature for bimodal neurons that exhibit MSI, and significant increases in multisensory enhancement were demonstrated for stages of adolescence at ages P160-200 and P200-240. Similar developmental increases in multisensory enhancement have also been reported in another study of PPr neurons in early alcohol-treated and control ferrets (Keum *et al.* , 2023). However, these data do not mirror the MSI levels reported for cats, where the magnitude of multisensory response change observed during development was not different from that seen in adults (Wallace *et al.* , 2006). It seems possible that these differences in results may be accounted for by the species, regional and methodological differences of these studies.

The present study also examined the levels of multisensory response change that were not significantly different from the most effective unisensory stimulus. It must be kept in mind that these non-integrative responses were evoked in bimodal neurons that were individually activated by visual and by somatosensory stimulation. As a consequence, both stimuli were effective when presented alone and, by definition, were presented within their excitatory receptive fields. Thus, the observed non-integrative effects could not have been induced by inhibitory effects of one stimulus being presented outside of its receptive field. Numerous reports have documented and examined non-integrated multisensory responses in bimodal neurons in a variety of neural areas (Perrault Jr *et al.* , 2005; Meredith *et al.* , 2021; Merrikhi *et al.* , 2022a; Merrikhi *et al.* , 2022b). The present study shows that such non-integrative multisensory effects were strongly influenced by development because, as depicted in Figure 5D, significant shifts were seen from primarily suppressive levels in infants to progressively higher levels of multisensory response change across the periods of adolescence. Furthermore, similar developmental shifts in non-integrative responses were observed a separate study of PPr neurons (Keum *et al.* , 2023). Ultimately, the present results show that both non-integrative and integrative response modes of bimodal neurons change during post-pubertal development.

We also examined the subthreshold form of multisensory neuron across the different age groups. Although small in occurrence (as also observed in other areas – (Meredith *et al.* , 2021; Merrikhi *et al.* , 2022a; Merrikhi *et al.* , 2022b), their proportions showed an increasing trend during development but no developmental pattern for changes in MSI levels were demonstrated. A similarly low incidence of multisensory response depression revealed the presence of the effect during development, but consistent changes across development could not be assessed.

In summary, the present study in ferret cortical area PPr identified patterns of appearance of multisensory neurons during development that were similar to those reported for cat cortex (Wallace *et al.* , 2006). However, unlike the cat AES regions, the magnitude of MSI in ferret PPr significantly increased across the post-pubertal developmental stages. Furthermore, bimodal neurons whose multisensory responses were not integrated showed a consistent shift from suppression in infants to progressively higher levels of multisensory response chage at the adolescent stages. These data, combined with the small presence of subthreshold multisensory neurons give insight into how the population of PPr neurons changes their responses to multisensory cues across pre- and post-pubertal development. How these changes, particularly during adolescence, are parlayed into the behavioral and perceptual tasks of the region are clearly a topic for future investigation.

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Figure legends

Fig. 1 : (A) Lateral view of the ferret cerebral cortex displaying the rostral posterior parietal cortex (PPr). The box shows the enlarged PPr area below, with the large dots representing the location of recording penetrations (not all displayed due to overlap). (B) The experiment involved multiple single-unit recordings of responses evoked by the presentation of a visual (white bar moved nasal-to-temporal across a screen), a tactile stimulus (1 g calibrated fiber moved across skin/hairs by an electronically controlled actuator), or the combination of both visual and tactile stimulation. (C) Examples of single-unit neuronal responses recorded after visual alone, tactile alone and combined visual- tactile stimulation. Top row shows the responses of a unimodal visual neuron (left) and a unimodal tactile neuron (right). The middle row depicts a bimodal PPr neuron that generated multisensory enhancement (left) (combined response significantly greater than most effective unimodal response) and another bimodal neuron (right) that generated multisensory response depression. Bottom row shows a subthreshold multisensory response where a combined stimulus significantly enhances (left) or depresses (right) activity with a stimulus from a different modality that is not effective when presented alone.

Fig. 2. Sensory and multisensory neurons in PPr show proportional changes from infancy and across adolescence. (A) The histograms indicate the average (± SE) proportion of a particular neuron response type (V=unimodal visual; T=unimodal tactile; S=Subthreshold multisensory; B=bimodal multisensory) du-

ring the different developmental epochs (yellow=infancy; blue=early adolescence; green=middle adolescence; red=late adolescence; see Methods for definitions). (B) The same data as in part 'A' except now plotted with lines connecting the different response types across development.

Fig. 3 : Responsiveness (average number of spikes/response ± SE) of PPr neuron types (Unimodal =unisensory visual and unisensory tactile; Bimodal multisensory; Subthreshold multisensory) across developmental stages (Infancy=yellow; early adolescence=blue; middle adolescence=green; late adolescence- red). For subthreshold neurons, only one subthreshold such neuron was identified in infancy. Univariate ANOVA followed by posthoc Bonferroni tests * = $p<0.05$; ** = $p<0.01$.

Fig. 4 : Spontaneous activity (spikes/sec ± SE; recorded 500 ms before sensory stimulation) in unimodal (unisensory visual and unisensory tactile), bimodal multisensory and subthreshold multisensory neurons across the developmental periods (Infancy=yellow; early adolescence=blue; middle adolescence=green; late adolescence=red). For the subthreshold category, only one neuron was identified in infancy. Univariate ANOVA followed by posthoc Bonferroni tests* = $p<0.05$; ** = $p<0.01$.

Fig. 5: A. Responses of PPr neurons to multisensory stimulation across the developmental stages. (A) Pie charts indicate the p roportions of bimodal neurons that exhibited significant multisensory integration in the different age groups. **B** . For bimodal neurons that demonstrate MSI, these box/whisker plots show the significant increase in the magnitude of MS enhancement. No enhancement was seen during Infancy; the Early adolescence group was significantly different from Mid and Late adolescence ones. KW followed by Post Hoc Mann Whitney tests, $p<0.01$ in both comparisons. Mid and Late adolescence groups were not different from each other. **C.** For bimodal neurons that failed to demonstrate significant MSI, these box/whisker plots show the significant increase in response levels across the developmental stages. KW followed by Post Hoc Mann Whitney tests, * = $p<0.05$. **D.** Cumulative probability plots of response changes after combined VT stimulation in all bimodal neurons (integrative and non-integrative) across four age groups. A Kruskall-Wallis non-parametric ANOVA (KW) followed by post-hoc Kolmogorov-Smirnov tests showed that all groups were significantly different from each other ($p<0.05$ for all comparisons). These data show that both integrative and non-integrative bimodal responses exhibit developmental changes that shift from primarily suppressive responses in neurons from infants to progressively higher response changes in adolescents.

Fig. 6: (A) For all subthreshold multisensory neurons, although present in each age group, their multisensory responses (box/whisker plots for each age group) do not show significant changes in magnitude across the different developmental stages. KW non-parametric ANOVA. Not significant. Only a single subthreshold neuron was observed during infancy (yellow circle). **B.** For subthreshold multisensory neurons that exhibited response enhancement, the bar graphs (± SE) show that the average magnitude of multisensory enhancement was not affected by developmental age. (Univariate ANOVA. Not significant). **C.**For subthreshold multisensory neurons that exhibited response depression, the bar graphs (± SE) show that the average magnitude of multisensory depression also was not affected by developmental age (Univariate ANOVA; Not significant).

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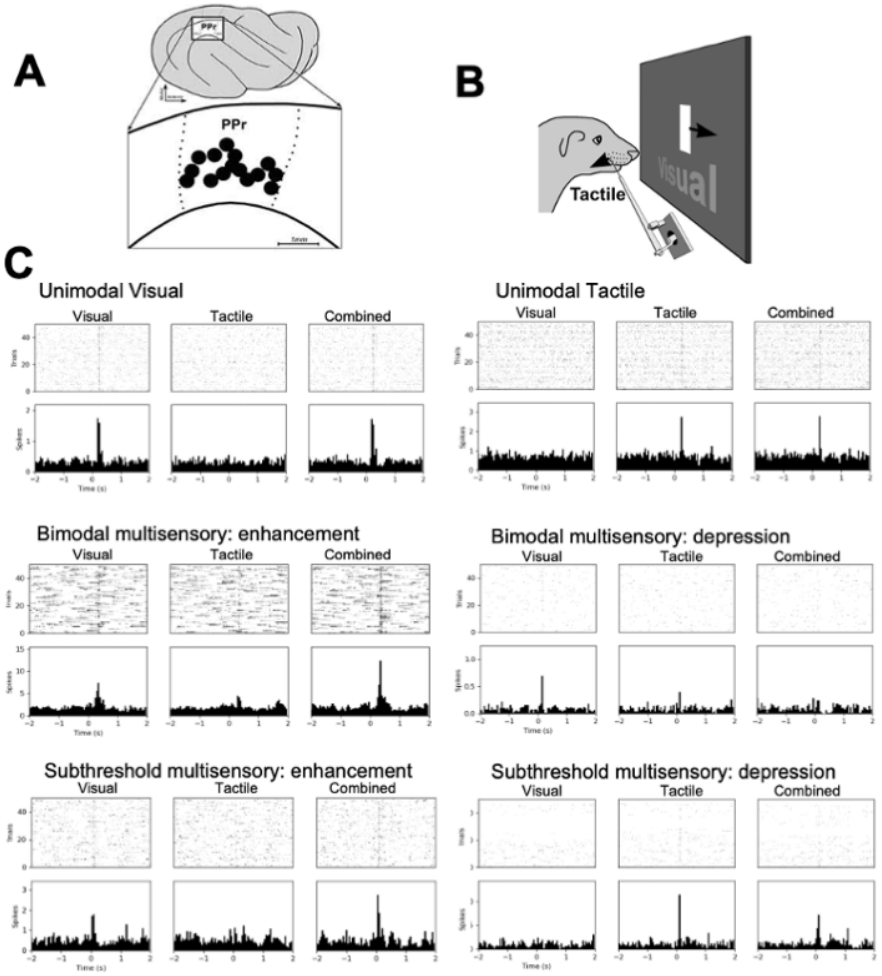

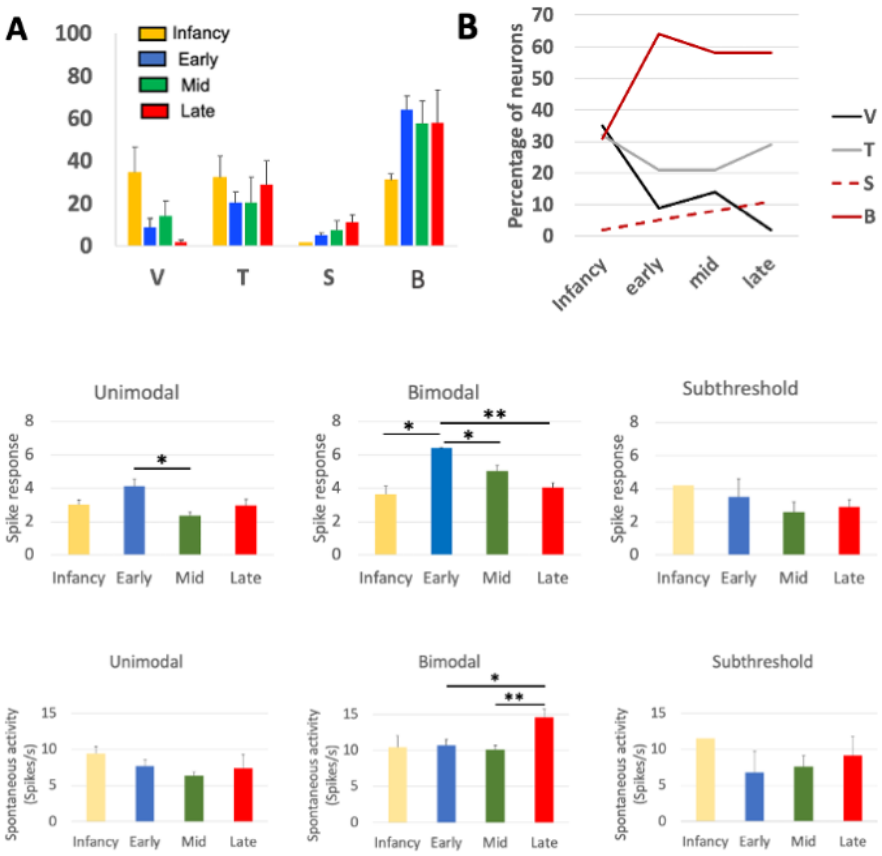

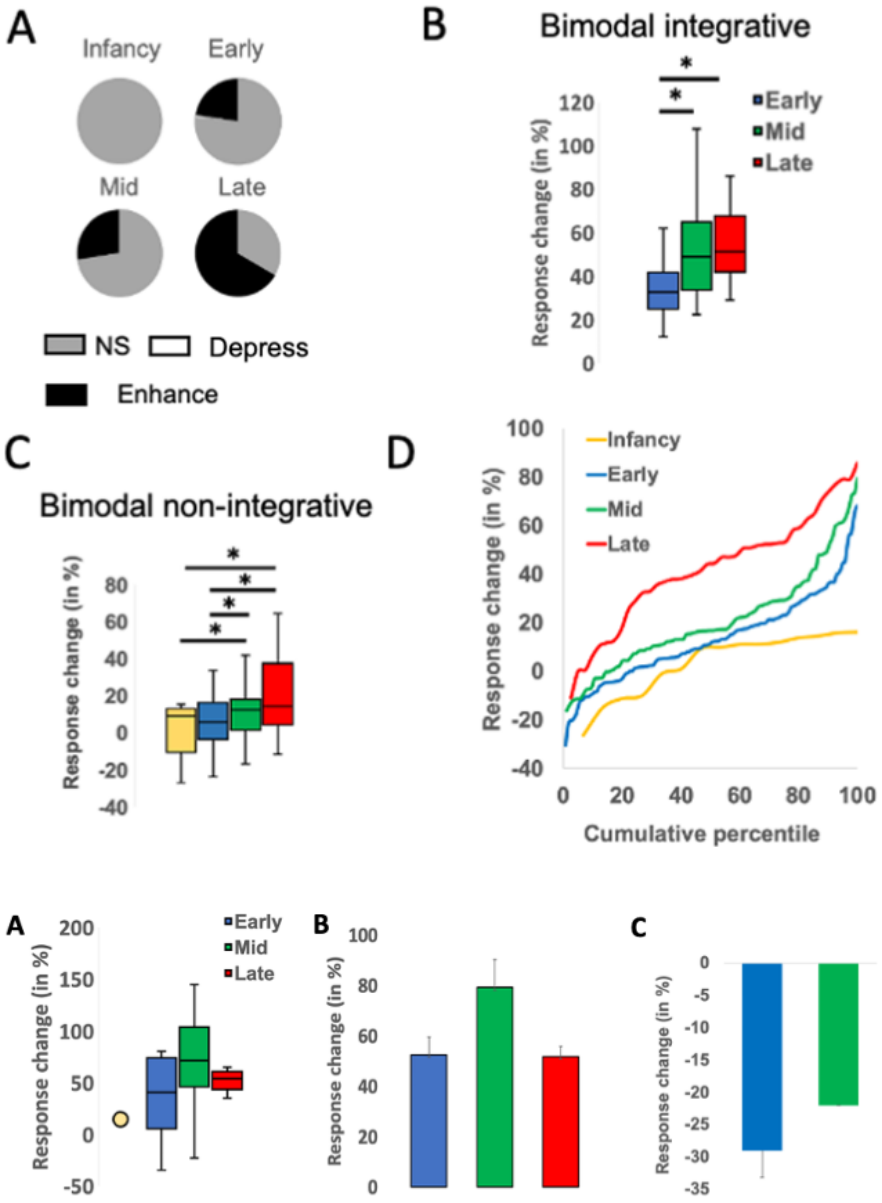

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