The Effect of Perceived Predation on the Neural Development of Convict Cichlids

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Abstract

The Effect of Perceived Predation on the Neural Development of Convict Cichlids.

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Elevated levels of background risk have been shown to elicit changes in behaviour, physiology, morphology, and cognitive function. While there is a growing body of research investigating how various aspects of the environment influence brain growth, research examining neuroplastic responses to local predation is lacking. Using lab-reared convict cichlids (*Amitatlania nigrofasciata)* as a model species, I tested the hypothesis that neuroplastic responses will vary between two levels of perceived predation risk in both juveniles and adults. In a series of laboratory trials, convict cichlids at two different ontogenetic stages (juveniles and adults) were exposed to either the alarm cues of injured conspecifics (high risk) or distilled water (low risk). When juvenile convict cichlids were exposed to high risk cues, they showed a significant increase in olfactory bulb size (19.7%) compared to the low-risk control. Additionally, all brain regions, when exposed to high risk cues, increased in size when compared to the low risk group: 13.5% in the telencephalon, 20.8% in the optic bulb, 11.9% in the cerebellum, and 18.2% in the hypothalamus. Overall the entire brain increased by 16.2% when compared to the low risk group, however no allometric growth of any single brain region was observed. Unlike the results seen in juveniles 1 day post treatment, examination of adult cichlid brains revealed no difference in olfactory bulb size, or any brain region, between the high and low risk groups. Furthermore, high risk juveniles that were given an 11-day latency period following treatment, showed no significant difference in any brain region, including olfactory bulb size, when compared to those given distilled water. Taken together these results suggest that juvenile cichlids may exhibit a bidirectional neuroplastic response to high risk cues and that these responses are ontogenetically constrained.

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Introduction

Predation is a pervasive selective pressure acting on prey populations (Lima & Dill, 1990; Priesser et al. 2005; Carreau-Green et al., 2008; Ferrari et al., 2009, 2010a), shaping their behaviour (Wisenden & Sargent, 1997; Foam et al., 2005a, 2005b; Gonzalo et al., 2012; Brown et al., 2014a), morphology (Brönmark & Miner, 1992; Brönmark & Pettersson, 1994; Chivers et al., 2007), and life history (Chivers et al., 1999). The capture and consumption of prey, which is termed the consumptive effect of predation, has a direct cost on prey fitness (Preisser et al., 2005). Although this direct cost of predation is high, there is an additional toll incurred via the struggle to avoid predators (Preisser et al., 2005; Jarvis, 2010; Peacor et al., 2011). These avoidance costs are referred to as the non-consumptive effects (NCE) of predation (Preisser et al., 2005; Peacor et al., 2011). They result from prey having to balance the avoidance of predators with the need to sustain other fitness-related activities (Helfman, 1989; Wagner & Luksch, 1998; Lima & Bednekoff, 1999). These activities include, but are not limited to, foraging (Foam et al., 2005a, 2005b; Dadda & Bisazza, 2006; Ferrari et al., 2010a), mating (Kats & Dill 1998; Kotrschal et al., 2015), and defending territories (Kim et al., 2011)

In response to predation pressure, most species can alter some aspect of their phenotype (Schoeppner & Relyea, 2005). This is known as a phenotypically plastic response: a single genotype that can produce multiple phenotypes under different environmental conditions (Smith & Smith, 2011). The ability to change phenotype allows individuals to minimize the potentially negative impacts of varying environmental conditions (Auld et al., 2009). Plasticity in response to predation has been demonstrated in behaviour, such as induced neophobia (Brown et al., 2013b, Joyce et al., 2016) and antipredator responses (Roh et al., 2004; Schoeppner & Relyea., 2005; Chivers et al., 2016b), as well as in life-history alterations that facilitate predator

avoidance at specific life stages (Chivers et al., 1999), and physiology as seen in the endocrine responses to increased perceived risk (Day et al., 2004). Increased predation has also been shown to induce morphological plasticity in prey species (Relyea, 2003; Hoverman & Relyea, 2007). Chivers et al., (2007) showed that goldfish (*Carassius auratus*) increased their body depth and weight in response to high risk, which was associated with increased survival during predation events. Similar results were observed in research conducted on the crucian carp (*Carassius carassius*) by Brönmark & Miner (1992) and Brönmark & Pettersson (1994). In addition, there is evidence that predator-induced morphological plasticity is reversible when the threat of predation is removed (Relyea, 2003; Chivers et al., 2007). For example, freshwater snails (*Helisoma trivolis*) show a reversal of predator-induced shell growth shortly after the predator is removed from their environment (Hoverman & Relyea, 2007). Prey often live in close proximity to their predators (Ward & Mehnerb, 2010). Morphological plasticity is becoming widely accepted as a means for individual prey to mitigate the risk posed by nearby predators.

Neuroplasticity

Different types of environmental information are processed by different regions of the brain in fish (Ebbesson & Braithwaite, 2012; Wagner, 2002). Olfactory bulbs process chemical cues (i.e. foraging and risk assessment information) from the surrounding environment (Ebbesson & Braithwaite, 2012; Kotrschal et al., 2012a). The telencephalon integrates spatial information (Burns & Rodd, 2008; Ebbesson & Braithwaite, 2012). A larger telencephalon may increase the accuracy and speed to make a decision (van der Bijl et al., 2015). The area of the brain that facilitates visual perception is the optic bulb (van der Bijl et al., 2015). The cerebellum is responsible for associative learning (Ebbesson & Braithwaite, 2012) and coordination of

movements (Kotrschal et al., 2012a). Lastly, the hypothalamus plays a major role in the regulation of hormones and neurochemicals (Blanton & Specker, 2007).

The relative size of each brain region corresponds to the individual's ecological niche (Näslund et al., 2012; Wagner, 2002). For example, reef fishes, irrespective of phylogenetic group, have large brains with well-developed telencephala and highly-foliated cerebella because of the complex environment that they inhabit (Yopak et al., 2012). This relationship indicates that environmental complexity requires a great deal of spatial processing and associative learning.

In general, the vertebrate brain is very plastic only in early life (Fernandez et al., 2011; Kotrschal et al., 2012a). The brains of fish, however, remain plastic even in adulthood (Zupanc, 2001; Mayer et al., 2011; Ebbesson & Braithwaite, 2012). Adult fish brains have an enormous potential for the neural cell proliferation that underlies neuroplasticity (Lema et al., 2005; Shumway, 2008; Fernández et al., 2011). For example, during any given two-hour period, adult brown ghost knifefish (*Apteronotus leptorhynchus*) have on average 0.2% of brain cells in the s-phase of mitosis (Zupanc, 2001). Similar neural cell proliferation was also observed in three species of adult teleost fish (Fernández et al., 2011). The brain-wide neural cell proliferation seen in fishes underlies their experience-dependent neuroplasticity (Shumway, 2008). As the environmental information being processed shifts, there is an associated change in brain development (Gittleman, 1994).

An increase in the amount of environmental information being processed is positively associated with brain development (Gittleman, 1994). The greater the extent to which an individual encounters the same environmental cues, the more the associated synapses will grow and strengthen via long term potentiation (LTP) (Nabavi et al., 2014). If a stressor, such as the

threat of predation, is consistently present, then the brain structures associated with predator-detection and avoidance are expected grow (Kotrschal et al., 2012b). However, an experiment by Gonda et al. (2010) showed that tadpoles (*Rana temporaria*) under conditions of low-density and high-predation developed smaller brains relative to those at high-density and high-predation and smaller brains than those at low-predation regardless of density (Gonda et al., 2010). In response to these unexpected results, the authors offered an explanation. They suggest that at low-density there is not enough group protection from a live predator. Less group protection means that the tadpoles must increase time sheltering from the predator, whose presence is constant throughout the experiment. The reduction in energy intake results in less energy available for brain development (Gonda et al., 2010).

The results of the above experiment indicate that the inability to obtain food can limit neural development, and there is no definitive effect of elevated risk by itself, stressing the importance of adequate feeding regimes during experiments. Indeed, zebra finch chicks (*Taeniopygia guttata*) subject to food deprivation develop smaller brains and smaller song repertoires than chicks exposed to a stable food source (Brumm et al., 2009). With the exception of the study by Gonda et al. (2010), there is virtually no available information regarding the influence of predation on neuroplasticity.

Environmental conditions can effectively influence the rate of cell proliferation in fish brains (Lema et al., 2005) despite the fact that neural processing is expensive, metabolically speaking (Laughlin et al., 1998; Marino et al., 2006; Monk et al., 2015). Increasing the size of the brain escalates the amount of energy required to maintain the Na/K ion concentration gradient across neural cell membranes (Laughlin et al., 1998). An enhanced nervous system must therefore provide an ecological benefit to compensate for the energy consumption (Monk et al.,

2015). In spite of the energetic cost, plastic reorganization of neural structures can benefit animals that inhabit areas with more socially available information (Lema et al., 2005) through enhanced sensory processing (Lefebvre et al., 1997). The ability of larger brains to process, store, and integrate copious amounts of environmental information has fitness benefits (Lefebvre et al., 1997; Lema et al., 2005; Sol et al., 2005), such as the heightened ability to recognize and thereby evade predation threats (Beston et al., 2017). Positive neuroplastic responses are likely the result of a balance between the constraining energetic costs of developing and maintaining a larger brain (Johnson, 1999) against the increased cognitive benefits (Kotrschal et al., 2013).

An important consequence of this balancing act is the bi-directionality of neuroplastic responses (Nabavi et al., 2014). If neurons are under-stimulated, such as when there is little to no specific stimulus and therefore little need for associated neurons, the synapses are expected to decrease in both number and size (Johnson, 1999), a process called long term depression (LTD) (Nabavi et al., 2014). A great example of this is seen in Atlantic salmon (*Salmo salar*). Näslund et al., (2012) showed that increased salmon brain size due to environmental complexity can disappear over time when the fish are transferred into barren tanks.

Specific environmental factors contributing to neuroplasticity are difficult to pinpoint (Lema et al., 2005). To date, most studies on the neuroplasticity of fishes have focused on the effect of visual environmental complexity (Kihslinger et al., 2006; Mayer et al., 2011; Kotrschal et al., 2012b; Näslund et al., 2012; Salvanes et al., 2013; also see Ebbesson & Braithwaite, 2012). Despite the fact that predation is such a predominant factor affecting prey species survival (Lima & Dill, 1990; Roh et al., 2004; Preisser et al., 2005), very little information is available regarding how brain morphology changes with varying levels of predatation signals. Assumptions on how neuroplasticity is related to high risk cues can only be made based on

behavioural evidence from several studies. Researchers such as Brown & Smith (1998), Ferrari et al., (2012), Brown et al., (2013c), and Mitchell et al., (2016) have examined the retention period of behaviours after conditioning prey to high risk. For example, woodfrog tadpoles (*Lithobates sylvaticus*) conditioned under high risk lost their neophobic response 11 days post-conditioning (Mitchel et al., 2016). Responding to predators that are no longer a threat would be detrimental over time (Brown et al., 2013c). These results indicate that anti-predator behaviour is being gained and then lost. If neurological processes underlie behaviours (Marchetti & Price 1989; Lefebvre et al., 2004; Lefebvre & Sol 2008), it is likely that the associated brain regions respond to elevated risk by growing in size, then diminishing once there is no longer an apparent risk.

Ontogenetic Constraints

Throughout ontogeny, most organisms are subject to changes in size, morphology (Persson et al., 2000), and predation risk (Kelley & Magurran, 2003). Size is a crucial component influencing an individual's vulnerability to predation (Joyce et al., 2016). During early stages of life, organisms are highly susceptible to predators (Kusch & Chivers, 2004). As prey become larger, they often exceed predator gape limitations (Brown et al., 2002) and may be better at outmaneuvering predators (Brown et al., 2013c). An individual's perception of risk may therefore be influenced by its developmental stage (Marcus & Brown, 2003; Brown et al., 2013a; Joyce et al., 2016).

Prey respond to acute risks differently based on their body size (Marcus & Brown, 2003; Harvey & Brown, 2004; Joyce et al., 2016). For example, faster growing individuals appear to lose their predator recognition retention at a faster rate (Brown et al., 2011b). This suggests that

responses to predators are retained only as long as the predator is still a threat (Brown et al., 2011a, 2013c), such as when the prey is younger and smaller (Ferrari et al., 2012).

Ontogenetic shifts in anti-predator behaviour have been reported in a number of species. For instance, yellow perch (*Perca flavescens*) show a size-dependent response to high risk (Harvey & Brown, 2004). Juvenile perch respond to alarm cues with anti-predator behaviour, while adult perch react to the same cues with foraging behaviour (Harvey & Brown, 2004). Similar results for size-based behavioural differences have been demonstrated by Marcus & Brown (2003) in pumpkinseed sunfish (*Lepomis gibbosus*), and also by Brown et al. (2002) in largemouth bass (*Micropterus salmoides*). Additionally, Joyce et al. (2016) conditioned convict cichlids to elevated-risk at three different life stages. Those conditioned to high risk in early life stages showed an induced neophobic response to novel odors, whereas adults under the same conditions did not.

Previous research has shown that both juvenile and adult fishes respond to high risk, although the behavioural response often differs. To date, no studies have specifically examined if ontogeny constrains neuroplasticity in response to high background risk. Even though Zupanc (2001) suggests that fish brains remain plastic throughout their lives, brain growth can vary depending on the life history stage (Kihslinger et al., 2006). If brain development is in fact linked to risk-induced behavioural responses, then there exists a necessity to investigate the limitations that ontogenetic stage imposes on neuroplasticity.

Thesis Goal

Plastic responses in morphology can be triggered by the alarm cues of injured conspecifics (Chivers et al., 2007; Ferrari et al., 2010b) as their presence is a reliable indicator of elevated predation risk (Hazlett, 2003; Ferrari et al., 2010b; Brown et al., 2013a; Gonzalo et al., 2012). Alarm cues are common among a wide variety of marine and freshwater fishes (Brown et al., 2013c), including cichlids and salmonids (Brown et al., 2002).

Using lab-reared convict cichlids (*Amitatlania nigrofasciata)* as a model species, I tested the hypothesis that neuroplastic responses will vary between two levels of perceived predation risk in both juveniles and adults. To test this hypothesis, I manipulated perceived predation risk levels using conspecific chemical alarm cues or a distilled water control.

First, by exposing juveniles to either elevated risk cues or low risk, I tested for differences in brain development between the two levels of perceived predation. An increase in the integration of environmental cues is positively associated with the growth of neural regions that detect said cues (Gittleman, 1994; Nabavi et al., 2014). The area that integrates chemical information is the olfactory bulb (Ebbesson & Braithwaite, 2012; Kotrschal et al., 2012a). By using conspecific chemical alarm cues to simulate predation risk I anticipated that perceived elevated risk will result in increased olfactory bulb size in juvenile convict cichlids, when compared to the low risk group. As I am using chemical cues to simulate elevated risk I do not expect increases in other brain region sizes as they do not integrate chemical information.

Furthermore, by removing the perceived risk followed by a low risk latency period, I investigated if the neuroplastic response to high risk cues can be lost. Nabavi et al., (2014) suggest that neuroplasticity is bi-directional. For this reason, I predicted that a latency period of 11 days post-conditioning will be associated with a cessation in juvenile olfactory bulb

development to a point where there is no difference between those conditioned under high risk and those under low risk. This predicted outcome is centered around behavioural research conducted on prey animals. Ferrari et al. (2012) and Mitchel et al. (2016) showed that behavioural responses to predation threats can diminish after 11 days.

Lastly adult cichlids were exposed to either high risk or low risk to investigate if the neuroplastic response to high risk cues is constrained by ontogeny. Although there is evidence that neophobia cannot be induced in adult cichlids (Joyce et al., 2016), fish brains are thought to remain plastic throughout their lives (Mayer et al., 2011; Ebbesson & Braithwaite, 2012). As such the third prediction of this thesis asserted that adults will show neuroplasticity similar to that of juveniles when exposed to high risk. Despite the ontogenetic differences in fish behaviours towards alarm cues, both juvenile and adult conspecifics still react in some way to alarm cues (Brown et al., 2002; Marcus & Brown, 2003; Harvey & Brown, 2004). This suggests that there is neural processing involved in cue detection regardless of age. The detection of alarm cues should therefore elicit neural development in the brain regions responsible for processing those cues: the olfactory bulbs. Due to a low sample size for adult cichlids, the effect of an 11 day latency period was tested only in juveniles.

The above predictions are rooted in the effect that predation may have on brain development. However, there exists an alternative hypothesis in the response to adding alarm cue. It could simply be that the addition of these cues adds complexity to the environment. The difference between the high and low risk may simply be due to the increase in socially available information (environmental complexity) in the high risk group. This alternative hypothesis extends beyond the scope of this experiment but should be kept in mind as an alternative explanation that can be tested in the future.

Methods

Juvenile convict cichlids (*Amatitlania nigrofasciata)* were reared from laboratory stock populations. The 38L holding tanks were kept barren at the time of hatching until the end of the experiment to minimize stimuli that can influence brain development (Näslund et al., 2012). Holding tanks were filled with dechlorinated tap water and held at constant conditions (~26^{o}C, pH ~7.2, 12:12 L:D cycle). Cichlids were fed daily with commercial flake food to satiation outside of the treatment times. This was done to account for feeding decreases that can occur from predator avoidance (Gonda et al., 2010).

Conspecific skin extract (alarm cue) was made to be used for the treatment phase (see experimental methods). Eighteen adult convict cichlids (6.9 cm ± 0.12 cm SL) were euthanized via cervical dislocation (in accordance with the Canadian Council on Animal Care and Concordia University Animal Research Ethics protocol #30000255). Skin fillets were removed from both sides of the donor convict cichlids and immediately placed into 100 mL of chilled distilled water. A total of 326 cm^2 of skin (diluted to a final volume of 2,178 mL) was collected. The alarm cue was frozen in 100 mL aliquots at -20°C until required.

Experiment 1: Response to perceived predation in juveniles

At 86 days post-hatching, on June 20 2017, a single brood of sample fish were randomly assigned to a treatment or control group with five replicates of each treatment. Each 38L treatment tank housed 28 cichlids (n_{total}=280), all of which were identified as juveniles, since cichlids do not reach maturity until six months post hatching (Ishikawa & Tachihara, 2010). Treatments were administered for 14 days at the same three times each day: 10:00am, 12:00pm, and 2:00pm.

The juvenile low-risk/control group (n_{LR}=140) received 10 mL of distilled water to control for the disturbance of adding a treatment. The juvenile high-risk/alarm cue group (n_{HR}=140) was given 10 mL of conspecific chemical alarm cue three times per day to simulate a nearby predation event. At the end of the 14-day treatment period, 14 juveniles from each of the experimental tanks (n_{HR}=70, n_{LR}=70) were haphazardly removed for analysis. The remaining fish were kept in their tanks to be used in *Experiment 2*. Mean (± SD) standard length at removal was 2.14 ± 0.40 cm. Standard length was not measured prior to treatments.

Fish were anesthetized in accordance with Concordia University Animal Research Ethics protocol number AREC30000255 using > 0.4 mL clove oil L^{-1} of water. Fish were preserved in 10% buffered formalin and stored cold for a minimum of 30 days to allow for adequate tissue fixation (Mu & Sanders, 2010). Standard body length (SL) was measured after anaesthetization and before dissection. These measures were taken using ImageJ software to the nearest 10^{-5} m.

Heads were mounted with the ventral side down in high-contrast wax on small dissection plates. Under a dissection microscope, surgical scissors were inserted into the eye socket posterior to the eye, and the dorsal region of the hyomandibular plate was severed on each side. Additional cuts made between the nares and from the nares to the anterior region of the eye-socket severed the frontal plate of the skull. The spinal cord was cut immediately posterior to the skull and needle-nose tweezers were used to fully remove the parietal and frontal plates of the skull thereby exposing the brain. The remaining debris was carefully removed, and surgical tweezers were used to fully remove the brain.

Images of the dorsal and ventral surfaces of the brain were taken using a three mega-pixel microscope camera. Cross-sectional measurements of each region were taken using ImageJ software to the nearest 10^{-5}m (*Figure 1*). 29.7% of olfactory bulbs, 10.3% of telencephala, 7.6%

of optic bulbs, 9.0% of cerebella, and 8.3% of hypothalami were removed from the analysis as they were damaged or unmeasurable.

Experiment 2: 11-day latency period for juveniles

This experiment was conducted between June 20 and July 14 2017. Only juveniles were examined due to the small number of available adults. Half of the fish treated in *Experiment 1* were left in the treatment tanks (n_{total}=140). They were kept for an 11-day latency period in order to determine if brain development reverts back to a low predation condition. During this time, no treatments were administered. At the end of the latency period, the remaining fish were removed for morphometric analysis. The procedure followed parts 2-5 of *experiment 1*. Mean (± SD) standard length at removal was 2.08 ± 0.45 cm. Standard length was not measured prior to treatments.

Experiment 3: Response to perceived predation in adults

At 12 months post-hatching, commencing March 13 2017, a single brood of sample fish were randomly assigned to a treatment or control group with 2 replicates of each treatment. Each 38L treatment tank housed 11 adult cichlids (n_{total}=44). The adult groups received the same treatment as the juveniles. At the end of the 14-day treatment period, all of the adults were removed for analysis. The procedure followed parts 2-5 of Experiment 1. Mean (± SD) standard length at removal was 6.65 ± 1.02 cm. Standard length was not measured prior to treatments.

Statistical analysis

Measurements were log transformed in order for the data to meet the assumptions of normality and homogeneity of variance. To determine the differences in brain growth between high and low risk groups I ran a linear mixed model ANCOVA using the *lm* function in R studio. Two different models were employed using either the sum of each brain region (all-brain) or

standard length (SL) as a cofactor. The dependent variable used was log transformed olfactory bulb surface area. In order to account for the effect that different tanks may have had on neural growth, tank number was included in the ANCOVA as a random factor. All data met the assumptions of normality (Shapiro-Wilk test) and equality of variance (Levene's test). Additionally, log transformed telencephalon, optic bulb, cerebellum, hypothalamus surface areas and the sum of all regions were analyzed as dependent variables (See *Appendix* 1 for SL as a covariate and *Appendix 2* for all-brain as a covariate). Percent differences between high and low risk groups were found for each brain region using the estimated marginal means for high and low risk.

To determine the repeatability of results, 20 bodies and 33 brains were randomly selected and re-measured by a colleague. A two-way mixed model intraclass correlation comparison between the first and second measurements was conducted to assess their absolute agreement (*Table 1*). Analysis yielded intraclass correlation average measures for standard length = 0.991 ($p < 0.001$), all-brain = 0.967 ($p < 0.001$), and olfactory bulb = 0.966 ($p < 0.001$).

Results

Experiment 1: Juveniles 1 day post treatment

Mixed model ANCOVA, utilizing SL as a covariate and tank number as a random factor, revealed a significant positive relationship between SL and olfactory bulb size ($F_{1,90} = 91.049$, $p < 0.001$). The same relationship can be seen between SL and the other four brain regions (*Table 2.1*). As SL increases, so does the size of the olfactory bulb (*Fig. 2.1*), as well as each other brain region measured (*Appendix 1*).

The results of *Experiment 1* were consistent with the first prediction of the thesis. Those that were exposed to high risk cues had 19.7% larger olfactory bulb sizes compared to those in the low risk group, when controlling for SL ($F_{1,8} = 9.776$, $p = 0.014$; *Fig 2.1*). The other four regions also showed significant increases in size when exposed to high risk (*Table 2.1*; *Appendix 1*). Indeed, when accounting for differences in SL, the overall brain size was 16.2% larger in cichlids exposed to high risk cues, when compared to the low risk group ($F_{1,8} = 15.116$, $p = 0.005$; *Fig. 2.4*).

There was no interaction between SL and treatment for any brain region except for the optic bulb ($F_{1,12\ 1} = 8.941$, $p = 0.003$; *Table 2.1*). As body size increases, the effect that high risk cues have on optic bulb size is less pronounced (*Appendix 1*). For the other four regions, including the olfactory bulb, the effect that treatment had on brain region growth did not depend on body size (*Table 2.1*).

Using a mixed model ANCOVA with the sum of all brain regions, "all-brain", as a covariate and tank number as a random factor, the relative size of each brain region in response to high versus low risk was determined. As stated above, olfactory bulbs were larger in the high risk group when SL was accounted for. Olfactory bulbs did not, however, grow proportionally larger than the rest of the brain in response to elevated risk cues (*Fig. 3.1*). In fact, no single brain region that was measured grew larger in proportion to the rest of the brain in response to the high-risk cues when compared to the low risk group (*Table 3.1*; *Appendix 2*).

Comparable to the model that used SL as a covariate, the all-brain model also yielded a significant positive relationship between all-brain size and olfactory bulb size ($F_{1,88} = 249.289$, $p < 0.001$). A similar relationship was found between all-brain size and the other regions measured (*Table 3.1*).

Interaction between risk level and all-brain size was found for only one brain region. There was no significant interaction between all-brain size and treatment on olfactory bulb, optic bulb, cerebellum, nor hypothalamus sizes (*Table 3.1*). However, as all-brain size increases, the effect that elevated risk has on telencephalon size decreases ($F_{1,87} = 13.028$, $p = 0.001$; *Appendix 2*).

Experiment 2: Juveniles 11 days post treatment

As in *experiment 1*, both SL and all-brain size had significant positive relationships with olfactory bulb size ($F_{1,114} = 73.451$, $p < 0.001$ and $F_{1,111} = 88.966$, $P < 0.001$ respectively). The same relationship is seen for all other brain regions with regard to SL (*Table 2.2*) and all-brain size (*Table 3.2*).

Consistent with the second prediction of this thesis, mixed model ANCOVA using SL as a covariate revealed that after an 11-day latency period, high-risk cues no longer had a significant effect on olfactory bulb size when compared to the low-risk group *(*$F_{1,8} = 1.562$, $p = 0.247$; *Fig. 2.2*). This suggests that the effect that high risk cues have on olfactory bulb size may not be long lasting. Additionally, there was no significant effect of treatment on olfactory bulb size when controlling for all-brain size ($F_{1,8} = 1.133$, $p = 0.318$; *Fig. 3.2*).

Indeed after 11 days without receiving treatment, no single region showed a significant difference in relative size between high and low risk groups when controlling for either SL (*Appendix 1; Table 2.2*) or all-brain size (*Appendix 2; Table 3.2*). The overall brain size also showed no difference in size between risk levels after 11 days without treatment when accounting for SL (*Fig. 2.5*).

No interaction was found between treatment and either covariate (SL or all-brain size) on any brain region.

Experiment 3: Adults 1 day post treatment

With an increase in SL, there is an associated increase in olfactory bulb size ($F_{1,36}$ = 42.916, $p < 0.001$), as well as in the other four brain regions measured (*Table 2.3*). Similarly, as all-brain size increases, so does every brain region measured including olfactory bulb size ($F_{1,34}$ = 75.302, $p < 0.001$; *Table 3.3*).

The results found using SL as a covariate were inconsistent with the initial prediction made for adult brain plasticity. There was no significant effect of high risk cues on adult olfactory bulb size when compared to the low risk group ($F_{1,2}$ = 1.251, p = 0.380; *Fig 2.3*). Exposure to high risk did not appear to affect the other regions of the brain (*Table 2.3; Appendix 1*) nor the overall size of the brain when compared to the low risk group (*Fig. 2.6*). There was no interaction observed between treatment and body size on any brain region (*Table 2.3*).

After applying all-brain size as a covariate, there was no proportional increase in olfactory bulb size ($F_{1,2}$ = 0.634, p = 0.509; *Fig. 3.3*) or any other region (*Table 3.3*; *Appendix 2*) when adults were exposed to high risk cues. Once again there was no interaction between all-brain size and treatment on any brain region size (*Table 3.3*).

Discussion

Results using conspecific chemical alarm cue as a proxy of predation risk provide support to the hypothesis that environments enriched with high risk cues can actuate neuroplasticity in juvenile convict cichlids. The same response however, does not occur in adults.

I found that environmental cues indicative of predation risk elicit a phenotypically plastic response in the brain anatomy of juvenile convict cichlids. When exposed to chemical alarm cues, they exhibited a 19.7 % increase in olfactory bulb growth compared to those in the distilled

water control group. This suggests that socially available cues regarding potential risk are related to neural growth. It remains to be determined, however, whether the observed differences between the high and low risk groups are the result of contrasting environmental complexity or varied predation risk.

It is important to note that the results of this experiment extend beyond the scope of the a-priori predictions (see appendix 1). In addition to olfactory bulb size, elevated risk had an effect on all other brain regions measured. When accounting for SL, the overall brain size was 16.2% larger in the high risk group compared to the low risk group. Although there was an increase in overall brain size, there was no significant allometric growth of any singular brain region after exposure to high risk cues.

The reasons for the growth seen in the other brain regions can only be speculated at this time. One explanation is that elevated risk alone encourages general neural growth compared to those in the control group. There is the possibility, however, that the addition of cues that imitate elevated danger may simply add a complexity factor to the environment, which in turn stimulates general brain growth. This alternative possibility must not be overlooked as it has been shown that environmental complexity can result in increased brain growth (Kihslinger et al., 2006; Mayer et al., 2011; Kotrschal et al., 2012b; Näslund et al., 2012; Salvanes et al., 2013). This thesis is the first study to examine how chemical alarm cues influence neuroplasticity. The explanation for why there are size differences between the high and low risk groups remains to be investigated. Whether they are due to increased complexity or the threat of predation is outside of the scope of this thesis. However, the morphological consequences of using chemical alarm cues are certain: they stimulate neural growth.

I observed that the use of chemical alarm cues promoted growth in optic bulb size by 20.8%. The optic bulbs are the area of the brain that facilitate visual perception (van der Bijl et al., 2015). These results therefore indicate that the integration of risk related environmental information via one receiving system may stimulate the growth of other sensory systems. The complementary growth of multiple processing structures could be explained by the fact that fish rely heavily on alternative cues to accurately assess their surroundings (Kelley & Magurran, 2003). Brown et al., (2011a) describe how individuals from high ambient background risk increase their vigilance towards secondary cues when exposed to low concentrations of alarm cue. Increased vigilance towards a secondary cue can allow prey to reliably assess risk (Foam et al., 2005a; Ward & Mehnerb, 2010; Brown et al., 2011a, 2014b). Results here provide substantiation for a morphological alteration behind a prey's ability to increase cue reliability in environments that are enriched with high risk indicators.

The telencephalon is the region responsible for integrating spatial information (Burns & Rodd, 2008; Ebbesson & Braithwaite, 2012). A larger telencephalon may increase the accuracy and speed to make a decision (van der Bijl et al., 2015). The high risk group had 13.5% larger telencephala compared to the low risk group. Supplementing habitat complexity with elevated risk signals may therefore potentially improve the rate and accuracy at which these fish make decisions about fitness related activities, including predator avoidance strategies.

An 11.9% increase in cerebellum size resulting from the addition of chemical alarm cues could aid in prey fitness, as it is the brain region in charge of coordinating movement (Kotrschal et al., 2012a). For example, Chivers et al. (2016a) showed that increased predation strengthens lateralization (turning bias) in yellow-and-blueback fusiliers (*Caesio* teres), which is associated with an increase in escape performance from predators. The alarm cue-related increase in

cerebellum size may therefore allow for more complex escape maneuvers. However, this remains to be tested.

Juveniles also showed an 18.2% increase in hypothalamus growth when the environment was enriched with high risk cues. This brain region is a key component of the hypothalamus-pituitary-thyroid (HPT) endocrine axis and plays a major role in regulating hormones and neurochemicals (Blanton & Specker, 2007). A larger hypothalamus is likely associated with an intense physiological response to chemical alarm cues that has yet to be explored fully. However, it is probable that the neuroplastic response of the hypothalamus is related to changes in somatic growth, metabolism, and life history (Blanton & Specker, 2007). For example, increased neurological connection between the hypothalamus and pituitary corresponds to greater control of gonad development (Scott, 1987). For this reason, it may be interesting to see how chemical alarm cues may influence age at maturity for convict cichlids.

Overall, larger brains are thought to increase cognitive ability (Kotrschal et al., 2015; 2013), which in turn improves monitoring and assessment of their environment (Sol et al., 2005, Lefebvre & Sol, 2008) and predation threats (van der Bijl et al., 2015). The associated increase in information acquisition likely provides a fail-safe system whereby prey fish are less prone to errors in decision making (Näslund et al., 2012).

The second prediction of this thesis presumed that 11 days after exposure to alarm cues, the difference in olfactory bulb size between high and low risk groups would diminish. Following a latency period, there was no longer an observable difference between treatments in olfactory bulb, or any other region of the brain. Indeed, the high and low risk groups showed similar overall brain size. There are two possible explanations that may explain these results. The first is based on work by Chivers et al. (2007) which showed that after discontinuing high risk

treatment, the body growth of goldfish showed the same pattern as the distilled water control group. After stimulation is interrupted, the brains of the high risk group could simply decrease in growth rate. The rate reduction would then allow the brain of the low risk group to catch up. Alternatively, what we are seeing could be bidirectional neuroplasticity. After the environment was no longer being enriched with high risk cues, the brains of the high risk fish reverted back to an under-stimulated/low risk condition, similar to the reversal in shell growth seen in freshwater snails (Hoverman & Relyea, 2007).

The third prediction of this thesis, was that adults exposed to alarm cues would have increased olfactory bulb size, compared to the control group. However, there was no apparent effect of alarm cue seen on olfactory bulb size. Additionally, I found no difference in neural growth between high and low risk for any brain region in adults. This indicates that brain development in adult cichlids is not affected by environmental enrichment via alarm cues.

Reasons for why adults do not exhibit neuroplasticity in this experiment are not known. It could be that they are exposed to damage release conspecific chemical alarm cues as a result of aggressive interactions, such as defending territories (Praw & Grant, 1999), during the year leading up to the experiments. Another explanation is the energetic cost of growing neural growth acting as a constraint. Adult fish will often trade off growth in favour of allocating more energy to reproduction (Ferrari et al., 2010b). If fish can trade off somatic growth, then it is likely that they also trade off neural growth for reproduction. If the neural growth seen in juveniles is in response to environmental enrichment rather than the threat of predation, then this explanation for the lack of adult neuroplasticity is very likely.

Alternatively, if brain growth is the result of predation risk, then the lack of difference between treatments in adults may be attributed to an ontogenetic niche shift. In this case, the

larger body size of adults may provide protection from predators (Brönmark & Miner, 1992; Brönmark & Pettersson, 1994; Chivers et al., 2007). By taking refuge in body size alone, adults may not need to respond to chemical alarm cues as it would be energetically costly (Brown et al., 2002; Marcus & Brown, 2003; Harvey & Brown, 2004). This explanation would imply that neuroplasticity in response to chemical alarm cues is ontogenetically constrained. It may also help to explain why juvenile cichlids exhibit induced neophobia whereas adults do not (Joyce et al., 2016).

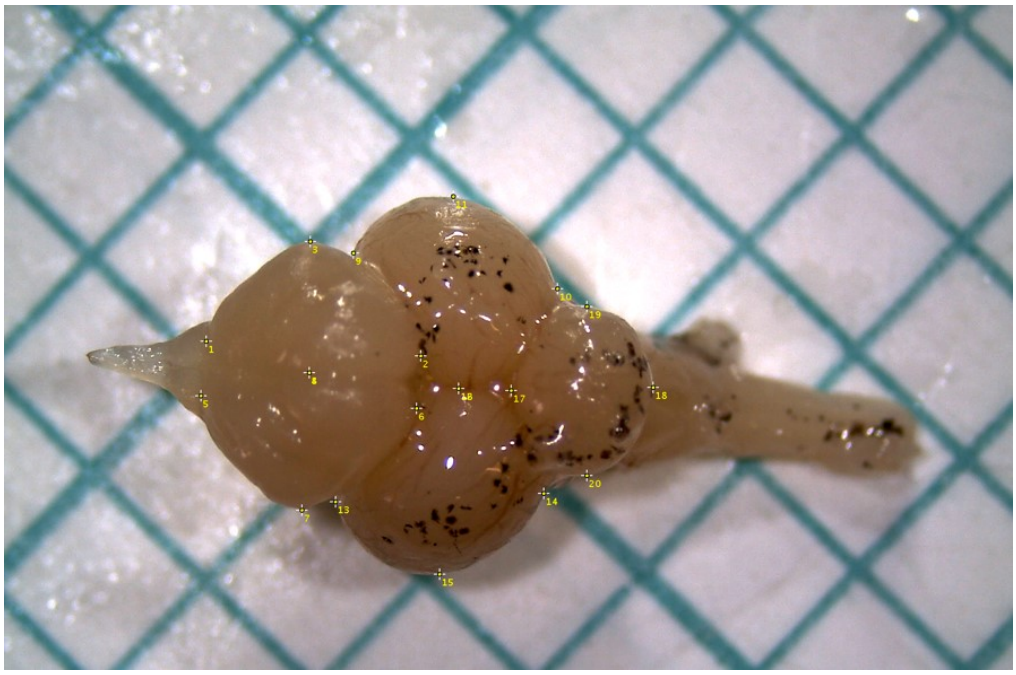
Here I have highlighted that neuroplasticity occurs in juvenile convict cichlids and that this plasticity may be bi-directional. I have shown that adult cichlids do not have a significant neuroplastic response to the alarm cues of injured conspecifics. It is likely that the neuroplastic reactions seen in juveniles could aid in predation responses, however we can't rule out that neural growth may simply be due to the enhanced environmental complexity. Regardless of the underlying reason, this thesis demonstrates that chemical alarm cues can stimulate neural growth.

Understanding how environmental cues affect neuroplasticity and cognitive ability at various life stages is important for the management fish populations (Ebbesson & Braithwaite, 2012). Previous studies have shown that a larger brain relative to body size is associated with higher cognitive ability (Burns & Rodd, 2008; Kotrschal et al., 2013; 2015), behavioural flexibility (Sayol et al., 2016), and increased survival (Sol et al., 2007; 2008). My findings may provide valuable insight for the hatchery industry as hatchery fish are often restocked for population enhancement and conservation (Brown et al., 2013a). This is important as numerous fish species, including many species of cichlids (Turner, 2007), have experienced population declines despite restoration efforts (Salvanes & Braithwaite, 2006). Globally speaking, cichlid species, such as tilapia, are very important freshwater food sources (Turner, 2007).

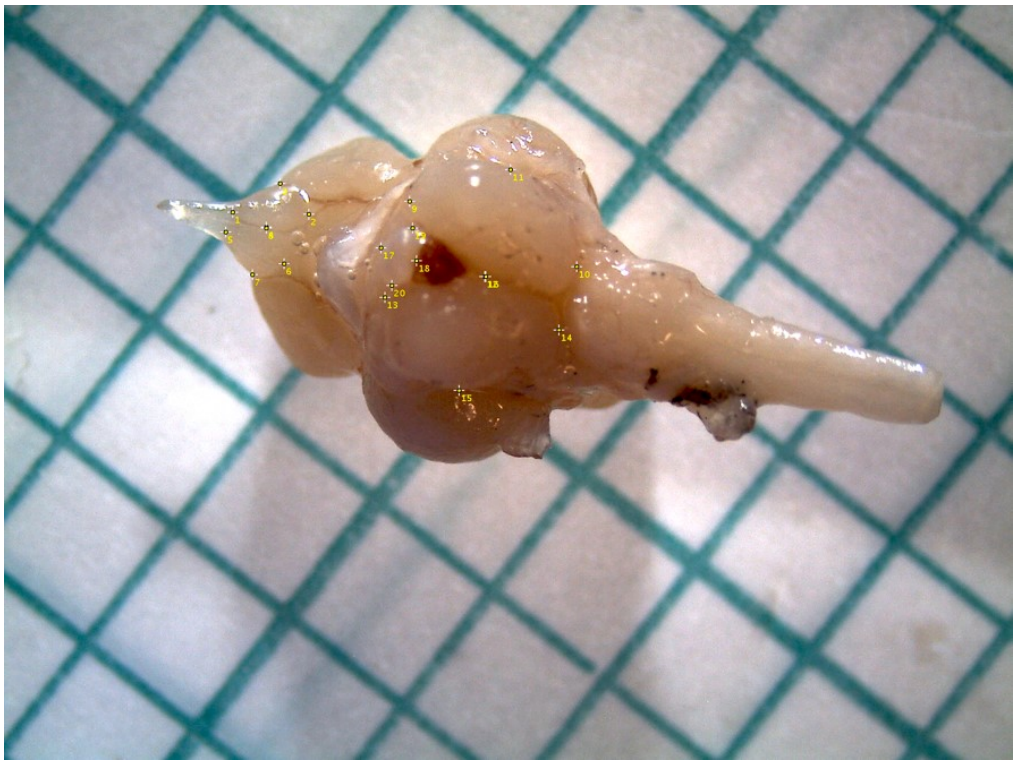
Lab-based results are often criticized due to a lack of ecological relevance (Lefebvre & Sol, 2008). Nevertheless, laboratory experiments are effective at removing environmental features (Lefebvre & Sol, 2008), which is important as we need to assess the effect that single environmental features have on brain size (Shumway, 2008). New investigational paradigms that explore the cognitive and survival advantages associated with neuroplasticity in both the laboratory and wild will not only add to my research but could be fundamental to the success of the hatchery industry. Additional experiments could demonstrate important relationships between neural development, behavioural changes, and associated survival advantages. Furthermore, the use of an environmentally irrelevant chemical cue as an additional control could help distinguish whether the observed increase in brain size is due to elevated predation risk or the result of environmental enrichment. Aside from the limitations already listed, measuring SL before and after the experiment would bolster my results by illuminating whether the observed differences in juvenile brain size were the result of (or related to) differential somatic growth. Growth rate data would show that individuals may have grown more in the high risk treatment resulting in an associated increase in brain growth.

Evidence for biological adaptations comes from studies on neuro-anatomy, neuro-physiology and behaviour (Chandroo, 2004). This thesis has contributed to the overall understanding of the neuro-ecology of a cichlid species. Convict cichlids likely experience two-way neuroplasticity in response to alarm cues as juveniles, but no neuroplastic response as adults. Although fish brains may remain plastic throughout their lives (Ebbesson & Braithwaite, 2012, Mitchel et al., 2016), the effect that chemical alarm cues have on brain morphology in convict cichlids is ontogenetically constrained.

Figure 1: Dissected Brain Views



Dorsal (top) view
-20 coordinate points placed on various brain regions. 1-4 Right telencephalon; 5-8 left telencephalon; 9-12 right optic bulb, 13-16 left optic bulb; 17-20 cerebellum



Ventral (bottom) view
-20 coordinate points placed on various brain regions. 1-4 left olfactory bulb; 5-8 right olfactory bulb; 9-12 left hypothalamus; 13-16 right hypothalamus; 17-20 pituitary.

Figure 2.1: Juveniles 1 day post treatment.
Scatterplot showing the difference in olfactory bulb size (mm^2) between high and low risk treatments, using standard length (mm) as a covariate. Low risk n=46, high risk n=56.

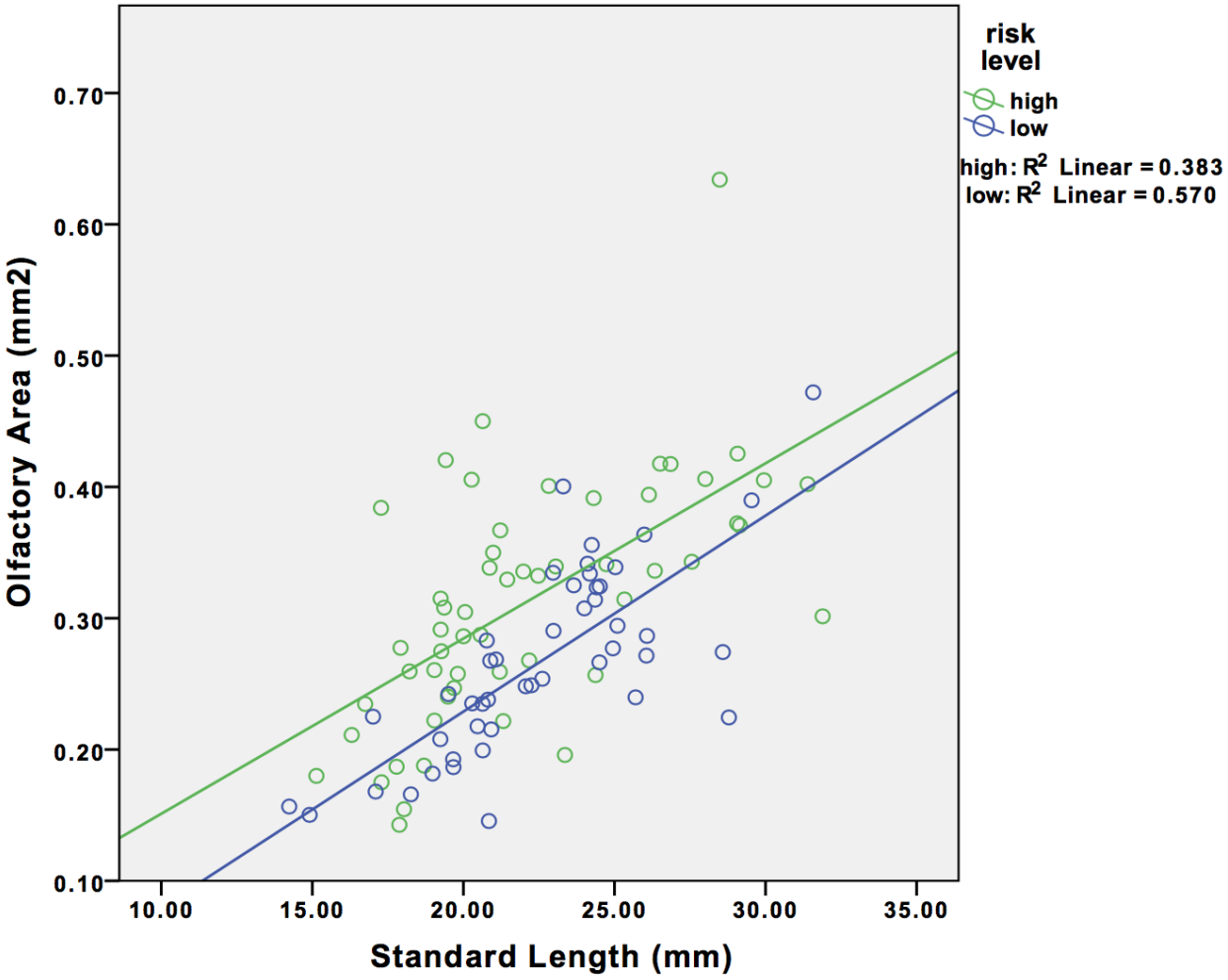


Figure 2.2: Juveniles 11 days post treatment.
Scatterplot showing the difference in olfactory bulb size (mm^2) between high and low risk treatments, using standard length (mm) as a covariate. Low risk n=63, high risk n=62.

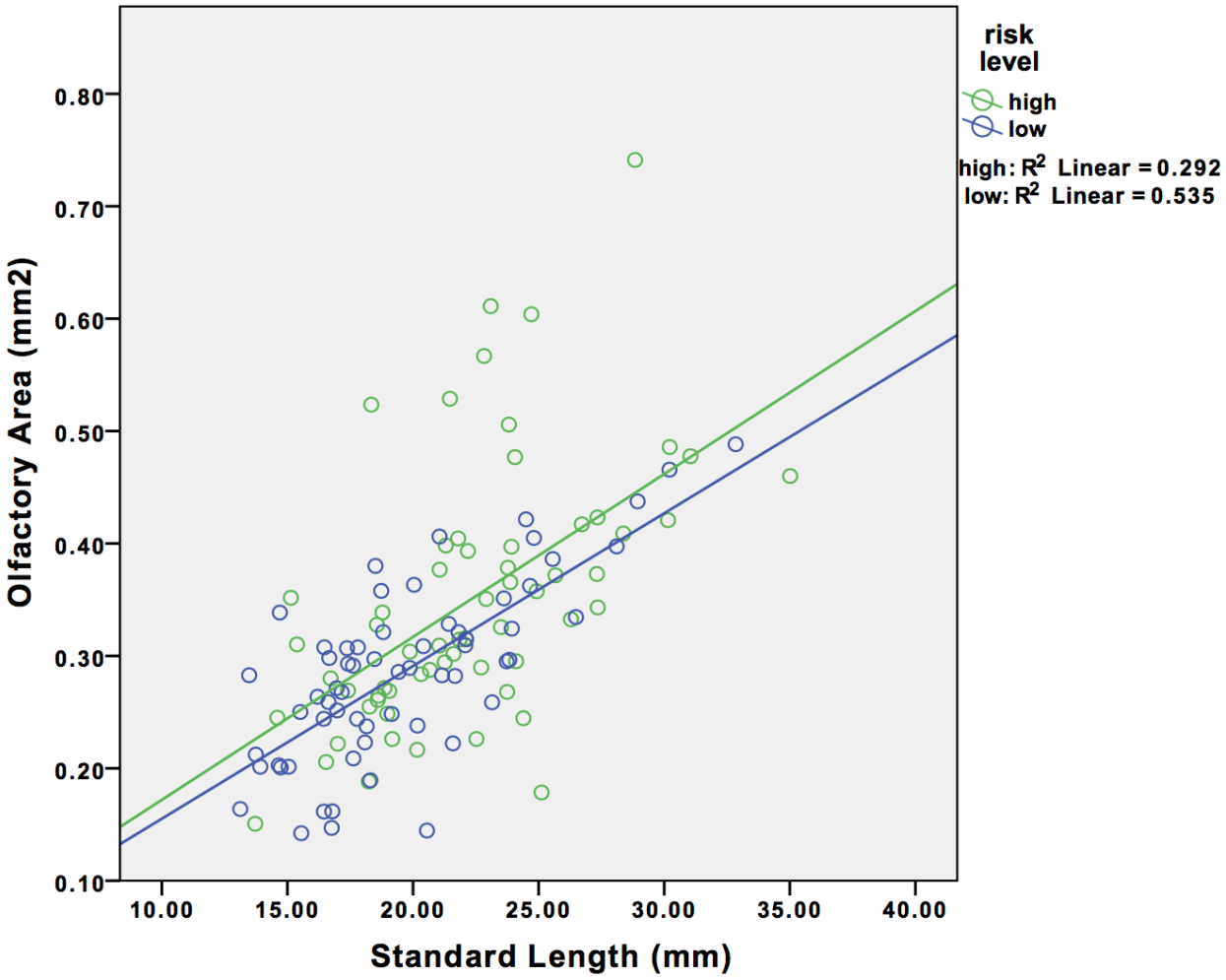


Figure 2.3: Adults 1 day post treatment.
Scatterplot showing the difference in olfactory bulb size (mm^2) between high and low risk treatments, using standard length (mm) as a covariate. Low risk n=22, high risk n=19.

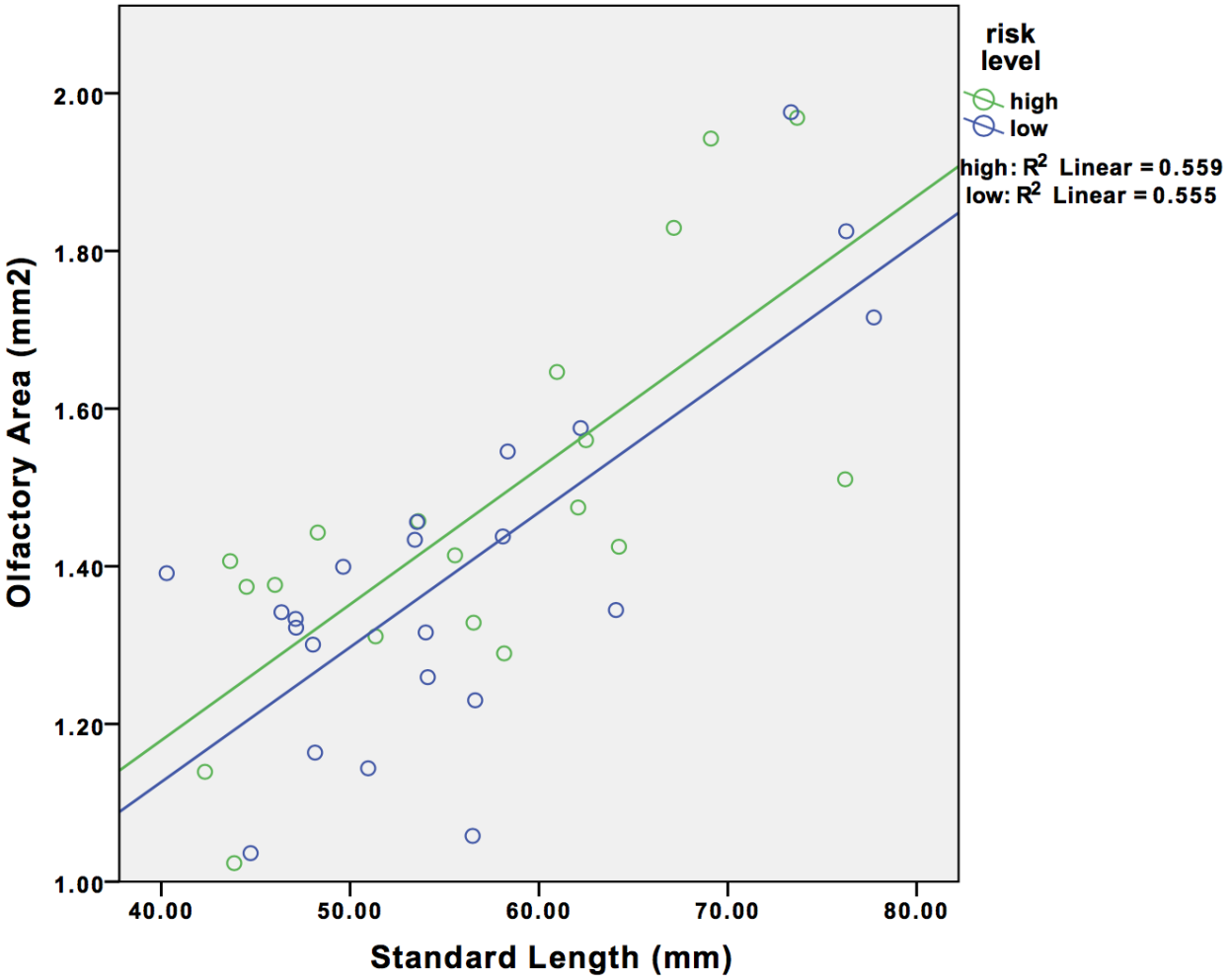


Figure 2.4: Juveniles 1 day post treatment.
Scatterplot showing the difference in overall brain size (mm^2) between high and low risk treatments, using standard length (mm) as a covariate. Low risk n=44, high risk n=55.

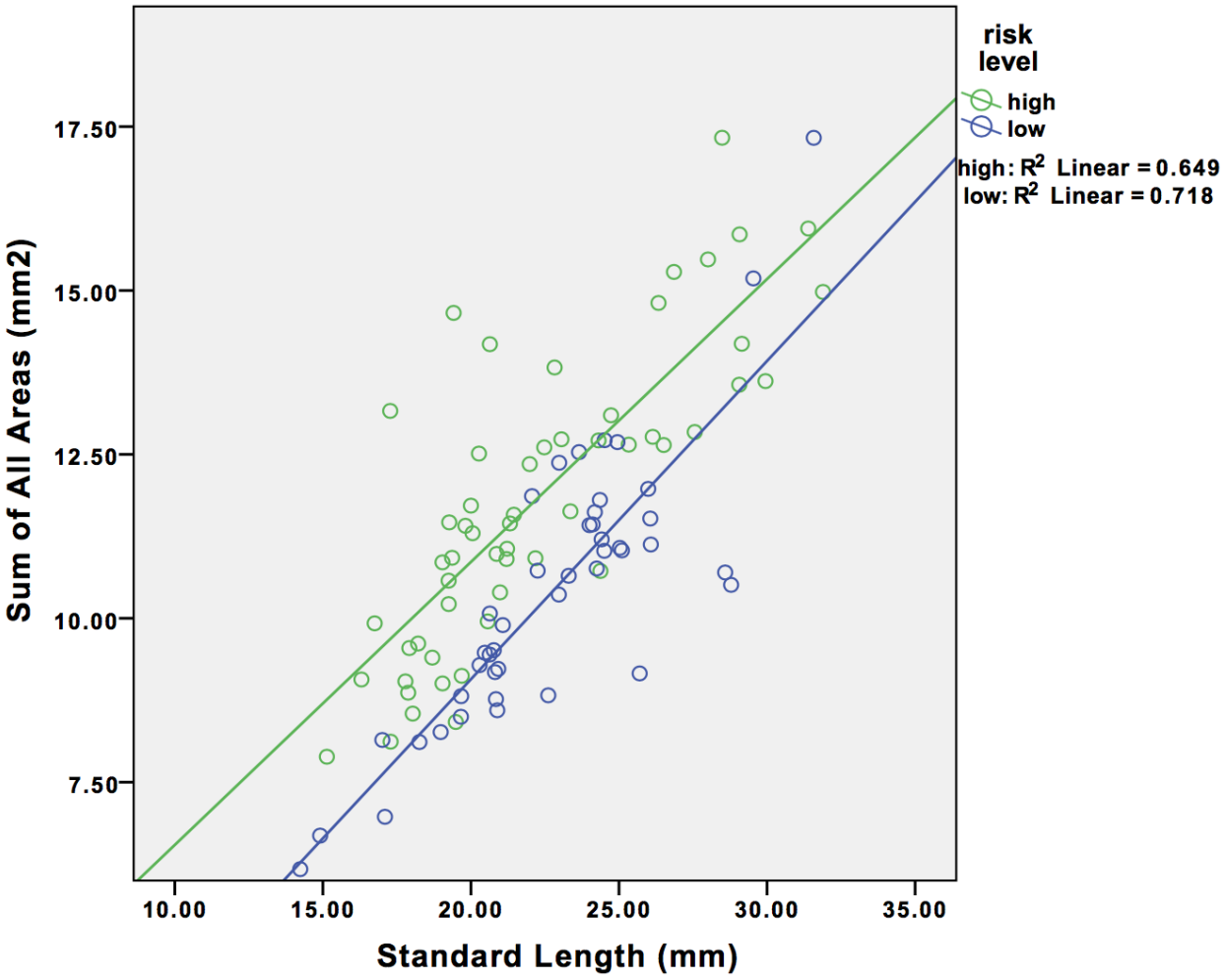


Figure 2.5: Juveniles 11 days post treatment
Scatterplot showing the difference in overall brain size (mm^2) between high and low risk treatments, using standard length (mm) as a covariate. Low risk n=61, high risk n=61.

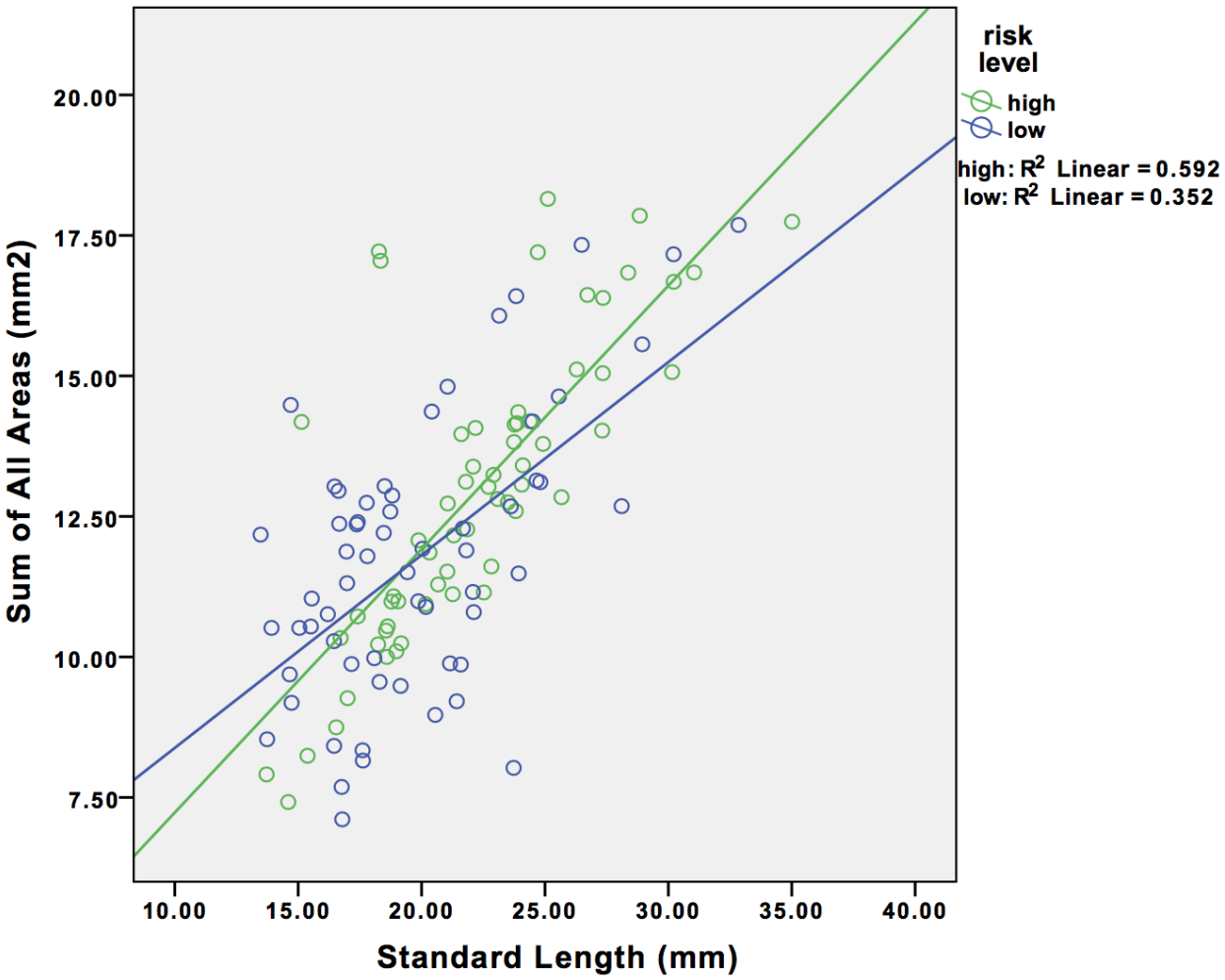


Figure 2.6: Adults 1 day post treatment
Scatterplot showing the difference in overall brain size (mm^2) between high and low risk treatments, using standard length (mm) as a covariate. Low risk n=22, high risk n=17.

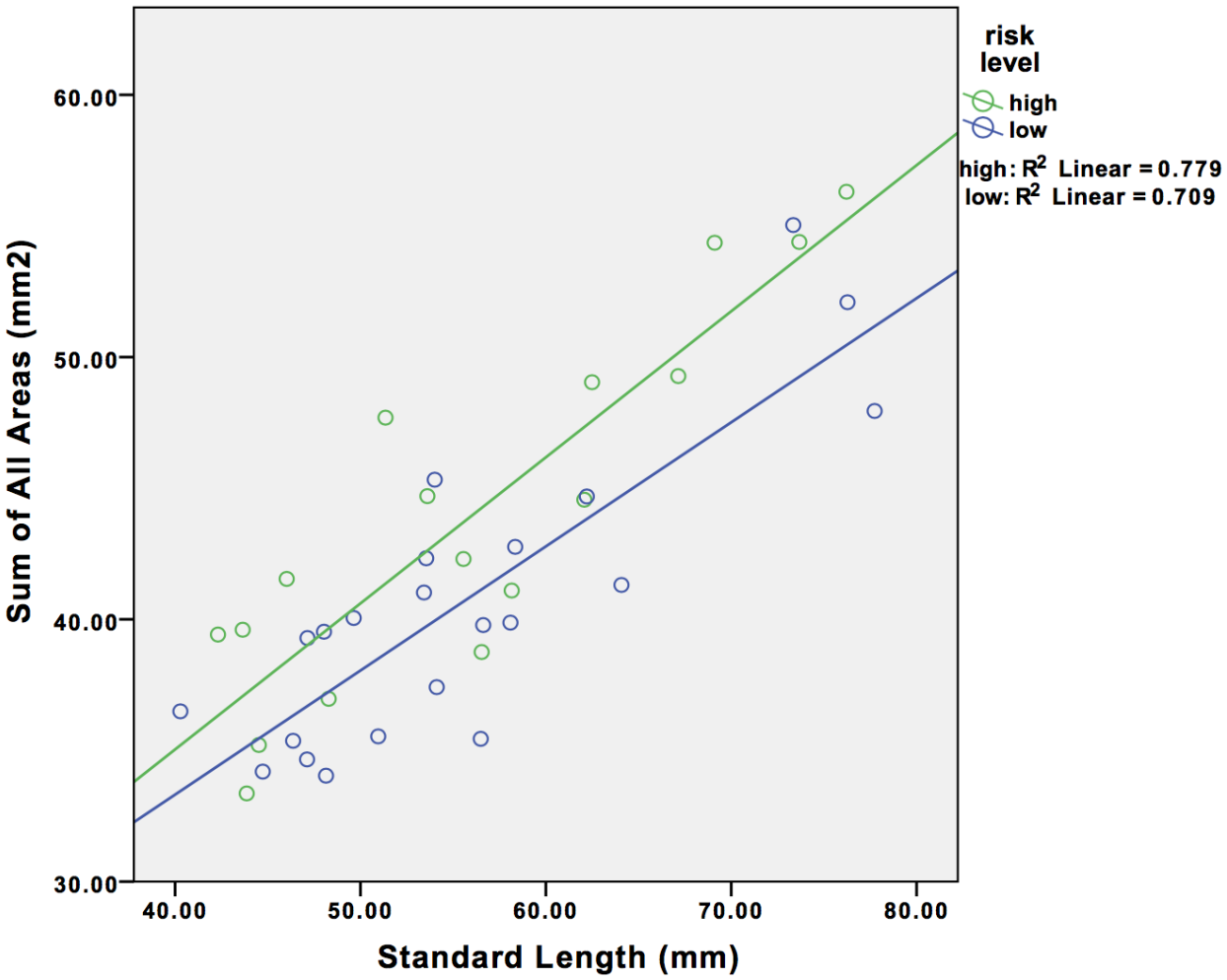


Figure 3.1: Juveniles 1 day post treatment
Scatterplot showing the difference in olfactory bulb size (mm^2) between high and low risk treatments, using "all-brain" size (mm) as a covariate. Low risk n=44, high risk n=55.

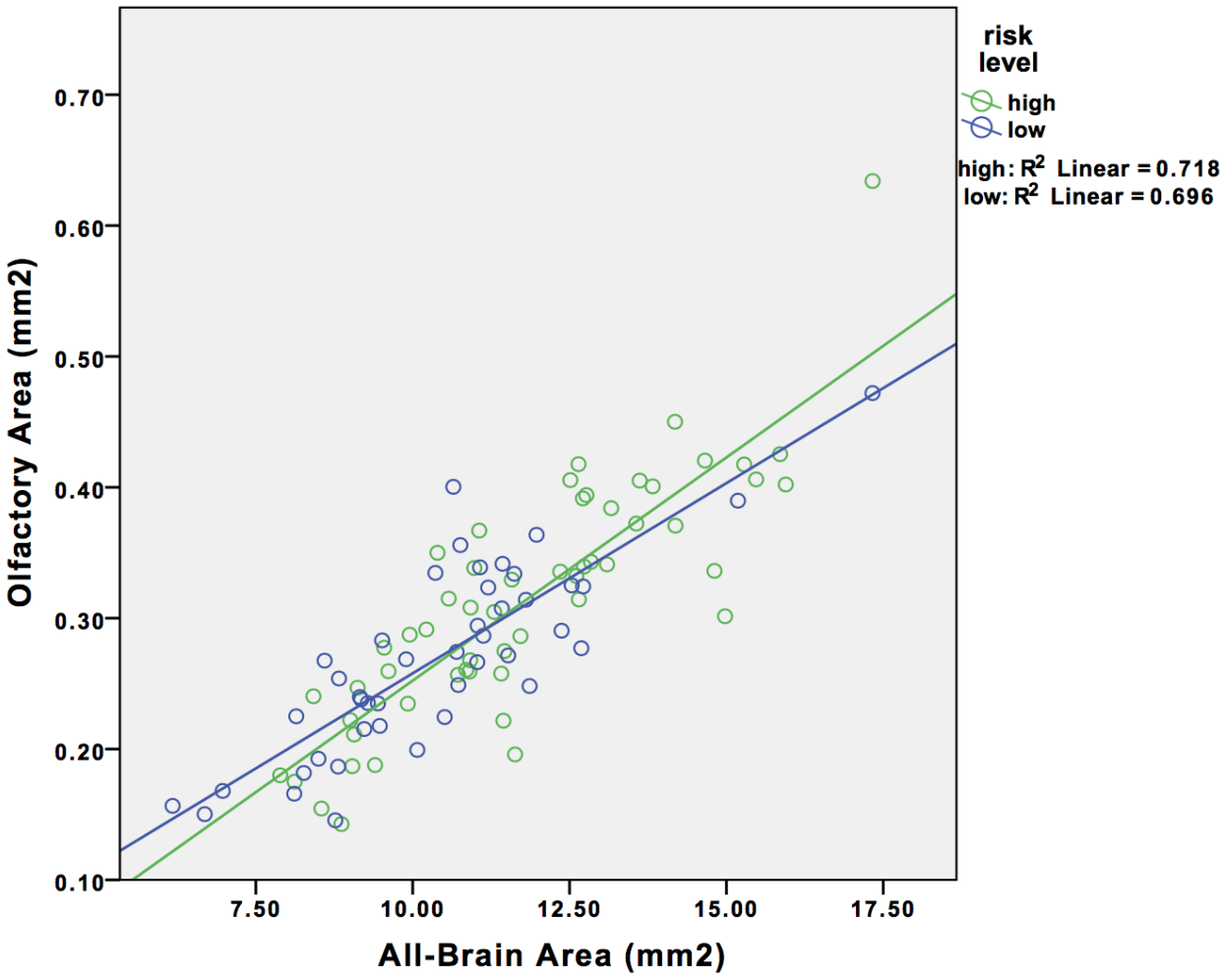


Figure 3.2: Juveniles 11 days post treatment
Scatterplot showing the difference in olfactory bulb size (mm^2) between high and low risk treatments, using "all-brain" size (mm) as a covariate. Low risk n=61, high risk n=61.

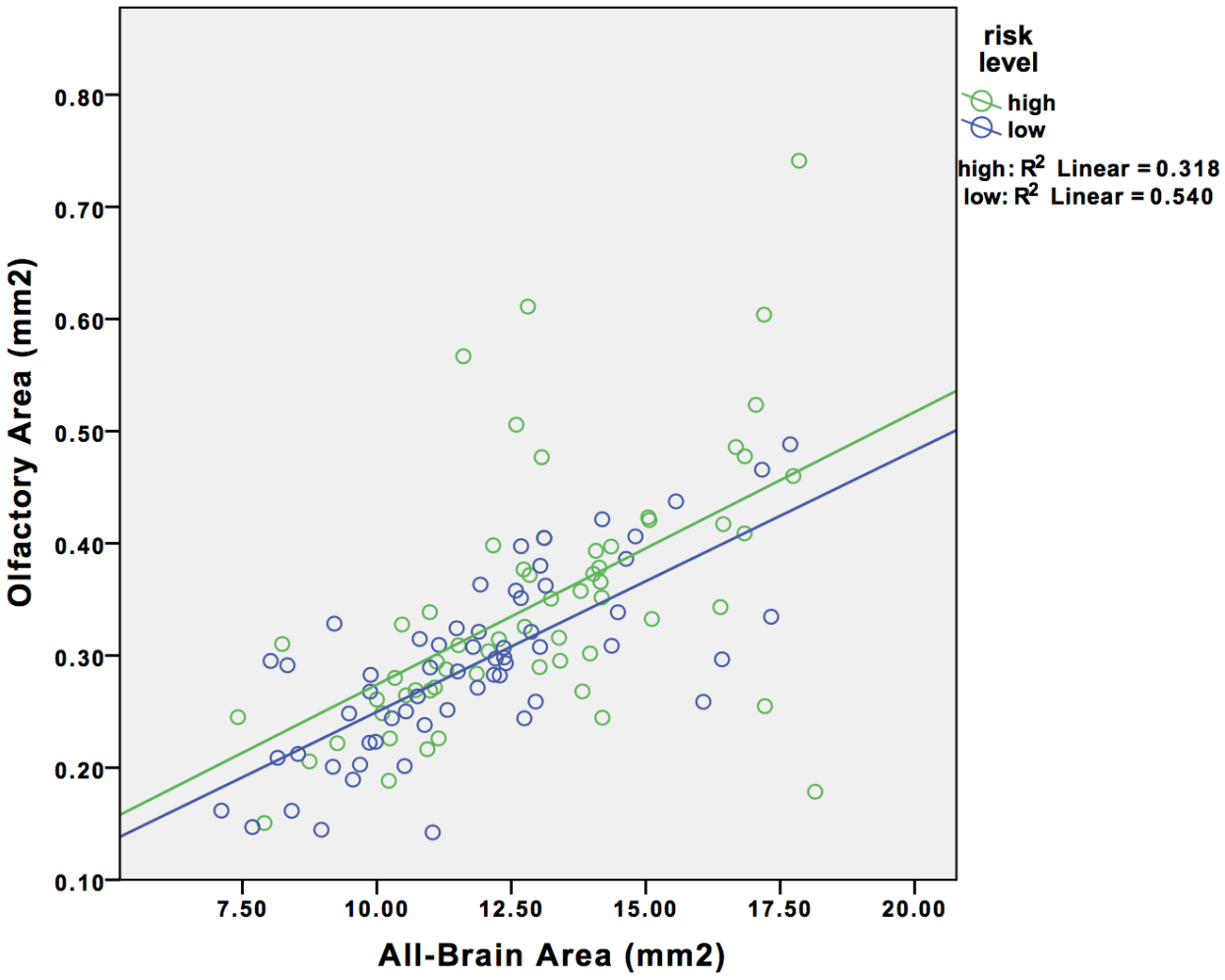


Figure 3.3: Adults 1 day post treatment
Scatterplot showing the difference in olfactory bulb size (mm^2) between high and low risk treatments, using "all-brain" size (mm) as a covariate. Low risk n=22, high risk n=17.

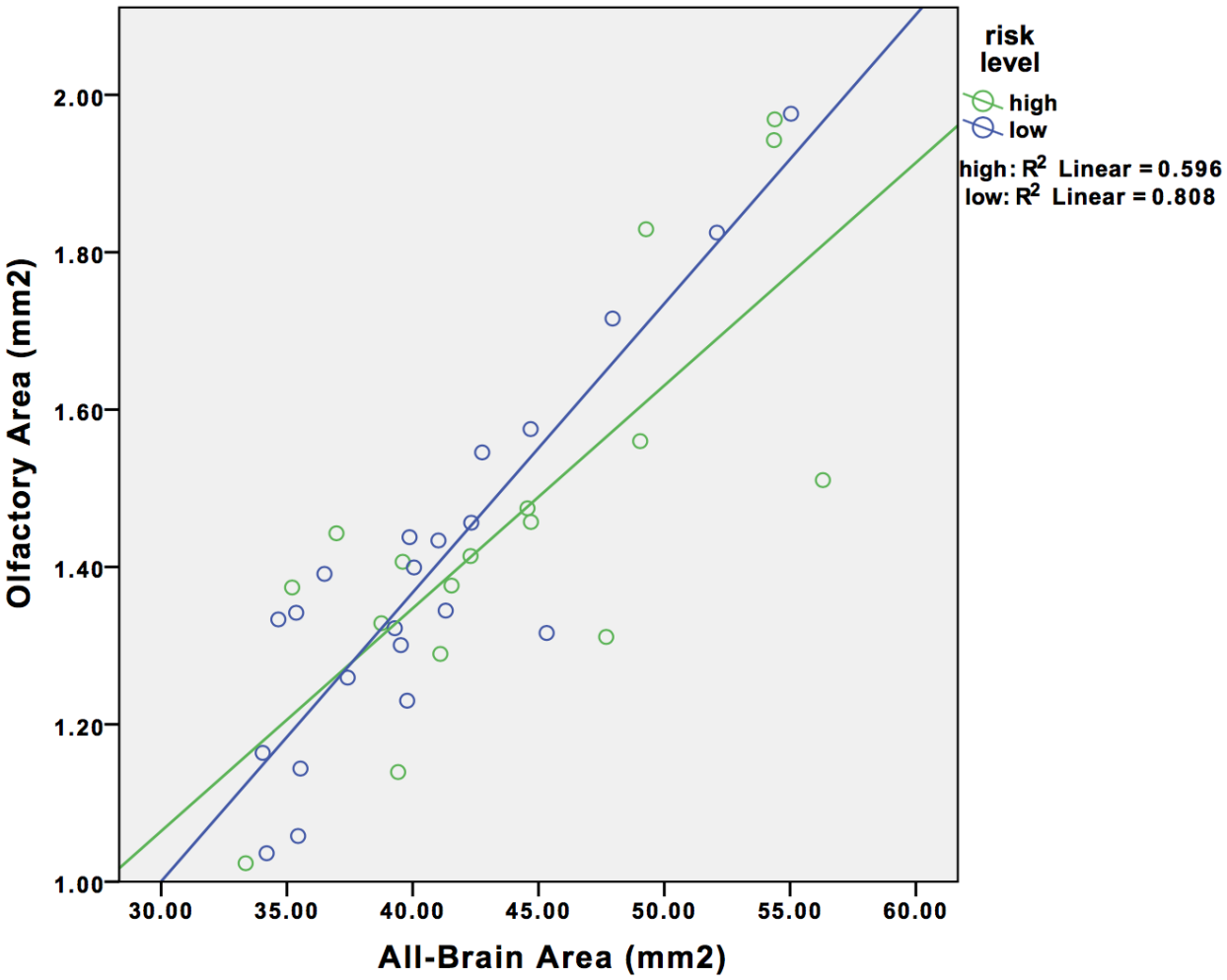


Table 1: Repeatability Measures. SPSS intraclass correlation. 20 bodies and 33 brains were selected at random and re-measured. 2-way mixed model was used to determine the absolute agreement of the measurements. Average measure correlation coefficient is provided to indicate the average consistency of the two measurements relative to each other.

Juveniles: 1 day post treatment	**Average measure (intraclass correlation coefficient)**	**CI (95%)**	**F test**
Olfactory Bulb	0.966	0.931 - 0.983	F31,31 = 29.028 (p=0.000)
Telencephalon	0.969	0.937 - 0.985	F32,32 = 34.263 (p=0.000)
Optic Bulb	0.959	0.916 - 0.980	F32,32 = 25.151 (p=0.000)
Cerebellum	0.961	0.923 - 0.981	F32,32 = 26.050 (p=0.000)
Hypothalamus	0.964	0.875 - 0.986	F31,31 = 38.876 (p=0.000)
Sum of All Regions	0.965	0.925 - 0.984	F31,31 = 32.185 (p=0.000

Table 2.1: ANCOVA results for juveniles 1 day post treatment. ANCOVA was used to test the effect of treatment on 5 brain regions and the sum of all regions using standard length as a covariate. Tank number was included in the model as a random factor.

Juveniles: 1 day post treatment	**F**	**df**	**p value**	**Sig. effect?**
Olfactory Bulb				
Standard length	91.049	1, 90	0.000	Yes
Treatment	9.776	1, 8	0.014	Yes
Interaction	1.199	1, 89	0.277	No interaction
Telencephalon				
Standard length	158.524	1, 118	0.000	Yes
Treatment	12.096	1, 8	0.008	Yes
Interaction	3.719	1, 117	0.056	No interaction
Optic Bulb				
Standard length	135.113	1, 121	0.000	Yes
Treatment	20.875	1, 8	0.002	Yes
Interaction	8.941	1, 121	0.003	**Sig. interaction**
Cerebellum				
Standard length	174.632	1, 120	0.000	Yes
Treatment	6.836	1, 8	0.002	Yes
Interaction	2.320	1, 119	0.130	No interaction
Hypothalamus				
Standard length	135.861	1, 121	0.000	Yes
Treatment	25.415	1, 8	0.001	Yes
Interaction	0.243	1, 120	0.623	No interaction
Sum of All Regions				
Standard length	138.632	1,88	0.000	Yes
Treatment	15.116	1,8	0.005	Yes
Interaction	3.259	1,87	0.075	No interaction

Table 2.2: ANCOVA results for juveniles 11 days post treatment. ANCOVA was used to test the effect of treatment on 5 brain regions and the sum of all regions using standard length as a covariate. Tank number was included in the model as a random factor

Juveniles: 11 days post treatment	**F**	**df**	**p value**	**Sig. effect?**
Olfactory Bulb				
Standard length	73.451	1,114	0.000	Yes
Treatment	1.562	1,8	0.247	No
Interaction	0.270	1,113	0.604	No interaction
Telencephalon				
Standard length	76.886	1,122	0.000	Yes
Treatment	1.513	1,8	0.254	No
Interaction	0.156	1,121	0.694	No interaction
Optic Bulb				
Standard length	84.199	1,123	0.000	Yes
Treatment	0.425	1,8	0.533	No
Interaction	0.043	1,122	0.836	No interaction
Cerebellum				
Standard length	124.823	1,122	0.000	Yes
Treatment	0.053	1,8	0.824	No
Interaction	0.325	1,121	0.570	No interaction
Hypothalamus				
Standard length	100.011	1,123	0.000	Yes
Treatment	0.030	1,8	0.867	No
Interaction	0.006	1,122	0.938	No interaction
Sum of All Regions				
Standard length	134.324	1,111	0.000	Yes
Treatment	0.072	1,8	0.794	No
Interaction	0.841	1,110	0.361	No interaction

Table 2.3: ANCOVA results for adults 1 day post treatment. ANCOVA was used to test the effect of treatment on 5 brain regions and the sum of all regions using standard length as a covariate. Tank number was included in the model as a random factor

Adults: 1 day post treatment	**F**	**df**	**p value**	**Sig. effect?**
Olfactory Bulb				
Standard length	42.916	1,36	0.000	Yes
Treatment	1.251	1,2	0.380	No
Interaction	0.004	1,35	0.948	No interaction
Telencephalon				
Standard length	57.647	1,39	0.000	Yes
Treatment	2.209	1,2	0.276	No
Interaction	1.147	1,38	0.291	No interaction
Optic Bulb				
Standard length	69.240	1,39	0.000	Yes
Treatment	3.366	1,2	0.208	No
Interaction	0.022	1,38	0.883	No interaction
Cerebellum				
Standard length	85.953	1,39	0.000	Yes
Treatment	0.023	1,2	0.893	No
Interaction	2.547	1,38	0.119	No interaction
Hypothalamus				
Standard length	47.882	1,35	0.000	Yes
Treatment	5.436	1,2	0.145	No
Interaction	0.047	1,34	0.830	No interaction
Sum of All Regions				
Standard length	96.973	1,34	0.000	Yes
Treatment	10.766	1,2	0.082	No
Interaction	0.735	1,33	0.397	No interaction

Table 3.1: ANCOVA results for Juveniles 1 day post treatment. ANCOVA was used to test the effect of treatment on 5 brain regions using all-brain as a covariate. Tank number was included in the model as a random factor

Juveniles: 1 day post treatment	**F**	**df**	**p value**	**Sig. effect?**
Olfactory Bulb				
All-brain size	249.289	1,88	0.000	Yes
Treatment	0.010	1,8	0.922	No
Interaction	0.079	1,87	0.779	No interaction
Telencephalon				
All-brain size	482.799	1,87	0.000	Yes
Treatment	10.739	1,8	0.011	**Yes**
Interaction	13.028	1,87	0.001	**Sig. interaction**
Optic Bulb				
All-brain size	665.537	1,88	0.000	Yes
Treatment	2.041	1,8	0.191	No
Interaction	2.791	1,87	0.098	No interaction
Cerebellum				
All-brain size	334.788	1,88	0.000	Yes
Treatment	4.429	1,8	0.069	No
Interaction	0.101	1,87	0.752	No interaction
Hypothalamus				
All-brain size	518.343	1,88	0.000	Yes
Treatment	1.028	1,8	0.340	No
Interaction	0.139	1,87	0.710	No interaction

Table 3.2: ANCOVA results for juveniles 11 days post treatment. ANCOVA was used to test the effect of treatment on 5 brain regions using "all-brain" as a covariate. Tank number was included in the model as a random factor

Juveniles: 11 days post treatment	**F**	**df**	**p value**	**Sig. effect?**
Olfactory Bulb				
All-brain size	88.966	1,111	0.000	Yes
Treatment	1.133	1,8	0.318	No
Interaction	2.682	1,110	0.104	No interaction
Telencephalon				
All-brain size	804.454	1,111	0.000	Yes
Treatment	3.790	1,8	0.087	No
Interaction	2.647	1,110	0.107	No interaction
Optic Bulb				
All-brain size	1395.934	1,111	0.000	Yes
Treatment	0.034	1,8	0.859	No
Interaction	0.822	1,110	0.367	No interaction
Cerebellum				
All-brain size	683.094	1,111	0.000	Yes
Treatment	0.590	1,8	0.465	No
Interaction	1,288	1,110	0.261	No interaction
Hypothalamus				
All-brain size	393.723	1,111	0.000	Yes
Treatment	3.024	1,8	0.120	No
Interaction	0.400	1,110	0.528	No interaction

Table 3.3: ANCOVA results for adults 1 day post treatment. ANCOVA was used to test the effect of treatment on 5 brain regions using "all-brain" as a covariate. Tank number was included in the model as a random factor

Adults: 1 day post treatment	**F**	**df**	**p value**	**Sig. effect?**
Olfactory Bulb				
All-brain size	75.302	1,34	0.000	Yes
Treatment	0.624	1,2	0.509	No
Interaction	1.420	1,33	0.242	No interaction
Telencephalon				
All-brain size	261.185	1,34	0.000	Yes
Treatment	0.850	1,2	0.454	No
Interaction	0.999	1,33	0.325	No interaction
Optic Bulb				
All-brain size	148.396	1,34	0.000	Yes
Treatment	0.019	1,2	0.904	No
Interaction	0.182	1,33	0.672	No interaction
Cerebellum				
All-brain size	108.712	1,34	0.000	Yes
Treatment	6.593	1,2	0.124	No
Interaction	0.118	1,33	0.733	No interaction
Hypothalamus				
All-brain size	96.973	1,34	0.000	Yes
Treatment	10.766	1,2	0.082	No
Interaction	0.735	1,33	0.397	No interaction

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Appendix 1: How Treatment Affects the Other Four Brain Regions Measured, Using Standard Length as a Covariate.

Results

The effect between treatments was assessed with a linear mixed model ANCOVA using the lm function in R studio. Standard length (SL) was used as a covariate and tank number was used as a random factor. The dependent variables, telencephalon, optic bulb, cerebellum, and hypothalamus surface areas were analyzed after log transformations in order to meet the assumptions of normality.

Experiment 1: Juveniles 1 day post treatment

In addition to the main results of this thesis, there was a significant effect of risk level on the other four brain regions measured. Compared to the low risk group, the cichlids that were exposed to high risk cues had 13.5% larger telencephala ($F_{1,8} = 12.096$, $p = 0.008$), their optic bulbs were 20.8% larger ($F_{1,8} = 20.875$, $p = 0.002$), cerebella were 11.9% greater in size ($F_{1,8} = 6.836$, $p = 0.031$), and hypothalamus was 18.2% bigger ($F_{1,8} = 25.415$, $p = 0.001$) when accounting for differences in SL. However, even though there was a main effect of risk on optic bulb size, there was significant interaction ($F_{1,121} = 8.941$, $p = 0.003$). As standard length increases the effect that risk has on optic bulb size decreases (*Figure A1.1*).

No Interaction was found for any other region. Additionally, every region measured had a positive relationship with SL (*Table 2.1*).

Experiment 2: Juveniles 11 days post treatment

Linear Mixed model ANCOVA revealed that after an 11-day latency period, there was no longer a significant difference between high and low risk on telencephalon size ($F_{1,8} = 1.513$, $p = 0.254$), optic bulb size ($F_{1,8} = 0.425$, $p = 0.533$), cerebellum size ($F_{1,8} = 0.053$, $p = 0.824$), or

hypothalamus size ($F_{1,8} = 0.030$, $p = 0.867$). Comparisons between treatments for each region can be seen in *Figure A1.2.*

There was no interaction between risk level and SL on any brain region (*Table 2.2*). Standard length significantly affected the size of all regions (*Table 2.2*). As SL increases, the size of each brain region also increases (*Figure A1.2)*.

Experiment 3: Adults 1 day post treatment

Compared to the low risk group, there was no significant effect of elevated risk on adult telencephalon size ($F_{1,2} = 2.209$, $p = 0.276$), optic bulb size ($F_{1,2} = 3.366$, $p = 0.208$), cerebellum size ($F_{1,2} = 0.023$, $p = 0.893$), or hypothalamus size ($F_{1,2} = 5.436$, $p = 0.830$) when SL was used as a random factor. Comparisons are visualized in *Figure A1.3*.

No interaction between SL and treatment was observed for any region. As SL increases, there is an associated increase in all four regions (*Table 2.3*).

Repeatability

The same methods were used to determine repeatability as in the methods section of the main thesis. 20 bodies and 33 brains were randomly selected and re-measured by a colleague. A two-way mixed model intraclass correlation comparison between the first and second measurements was conducted to assess their absolute agreement (*Table 1*). Analysis yielded intraclass correlation average measures for telencephalon = 0.969 ($p < 0.001$), optic bulb = 0.959 ($p < 0.001$), cerebellum = 0.961 ($p < 0.001$), and hypothalamus = 0.964 ($p < 0.001$).

Figure A1.1: Effect of treatment on four brain regions and the sum of all regions in juveniles 1 day post treatment. Standard length is used as a covariate.

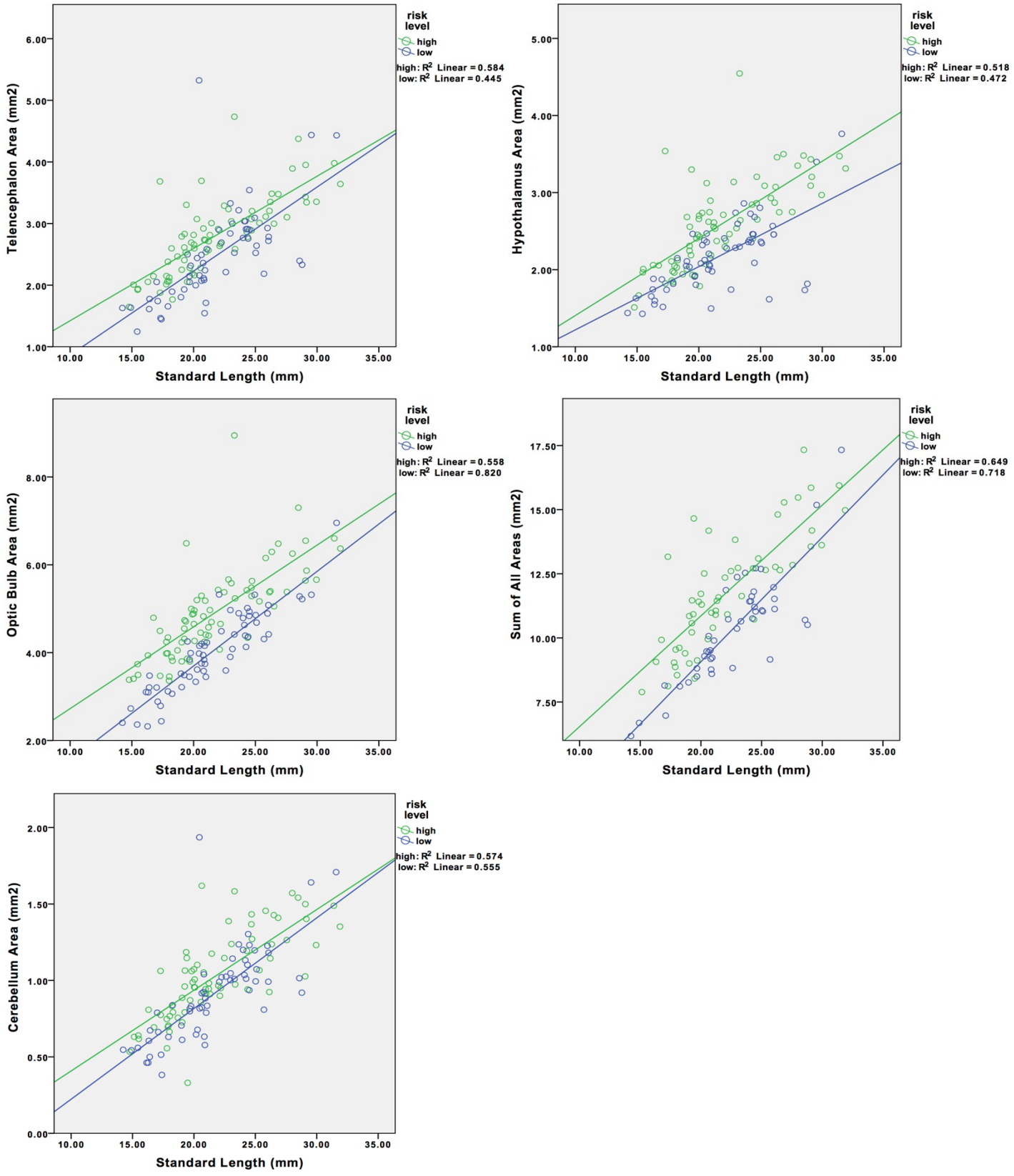


Figure A1.2: Effect of treatment on four brain regions and the sum of all regions in juveniles 11 days post treatment. Standard length is used as a covariate.

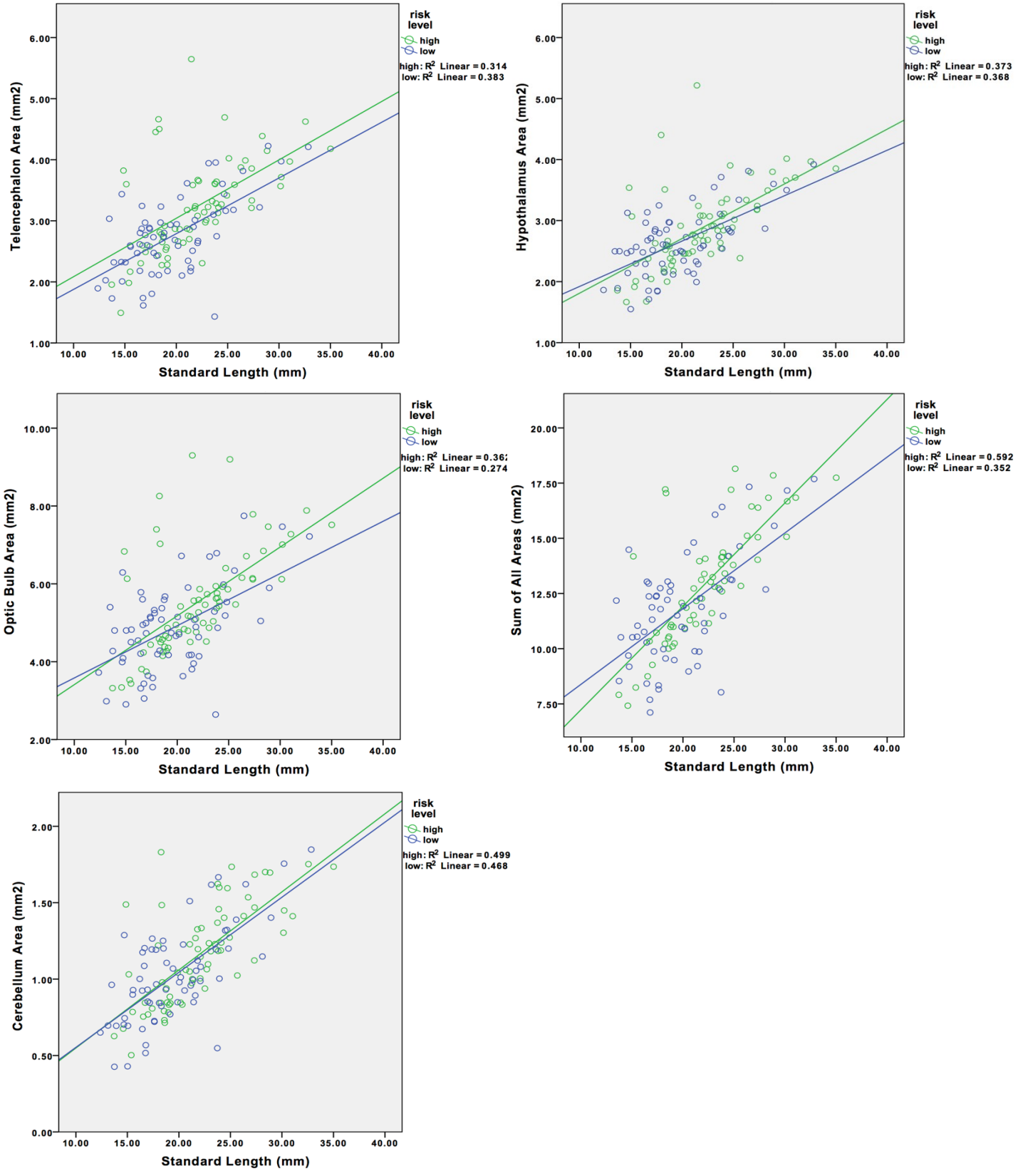
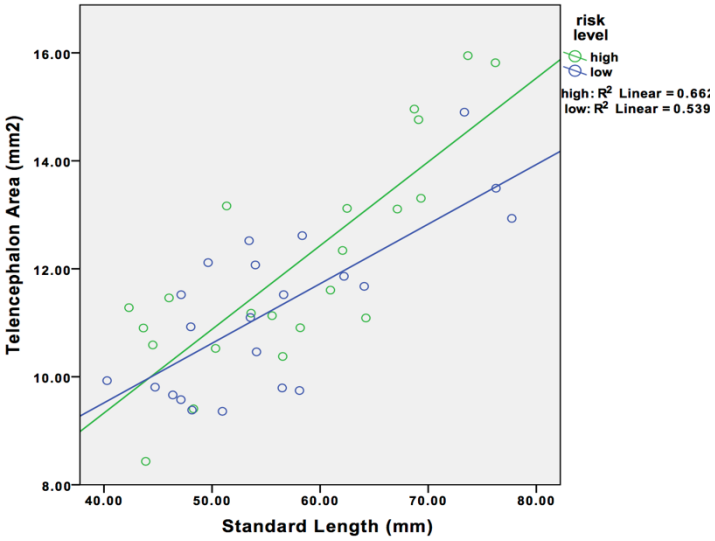
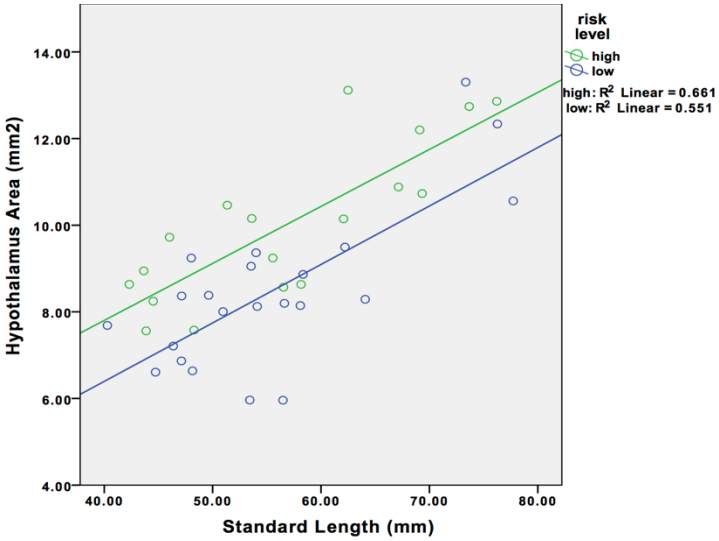
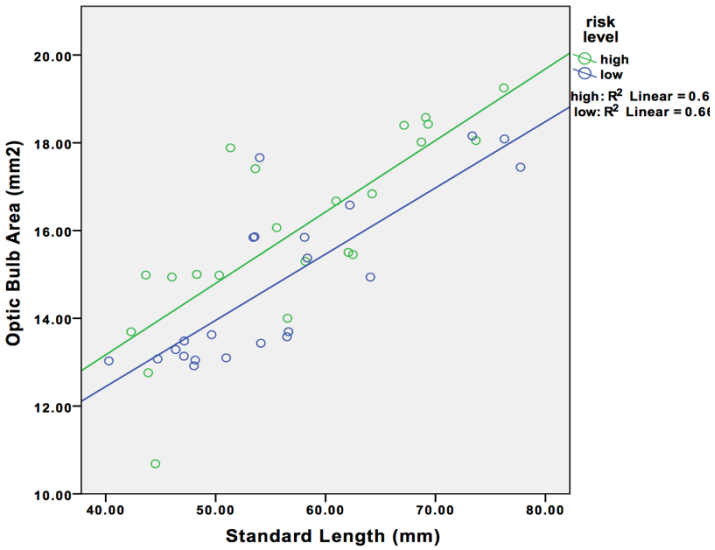
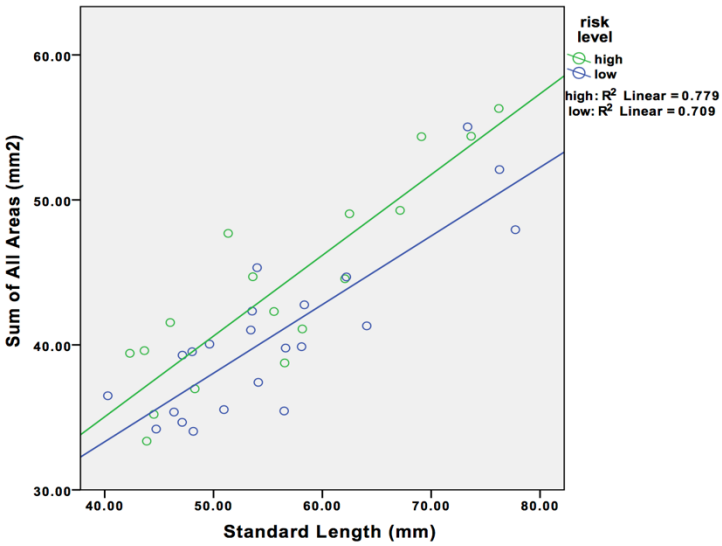


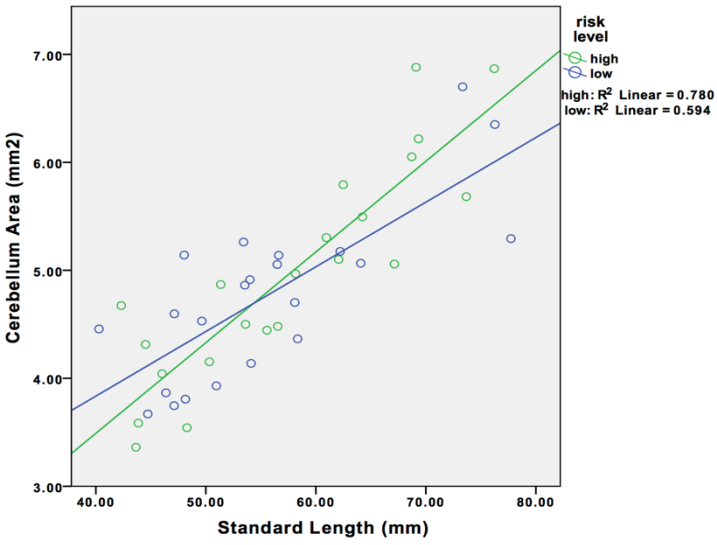
Figure A1.3: Effect of treatment on four brain regions and the sum of all regions in adults 1 day post treatment. Standard length is used as a covariate.











Appendix 2: How Treatment Affects the Other Four Brain Regions Measured, Using All-Brain as a Covariate.

Results

The effect between treatments was assessed with a linear mixed model ANCOVA using the lm function in R studio. The sum of all brain regions measured (all-brain) was used as a covariate and tank number was used as a random factor. The dependent variables, telencephalon, optic bulb, cerebellum, and hypothalamus surface areas were analyzed after log transformations in order to meet the assumptions of normality.

Experiment 1: Juveniles 1 day post treatment

In addition to the main results of this thesis, linear mixed model ANCOVA revealed that elevated risk did not result in increased proportional growth in optic bulb ($F_{1,8} = 2.041$, $p = 0.191$), cerebellum ($F_{1,8} = 4.429$, $p = 0.069$), or hypothalamus ($F_{1,8} = 1.028$, $p = 0.710$) sizes when all-brain size was used as a random factor when compared to the low risk group (*Fig. A2.1*).

Although there was no main effect of risk level on telencephalon size ($F_{1,8} = 10.739$, $p = 0.011$), there was a significant interaction ($F_{1,87} = 13.028$, $p = 0.001$). As all-brain size increases, the effect that high risk cues have on telencephalon size decreases (*Fig. A2.1*). No Interaction was found for any other region (*Table 3.1*). All regions measured had a positive relationship with all-brain size (*Table 3.1*).

Experiment 2: Juveniles 11 days post treatment

Similar to juveniles 1 day post treatment, after an 11-day latency period, there was still no significant proportional growth in telencephalon size ($F_{1,8} = 3.790$, $p = 0.0.087$), optic bulb

size ($F_{1,8} = 0.034$, $p = 0.859$), cerebellum size ($F_{1,8} = 0.590$, $p = 0.465$), or hypothalamus size ($F_{1,8} = 3.024$, $p = 0.120$) when all-brain size was used as a covariate (*Figure A1.2*).

No interaction between all-brain size and treatment was observed for any region (*Table 3.2*). As all-brain size increases, there is an associated increase in all four regions (*Table 3.2*).

Experiment 3: Adults 1 day post treatment

Utilizing all-brain size as a covariate, there was no effect of high risk cues on adult telencephalon size ($F_{1,2} = 0.850$, $p = 0.454$), optic bulb size ($F_{1,2} = 0.019$, $p = 0.904$), cerebellum size ($F_{1,2} = 6.593$, $p = 0.124$), or hypothalamus size ($F_{1,2} = 1.622$, $p = 0.331$) when compared to the low risk group (*Figure A2.3*).

There was no interaction between risk level and all-brain size on any brain region (*Table 3.3*). As all-brain size increases, the size of each brain region also increases (*Table 3.3)*.

Figure A2.1: Effect of treatment on four brain regions juveniles 1 day post treatment. "All-brain" is used as a covariate.

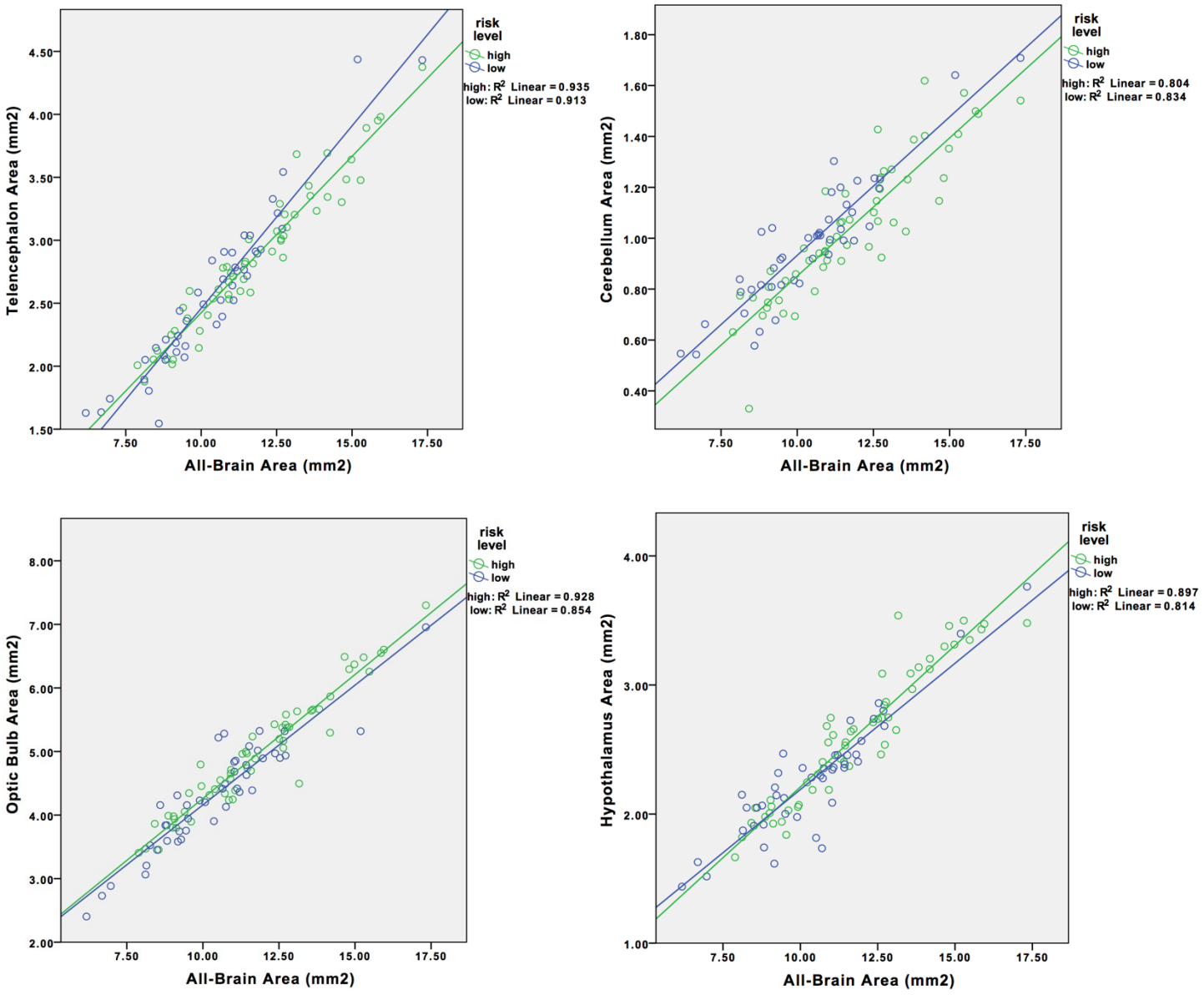


Figure A2.2: Effect of treatment on four brain regions juveniles 11 days post treatment. "All-brain" is used as a covariate.

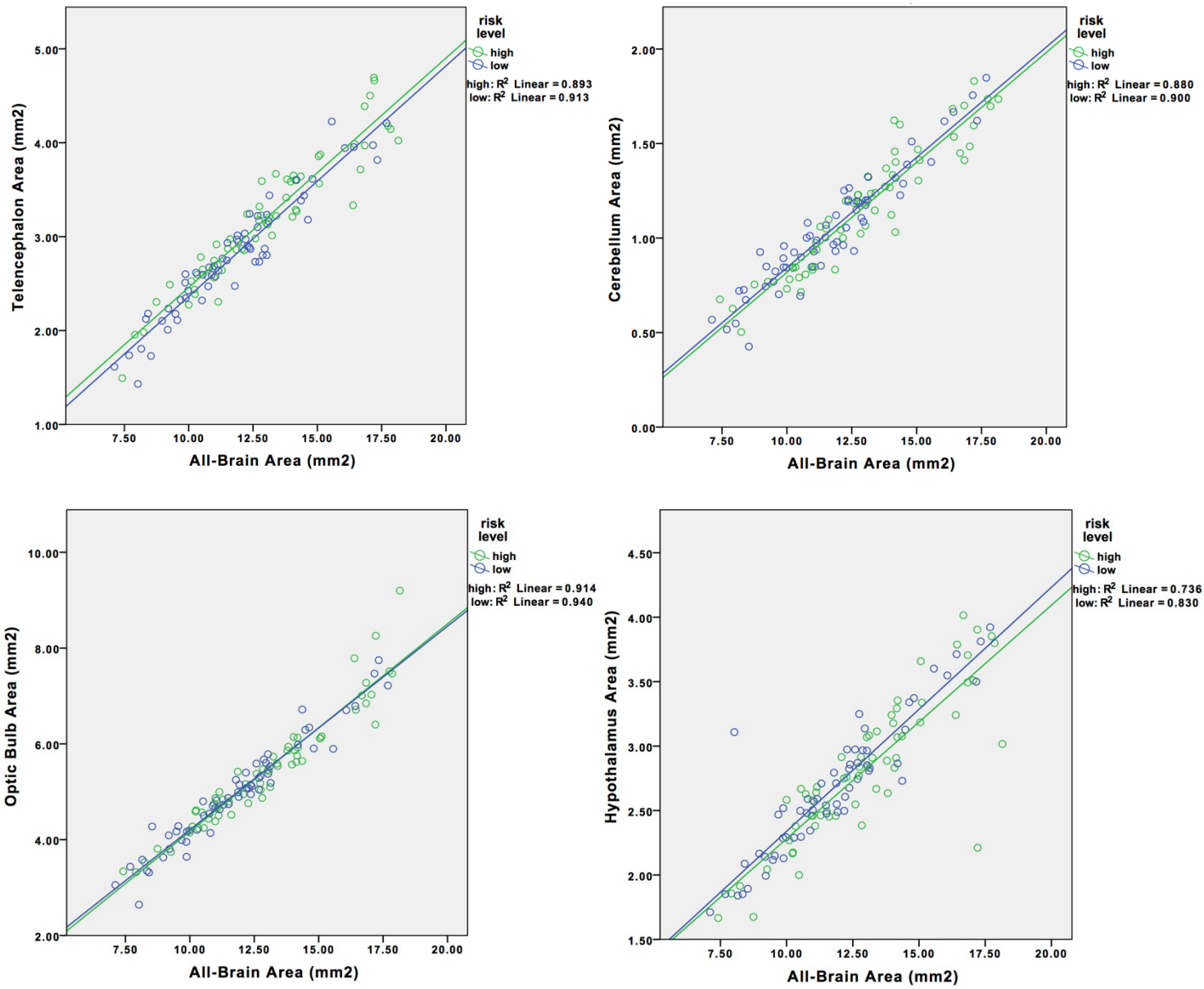


Figure A2.3: Effect of treatment on four brain regions adults 1 day post treatment. "All-brain" is used as a covariate.