EYE MOVEMENTS AND PUPIL REACTIONS DURING AFFECTIVE PICTURE VIEWING
(Settore scientifico-disciplinare: M-PSI/02)

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ANNO ACCADEMICO 2005/2006
Acknowledgments:

Nothing included in this work could have been done without the help of the members of the CSEA/Center for the Study of Emotion and Attention in Gainesville, FL, directed by Professor Peter J. Lang. In particular, I must express my deep gratitude to Professor Margaret M. Bradley, for her tireless support and patient enlightenments all the way through the recording, analysis, and interpretation of the data contained in this investigation. To Professor Lang and Professor Bradley I must convey my wonder for their exclusive ability to give the Center a combination of rigorous methodology, enthusiasm for science, and passion for “gustarsi la vita”.

I also personally thank each one of the researchers I had the pleasure to work with, and anyone from the CSEA who helped this inquiry asking questions I did not know how to answer.

To Professor Maurizio Codispoti and Professor Francesco Versace my endless gratefulness for supporting me and for allowing me to be part of the CSEA research group.

To Professor Carlo Semenza, my deep appreciation for his trust and open-mindedness throughout the years I spent abroad during my PhD program.

Finally, to the psychophysiology research group directed by Professor Jaime Vila Castellar, my deep thankfulness for warmly welcoming me in Granada, Spain, during the last month of this work.
To the beauty of distant worlds that find ways to meet
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1 A bi-dimensional approach to emotions and motivation

1. What are emotions... or what is emotion?

Since human beings have been able to express themselves through the use of language, songs, poems, and novels have been filled with words like 'joy', 'love', 'hate', 'fear', 'desire', 'anger'. Each one of us has an intuitive idea, typically related to our personal experience, of what each of these terms refers to.

Nonetheless, it is likely that if someone would ask us to explain what emotion is, we might feel embarrassed, maybe even ashamed, realizing how difficult it can be to define a concept we use so frequently. We should not, however, feel so uncomfortable: As Davis & Lang (2003) pointed out, "fundamental concepts are often the hardest to define", and for centuries philosophers and, more recently, scientists, have striven to find a satisfactory definition of emotion (LeDoux, 1996).

Descartes (see Panksepp, 1982) identified wonder, joy, desire, hate, and sadness as basic emotions, and it is interesting (Bradley, 2000) that the Dutch philosopher was the last theorist to identify a larger number of positive emotions. This is one possible approach to the study of emotion, according to which emotion is better conceptualized as a series of discrete, unique emotional states, not necessarily linked to each other. Language can characterize large numbers of emotional states (in Fehr & Russel, 1984, participants labeled up to 197 different emotional states). Therefore, one of the main goals of this perspective has been reducing the number of items included in these lists, in order to identify primary, fundamental emotions (Izard, 1977; Oatley & Johnson-
Laird, 1987). However, investigators have not yet been able to reach an acceptable consensus, and it is possible that there might be as many different lists of ‘basic’ emotions as there are researchers.

As a consequence, even if this viewpoint has its advantages and it is thus far the most common also in psychophysiology (Bradley, 2000), it is however not parsimonious (Bradley, Greenwald, & Hamm, 1993).

Indeed, human beings are capable of expressing emotion through highly variable response systems. Lang (1993) highlighted that emotion has three main measurable output systems: Language, behavior, and physiological reactions.

2. The database of emotion: language, behaviors, and physiology

Even if language is most likely an exclusive characteristic of human beings, emotional expressions can be found in several species (e.g., threat or distress cries, sounds of satisfaction or sexual passion). In humans, language is commonly employed for evaluative reports (e.g., self-ratings and descriptions of feelings and attitudes; Lang, Bradley, & Cuthbert, 1997).

Despite the abovementioned diversity in the linguistic expressions of emotion, Wundt (1896, in Lang, Bradley, & Cuthbert, 1997) identified fewer dimensions as being able of describing differences in affective meaning of stimuli like words, objects, events. These dimensions, according to Wundt, were pleasure (Lust), tension (Spannung), and inhibition (Beruhigung). In the same direction, Osgood and colleagues (Osgood, Suci, & Tanenbaum, 1957) found that most of the variance in emotional descriptors was explained by two bipolar dimensions: Affective valence, ranging from attraction/pleasure to aversion/displeasure, and activation, ranging from calm to aroused. These same dimensions were observed also in non-English speaking cultures, and using non-verbal stimuli (from sonar signals to aesthetic paintings). Moreover, more recent works on natural language categories (Ortony, Clore, & Collins, 1988) also
suggested that two superordinate categories, positivity and negativity (corresponding to pleasant states and unpleasant affective states, respectively), organized people’s knowledge about emotions.

Therefore, superordinate motivational factors of affective valence (positive or negative) and arousal seem to organize emotion reports in human beings.

**Behaviors**, or functional behavioral sequences, like fight, flight, sexual approach or threat displays, are the typical way different emotional states are expressed by humans and non-humans.

Schneirla (1959) suggested that behaviors of primitive organisms can be fully described by responses of approach to appetitive stimuli, and withdrawal from nociceptive stimuli. Unconditioned reflexes are the most basic of these self-protecting behaviors. Konorski (1967), distinguished between preservative (e.g. ingesting, copulating, nurturing) and protective (e.g. escaping) unconditioned reflexes based on their motivational role. Moreover, Konorski considered arousal the degree of activation in both preservative and protective reactions. Subsequently, Dickinson & Dearing (1979), developing Konorski’s dichotomic model, proposed that these two opponent aversive and attractive motivational systems were activated by distinct unconditioned stimuli and reciprocally inhibited.

Studying animal behavior within motivationally contradictory contexts (the same location for appetitive and aversive reinforcers), Miller (1951, 1959) observed that increasing the physical proximity to the goal location caused an increase in the strength of motivational activation. That is to say, when the animal was far from the motivationally relevant site approach prevailed, thus it moved towards the goal. However, moving closer to the motivational goal, avoidance prevailed, therefore the animal either escaped from the goal or most of the times simply stopped halfway. In these conflicting situations, resulting in the absence of overt behaviors, presumably arousal/activation increases. It must also be noted (see figure below, from Lang et al 1997) that the avoidance gradient is steeper than the approach gradient, thus indicating
that the tendency to avoid aversive stimuli is stronger than the tendency to approach appetitive stimuli.

Illustration 1.1: Graphic representation of Miller's approach-avoidance conflict. "The tendency to approach is the stronger of the two tendencies far from the goal, whereas the tendency to avoid is the stronger of the two near the goal. Therefore, when far from the goal, the subject should tend to approach part way and then stop. In short, he should tend to remain in the region where the two gradients intersect" (Miller, 1959). Caption from Lang, Bradley, & Cuthbert, 1997.

In general, overt motivated behaviors at least in animals can be described as ranging from approach to avoidance, where the intensity of the response depends on the degree of activation of each motivational system.

Motivated behavior in humans is of course more creative, less predictable, and more adaptive than in other species (Davis & Lang, 2003). Apart from being usually accompanied by corresponding reported feelings, human emotions (Bradley & Lang, 2000a) are less straight-forward than those of more primitive organisms. The evolution
of the brain, specifically that of the cerebral cortex, made humans capable of displaying wider behavioral repertoires, so the same motivationally relevant stimulus can result in a variety of behavioral responses. Moreover, human beings seem better suited than other species for delaying behaviors whenever long-term tactics are preferable, that is to say, they are capable of a more flexible use of time. In this sense, for example, the proximity of an appetitive stimulus in humans does not necessarily prompt an approaching behavior, if the individual judges long-term tactics as more likely to be successful. In addition, individuals can, if necessary, completely abstain from any overt response, however, also in this event affective reactions are not easily completely inhibited or suppressed (Bradley, 2000). As an example (Bradley, 2000), we might avoid reacting to the provocations of a colleague if they happen in a socially inappropriate situation. However, our body might still react as if we actually took action.

For these different reasons emotions have been regarded as dispositions to action (Bradley & Lang, 2000a; Lang, Bradley, & Cuthbert, 1997), also considering that, especially in human emotions, they are not inevitably triggering overt behaviors. In line with this perspective, it is interesting (Bradley, 2000) that both terms emotion and motivation come from the Latin verb movere (to move). In general, motivationally relevant stimuli, either appetitive or aversive, prompt a series of metabolic changes in muscles, glands, viscera of the organism, which correspond to physiological reactions occurring when the organism is preparing for real action execution.

In this sense, physiological reactions, changes in somatic muscles and in the viscera, are regarded as an evolutionary support to overt acts and as a way to display affections (e.g., smiling, frowning, blushing) (Lang, Bradley, & Cuthbert, 1997). The organization of emotional responses into three systems (emotional language, overt behavior, physiology) is an oversimplification of the way motivational systems might take action (Bradley & Lang, 2000a). However, if we examine emotions
from the perspective of natural science (Bradley & Lang, 2000a), language, behavior, and physiology are the measurable database of emotions (Lang, Bradley, & Cuthbert, 1997). Accordingly, the main goal of research in motivation and emotion should be proposing a theory capable of integrating these different response systems (Bradley & Lang, 2000a), even if the task is made harder by the fact that these indices are usually not covarying with each other (Lang, 1968).

3. A dimensional approach to emotion

Lang and associates (Bradley, 2000; Bradley & Lang, 2000a; Lang, 1995; Lang, Bradley, & Cuthbert, 1990, 1997) advocated a biphasic and dimensional approach to emotion, where linguistic, behavioral and physiological responses are the result of the activation of two separate motivational system, one appetitive and one aversive/defensive. Within this perspective arousal represents the degree of activation of each motivational system, and therefore does not constitute a separate substrate. Emotions are viewed as products of Darwinian development, thus expressions, acts, physiological reactions which promote the survival of the individual and of its specie, either facilitating the approach to appetitive stimuli (like food or possible mating partners), or assisting in avoiding/attacking aversive stimuli (like threatening events or individuals).

According to this view, appetitive and aversive motivational systems are the basis of every possible emotion, and variability in the expression of emotions comes from specific contextual demands. Indeed, one of the main problems in the study of emotion has been the lack of control over the context in which affective reactions are evoked (Bradley, 2000; Lang, Bradley, & Cuthbert, 1997).

Emotional context: the International Affective Picture System

Looking at pictures is a ubiquitous human activity (Lang, Bradley, & Cuthbert, 1997). People generally enjoy looking at pictures both because it is simply pleasant to do so, and because pictures are an immediate source of information. In fact pictorial
stimuli, thus also photographs, convey a symbolic/representational meaning which matches properties of the real object or event they represent. Therefore pictures can activate the same network of cognitive representations and emotional responses associated to the real stimulus. Affective reactions caused by photographs are, in turn, similar to those prompted by the real stimulus, and affective judgments associated to a real object are expected to parallel those associated to its representation (Lang, Greenwald, Bradley, & Hamm, 1993).

Lang and associates developed a set of visual (International Affective Picture System/IAPS; Lang, Bradley, & Cuthbert, 2005), acoustic (International Affective Digitized Sounds System/IADS; Bradley & Lang, 1999a, 2000b), and verbal stimuli (Affective Norms for English Words/ANEW; Bradley & Lang, 1999b) which, over the past years, turned out to be able to reliably evoke a wide range of psychological and physiological emotional responses. Visual stimuli, in particular, included emotionally evocative color photographs, covering a wide range of semantic contents (babies, opposite-sex nudes, romantic couples, sport events, food, nature scenes, household objects, snakes, spiders, guns, mutilated bodies).

This greatly diverse set of images has been labeled International Affective Picture System (IAPS), and constitutes a standardized set of internationally accessible emotional stimuli. One of the goals of creating such a database was in fact also to allow comparisons among emotion studies conducted in different laboratories (Bradley, Greenwald, & Hamm, 1993). New photographs have been regularly added to the first set of pictures (Lang, Oehman, & Vaitl, 1988), and the IAPS, to date, includes approximately one thousand images (Bradley, Cuthbert, & Lang, 2005).

3.1. Verbal reports: the Self-Assessment Manikin

With the goal of providing normative ratings and standardizing these emotionally evocative images, Lang and colleagues asked large samples of subjects to
rate their personal experience while viewing each picture, according to two independent dimensions of affective valence and arousal\(^1\). For this purpose they repeatedly employed an instrument called Self-Assessment Manikin (SAM; Lang, 1980). This evaluation tool directly assesses the pleasure and arousal associated in response to the specific object or event. SAM employs non-verbal, graphic depictions, which make it usable also with non-English speaking cultures and not linguistically sophisticated populations (like, for example, children and aphasic patients). As can be seen in the following figure, SAM ranges from smiling to sad/frowning to represent the valence dimension. On the contrary, SAM looks either sleepy or with wide-opened eyes, along the arousal/activation dimension. SAM ratings have a high correlation (Bradley & Lang, 1994) with more time-consuming evaluation tools, like Mehrabian and Russell’s semantic differential scale (Mehrabian & Russell, 1974), which includes a greater number of factors.

\[\text{Illustration 1.2: SAM/Self-Assessment Manikin (Lang, 1980) non-verbal representations to obtain ratings of hedonic valence (upper part) and arousal (lower part).}\]

SAM valence and arousal ratings define the position of each picture in the two-dimensional affective space created by covarying valence and arousal dimensions (see figure below).

\(^1\) A further dimension, called dominance, was included in earlier works. Since the beginning this dimension seemed to account for a smaller portion of the variance, compared to hedonic valence and arousal, and significantly covaried with arousal ratings. For this reason it has not been included in more recent analyses (Bradley, 2000).
Illustration 1.3: The two-dimensional affective space defined by the mean pleasure (y axis) and arousal (x axis) ratings for IAPS pictures, and the location of some specific picture contents, based on the mean arousal and pleasure ratings for the exemplars in each picture content. Vectors indicate the hypothetical appetitive and defensive motivational systems that organize affective evaluations. Figure and caption from Schupp, Cuthbert, Bradley, Hillman, Hamm, & Lang, 2004.

The overall ‘boomerang-shaped’ distribution for all of these pictures suggested that two separate vectors extend from a non-arousing, neutral area (in the left central part of the graph) towards a high-arousing pleasant area (right upper) and towards a high-arousing unpleasant area (right lower part). Other characteristics of this affective space have been identified (Bradley, 2000; Bradley & Lang, 2000a): 1) Each affective dimension is fully covered, indicating that these stimuli are able to evoke emotional reactions fully ranging across pleasure and arousal dimensions. 2) Despite repeated
efforts to fill emptier areas in this affective space (for example, attempting to find highly unpleasant stimuli which are also low in arousal), the boomerang-shaped distribution remained stable across several experiments and years of research. 3) Stimuli judged as neutral in the valence dimension are not associated with high values on the arousal dimension.

When participants were asked also to rate how interesting the pictures were (Bradley, Greenwald, & Hamm, 1993), high arousing images, either pleasant or unpleasant, were designated as the most interesting pictures, and a high correlation between arousal and interest level was found ($rs$ from .76 to .87).

It is relevant to notice that the correlation between arousal and pleasure ratings is higher for unpleasant than pleasant stimuli, indicating that unpleasant stimuli are more easily activating the aversive/defensive system, while pleasant stimuli are in general less strongly activating the appetitive system. In addition, when low-arousing neutral stimuli are presented, there is a tendency to rate them as slightly positive (Bradley, 2000; Lang, Bradley, Cuthbert, 1997). These affective ratings therefore closely parallel the approach/avoidance behavioral response Miller (1959) observed in his animal studies. As mentioned earlier, the avoidance gradient is steeper than the approach gradient (indicating, from a motivational perspective, stronger defensive activation). Both these lines of research have been interpreted as indicating different response bias when affective stimuli are presented. A negativity bias is revealed by a tendency to respond more strongly to aversive stimuli, while a positivity offset lies beneath the weak inclination to activate the appetitive system, therefore approaching the stimulus, whenever the stimulus is neither appetitive nor aversive (Cacioppo & Berntson, 1994; Ito, Cacioppo, & Lang, 1998).

IAPS ratings proved to be both stable and reliable (Bradley, Greenwald, & Hamm, 1993): Not statistically significant differences were found when the same picture was presented in different sets of pictures or in different experiments. In addition, IAPS pictures were rated using both paper-and-pencil and computerized SAM
versions, but the administration format did not influence affective ratings. Viewing condition did not affect IAPS ratings either: There was a high correlation between ratings provided by subjects gathered in a classroom and by participants individually evaluating each stimulus in a laboratory.

It is particularly interesting that the same boomerang-shaped distribution has been found for acoustic stimuli forming the IADS (Bradley & Lang, 2000b), and for verbal stimuli included in the ANEW (Bradley & Lang, 1999b).

It has been formerly mentioned that one of the main goals underlying the creation of the IAPS database, was the possibility of using these same stimuli in different cultures and across different laboratories. Still, since the earliest works on this set of stimuli, it has been pointed out (Bradley, Greenwald, Hamm, 1993) that a very specific population was the source of IAPS affective normative ratings: Undergraduate students from the University of Florida. For this reason, in several European countries (Germany, Italy, Spain, Sweden) research groups administered the same IAPS stimuli to non-American students. Preliminary results (Bradley, Greenwald, Hamm, 1993) did not reveal substantial differences for affective ratings. More recently, psychophysiology research groups in Spain and Brazil provided cross-cultural validations of stimuli coming from the IAPS. High correlations between affective ratings obtained from Spanish compared to American university students indicated that IAPS emotional evocative power is robust to both cultural and geographic distance (Moltó, Montañés, Poy, et al., 1999; Vila, Sánchez, Ramírez, et al., 2001).

In summary, stimuli taken from the IAPS proved to be a stable, reliable, effective tool to evoke participants’ verbal emotional reaction, and there are undeniable methodological advantages, but also some caveats, in the use of stimuli taken from the IAPS in emotion and attention research.

Picture viewing is a typically passive activity, which implies the lack of local action (Lang, Bradley, & Cuthbert, 1997). This is a critical aspect especially in
psychophysiology research, where it is mandatory to reduce as much as possible any
'noise' caused by motor interference. Moreover, physical characteristics of pictorial
stimuli can be easily controlled and this guarantees the exact reproduction within and
between experiments and laboratories. As a result, stimuli taken from the IAPS provide
a controlled context for the study of emotional reactions to affectively relevant stimuli.

In this sense, it is important that the IAPS catalogue includes also neutral
stimuli, which are capable of indicating behavioral and physiological responses
connected to the task itself. It is indeed the comparison between reactions caused by
affective and neutral stimuli that indicates whether an emotion is associated or not with
behavioral or physiological changes (Bradley, 2000).

Also in the event in which differences between responses to neutral and
affective IAPS stimuli are observed, it must be borne in mind that results are limited to
the specific picture viewing context, and cannot, therefore, automatically be extended to
other emotional contexts (Bradley, 2000). As an example, it has been found that if
emotional stimuli, either pleasant or unpleasant, were presented in the context of
emotional picture perception caused a cardiac pattern different from that observed
during emotional imagery. In particular, while heart rate accelerated during imagery of
both pleasant and unpleasant events (Levenson, 1992), during emotional picture
perception heart rate decelerated only for unpleasant stimuli while accelerated during
pleasant pictures (Lang, Bradley, & Cuthbert, 1990). In general, the fact that specific
stimulus and task characteristics are associated with specific psychophysiological
reactions has been labeled by Lacey (1967) ‘stimulus specificity’.

3.2. Behavioral measures: Reaction times & picture viewing time

As just mentioned, one of the main advantages of employing IAPS stimuli is
that they do not require any overt behavior from the participant, according to a typically
passive, free-viewing context. However, there have been some experiments in which
behavioral measures were collected.
Data suggest (Bradley, Greenwald, Petry, & Lang, 1992) that reaction times to non-aversive probes are slower when probes are presented while participants are attending emotional stimuli. More recent data, in which different emotional categories were distinguished (Buodo, Sarlo, & Palomba, 2002), indicated that both pleasant and unpleasant high-arousal affective pictures (sexually explicit erotica and mutilations) obtained longer reaction times compared to less arousing stimuli. It seems noteworthy that in both studies probes presented during unpleasant stimuli tended to be associated with even slower reaction times.

These results were interpreted as indicating that fewer resources are available for the secondary task (responses to the probe) when the primary task comprises emotional stimuli. Thus affective stimuli might be better in engaging viewers' attention.

In a free-viewing context, when participants were asked to determine IAPS images viewing time (Hamm, Cuthbert, Globisch, & Vaitl, 1997; Lang, Greenwald, Bradley, & Hamm, 1993), emotional pictures, either pleasant or unpleasant, were viewed longer than neutral images. These data, therefore, seem against the possibility of 'stimulus rejection' which has been hypothesized (Lacey & Lacey, 1970) when unpleasant stimuli are presented. However, Hamm and associates data indicated also that in conditions of high distress (e.g. showing phobia-related stimuli to a group of phobics), highly phobic participants had significantly shorter viewing times during the presentation of their personal aversive material. This was therefore interpreted as indicating that aversive stimulus rejection can be observed only in the presence of extreme participant's discomfort. See following figure exposing Hamm, Cuthbert, Globisch, & Vaitl (1997) results.
Illustration 1.4: Pleasant, neutral, and unpleasant images, and pictures of snakes and spiders taken from the IAPS set were presented to normal and spider/snake phobic subjects. Subjects went through the slides at their own pace and viewing time was recorded. Animal phobics viewed their phobia-related object for a shorter time compared to controls. Figure from Lang, Bradley, & Cuthbert, 1997.

3.3. Psychophysiological measures

The main goal behind the creation of the IAPS database was to employ physically controllable and emotionally evocative stimuli in psychophysiological research. Since the earliest efforts (Lang, Oehman, & Vaitl, 1988), the IAPS catalogue has been utilized in a substantial number of studies examining both peripheral (e.g. facial muscles activity during pictures and aversive probes, skin conductance response, cardiac activity) and central psychophysiological measures (directly recording cortical activity with visual evoked potentials, or indirectly measuring changes in blood activity using positron emission tomography/PET and functional magnetic resonance imaging/fMRI).

The theoretical framework in which these researches were conducted by Lang and associates assumes that the organization of emotion in the brain involves two motivational systems, one appetitive and one aversive, and that this bi-dimensional organization is expected to be reflected in physiological responses to affective stimuli, so that peripheral and central reactions should covary significantly either with affective valence or with arousal ratings.
What follows is a review of main results emerged thus far.

3.3.1. Peripheral measures

3.3.1.1. Activity in facial muscles: Corrugator and zygomatic muscles

The activation of facial muscles involved in the display of facial expressions is one of the most obvious behaviors resulting from the processing of emotional stimuli (Bradley, 2000; see Tassinary & Cacioppo, 2000, for a review on surface electromyography). Since Darwin's first observations of emotional displays in different cultures (1873), it has been suggested that the way we use our face to convey our feelings might be shared cross-culturally. More recent data partially supported this hypothesis (Ekman & Freisen, 1986). However, the possibility that activity in facial muscles might be-at least partially- under voluntary control and strongly influenced by social context (Fridlund, 1991) recommends caution when interpreting results in terms of motivational systems involvement.

The activity of corrugator supercilii muscles is involved in the lowering and contraction of the brows, which is commonly regarded as an indication of distress (Fridlund & Izard, 1983; Larsen, Norris, & Cacioppo, 2003). Therefore, whenever stimuli judged as unpleasant are presented, motor units in corrugator muscles are expected to fire, even if firing is not sufficient to produce a visible frown (Bradley, 2000). The presentation of stimuli taken from the IAPS (Bradley, & Lang, 2000a) causes significant corrugator muscles contraction during affectively negative stimuli. A modest response (slightly above baseline) is visible when neutral stimuli are displayed, while during stimuli rated as highly pleasant corrugator muscles activity indicates relaxation (below baseline response). Correspondingly, a significant linear relationship is observed between affective valence ratings and corrugator muscles activity (Bradley, Greenwald, & Hamm, 1993), and this significant correlation is found in more than 80% of the participants (Lang, Greenwald, Bradley, & Hamm, 1993). More recent analyses
(Bradley, Codispoti, Cuthbert, & Lang, 2001), which included distinctions among specific affective contents, indicate that corrugator muscles are contracting the most for pictures depicting contaminations (e.g., cockroaches on food) and mutilations, while relaxing the most when participants view pictures of families. (See figure below, top graph).

*Zygomatic* muscles activity results in the cheeks being drawn back and tightened (Bradley, 2000). If motor units in these muscles are sufficiently activated, the person is smiling. Thus, we can expect these muscles to fire when stimuli judged as pleasant are displayed. Indeed, when viewing IAPS pictures the strongest zygomatic activity is found during pleasant material (Bradley & Lang, 2000a), particularly when these pictures depict families (Bradley, Codispoti, Cuthbert, & Lang, 2001). (See following figure, bottom graph). In general a significant linear correlation is observed between pleasure ratings and zygomatic muscles response (Bradley, 2000; Bradley, Greenwald, & Hamm, 1993). However, a slight increase in zygomatic activity can be found also when participants are looking at material rated as highly unpleasant (Bradley, 2000; Bradley & Lang, 2000a), and the majority of this response is accounted for by pictures depicting mutilations (Lang, Greenwald, Bradley, & Hamm, 1993). Therefore, also a significant quadratic correlation emerges between hedonic valence ratings and zygomatic muscles activity, although reliably smaller than the linear relationship.

In general, as a consequence of being influenced by social context, facial muscles show strong and reliable gender effects, suggesting that in most cases women are more facially expressive than men (Bradley, 2000; Bradley, Codispoti, Sabatinelli, & Lang, 2001). For example, zygomatic muscles activity showed a significant linear relationship with pleasure ratings in approximately two-thirds of female participants, while only one quarter of males responded in line with hedonic valence ratings (Bradley, Greenwald, & Hamm, 1993; Lang, Greenwald, Bradley, & Hamm, 1993).
Illustration 1.5: Mean changes in corrugator electromyographic (EMG) activity (top) and zygomatic activity (bottom) when viewing specific picture contents. Specific stimulus contents are ordered by rated arousal within the pleasant (left to right: high to low arousal) and unpleasant (right to left: low to high arousal) picture sets. Open bars represent pleasant picture contents; hatched bars represent same-sex erotica; solid bars represent unpleasant picture contents. Figure and caption from Bradley, Codispoti, Cuthbert, & Lang, 2001.

3.3.1.2. Cardiac response: Heart rate

The heart is innervated by both the sympathetic and the parasympathetic branch of the autonomous nervous system and, as a consequence, its activity reflects the activation of either system (Bradley, 2000; Brownley, Hurwitz, & Schneiderman, 2000). In the following figure cardiac activity during 6 s of picture viewing can be observed (cardiac activity is measured as number of heart beats per minute/bpm). Heart rate waveforms during pleasant, neutral, and low-arousal unpleasant picture viewing show a typically triphasic pattern: An initial deceleration is followed by a brief acceleration, and a subsequent secondary slowing (Bradley, Lang, & Cuthbert, 1993; Lang, Bradley, & Cuthbert, 1997). This triphasic pattern has been observed also in response to simple
novel stimuli and recognizable tones among variable stimuli (Lang & Hniatow, 1962), and it is, therefore, been interpreted as a general index of attention/orienting towards stimuli (Graham & Clifton, 1966). However, when pictures with an aversive content are presented, initial cardiac deceleration is maintained throughout picture presentation time (Bradley, 2000; Bradley, Codispoti, Cuthbert, & Lang, 2001; Bradley, Lang, & Cuthbert, 1993; Lang, Bradley, Cuthbert, 1997), and this effect cannot be attributed to attention or arousal, because interest and arousal ratings are the same for pleasant and unpleasant stimuli (Bradley, Lang, & Cuthbert, 1993). Moreover, cardiac deceleration after the exposure to highly fearful stimuli has been repeatedly found also in animals (‘fear bradycardia’, in Campbell, Wood, & McBride, 1997), and it is thought to reflect parasympathetic system dominance (Lang, Bradley, & Cuthbert, 1997). On the contrary, highly pleasant images prompt the greatest secondary acceleration (Bradley, 2000).

When specific picture contents were investigated (Bradley, Codispoti, Cuthbert, & Lang, 2001), pleasant stimuli depicting highly arousing erotic couples caused the greatest initial deceleration, while low-arousing pictures depicting nature scenes elicited the smallest initial deceleration. On the contrary, all unpleasant stimuli resulted in a deceleration pattern, independent from the specific picture content.

It is interesting that in conditions of elevated distress, such as when phobic individuals are presented with their object of fear, participants showed cardiac acceleration during unpleasant stimuli perception (Hamm, Cuthbert, Globisch, & Vaitl, 1997). Therefore, while cardiac deceleration when controls are viewing unpleasant stimuli has been interpreted in terms of parasympathetic system dominance, cardiac acceleration in phobic individuals seemed to suggest a greater engagement of the sympathetic nervous system branch for this specific population.
Illustration 1.6: Averaged waveforms for heart rate change when viewing pleasant, neutral, and unpleasant pictures (left) indicate a sustained deceleratory response for unpleasant pictures but a triphasic (deceleratory, acceleratory, deceleratory) response when viewing pleasant pictures. Separate cardiac waveform plots when viewing specific pleasant (top right) and unpleasant (bottom right) picture contents indicate that sustained deceleration is obtained for all unpleasant contents, whereas the triphasic waveform predominates for pleasant contents. Figure and caption from Bradley, Codispoti, Cuthbert, & Lang, 2001.

3.3.1.3. Sympathetic response: Skin conductance

Sweating activity, as revealed by increases in electrodermal conductivity, is regarded as a reliable index of reactivity to arousing stimuli (Lang, Bradley, & Cuthbert, 1997). Skin conductance is commonly recorded on the palm of the hand. Contrary to the cardiac system, the electrodermal system is thought to be exclusively innervated by the sympathetic nervous system (Bradley, 2000; Bradley & Lang, 2000a;
see Dawson, Schell, & Filion, 2000, for a review on electrodermal system. Accordingly, when displaying pictures taken from the IAPS, skin conductance increases with higher arousal ratings for both pleasant and unpleasant stimuli (Bradley, Lang, & Cuthbert, 1993; Lang, Bradley, Cuthbert, 1997). Thus, a significant linear correlation between skin conductance and arousal is typically observed (Bradley, Greenwald, & Hamm, 1993). In figure below it is possible to observe skin conductance reactions to different picture contents (Bradley, Codispoti, Cuthbert, & Lang, 2001): Larger responses are observed for highly arousing stimuli either pleasant (erotic couples and opposite sex erotica), or unpleasant (mutilations, animal attacks, and human attacks).

Illustration 1.7: Mean skin conductance changes when viewing specific pleasant, neutral, and unpleasant picture contents are illustrated. Specific stimulus contents are ordered by rated arousal within the pleasant (left to right: high to low arousal) and unpleasant (right to left: low to high arousal) picture sets. Open symbols represent pleasant picture contents; shaded symbols represent neutral picture contents; solid symbols represent unpleasant picture contents; x represents same-sex erotica. Figure and caption from Bradley, Codispoti, Cuthbert, & Lang, 2001.

3.3.1.4. Motivational priming: Reflexive reactions to aversive startling probes

The last peripheral measure taken into consideration is related to the physiological and behavioral responses (startle reflex) evoked by the aversive and abrupt presentation of a startling stimulus (startle probe).
Lang and associates' dimensional approach to emotions assumes that the subcortical activation of either the aversive or the appetitive motivational system has a modulatory effect on the processing of oncoming stimuli. That is to say, when one of the two motivational systems is activated, a network of associations, representations, and action programs connected to the triggered system are also primed. As a result, these processes, which are consistent with the activated motivational system, have a higher probability to be accessed, and this, in turn, would cause also greater response strength. Conversely, non consistent brain processes are less likely to be accessed, and, accordingly, cause physiologically weaker reactions (motivational priming hypothesis, in Bradley & Lang, 2000a; Lang, 1995; Lang, Bradley, & Cuthbert, 1990, 1997). As an example, when an individual is already aversively primed (like during an unpleasant affective state), responses to similarly aversive stimuli will be stronger and more likely to occur, while responses to pleasant, appetitive cues, not consistent with the motivational framework, are expected to diminish or not occur at all. Therefore, responses to aversive or appetitive cues are either augmented or diminished according to a motivational match/mismatch with the ongoing motivational state.

Some reflexes, being basic and primitive defensive responses, are expected to manifest this motivational priming. In most mammals, the presentation of an abrupt sensory event results in a cascade of physiological and behavioral reflexive reactions, which as a whole constitute the “startle reflex” (Lang, Bradley, & Cuthbert, 1997). As can be seen in figure A below, reflexes to aversive startling noises have originally been examined in animals (rats), where reactions to an abrupt noise (a toy gunshot) were augmented in an aversive condition (threat of shock) compared to a neutral, non aversive condition (Brown, Kalish, & Farber, 1951). Davis and associates (Davis & File, 1984), studying the startle reflex in rats, were able to identify a specific anatomical pathway involved in the response to startling probes. This primary basic pathway (Davis, Gendelman, Tischler, & Gendelman, 1982) is supposed to be initiated whenever an abrupt stimulus is presented to rats' ears. It involves an afferent connection from the
cochlear nucleus to the reticular formation, and an efferent connection from the reticular formation to the reflex effectors (through spinal neurons). This primary startle circuit is regulated by basic stimulus parameters such as its intensity and abruptness. Thus, another secondary circuit is assumed to underlie fear-learned startle potentiation during aversive conditions, and it is supposed to include projections to the reticular formation from the central nucleus of the amygdala (Bradley, Cuthbert, & Lang, 1999; Davis & Lang, 2003).

Illustration 1.8: (A, from Brown, Kalish, & Farber, 1951) Startle reflexes are potentiated in animals when a cue signals shock, compared with a no-shock condition. (B, from Simons & Zelson, 1985) Startle reflexes are inhibited when human subjects look at an interesting compared with neutral pictures. (C, from Vrana, Spence, & Lang, 1988) Startle reflexes are potentiated when viewing unpleasant pictures as well as inhibited when viewing pleasant pictures, compared with neutral materials, producing affective modulation of the startle reflex. Figures and caption from Bradley, Cuthbert, & Lang, 1999.

In humans, corresponding pathways are hypothesized to be involved in primary and secondary (fear-modulated) startle responses (Lang, Bradley, & Cuthbert, 1998).

Human startle reflex is commonly measured by recording the rapid eye closure which follows the presentation of the startle probe. Electrodes positioned below the
lower eyelid monitor the EMG activity of the orbicularis oculii muscle, and measure the size (magnitude) of the response to the startling probe. Startle probes are usually abrupt (with an almost instantaneous rise time) and fast (50 ms) burst of 90-100 dB white noise. These acoustic stimuli are typically rated as extremely unpleasant (Bradley, Lang, & Cuthbert, 1993) and are usually presented binaurally (Lang, Bradley, & Cuthbert, 1997). The startle reflex occurs extremely rapidly, and this allows the recording of the blink response without interfering with the ongoing task (Bradley & Lang, 2000a).

Studies of human attention during the presentation of startling noises (Simons & Zelson, 1985) indicated that startle reflex diminished during interesting compared to duller backgrounds. This seemed therefore to indicate that an increase in attention caused startle inhibition. (See figure B in previous illustration).

However, also humans, as well as animal subjects, showed startle reflex potentiation following the exposure to aversive stimuli (shock) (Greenwald, Bradley, Cuthbert, & Lang, 1998), suggesting that mechanisms underlying human fear-learned startle potentiation might be similar to those hypothesized in animal studies.

To test the motivational priming hypothesis Vrana, Spence, & Lang (1988) and Bradley, Cuthbert, & Lang, (1990) presented startling noises while participants viewed affective pictures taken from the IAPS. As mentioned earlier, emotional priming theory predicted that the match or mismatch between stimulus affective meaning and ongoing motivational state caused blink reflex potentiation or inhibition, respectively. So, the presentation of the startle probe (aversive stimulus) during unpleasant images (aversive state) was expected to cause startle potentiation (affective match condition). On the contrary, the presentation of the startle probe (aversive stimulus) during pleasant pictures (appetitive state) was expected to cause startle inhibition (affective mismatch condition).

In line with the emotional priming hypothesis, Lang and associates found that startle reflex was smaller if the probe was presented during pleasant pictures viewing,
while it was larger during unpleasant stimuli. The response to neutral stimuli fell in between (See figure C in previous illustration). These results, indicating affective modulation of the blink reflex, have been replicated several times by different research groups (for reviews on affective startle modulation, see Bradley, Cuthbert, & Lang, 1999, and Lang, Bradley, & Cuthbert, 1990).

Startle reflex during the presentation of affective stimuli has been extensively investigated, examining the effect of changes in properties of the startle probe and of the affective foreground (here, pictures).

Effects on startle reflex affective modulation of changes in startle probe properties.

1) Intensity of the probe. As mentioned earlier, startle reflex can be obtained with different probe intensities, ranging from 80 to 105 dB (Cuthbert, Bradley, & Lang, 1996). While the magnitude of the response to the startle probe increases with higher probe intensities, startle reflex affective modulation is found for both weaker and stronger probe intensities.

2) ‘Rise time’ of the probe. The speed with which an acoustic stimulus reaches its maximum intensity is called ‘rise time’. It is mandatory, for the startle reflex to occur, that the startle probe rise time is almost instantaneous (Lang, Bradley, Cuthbert, 1998).

3) Probe modality. The presentation of a startling probe in different sensory modalities (Bradley, Cuthbert, & Lang, 1990) has been a critical test to confirm that startle affective modulation was better explained by the motivational priming hypothesis than by the attention hypothesis advanced by Graham and associates (Anthony & Graham, 1985; Hackley & Graham, 1984). The attentional theory predicted that startle size was determined by the ongoing attentive state.
Accordingly, startle reflex potentiation was expected if the startle probe was presented in the same sensory modality of the ongoing task, where attentional resources were allocated. On the contrary, with sensory modality mismatch (attentional resources allocated to one modality while the probe was presented in the other) startle inhibition was predicted. The *motivational theory*, instead, highlighted the importance of the ongoing motivational state, determined, in this paradigm, by current IAPS picture valence. Within this framework, probe modality should not matter, while a match/mismatch between aversive startle valence and ongoing affective state should control startle size. Data observed by Bradley, Cuthbert, & Lang, (1990) were in line with the latter hypothesis. During the typical IAPS pictures viewing task, affective modulation of the startle reflex was observed after the presentation of both acoustic and visual startle probes. Moreover, for both startle modalities smaller blink sizes were observed when participants viewed pleasant pictures, while larger blink sizes were found during unpleasant images.

4) Attention to probes. Cuthbert, Schupp, Bradley et al. (1998) found that startle reflex affective modulation was the same independently from whether participants were instructed to attend or not attend to the probes.

5) Probe timing. Startle probes are typically presented at variable intervals after picture onset, also to avoid potential anticipation patterns in participants. Besides, different temporal intervals allow investigating when startle reflex affective modulation begins to appear (Bradley, Cuthbert, & Lang, 1999; Bradley, Lang, & Cuthbert, 1993). When the startle probe is presented right after picture onset (< 500 ms), startle reflex is generally inhibited, according to a phenomenon called ‘prepulse inhibition’ (Anthony & Graham, 1985; Hackley &
Graham, 1984). In general, prepulse inhibition indicates blink reflex decrements when non-startling stimuli are presented immediately before the startle probe. Using IAPS pictures, Bradley, Cuthbert, & Lang (1993) found a greater prepulse inhibition for more arousing stimuli, either pleasant or unpleasant, compared to neutral images. Beyond the prepulse inhibition time interval (up to 500 ms from picture onset), startle reflex emotional modulation occurs and it is maintained throughout picture viewing (in Lang and associates experiments, typically 6 s). On the contrary, probes presented after picture offset are not influenced by the valence of the preceding picture (Lang, Bradley, & Cuthbert, 1998).

Effects on startle reflex affective modulation of changes in IAPS picture properties.

1) Pictures aversive/appetitive evocative power. The motivational priming hypothesis predicts that a greater engagement of either the appetitive or the motivational system, revealed by IAPS pictures higher arousal ratings, should increase the strength of the response output. To test this hypothesis Cuthbert, Bradley, & Lang (1996) presented pictures taken from the IAPS catalogue, which varied in both rated pleasure (pleasant, neutral, and unpleasant) and arousal (low, moderate, and high). They found that startle reflexes were greater for high arousing stimuli, which prompted the greatest startle reflex inhibition (during pleasant pictures) and potentiation (during unpleasant images).

2) Picture presentation. As earlier mentioned when exposing the effects of different startle probe timings, startle reflex affective modulation occurs during the whole picture presentation period, while it is not observed after picture offset (Bradley, Cuthbert, & Lang, 1999;
Bradley, Lang, & Cuthbert, 1993). This could suggest that pictures have to be perceptually available to prompt startle reflex affective modulation. Codispoti, Bradley, & Lang (2001) presented IAPS pictures only for 500 ms, while startle probes were delivered later on. For up to 3 s after picture offset affective modulation of startle probes was analogous to that obtained with longer presentation times (Bradley, 2000; Bradley, Lang, & Cuthbert, 1993). This indicates that stimuli were still being processed, even if they were not perceptually available, and that this made possible startle reflex emotional modulation. Thus, as a general rule, emotional processing is sufficient for the motivational priming to take place, while the actual presence of the emotion-evoking stimulus is not necessary.

3) Pictures repetition. In both animal and human subjects, startle reflex decreases with repeated startle probe delivery (see Sanford, Ball, Morrison et al., 1992; Rimpel, Geyer, & Hopf, 1982, for animal and human studies, respectively). This habituation process is presumed to involve the primary startle circuit. Bradley, Lang, & Cuthbert (1993) repeatedly presented a small group of IAPS pictures, while startle probes were delivered (6 pictures presented for 12 times). As they expected they found that blink reflex size diminished with repeated presentation, however affective modulation did not significantly change across trials (see following illustration). The fact that startle reflex habituated, while emotional modulation did not, has been interpreted as in favor of the involvement of different startle circuits, one basic and one emotionally-modulated (Davis & File, 1984).
The startle reflex results we just exposed are specifically concerning reactions to affective pictures taken from the IAPS catalogue. However, significant emotional modulation of the startle reflex has been observed using a wide range of stimuli, including videos (Jansen, & Frijda, 1994), odors (Miltner, Matjak, Braun, et al., 1994; Ehrlichman, Brown, Zhu, & Warrenburg, 1995), sounds (Bradley & Lang, 2000b), texts (Vrana & Lang, 1990). In all these studies blinks were larger when participants were exposed to unpleasant stimuli, but smaller when pleasant stimuli were presented. Overall, this is in favor of the hypothesis that affective priming is not depending on the specific context, but instead reflects the involvement of different motivational systems.
Beginning this extremely brief review about startle reflex affective modulation, it has been pointed out that EMG blink is simply the most commonly examined startle reflex component and that a cascade of physiological and behavioral responses follow the presentation of startling probes. Accordingly, we might expect other startle reflex components to be modulated by the ongoing affective state.

Within an imagery study, Vrana (1995) investigated EMG blink, skin conductance, and cardiac responses to a startling tone, while participants imagined emotional, pleasant or unpleasant, and neutral situations. He found that both blink and skin conductance responses to the probes were modulated by the ongoing affective state. In particular, Vrana found larger skin conductance responses to unpleasant compared to neutral and pleasant conditions. Cardiac pattern, on the contrary, did not reflect the ongoing motivational state. However, before concluding that skin conductance response to a startling probe always shows affective modulation, while heart rate does not, it must be kept in mind that specific tasks are associated to specific physiological responses (‘stimulus specificity’, Lacey, 1967). Thus, startle reflex responses during an imagery task might be different from reactions obtained during picture viewing.

Some studies investigated also cortical evoked potentials following the presentation of startling probes (Schupp, Cuthbert, Bradley, et al., 1997). In the context of IAPS picture viewing, the presentation of a probe prompted a large P300, which was smaller for emotional compared to neutral stimuli. The fact that the P300 component was reduced for both pleasant and unpleasant stimuli and that the size of the P300 is typically varying depending on attentional engagement (Donchin & Coles, 1988), suggested that the cortical response to startling probes might be influenced by the engaging (arousing and attentive) value of the ongoing stimulus, more than its specific

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2 Following a tone-cued imagery procedure, participants first read a pair of cards, each describing a different emotional situation. Then, different frequency tones signaled which of the two cards participants had to imagine for 8 s (“as if you were actually in the situation and participating in the events described”). Another tone signaled the end of the imagery period in this study the same emotional situation was imagined six times (Vrana, 1995).
valence. Confirming this hypothesis, when the cortical impact of specific picture contents was investigated (Schupp, Cuthbert, Bradley, et al., 2004), a clear effect of content arousal value was found, indicating smaller P300 amplitudes for more arousing contents (specifically, erotica for pleasant stimuli, threat and mutilation for unpleasant stimuli).

3.3.2. Bumping into aversive stimuli: The defense cascade

The concept of ‘stimulus specificity’ was introduced by Lacey (1967) to describe the fact that specific stimuli are associated with specific physiological responses. This, within the dimensional approach to motivations suggested by Lang and associates, implies that motivationally relevant events are causing physiological changes which are specific to each response system. As an example, we just observed that skin conductance is increasing to both pleasant and unpleasant pictures, while heart rate is reacting differently to positively and negatively affected stimuli.

Furthermore, each response system is expected to react in its own way to increases in activation in each motivational system (in Lacey’s words ‘directional fractionation’, 1967). Again, this has been visible using stimuli taken from the IAPS catalogue: Unpleasant stimuli prompt sustained cardiac deceleration in normal healthy subjects (similar to ‘fear bradycardia’ observed in animals). On the contrary phobic patients, for whom unpleasant stimuli become especially aversive, show cardiac acceleration.

Overall, a wider view on peripheral physiological responses to emotional stimuli highlights not a single reaction but a cascade of behavioral and physiological events following the presentation of motivationally relevant stimuli. Moreover, increases in activation (increased stimulus proximity in Miller’s ‘conflict theory’, 1959) can differentiate specific response systems.

Several theorists suggested that within the aversive context it is possible to differentiate several stages, varying according to both the degree of motivational
activation, and the specific behavioral responses associated (Fanselow & Lester, 1988; Masterson & Crawford, 1982; Tinbergen, 1951). Defensive response, according to this perspective, varies depending on predator imminence. In line with these hypotheses and based on data obtained using the picture perception paradigm, Lang and colleagues (Bradley, 2000; Bradley & Lang, 2000a; Lang, Bradley, & Cuthbert, 1997) proposed that aversively relevant stimuli result in a defense cascade. Depending on the degree of motivational activation, physiological responses included in the defense cascade range from indicating generic orienting to a defensive response. (See figure below for a schematic outlook at the defense cascade).

Illustration 1.10: A schematic presentation of the defense response cascade generated by increasingly arousing aversive stimuli. See paragraph below for more details. Figure and caption from Bradley, Codispoti, Cuthbert, & Lang, 2001
During a low-arousing motivational state (left side of the schema), physiological responses to aversive stimuli are the same as those to appetitive stimuli: Heart rate decelerates, suggesting parasympathetic engagement, while slight and not reliable increases in skin conductance can be observed. Both heart rate and skin conductance will increase for progressively more arousing stimuli. In general, responses at this stage are those typically associated with the orienting response, where more attentive resources are allocated to a stimulus judged as relevant. Accordingly, startle reflex is inhibited, as expected when participants are attending low-arousing yet interesting stimuli.

With more and more motivational activation (higher arousal ratings, analogous to higher predator/threat imminence) heart rate keeps on decelerating, while skin conductance response gets larger and larger. With progressively stronger heart rate deceleration and while the individual is focusing on the relevant stimulus, we begin observing startle reflex potentiation. Therefore, any stimulus which is aversive enough to defensively prime the individual causes a supranormal reaction to the startle probe (Bradley & Lang, 2000a). Defense response is typically characterized by tachycardia. It has been noted (Cook & Turpin, 1997) that heart rate accelerates only right before action, thus, in the defense cascade model, tachycardia is assumed to occur only at the peak of defensive activation (highest threat imminence). This does suggest, as a result, that phobic individuals are fastest in reaching highest activation stages compared to controls.

3.3.3. Central measures

3.3.3.1. Cortical brain activity: Visual evoked potentials

When electrical brain activity is recorded while participants look at IAPS picture, specific event-related potentials can be observed (see Ray, 1990; Fabiani, Gratton, & Coles, 2000 for reviews on fundamentals of electrocortical recording). In particular, high-arousing stimuli, either pleasant or unpleasant, cause a positive slow
wave activity (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Palomba, Angrilli, & Mini, 1997; Schupp, Cuthbert, Bradley, Cacioppo, Ito, & Lang, 2000). This cortical response to emotional stimuli begins 200 ms after picture onset and reaches its peak approximately 1 s from picture presentation (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000). In this specific study images were displayed for 6 s. The same late cortical reactivity to emotional stimuli was confirmed with shorter lasting picture presentation times (1.5 s in Schupp, Cuthbert, Bradley, Cacioppo, Ito, & Lang, 2000), and using high-density sensor arrays (Keil, Bradley, Hauk, Rockstroh, Elbert, & Lang, 2002; Schupp, Junghoef er, Weike, & Hamm, 2003a). The location of this late positive potential (LPP) is the posterior parietal and occipito-temporal cortex, and the topography is similar for high and low-sensor density studies (Keil, Bradley, Hauk, Rockstroh, Elbert, & Lang, 2002; Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000, respectively). In general, cortical potentials (measured at the positive response peak, between 400 and 700 ms) show a high correlation with IAPS arousal ratings (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000).

Specific evoked potentials to emotionally engaging stimuli have also been found with 10 Hz flickering color pictures presentation procedures (‘steady-state’ visual evoked potentials, in Keil, Moratti, Sabatinelli, Bradley, & Lang, 2004), and with shorter picture presentation periods (Junghoef er, Bradley, Elbert, & Lang, 2001), suggesting in the latter case that emotional modulation might occur also in the absence of complete perceptual processing.

Moreover, in addition to the late positive potentials, typically occurring between 400 and 700 ms from picture onset, recent data have found affective modulation also in earlier time windows, 100-300 ms from picture presentation (Junghoef er, Bradley, Elbert, & Lang, 2001; Schupp, Junghoef er, Weike, & Hamm, 2003a; Schupp, Junghoef er, Weike, & Hamm, 2003b). This might indicate that the stimulus arousal value might modulate even initial visual cortex processing.
3.3.3.2. Brain imaging studies: PET and fMRI data

Brain imaging techniques have also been employed to investigate affectively modulated brain activity with spatially more accurate methods, like positron emission tomography/PET and functional magnetic resonance imaging/fMRI. Obtained results are mostly in line with those emerged using visual evoked potentials/VEP. Before exposing brain imaging main results, it must be kept in mind that while VEP directly measure electrical brain activity from the scalp surface, both PET and fMRI record brain activity indirectly, detecting variations in the local amount of cerebral blood flow.

PET studies (Lane, Reiman, Bradley, et al., 1997; Lane, Chua, & Dolan, 1999) indicated that emotional pictures taken from the IAPS, either pleasant or unpleasant, caused a significantly greater activity in the thalamus and in the medial prefrontal cortex compared to neutral stimuli. Moreover, for females unpleasant stimuli elicited larger extrastriate visual cortex activity (Lane, Reiman, Bradley, et al., 1997), while for males (Lane, Chua, & Dolan, 1999) highly arousing pleasant images caused the largest response in the same brain area. However, before drawing conclusions about gender differences in visual cortex reactivity, it must be noted that for this group of females skin conductance values while viewing positive stimuli were significantly smaller than during unpleasant stimuli. Therefore, this indicates that for these women pleasant stimuli were not as arousing as unpleasant stimuli.

When the fMRI method has been employed to record brain activity during IAPS pictures viewing, it confirmed and extended results indicating a larger activation for emotionally relevant stimuli (Lang, Bradley, Fitzsimmons, et al., 1998). In particular, only emotional stimuli caused larger responses in the occipital gyrus, in the right fusiform gyrus, and in the right inferior and superior parietal lobules. Moreover, occipital activation was greater for stimuli which were higher in arousal values, specifically for those depicting mutilations, human threats, and erotica (Bradley, Sabatinelli, Lang, et al., 2003). When brain responses of control subjects were
compared to those of particularly fearful participants (Sabatinelli, Bradley, Fitzsimmons, & Lang, 2005), the activity in the amygdalas and in the inferotemporal visual cortex increased for both groups with rated picture arousal. Additionally, fearful individuals showed significant increases in the activation of both these structures compared to non-fearful individuals.

Illustration 1.11: Example of brain imaging results using images taken from the IAPS catalogue. Sites of functional activity during processing of pictures with different affective content, compared with the inter-picture interval, as determined from averaging functional maps across subjects. Clusters meeting criteria are depicted in red and yellow, with yellow indicating a larger difference. **First and fourth columns:** Examples of color and grayscale versions of exemplars in each of the employed picture contents. **Second column:** All picture contents show activity centered on the calcarine fissure, whereas only emotionally arousing pictures (e.g., erotica, threat, mutilation) also show bilateral activity in the occipital gyrus. **Third column:** The extent of functional activity in the bilateral fusiform gyrus varies, with emotionally arousing pictures prompting more activity than pictures rated lower in arousal. Figure and caption from Bradley, Sabatinelli, Lang, Fitzsimmons, King, & Dasai, 2003.
3.3.4. Factor analysis: Affective patterns in physiological responses

Taken together, linguistic, behavioral, and physiological responses during picture perception appear to respond either to the general level of motivational activation (arousal) or to the positive or negative affective meaning attributed to the stimulus (hedonic valence). To further test this hypothesis, Lang and his associates (e.g., Bradley, 2000; Lang, Bradley, & Cuthbert, 1998; Lang, Greenwald, Bradley, & Hamm, 1993), conducted several factor analyses of linguistic, behavioral, and physiological measures. These analyses consistently resulted in a two factors solution, with very low cross-loadings for all measures. The first factor included high loadings for pleasure ratings, heart rate change, corrugator and zygomatic muscles EMG activity, suggesting a primary valence factor, sensitive to the aversive and appetitive stimulus value. The second factor involved high loadings for arousal ratings, interest ratings, viewing time, skin conductance, and cortical slow wave, implying a factor connected to activation, arousal. It is noteworthy that factor analyses repeatedly individuated these two main factors. The stability of these results seems therefore to support the existence of two independent motivational determinants, which underlie the variable outputs of the three main emotional response systems (language, overt acts, and physiology).

3.3.5. Ruling out perceptual features effects

Before summarizing the dimensional approach to emotions, it is important to remark that picture color cues (like red indicating the presence of blood) did not explain this pattern of peripheral physiological responses. Indeed, analogous peripheral and central reactions have been observed using grayscale versions of the IAPS stimuli (Bradley, Codispoti, Cuthbert, & Lang, 2001; Junghoefer, Bradley, Elbert, & Lang, 2001, respectively). Moreover, as earlier remarked, also a brief presentation (500 ms) of the affective stimuli (Codispoti, Bradley, & Lang, 2001) was effective in causing the same emotional modulation of skin conductance, corrugator muscles, and startle reflex
typically observed using longer (6 s) picture displays. (This was, however, not true for heart rate affective modulation, which seemed to depend on the sustained presence of the stimulus).

Overall, both results indicate that neither some specific perceptual features nor the actual presence of the stimulus are necessary or sufficient to explain affective modulation of peripheral and central physiological variables.

4. Summarizing...

The dimensional approach to emotion suggested by Lang and colleagues can convincingly explain a considerable amount of peripheral and central physiological data. This theoretical framework assumes that emotions are the result of primitive adaptive reflexes and physiological responses, which evolved to promote the survival of the individual and its specie. These adaptive and evolutionarily helpful acts could either protect the organism from potential threats (preparing it to fight or flight) or promote approaching behaviors towards rewarding stimuli. Accordingly, two motivational systems are expected to underlie basic approach and withdrawal behaviors, one appetitive and the other aversive.

In humans, emotions are viewed as dispositions to action, highlighting the fact that most of the times motivational engagement does not result in overt behaviors. Additionally, the elemental distinction between approach and behavior could appear not able to cover the huge repertoire of emotional acts of which human beings are capable. However, Lang and colleagues, proposed that human beings measurable emotional response systems essentially comprise language, overt behaviors, and physiological responses. Their data indicated that all these output systems might be organized around the appetitive and aversive motivational systems. Thus, they suggest that the frequently observed lack of covariation between these output systems might be due to the absence of a stable context in which emotions are evoked. For this reason, their research group has been developing several sets of standardized stimuli (mostly pictures, but also sounds and words) capable of evoking a wide range of emotions. Large samples of
subjects rated each of these stimuli, allowing Lang and associates to keep under control the linguistic output of emotion. In addition, the use of pictorial stimuli, implying a passive viewing context, limited behavioral responses.

They repeatedly found that peripheral and central psychophysiological responses to emotionally relevant stimuli support a bidimensional view of emotions, indicating that specific output systems react differently to the level of activation of either the appetitive or the aversive motivational system. (Factor analysis repeatedly supported this hypothesis, organizing linguistic, behavioral, and physiological measures around two factors, identifiable with valence and arousal).

In turn, the catalogue of affective stimuli created and constantly updated by Lang and colleagues proves to be a reliable tool to evoke emotional reactions, providing standardized normative affective ratings which can be easily used by laboratories worldwide. In addition, the dimensional theory of emotions simplifies the approach to the multivariable and extremely creative human database of emotion, while the methodological perspective chosen by Lang and associates allows easier correlations among different linguistic, behavioral, and physiological indices.
2 Ocular movements: perceptual and emotional scene processing

1. Visual system anatomy: From the eyes to the brain

It has been stated elsewhere (Palmer, 1999) that the modern era in the study of optical physiology began in the XI century, when the Arabian philosopher Al-hazen intuited that the optimal way of thinking about the human eye was as a pinhole camera (or camera obscura), in which it is the light, entering the hole and being then reflected on the back surface of the camera, to create the images we ‘see’. This intuition, developed in the XVI and XVII century by Giovanni Battista della Porta and Kepler, simplified the interpretation of human optics, allowing researchers to think about the eyes as mere optical devices. Of course, despite the practical usefulness of this metaphor, the complexity of the human visual system is way beyond that of any device implemented thus far (Gregory, 1998).

The eyes are located approximately 6.5 cm apart and firmly positioned within the skull’s eye sockets. In figure below we can observe the section of a human eye. All of the objects we are looking at reflect light which enters the eyeball through a bulge on the eye’s surface called cornea. The cornea is the first tool the human body uses to bend the rays of light toward the center of the eye. The cornea does not receive its nutrients from blood, but from the continuously renewed liquid set between the cornea and the iris muscles called aqueous humor. Afterward, the light passes through the pupil, a hole in the pigmented smooth muscles of the iris, and then it goes through the lens. The
portion of the eyeball behind the lens contains numerous capillary blood vessels and it is filled with a liquid called *vitreous humour*.

**Illustration 2.1: The human eye and its main components.**  
*Figure from Gregory, 1998.*

The function of the lens is to accommodate, that is to say, adjust its own size to the distance of the objects we are looking at, so that the light is hitting exactly the surface of the back of the eye, the retina. (Hyperopy and myopy are widespread lens diseases in which the lens does not properly accommodate, putting the image focus respectively behind and in front of the retina). To be accommodated, the lens undergoes the action of the *ciliary muscles*: To focus on close objects they contract, thus also thickening the lens, while to focus on distant objects these muscles relax, thus making the lens thinner and capable of focusing distant objects on the surface of the retina.
The retina contains millions of tiny photoreceptors called rods and cones. These photoreceptors, first described by Treviranus in 1835 (Gregory, 1998), are not on the surface of the retina: As figure below shows, photoreceptors are placed ‘upside-down’ behind a network of blood vessels, nerve fibers and supporting cells which together constitute the layer called ‘retina’. Rods and cones have been named this way because of their shape, more clearly distinguishable on the periphery of the retina, where there are fewer photoreceptors.

Illustration 2.2: Structure of the retina. Light travels through layers of blood vessels, nerve fibers and supporting cells to the sensitive receptors (rods and cones). These lie at the back of the retina, which is thus functionally inside-out. The optic nerve is not, in vertebrate eyes, joined directly to the receptors, but is connected via layers of highly interconnected cells, which are part of the brain lying in the eyes. Figure and caption from Gregory, 1998.

Our eyes contain way more rods (ca. 120 millions) than cones (ca. 8 millions), and while rods are everywhere in the retina except at its very center, cones are especially concentrated in the center of the retina, which is called fovea. The fovea contains only cones and it is the area on which it strikes the very center of our visual field (approximately 2 degrees of visual angle, Rayner, 1998). The reason for this complementary displacement of human photoreceptors lies in rods and cones different
functions. As Farah (2000) pointed out, the visual system is characterized by an incredibly high level of 'division of labor', also called 'partitioning', 'multiplexing', or 'parallelism', which begins with the functions of these two receptors: Rods have a greater sensitivity, giving low resolution images of external stimuli under conditions of low illumination (scotopic conditions), whereas cones are particularly responding to details, giving a better resolution of external stimuli under normal illumination (photopic) conditions. Moreover, cones can be divided into three types, each one absorbing a different wavelength of light within a process called 'pigment bleaching'. The separate response of each kind of cones combines to give us the perception of different colors.

Since cones are located especially in the center of the retina (fovea and nearby area, parafovea), we have the best visual resolution and color perception exactly in the center of our visual field.

At this point it is also important to keep in mind that 1) after the action of these photoreceptors the light coming from the object has already been processed and replaced by a retinotopic map of differences between darker and lighter regions; and 2) because of the way light behaves within any camera obscura, object's right/left and up/down coordinates are already inverted at the level of retinal photoreceptors.

Since the first synapse with photoreceptors, the bundle of neural channels which constitutes the optic nerve is actually splitting the external stimulus image between M and P channels, respectively going toward the magnocellular and parvocellular layers of the lateral geniculate nucleus (LGN). The M ganglion cells have broader axons which allow them to conduct faster; they receive inputs from a bigger number of receptors and are characterized by a better light sensitivity/temporal resolution (they use a larger sample of inputs for a shorter amount of time), but also by a low spatial resolution. For these characteristics, M cells seem to be better equipped for the perception of motion. P ganglion cells, on the contrary, have smaller, thus slower, axons. They receive inputs from a smaller number of receptors and are characterized by a good spatial resolution,
but also by a low temporal resolution (they use a smaller number of inputs for a longer amount of time), they also respond to colors. For these characteristics they appear to be more suitable for object recognition.

After the optical decussation at the level of the optic chiasm, these two sets of cells constitute the first portion of the geniculostriate pathway, which passes through the lateral geniculate nucleus (LGN) and ends in the primary visual cortex (see following figure). After the optic chiasm decussation, a smaller portion of cells constitute the collicular pathway (later examined), ending in the superior colliculi.

Illustration 2.3: Information from the inner (nasal) portion of each retina crosses over to the opposite side of the brain receives input only from the opposite half of the visual field. The representation of the central portion of the visual field receives disproportionate representation in visual cortex. Figure and caption from Palmer, 1999.
Within the lateral geniculate nucleus, the aforementioned magnocellular and parvocellular layers keep the same functional partitioning between M and P cells: Magnocellular layers have the best temporal resolution, while parvocellular layers have the best spatial resolution, even if there is no total independence between the functions of these two systems. At the level of the LGN there is still a retinotopic organization of the image, which is kept also in the LGN major projection towards the primary visual cortex. Moreover, at cortical level it is also possible to observe a distortion of the cortical representation of the image, such that neurons firing for the central degrees of visual field cover a larger cortical area than the more peripheral degrees of visual field (this phenomenon has been labeled ‘cortical magnification’ by Tootel, Silverman, Switkes, & De Valois, 1982).

One of the biggest steps forward in the understanding of the anatomy and functional organization of the primary visual cortex comes from the neurophysiological studies of Hubel and Weisel (Hubel, 1988; Hubel & Weisel, 1962). These physiologists, using extremely simple visual stimuli (e.g., differently oriented bars), recorded single cells activity from non-human visual cortices. They originally described three different groups of cortical cells, each one responding to a different kind of stimulus characteristic: 1) ‘simple’ cells, responding to changes in specific edges location and orientation (meaning, some ‘simple’ cells respond only to a specific orientation, others only to another specific orientation); 2) ‘complex’ cells, reacting in a more complex way to more complicated stimuli; 3) ‘hypercomplex’/‘endstopped’ cells, responding selectively to specific lengths.

Moreover, they observed that cells responding to the same specific aspect of the stimulus were located in columns (so that, if a cell responded to a specific orientation, the one perpendicularly below responded to the same orientation), while moving horizontally through the cortical surface, cells were responding to progressively different orientations. In addition they noticed that groups of neurons were responding
to one eye’s stimulation, and were placed adjacent to groups responding to inputs from the other eye. Approximately twenty columns, including all possible orientations with both eyes input, formed what they called a ‘hypercolumn’, which processed the information coming from a small portion of the retina.

After Hubel and Wiesel indicated this unexpectedly high level of segregation within the visual cortex, some rigidly reductionist theories suggested the possibility of extending these results to more complex visual stimuli, up to the point of hypothesizing single cells responding to extremely specific complex and unique visual stimuli (“grandma’s cells” are the example typically described). However, the high level of partitioning from the retina up to the primary visual cortex and the fact that retinotopy is encountered at several progressive stages of visual information processing should not be taken as an indication of totally independently segregated analysis of stimulus characteristics (see Farah, 2000): Interconnections among areas devoted to different stimulus aspects can be observed at different levels of the visual system, so that structures typically responding to some specific aspects of the stimulus are also connected to structures typically reacting to others.

As Farah pointed out (2000), to avoid inappropriate inferences from Hubel and Wiesel’s results it is mandatory to bear in mind that visual stimuli used to elicit these neurocellular responses in non-human subjects were extremely simple ones. This suggests that we can expect the human visual system to have a higher level of organization and complexity compared to non-humans. In turn in human beings the visual cortex arrangement is possibly characterized by a higher number of interconnections among areas involved in different functions. Therefore, additional and long-term research is still needed in the attempt of understanding how the human visual system might be responding to natural, complex images.
2. **Anatomy of eye movements control**

Whenever needed, human eyeballs move to put visual stimuli in the center of our visual field, allowing us to fixate them or examine their specific details.

Eye movements are controlled by a system of six different ocular muscles. These muscles, labeled superior and inferior oblique, lateral and medial rectus, superior and inferior rectus, are depicted in figure below (Gregory, 1998). They are coupled and characterized by reciprocal action, such that if one muscle contracts the other one is relaxed, and each couple of muscles takes care of a specific movement of the eye (Guyton, 1991).

*Illustration 2.4: Oculomotor muscles. The eyeball is maintained in position in the orbit by six muscles, which direct the gaze to any position, and allow the eyes to converge at varying distances. The muscles are under constant tension, forming a delicately balanced system. Figure and caption by Gregory, 1998.*

- **Superior and inferior oblique muscles** keep our visual field in a properly stable vertical position, compensating for the movements of the head and of the whole body;
- when the *lateral or medial rectus muscle* contracts, our eyes move horizontally from one to the other side of our visual field;
- on the contrary, the *superior and inferior rectus muscles* contract to move our eyes along the vertical plane.
The activity of these extraocular muscles is controlled by three cranial nuclei, located in the brainstem and sometimes called ‘gaze centers’ (Palmer, 1998). It seems noteworthy that the gaze centers are not acting on ocular muscles based on the pairings we just described: The oculomotor cranial nucleus, through the III cranial nerve, controls the activity of four out of six ocular muscles (inferior oblique, superior and inferior rectus, and medial rectus), while the trochlear nucleus controls only the superior oblique muscle, and the abductor nucleus controls only the lateral rectus muscle. Therefore, together with a reciprocal action of each couple of muscles, we observe a cranial nucleus in charge of the activity of an entire group of ocular muscles versus two separate cranial nuclei each one regulating the activity of a single muscle, devoted to a specific movement of the eye. The ultimate reason for this organization is a finer control of eye movements. In addition, the medial longitudinal bundle provides interconnections among these three gaze centers, thus contributing to the combined action of this set of muscles. Additionally, it is relevant for the processing of visual information that the medial longitudinal bundle receives inputs about the balance of the whole body from the VIII cranial nucleus (vestibular nerve).

Cortical projections deliver high-level perceptual and cognitive information to the gaze centers: Frontal areas of the cortex project to the pretectal area, while occipital areas project both to the pretectal area and to the superior colliculi. Lesion studies revealed that if the premotor frontal areas are disrupted, the patient is not able to move the focus of a fixation to a different target, and for this reason these cortical areas were thought to be involved in the voluntary control of fixations. On the contrary, the disruption of the secondary visual areas causes an inability to fixate on any target, thus these areas were thought to be involved in the involuntary control of fixations (Guyton, 1991).
3. **Ocular movements measurement**

The set of ocular muscles described above allows us to execute a series of typical movements of the eyes. These movements can be distinguished based on their speed, duration, and, most of all, goal and specific context in which they are generated (Rayner, 1998). Typically, between one ocular movement and the next, we are fixating an object.

Fixations are commonly defined as the amount of time during which the eyes remain relatively still (see ‘nystagmus’ definition below). On average they last for about 200-300 ms, but their duration varies depending on the specific task subjects are performing (Rayner, 1998).

When defining what a fixation is, we labeled it as a period of time during which the eyes are *relatively still*. The reason for this specification is that ocular muscles are never completely still, being the fixations characterized by a small yet constant tremor labeled nystagmus. It is not totally clear what these tremors are for, although it has been suggested they might keep retinal neurons from constantly firing. While recording eye movement data nystagmi are commonly considered as ‘noise’ and, accordingly, excluded from data analysis.

Saccades are the most common kind of eye movement and occur whenever we examine a scene, read a text, or look for an object. These are rapid movements of the eye (according to Goldberg, Eggers, & Gouras, 1991, up to 900 degrees per second) which take about 150-200 ms to be planned and executed (about 30 ms for the sole movement). They have been described as ballistic movements, meaning that once they started, their trajectory can’t be changed (Palmer, 1998). The speed of each saccade is a monotonic function of the distance the eyes have to cover, thus a saccade that covers a longer distance will take longer to occur. In addition, saccade’s velocity varies during the saccade itself: It reaches its maximum speed right before half of the saccade has been executed, and then it slows down prior to reaching the target (Rayner, 1998). The distance that needs to be covered (saccade size) affects also the duration of the saccade:
A 2 visual degrees saccade, typical of reading, lasts less than a 5 visual degrees saccade, typical of scene perception (Abrams, Meyer, & Kornblum, 1989).

Rayner (1984, 1988) reviewed saccades' size and fixations duration in a series of papers related to various cognitive tasks. He reported that these oculomotor components vary depending on the specific task the subject is performing. When we silently read a text we make shorter fixations (lasting on average 225 ms) and smaller saccades (moving for 2 degrees of visual angle, roughly corresponding to 8 letters) compared to when we are exploring a scene. During scene perception we fixate on average for 330 ms, and we also move across wider areas of the scene (average saccade size is 4 degrees of visual angle).

While we make a saccade we have a lower sensitivity to visual inputs, a phenomenon called saccadic suppression (Matin, 1974). It has been hypothesized (Uttal & Smith, 1968) that the reason saccadic suppression occurs is that the speed of the saccade would not allow us to perceive more than a blur of the external stimuli. This phenomenon has been the object of considerable debate, particularly in the context of written text processing, concerning both whether during a saccade we keep on acquiring new upcoming information, and whether we keep on cognitively processing the information we already acquired (for a more recent review, see Diamond, Ross, & Morrone, 2000).

Pursuit eye movements are slow ocular movements we typically make to follow the trajectory of a moving target. If this ocular movement is not quick enough to track a fast target, it is usually followed by a saccade.

Vergence eye movements occur when we try to focus on a particularly close object, so that we need to move our eyes one towards the other.

Vestibular eye movements take place after we rotate our head or move our entire body: The goal of these ocular movements is to compensate for our body movements and maintain our visual focus aligned with them.

Occasionally, also drifts and micro-saccades can be detected within less predictable situations: The first are small, slow movements of the eyes, supposedly
consequences of the nervous system not perfectly controlling the oculomotor system, the latter occur right after drifts, to go back to the initial eye position.

Given the specific visual stimulus context in which some of these ocular movements are generated (moving targets for slow pursuit eye movements, approaching targets for vergences, movements in viewer’s body and/or head for vestibular eye movements), and given also that some ocular movements, like drifts and micro-saccades, are in most of the experiments discarded from the analysis and treated as ‘noise’ (Henderson, Weeks, & Hollingworth, 1999), it should not be surprising that for the purpose of scene viewing, in which the viewer is examining a static image, only saccades and fixations are taken in consideration.
4. \textit{Historical eras in eye movement research}

According to Rayner (1998) we can identify three separate stages in the history of research relating ocular movements to cognitive processes.

The first era, right after the first observations on ocular behavior during reading by Javal in 1879 (Huey, 1908, cit. in Rayner, 1998), cover the period until the 20s. Rayner reports that this is the era in which some basic ocular phenomena are described, like saccadic suppression (drop in the information intake while a saccade is executed), saccade latency (time period to initiate, plan a saccade), and size of perceptual span (maximum number of items –letters, objects- we can process with only one fixation).

The following period, from the 20s to the late 50s, is characterized by the affirmation of the behaviorist movement in American experimental psychology. During this phase, researchers adopt a more applied approach to the study of eye movements, while some classic works about eye movements in reading (Tinker, 1946) and scene perception (Buswell, 1935) are published. After this era, as reported by Rayner, and up to the middle of the 70s, there is a hiatus in the study of cognitive processes using ocular measures, and only few eye movement researches are conducted.

Rayner states that the more recent era begins in the middle of the 70s, and it owes most of its steps forward to advances in ocular recording technologies, with increased eye-trackers’ spatial and temporal resolution, together with decreased troubles in recording ocular data. Moreover, several eye movements recording techniques are developed (Mueller, Cavegn, d’Ydewalle, & Grooner, 1993). These field-related improvements happened together with a general progress in computers’ computational capability, in turn resulting in the possibility of interfacing laboratory computers with eye-tracker systems, collectively allowing an incredibly larger amount of data to be stored and analyzed.

It seems noteworthy that the vast majority of the current knowledge regarding eye movement behavior during cognitively complex tasks is coming from research in
reading (Henderson & Hollingworth, 1998). Only during the last fifteen/twenty years there has been a growing interest in deepening eye movement behavior during scene viewing.
5. **Eye movements during scene viewing**

Whenever we are trying to analyze how a visual stimulus is processed by our visual system, we can approach the problem from three different perspectives (Henderson & Hollingworth, 1999). We can be interested in how our visual system extracts low-level visual features (edges, texture, color, depth...) that are characterizing the object from a purely physical, perceptual point of view: This is the approach of *early/low-level vision*, typically exemplified by Marr's researches (Marr, 1982).

We can also focus on the “extraction of shape and spatial relations that can be determined without regard to the meaning, but that typically requires a selective or serial process” (Henderson & Hollwingworth, 1999): This is labeled the *mid-level vision* approach (Ullman, 1996).

Otherwise we can try to understand the relationship between the presentation of complex visual stimuli and high-level processes (including creation of stimulus representation, recognition, memories of the stimulus, knowledge of its function and meaning, expectancies...): This is the *high-level* approach to vision, which implies an interaction between perceptual and cognitive aspects of visual stimulus processing.

We will review some of the most classic works in the study of ocular behavior during scene viewing. As we will be able to appreciate, most of these studies adopt a high-level approach to the vision of static, visually complex, naturalistically plausible images, assuming an active role of the perceiver (Henderson, Weeks, & Hollingworth, 1999). Considering the processing of real-world scenes, we must indeed assume that the viewer has some kind of mechanism to actively select the external upcoming information, because our cognitive system and brain structures cannot fully process every visual stimulus presented in our visual field (Parkhust, Law, & Niebur, 2002). Eye movements and progressive fixations can be regarded as an expression of this selective process, and the necessity of visual information selection is even more evident.
if we consider that it has been estimated (Fischer, Karsh, Breitenbach, & Barnette, 1983) that we make approximately 230,000 fixations a day.

5.1. First researches about ocular behavior during scene viewing

The first exploratory research about fixation placement during scene viewing was published in 1935 by Buswell. Buswell presented 55 color pictures of different artworks to 200 participants, asking them to perform different kinds of tasks (Gould, 1976; Henderson & Hollingworth, 1998). What Buswell noticed was that participants were looking more frequently at some specific regions, which appeared to be highly informative. For example, when viewing “*Un dimanche après-midi à l’Île de la Grande Jatte*” (Sunday on the island of la Grande Jatte) by George Seurat (see illustration below), Buswell reported that viewers regularly fixated people’s faces and people’s hands, more than less informative background regions. These observations are the first documented evidence of a correlation between eye movement patterns and regions of interest in a scene, that is to say, a relationship between the amount of information contained in a specific region of a scene and the probability that a fixation will land on that same region.

*Illustration 2.5: George Seurat’s “Un dimanche après-midi à l’Île de la Grande Jatte” used by Buswell (1935) for his studies of ocular movements behavior.*
In 1967, more than thirty years after Buswell’s study, two important researches in scene perception were run, one coming from the Russian investigator Yarbus, the other from Mackworth and Morandi.

Yarbus' main accomplishment (1967) was to help understanding the role of viewer’s intentions and goals on eye movement execution. He presented color pictures of paintings and artworks for variable periods of time (up to 30 minutes to look at one image), asking his subjects to freely explore the pictures or perform different kinds of tasks. He observed that the instructions he gave changed the viewer’s pattern of fixations.

In figure below there is a typical example of different eye movements while participants viewed a picture of a painting (I. E. Repin’s “An unexpected visitor”). In panel 1 we can see participant’s ocular pattern after Yarbus did not give any instruction (fixations on faces, but also on other details in the scene). Panel 2 corresponds to ocular behavior following Yarbus’ request to guess the economical status of the depicted family (more fixations on clothes than faces), whereas panel 3 corresponds to the eye movement pattern after Yarbus asked the viewer to determine the ages of the characters in the scene (more fixations of faces than on other details). In general, Yarbus concluded that viewer’s intentions and goals determine the specific fixations strategy that will be executed based on the most informative regions for that specific goal. Therefore viewers must have an active role in selecting picture’s information through different eye movement patterns.
Illustration 2.6: Repin's "An unexpected visitor" painting used by Yarbus (1967) for his pioneering investigations about modifications in ocular movement patterns depending on experimenter's instructions. Figure from Gould, 1976.

Mackworth and Morandi (1967) analytically explored the relationship between the amount of information portrayed in a specific region of a scene and the probability that a fixation will land on that same region. First they divided two color photographs into 64 square regions. Later they asked a group of subjects to rate the degree of 'informativeness' of each region, asking them to base their scores on how easy they thought it would have been to recognize the same region later. Next, the two pictures were presented simultaneously for 10 seconds to a different group of subjects, whose task was to examine the pictures deciding which one they preferred (picture-preference task). Mackworth and Morandi found that the number of fixations on each region (fixation density) was related to the other viewers' informativeness ratings, so that regions which were rated more informative had consistently a higher number of fixations. On the contrary, regions rated scarcely informative by independent viewers
often did not receive any fixation at all. The lack of fixations on poorly informative regions seemed to suggest that observers were able to distinguish relevant from not relevant areas without directly looking at them, that is to say, observers could use peripheral vision (out of foveal focus) to extract semantic information.

Additionally, Mackworth and Morandi reported that viewers were as likely to fixate informative areas within the first 2 seconds of pictures presentation, as within other successive 2 s intervals, and this observation seemed in favor of an early peripheral semantic relevance analysis.

Antes in 1974 brought other evidences in favor of the effect of region’s informativeness (relevance from a semantic point of view) on fixation placement, and, as consequence, of an early access to semantic information. As previously done by Mackworth and Morandi (1967), after selecting monochrome shaded drawings (mostly taken from the Thematic Apperception Test), Antes asked an independent group of viewers to score the informativeness of each region in the drawing. In this study ‘informativeness’ was defined as the contribution of that specific region to the overall meaning of the picture. Successively, the same drawings were presented for 20 seconds to a different group of participants for a picture-preference task, while ocular movements were recorded. Antes replicated Mackworth and Morandi’s (1967) main result, finding a high correlation between informativeness ratings and fixations placement. Moreover, he observed that the first fixations viewers made consistently took place on more informative, compared to less informative, regions.

Overall, these first studies pointed out the active role of the viewer in selecting relevant information from a scene (Yarbus, 1967), and supported a positive linear correlation between the amount of information depicted in a specific region of a scene and the probability that a fixation landed on that same region (Antes, 1974; Buswell, 1935; Mackworth and Morandi, 1967). Moreover, data seemed to indicate that semantic information was accessible early during a scene visual processing (Antes, 1974; Mackworth and Morandi, 1967).
5.2. Loftus and Mackworth’ research

Despite the relevant and stimulating results emerged in these first researches, according to Loftus and Mackworth (1978) it was still not clear what was causing subjects’ judgment of informativeness. Mackworth and Morandi (1967) hypothesized that subjects might have considered informative (and therefore fixated more often) areas of ‘physical discontinuity’, thus implying a strictly perceptual explanation of participants’ informativeness judgment. Loftus and Mackworth (1978), on the contrary, suggested that a region can be informative both for physical and for cognitive reasons. Therefore, with the goal of studying informativeness from a cognitive perspective, they stated that an object was informative whenever it had “a low a priori probability of being in the picture, given the rest of the picture and the observer’s past history”. The example they gave was that a tractor in a farm would be considered a low informative object, given its high probability of being in the scene, while an octopus in the same scene would have been highly informative. Subjects’ task was to inspect each line-drawn scene in order to be ready for a later recognition task. Their results showed that subjects were looking at informative objects earlier during scene presentation time, more often (meaning, a greater number of fixations landed on informative areas), and for longer fixation durations (this suggesting a deeper processing of informative objects).

When trying to explain these results, Loftus and Mackworth referred to data coming from Biederman (1972), who presented jumbled versus normal pictures, and Potter (1975), who presented pictures at a high presentation rate. Data coming from both these authors suggested that subjects are able to quickly extract the gist of a scene, also when there is not enough time to perform several fixations. They both concluded that for this reason local perceptual features are not enough to explain the global process of real-world scene recognition.

However, subsequent data have questioned Loftus and Mackworth results.
In 1979 Friedman showed line drawings of real-world scenes to a group of subjects who prepared for a memory test. During an earlier stage of the experiment an independent group of subjects rated objects in each scene as more or less likely within the context of that scene. Friedman did not find an effect of objects semantic likelihood on fixation density, so it was not replicated that an increase in the informativeness of a region corresponded to a greater number of fixations on that region. Nonetheless, Henderson, Weeks, & Hollingworth, (1999) proposed that the reason why Friedman could not replicate Loftus and Mackworth (1978) effect was that her manipulation of the experimental stimuli was too weak, and in her study informative objects were not totally unlikely (like Loftus and Mackworth’ octopus in a farm), but only somewhat less likely than the others.

5.3. More recent investigations about ocular behavior during scene viewing

Some years later, De Graef, Christiaens, and d’Ydewalle (1990) were also not able to replicate Loftus and Mackworth result that informative regions are accessed earlier during scene viewing. De Graef and colleagues presented line drawings in a visual search task: Subjects had to look for non-objects, so meaningless object-like figures. Semantic informativeness was manipulated as in Loftus and Mackworth experiment, so that predefined meaningful target objects were included in the scene together with these non-objects, and target objects could be either semantically inconsistent (thus informative) or consistent (not informative). Informative objects were not fixated earlier during scene viewing, indeed during the first 8 fixations informative and not informative objects had the same probability to be fixated. However it should be noticed that the visual complexity of stimuli included in these two studies was not the same, and while De Graef and colleagues presented line-drawings obtained from real-world photographs (therefore with a higher number of details), stimuli employed by Loftus and colleagues consisted of scenes with a smaller amount of objects. It is possible, as Henderson argued (Henderson, Weeks, & Hollingworth, 1999), that when
viewers are presented a more complicated image they can't extract quickly enough semantic information, and as a result, they have first to rely on low-level perceptual features.

Mannan, Ruddock, & Wooding (1995) brought some other data in favor of the fact that first fixation placement is based solely on perceptual features. In their study they presented grayscale pictures of real world scenes for 3 seconds, using high and low-pass filters to manipulate the quality of the visual information available. Results indicated that even when participants could not understand what the picture depicted, the placement of the first fixation in the low-pass filtered condition was the same as in the unfiltered condition, thus suggesting that the meaning of the picture did not play any role in the initial fixation placement. Therefore, these data seemed in favor of initial picture information processing based solely on perceptual features. However, when in a successive experiment Mannan, Ruddock, & Wooding (1996) attempted to define which ones of seven different physical features were modulating initial fixation placement, only edge density was to some extent reliable in predicting fixation placement, and even for this factor the effect was weak.

In an attempt to clarify these contradictory results and understand ocular behavior not only during initial fixation placement, but during the whole image presentation period, Henderson, Weeks, & Hollingworth, (1999) presented 24 line-drawings of complex, real-world scenes to 18 participants. As previously done by Loftus and Mackworth (1978) and De Graef et al. (1990), scenes informativeness was manipulated by introducing consistent (not informative) or inconsistent (informative) objects, independently drawn for each scene. Informative and not informative objects

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3 For each region contributing to an image, Mannan and colleagues (1996) analyzed these seven physical features: Luminance maxima—highest luminance points—, luminance minima—lowest luminance points—, image contrast, maxima of local positive physiological contrast, minima of local negative physiological contrast, edge density, and high spatial frequency.

4 The 24 line-drawn stimuli used by Henderson and colleagues (1999) were modified from those already employed by De Graef et al. (1990). To obtain these drawings the authors first took 24 pictures of real-world scenes, later they projected these pictures onto a screen, and sketched the most important image contours to obtain a line-drawn real-world scene.
were placed in the same area of the scene, and the specific 'target area' delimiting informative and not informative objects had the same size in the two conditions. Subjects saw each scene in both conditions, and participants' task was to decide whether all of the objects "fit in the scene or not", while preparing for a memory test (which was actually never administered). They could take up to 15 s before giving their response. Henderson and colleagues examined a number of oculomotor responses. These responses can be grouped into variables regarding initial ocular behavior, and variables regarding the amount of stimulus processing (fixation density and processing time). The results of this study indicated that while initial processing was not affected by semantic information (meaning that informative targets were not more likely to be initially fixated compared to not informative targets, against Loftus and Mackworth hypothesis), later there was a robust effect of semantic information on the amount of processing, such that informative targets received a greater number of fixations and were processed (fixated) for a longer amount of time (in line with Loftus and Mackworth results).

5.4. A Saliency Map during scene viewing

Trying to account for these results and extending Morrison's model of eye movements in reading (1984), Henderson and colleagues (Henderson, 1992; Henderson, Weeks, & Hollingworth, 1999), presented their model of ocular behavior during scene viewing, the Saliency Map Framework during scene viewing. The hypothesis is that low-level stimulus features (like luminance, contour density, color, and so forth) are

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5 Initial Fixation Placement variables: probability of immediate target fixation; number of fixations before looking at target object (informative or not informative, depending on the condition); size of the first saccade to the target.
Fixation Density variables: number of entries = number of times the eyes moved from an area outside the target region to the target region, thus indicating the number of refixations after the target had been identified.
Processing Time variables: first-pass gaze duration = overall sum of time spent fixating the target object from when it was first fixated (first entry) until the eyes stopped fixating the target object (first exit); first-pass gaze fixation count = number of fixations on target object between first entry and first exit; second-pass gaze = sum of the time spent fixating the target object from when it was fixated for the second time (second entry) until the eyes stopped fixating the target object (second exit); total fixation duration = sum of all fixations to the target region; total fixation count.
determining an early parse of the scene into areas characterized by different degrees of salience. The authors specified that also viewer’s task can affect the initial salience map, but only if this effect is based uniquely on perceptual features. (This point does not seem to be completely clear, because if depending on the task the viewer follows a different strategy to explore the perceptual features of an image, this effect of the task seems, in turn, to imply a higher-level, cognitive approach to scene viewing). This initial perceptually-based salience map gives the highest ‘saliency weight’ to one region (in line with this hypothesis, see also Einhauser & Koenig, 2003, ), which is the one the eyes will fixate first. The amount of time the eyes spend on that region is determined by the time needed to successfully process the depicted information, with a concomitant perceptual and cognitive analysis. If the area is successfully processed, then the saliency weight for that area will decrease, and the eyes will move to the area with the next highest saliency weight. If, on the contrary, processing is not successful (because it is too slow or incomplete), refixations on that region and in nearby areas will occur. Thus, according to the model, there is a high probability that regions which are difficult to analyze, for either perceptual or cognitive reasons, will receive more refixations.

This model of ocular behavior during scene viewing is consistent with models of ocular behavior during reading (Rayner and Pollatsek, 1992), and with other strictly bottom-up models of ocular behavior during initial viewing of complex natural and artificial scenes (Parkhurst, Law, & Niebur, 2002).

6. Methodological issues

After this brief review of some of the most important studies in the field of complex, natural scene viewing, it should be evident that there is not a clear and unique way to approach the problem of describing and understanding eye movements during scene perception. Indeed, there is a considerable amount of factors which differ from one research to the other and, consequently, make it difficult to compare and generalize results.
6.1. What is a scene?

Henderson and Hollingworth (1999) pointed out that quite frequently studies about the perception of complex, natural images did not give any definition of ‘scene’, implying an intuitive grasp of its meaning. Henderson and colleagues attempted to specify that what they called ‘scene’ was “a semantically coherent (and often nameable) view of a real-world environment comprising background elements and multiple discrete objects arranged in a spatially licensed manner”. Furthermore, they added that for ‘background elements’ they meant larger, not movable structures such as walls or mountains, whereas for ‘objects’ they meant smaller, movable items. Despite being aware that this definition did not avoid misinterpretations either, (can a zoomed view of a box of paperclips on a desk be called ‘a scene’?), Henderson et al. still adopted this ‘imprecise, intuitive, and not wholly satisfying’ scene definition (Henderson & Hollingworth, 1999), providing with examples of visual stimuli they employed in several researches (human-scaled environments, like a living-room, a kitchen, a bar) to specify their idea of a ‘scene’.

It is important to notice that their intuitive definition of what a scene is does not include human (nor non-human) beings in the scenes, and that the kinds of stimuli these authors presented belong to a limited class of environments (interiors).

As a result, the ecological validity of their studies might be limited, considering that it still remains to be demonstrated that ocular behavior while viewing these specific environments can be extended to other types of real-world images.

6.2. Image type: how to present visual stimuli

Since the first studies in scene perception, there have been several ways of presenting pictorial stimuli to subjects. Images have been shown within pictures of color paintings (Buswell, 1935; Yarbus, 1967), real-world color pictures (Mackworth & Morandi, 1967; Parkhurst, Law, & Niebur, 2002), black and white line-drawings (De Graef, Christiaens, & d’Ydewalle, 1990; Friedman, 1979; Henderson & Hollingworth,
1998, 1999; Henderson, Weeks, & Hollingworth, 1999; Loftus & Mackworth, 1978), computer-rendered 2D images (Henderson & Hollingworth, 1998). It seems also relevant that the great majority of these studies reports only few examples of the employed stimuli, making it arduous for the reader to fully understand how the actual stimuli looked like, and consequently to draw conclusions about the reasons which led to not consistent results among some of these researches.

Of course, choosing the same image type does not necessarily mean images will be depicted in the same way. As an example, both Loftus & Mackworth (1978) and De Graef, Christiaens, & d’Ydewalle (1990) used black and white line-drawings, but, as mentioned earlier, while in the first experiment line-drawings included only few scattered objects, in the latter more complex natural scenes were employed, and this partially accounted for differences in obtained results.

Probably the most relevant issue regarding image type is that it has not yet been proven that different image types have the same effect on ocular behavior, that is to say, we do not know, for example, whether results obtained with line-drawings can be extended to real-world pictures viewing. In a review about scene perception, Henderson and Hollingworth (1998) referred to an unpublished study they conducted to verify the influence of different image types on eye movement behavior (Henderson & Hollingworth, 1997). In this experiment, they presented real-world scenes as line-drawings, color photographs, and computer-created 2D color images. The authors affirmed that despite some differences in ocular behavior when images were presented in different ways, the results tended to indicate the absence of an image type effect on ocular behavior. Unfortunately Henderson and Hollingworth (1998) did not report any of the statistical analyses they conducted, and, accordingly, their data cannot be used to draw any conclusion regarding this issue.

6.3. Task

Since Yarbus experiments (1967) it has been reliably proven that different instructions cause different eye movement patterns, therefore that viewer’s intentions
have a strong effect on ocular behavior. As an additional example, Schoonard, Gould, & Miller, (1973) compared the ocular behavior of highly trained IBM visual inspectors with that of less expert visual inspectors: They found that a higher degree of familiarity with the stimuli was associated with reliably faster and fewer fixations.

In the only study we could find comparing scene viewing behavior while performing two different tasks, Henderson, Weeks, & Hollingworth (1999) judged the effects of a memory task against those of a visual search task, finding fewer fixations before reaching the target in the visual search task. However, there might be alternative explanations for these results: first, task was not the only difference between one study and the other; second, it is possible that the fewer fixations in the visual search task were caused not by the task itself, but by a greater time pressure during this compared to the memory task (see following section, 'stimulus viewing time').

Despite the knowledge of task-dependent effects on ocular behavior, most of the studies in scene perception did not employ free viewing tasks (e.g., preparation for a memory task in Friedman, 1979; Loftus & Mackworth, 1978; Henderson, Weeks, & Hollingworth, 1999. Visual search task in Henderson, Weeks, & Hollingworth, 1999. Image-preference task: Antes, 1974; Mackworth & Morandi, 1967). Parkhurst, Law, & Niebur (2002), choosing a free viewing task, motivated their choice explaining that they wanted both to avoid any top-down effect on ocular behavior, and to most closely approximate natural viewing conditions.

6.4. Stimulus viewing time

There has been a high degree of variability in the amount of time subjects were allowed to explore visual stimuli: From 200 ms allowed by Henderson, Hollingworth, & Weeks (1996) up to 30 minutes of picture vision in some of Yarbus (1967) experiments. In general, there is not a clear standard, and, apart from the clearly excessive 30 min per picture proposed by Yarbus, the typical viewing time range is between 3 s and 15 s.
There are evidences suggesting that subjects might implement different strategies in the presence of considerable time pressure. In particular, when images are shown for an amount of time smaller than that typically required for a fixation, subjects might still be able to extract the gist of the scene (Biederman, 1972; Codispoti, Bradley, & Lang, 2001; Junghoefer, Bradley, Elbert, & Lang, 2001; Potter, 1975). It seems noteworthy that with substantial time pressure (200 ms stimulus presentation time), also Henderson and colleagues (Henderson, Hollingworth, & Weeks, 1996, cit. in Henderson, Weeks, & Hollingworth, 1999) obtained data indicating a scene gist effect on early objects processing, despite the majority of their data went in the opposite direction, and despite their model of ocular behavior during scene perception did not take into account the possibility of extracting semantic information before the first fixation placement.

6.5. Image content

As previously mentioned regarding image type, there is not a clear agreement about which image content should be preferable in scene perception studies, and accordingly, a variety of visual stimuli have been employed: Both images of outdoors (Parkhurst, Law, & Niebur, 2002) and indoors (De Graef, Christiaens, & d’Ydewalle 1990; Henderson, Weeks, & Hollingworth, 1999), including human beings (Buswell, 1935; Yarbus, 1967) or not (Henderson & Hollingworth, 1998, 1999). It has been already mentioned that viewers tend to fixate more frequently contours compared to homogeneous areas of a picture, and objects depicted as in motion compared to static ones (Gould, 1976). But, as Mannan and colleagues pointed out (1996), it is not completely clear which perceptual features are able to reliably predict initial fixation placement. Moreover, in the event human beings are included in an image, eyes and hands are fixated more often (Yarbus, 1967), and sometimes this has been interpreted not as a purely perceptually-driven effect, but as a tendency towards looking at areas conveying more meaning (Gould, 1976).
Despite these general and not necessarily precise predictions about ocular behavior in the presence of some specific stimuli, we still do not know the effects of different image contents on ocular pattern (Henderson & Hollingworth, 1998). Therefore it might be important to deepen this issue, either comparing results using different contents (e.g., ocular behavior using outdoors versus indoors, and so forth), or having access to a database of images able to include different image contents at the same time, and, as a result, distribute image contents effect among different experimental conditions. These requirement are clearly met by the IAPS catalogue (Lang, Bradley, & Cuthbert, 2005) which represents a database accessible to different research groups, and which therefore allows investigators to compare and generalize results across different laboratories.

6.6. Number of trials and number of participants

Since the first studies in ocular behavior during scene perception, investigators noticed a high degree of inter-individual variability in viewing patterns (Yarbus, 1967), together with a tendency towards intra-individual stability across successive sessions (Noton & Stark, 1971).

Regarding this within-subjects stability, Stark and colleagues (Noton & Stark, 1971) hypothesized that when the same image is presented several times, subjects tend to repeat the same order of fixations, which is repeated also when a recognition task is given. This suggested that spatial characteristics of eye movement pattern might be important for visual learning and recognition (so called ‘scanpath theory’). However, even if other laboratories confirmed that ocular patterns tend to be similar in the same participant, it has been found that intra-individual stability is not necessary to guarantee accurate recognition (Mannan, Ruddock, & Wooding, 1995, 1996).

In a more recent paper Andrews & Coppola investigated intra-individual variability in spatial and temporal patterns of eye movements while participants viewed different environments (Andrews & Coppola, 1999). Their data indicated a significant endogenous influence on oculomotor control, so that some basic idiosyncratic
characteristics could be identified in each subject. These individual differences in ocular behavior patterns were interpreted as the result of idiosyncratic differences in stimulus processing time, thus longer fixations corresponded to longer processing time.

Despite the observation of inter- and intra-individual differences in eye movement patterns, the use of only few trials per condition—not associated with an increase in the number of participants per condition—has been observed also in recent papers (2 trials in Mackworth & Morandi, 1967; 10 trials per condition in Henderson and Hollingworth, 1997; 3 trials in Horley et al., 2003). In addition, in some researches only few subjects have been included in the ocular data analysis (4 subjects in Parkhurst, Law, & Niebur, 2002; 8 subjects in Henderson & Hollingworth, 1997), hampering the possibility of drawing reliable conclusions from these studies.

6.7. How to report ocular variables

The brief review of studies about ocular behavior during scene viewing should have elucidated how there is not a preferable and shared way to present eye movement variables. The same researchers involved in this field expressed concern about the lack of uniformity in recording, evaluating, and reporting eye-tracker data. In 1998 Inhoff & Radach conducted an informal survey on a group of researchers habitually utilizing eye movement measures: All of these investigators expressed the need of further discussions about measurement-related and methodological issues in ocular behavior studies. In addition, they highlighted the need of deepening the functional interpretation of specific oculomotor events.

6.8. Different eye-tracker technologies

The issue of comparing results coming from data collected with different eye-tracker systems (Muller, Cavegn, d’Ydewalle, & Grooner, 1993) arises especially when we contrast older and more recent studies. As an example, according to Henderson, Weeks, & Hollingworth (1999) data on fixation placement based on eye-tracker systems
with low spatial resolution (in Loftus & Mackworth, 1978: Spatial resolution greater than 1 degree of visual angle) could have included mistakes in the definition of the location, and therefore caused a bias in the results. The relatively recent improvement in the spatial and temporal resolution of ocular recording tools (Muller, Cavegn, d'Ydewalle, & Grooner, 1993; Rayner, 1998; Richardson & Spivey, 2004) made it possible to obtain more reliable data, and in general current technologies allow good temporal and spatial resolution, in turn making it easier and more reliable to compare results from different eye-tracking systems. However, the extent to which the resolution of an ocular recording system is sufficient or not ultimately depends on the ocular variable under examination. As an example, low temporal accuracy can still be appropriate if we want to record the overall number of fixations during scene viewing, while it is not recommended if we are interested in recording first saccade latency.
7. **Ocular behavior and emotional visual stimuli**

Within an evolutionary approach to motivated selective attention, individuals who are capable of responding faster to relevant stimuli from the external environment will be also those better equipped for survival and, as a consequence, reproduction. An earlier location of rewarding stimuli—like mating partners or foraging areas—will elicit appropriate appetitive behaviors, while a prompt detection of any potentially threatening external stimulus will, if necessary, result in behaviors like escape or fight. Both these behaviors would, in turn, favor the survival of the individual and its specie.

Accordingly, unexpected relevant events cause the interruption of any ongoing process and request a specific elaboration (Oehman, 1979). The prompt detection and interpretation of any sudden stimulus is accompanied by a phasic psychophysiological activation, which has been labeled ‘orienting response’ (Graham, 1992; Sokolov, 1963). The ultimate goal of the orienting response is to facilitate the elaboration, and in turn, the appropriate behavioral response to this sudden external stimulus.

7.1. **Early detection of life-threatening stimuli**

Within this framework it has been proposed (Oehman, Flykt, & Esteves, 2001) that fear-relevant stimuli might be a special kind of stimuli, with a particularly effective ability of capturing attention. To verify their hypothesis, Oehman and colleagues (2001) reprised and deepened a method employed by Hansen & Hansen (1988), presenting their participants with 3 x 3 matrices, in which either each cell contained always the same kind of stimulus, or all cells but one contained the same group of stimuli. Viewers’ task was to press different buttons if they believed stimuli were all the same or not. Stimuli included in the matrices were color pictures of fear-relevant (spiders or snakes) or not-relevant stimuli (flowers or mushrooms).

Oehman observed that subjects were reliably faster in detecting fear-relevant stimuli. Additionally, response speed for threatening stimuli both remained the same when the size of the matrix was increased by one unit, and was not affected by the
location of the stimulus in the matrix (overall suggesting, if not a parallel, at least an extremely efficient process). Moreover, highly fearful subjects showed a stronger effect of their object of fear compared to low fearful subjects.

These results were replicated and extended using schematic faces as stimuli (Fox, Lester, Russo, et al., 2000; Oehman, Lundqvist, & Esteves, 2001; Tipples, Atkinson, & Young, 2002), thus confirming an advantage for the processing of threatening stimuli.

Other paradigms were also used to assess this earlier detection of fear-relevant stimuli. Mogg & Bradley (1999) presented results in line with this threat advantage hypothesis, utilizing a modified version of the dot probe task (Posner, Snyder, & Robertson, 1980). They presented pairs of photographs of faces of individuals, matched so that if one face expressed an emotion (happy or threatening), the other did not (showing a neutral expression). Both stimuli were briefly presented and then masked, according to a low awareness presentation mode, and successively subjects had to press different buttons if a probe appeared where the left or the right stimulus was presented. Participants were faster if the probe was presented below a threatening face. In a slightly modified version of this task, including faces labeled as threatening, sad, happy, and neutral, Bradley, Mogg, and Millar (2000) recorded also whether the first eye movement was directed towards the fear-relevant stimulus or not. Obtained results were not really clear in this regard, and did not fully support a threat advantage either for response speed, or for the direction of the gaze. Even so, it appears that the task employed by Bradley and colleagues was not the most appropriate to assess whether eye movements are preferentially directed towards fear-relevant stimuli, mostly because in the dot probe task eye movements are worsening the performance instead of improving it. For this reason, few subjects were moving their eyes towards any of the stimuli, and therefore only a small amount of eye movement data was used for the analysis.
Further modifying the dot probe task, Mathews, Fox, Yiend, & Calder (2003) used the direction of the gaze as a cue in a dot probe task. They used pictures of individuals, taken from the Ekman & Friesen database (1976). Each picture, depicting a neutral or a fearful expression, was first presented with the gaze directed ahead, then with the gaze directed either to the right or to the left. Participants, high and low anxious volunteers, had to press different buttons if the probe (a letter) appeared to the left or to the right. The authors found that high anxious people were faster in detecting a fearful expression, while in this study controls did not show any facilitation for fear-relevant stimuli.

Results presented thus far examined reaction time data, and overall suggested a faster detection of fear-related stimuli, indicating that our attentive system might be specifically primed to respond in a prompt manner to life-threatening cues (Oehman, Flykt, & Esteves, 2001; Oehman, Lundqvist, & Esteves, 2001). On this account, it seems reasonable to expect that this earlier detection of fear-relevant stimuli might also be accompanied by preferential movements of the eyes towards external alerting cues. There are some evidences in this regard.

Mogg, Millar, & Bradley (2000) displayed pairs of pictures of faces to depressed, generalized anxiety disorder patients (GAD), and controls, while recording their ocular behavior. Pictures depicted “threatening”, “sad”, “happy”, or “neutral” faces. GAD patients had a greater proportion of initial fixations towards threatening faces, and in addition these initial fixations were faster for GADs.

These appear as promising results, suggesting that different ocular patterns might be present in different populations of patients when threatening stimuli representing general threats or their personal fear are displayed.
7.2. Eye movements and threatening stimuli: Gaze avoidance or information seeking?

With the explicit goal of investigating ocular behavior when threatening stimuli were presented, Hermans, Vansteenwegen, & Eelen (1999) showed pairs of photographs representing flowers and spiders to controls and spider anxious subjects. Images were presented for 3 seconds, and eye movements were recorded throughout pictures presentation, while participants freely viewed the images (fake sensors were placed to mislead participants about the real purpose of the study). Ocular data were examined as proportion of viewing time on ‘spider area’. Both controls and spider anxious subjects looked more at spiders at the beginning of picture presentation, but successively their ocular behavior diverged: While controls further increased the proportion of fixations on spiders, afterward keeping a constant high amount of fixations on the threatening stimulus, spider anxious subjects showed a constant and progressive decrease in the proportion of fixations to the stimulus they feared (which was interpreted in terms of ‘gaze avoidance’ patterns towards the unpleasant stimulus).

As a whole, these results indicate that ocular behavior while viewing threatening stimuli supports the ‘threat advantage’ hypothesis (Oehman, Flykt, & Esteves, 2001; Oehman, Lundqvist, & Esteves, 2001), revealing for both controls and anxious participants an initial greater processing of fear-relevant stimuli compared to non-relevant stimuli. Afterward, only anxious individuals implement a gaze avoidance pattern, progressively fixating more and more away from their personal-fear stimulus, controls, on the contrary, keep on fixating the threatening stimulus, possibly to gather more information.

Further data indicated gaze avoidance patterns in phobic individuals. We already mentioned, when discussing the dimensional approach to emotion (see section 3.2, in the first chapter), Hamm and associates results (Hamm, Cuthbert, Globisch, & Vaitl, 1997), suggesting gaze avoidance patterns in phobic individuals. They presented phobia related pictures (many of which were taken from the IAPS database) to small animal.
and blood phobics. Participants determined picture time exposure, pressing a button whenever they chose to stop picture viewing: Phobics consistently viewed fear-related pictures for shorter periods compared to controls.

Horley (2003) presented social phobics with pictures of an individual with a neutral expression or conveying sadness or happiness, expecting social phobics to avoid their object of fear (faces depicting negative emotions). Overall, social phobics had a smaller amount of fixations on both 'neutral' and 'sad' expressions compared to 'happy' expressions. However, when only the area around the eyes was analyzed, social phobics had a significantly smaller number of fixations on 'sad' faces compared to the other faces. Thus, these results seemed partially in line with those obtained by Hermans and colleagues (1999). Conversely, it must also be remarked that only three stimuli were shown in this study. Additionally, facial expressions employed as 'sad' and 'neutral' faces did not substantially differ from each other, suggesting a weak stimuli manipulation. The fact that stimuli might not have been sufficiently emotional evocative is also hinted by the overall lack of difference between the number of fixations to 'sad' and 'neutral' faces.

In this regard, it should be noticed that schematic faces and pictures of faces might not be able to elicit emotional reactions as other kinds of stimuli do. Emotional faces are not judged as highly pleasant or unpleasant stimuli, and have lower arousal ratings compared to hedonically relevant pictures taken from the IAPS (Lang, Bradley, Fitzsimmons, et al., 1998). Moreover, fMRI data specified that pictures of faces cause a smaller response in the visual cortex compared to emotional pictures taken from the IAPS database (Lang, Bradley, Fitzsimmons, et al., 1998). With this in mind, we will now expose some data about ocular behavior when viewing more arousing and hedonically evocative stimuli.
7.3. Eye movements and emotional stimuli

With the goal of studying memory for emotional and neutral events, Christianson, Loftus, Hoffman, & Loftus (1991) recorded eye movements while administering a sequence of 15 pictures. The 8th picture was the target of a memory test which was run some minutes later. The target picture depicted either a woman riding a bike (neutral condition), or the same woman ‘lying on the ground beside her bike, bleeding from a head injury’ (unpleasant emotional condition), or carrying the bike on her shoulder (unusual condition). In a between-subjects design, each condition was seen by 54, 56, and 53 participants, respectively. Results indicated that subjects had a greater number of fixations when viewing the unpleasant emotional stimulus compared to the neutral and unusual stimuli.

In the same direction, but by means of both pleasant and unpleasant emotional stimuli taken from the IAPS (International Affective Pictures System, Lang, Oehman, & Vaitl, 1988), Lang, Greenwald, Bradley, & Hamm (1993) found that in a task in which picture exposure was determined by participant’s discretion, subjects viewed emotional stimuli, either hedonically pleasant or unpleasant, for a longer time compared to neutral stimuli. This suggested that, in general, the arousing value of the picture determines the amount of time participants spend looking at it, more than its specific negative or positive content.

Recently, there have been some works investigating ocular behavior in conditions in which emotional and neutral stimuli had to compete for a reduced amount of attentive resources.

In a series of experiments Calvo and Lang (2004) investigated whether semantic processing can occur even if participant is not foveally fixating the pictures. Hence, they presented pairs of emotional-neutral pictures taken from the IAPS for short amounts of time (150, 300, 450, 900 ms, and 3s). Overall, results indicated that when both emotional (either pleasant or unpleasant) and neutral pictures were presented, participants were more likely to fixate emotional pictures first. Moreover, for shorter
picture presentation times (150, 300, and 450 ms) emotional pictures were more accurately identified than neutral images, suggesting that when a limited amount of attentional resources are available, motivationally relevant stimuli are more likely to be processed.

In a different study, Calvo and Lang (2005) examined the time course of ocular behavior while presenting pairs of pictures for 3 s. Pictures depicted either emotional (pleasant or unpleasant) or neutral scenes. They found that during the first 500 ms of picture presentation emotional stimuli were attended more (higher 1st fixation probability, longer fixations duration) than neutral stimuli. However, this preference for emotional stimuli was not evident in the remaining viewing time.

Additionally, with the goal of investigating whether ocular behavior changed for different unpleasant contents, Calvo and Lang displayed again pairs of emotional-neutral pictures for 3 s, but in this experiment emotional images could either have a threatening content (e.g., an aimed gun) or depict injuries. Results indicated that during the first 500 ms both threat and injury pictures were attended more than neutral stimuli, while later ocular behavior diverged: Stimuli representing threat kept on being fixated, while stimuli representing injuries were attended less.

Both these studies suggested that when emotional and neutral stimuli have to compete for limited resources, the processing of emotional stimuli is facilitated. Calvo and Lang’s results (2004, 2005) showed that emotional stimuli in general, not only those threat-related are attended more extensively. In turn, it appears that based on these data the ‘emotionality hypothesis’ advanced by Calvo and Lang (2004, 2005) might be preferable to the threat-advantage hypothesis (Oehman, Flykt, & Esteves, 2001; Oehman, Lundqvist, & Esteves, 2001).

However, data from a second study by the same authors (Calvo and Lang, 2005) illustrated a different ocular behavior when participants are viewing unpleasant stimuli with qualitatively different contents (threat vs. injury). While initially both images depicting threat and images depicting injuries are equally likely to be fixated, later on
viewers tend to fixate threatening images longer than images depicting injuries. The authors interpreted these results from an evolutionary motivational point of view: When first examining visual stimuli, it is important to promptly pay attention to any potentially relevant stimulus, using a basic good/bad categorization, but without identifying specific stimulus details. Afterward, stimulus identification takes place and the viewer can decide whether it is more useful, from an adaptive point of view, to keep on looking at it or not. From this perspective, while a threat, regarded as an imminent, uncertain danger, does need to be further examined, injuries, considered as already occurred events, do not pose any ‘real’, upcoming danger, so the viewer can stop gathering information about these stimuli.

These recent studies by Calvo and Lang (2004, 2005) are, to the best of our knowledge, the only recent works investigating ocular behavior patterns while viewing emotion-evoking stimuli. The same authors emphasized that the fact that stimuli were presented in pairs might be a limitation to generalizing these results to free-viewing conditions, where there is no competition for the same attentive resources and participants can freely examine one picture at a time.

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6 In fact, two recent studies investigated ocular behavior of some specific populations while viewing emotional and neutral pictures, displayed one at a time. In LaBar, Mesulam, Gitelman, & Weintraub (2000) pictures taken from the IAPS database were presented to early-stage Alzheimer’s patients, while Quirk & Strauss (2001) showed IAPS pictures to schizophrenic patients. Considering the specificity of these diseases and, as a consequence, the difficulties in extending these data to healthy participants, results from these two studies are not reported in details.
3 Pupil diameter: emotional and cognitive modulation

1. Pupil diameter studies: Historical background

Against all odds, Mickey Mouse and Marilyn Monroe’s faces have something in common which might have helped them appearing cute and attractive (respectively...): Big eyes.

For centuries women seem to have known this ‘trick’ to look more appealing, since for centuries it has been reported that drops of a drug named ‘belladonna’ were administered locally to widen the size of young ladies’ pupils (Steinhauer & Hakerem, 1992).

Apart from this daily life knowledge, during the XVIII century Fontana (1765) recorded the first observations regarding pupil’s reactions to external stimuli. In particular, he noticed that upon awakening pupils dilated despite strong environmental illumination (Hess, 1972). One century later some German physiologists (Westphal, 1863; Schiff, 1874; Mentz, 1895) performed the first systematic studies of pupillary reactions to nonvisual stimuli, investigating pupil response to drugs (chloroform), to acoustic and painful stimuli, and to mathematical problems. Loewenstein (1920) and Bumke (1911) further deepened these preliminary data indicating pupillary responses to mental tasks. As a result of his studies, Oswald Bumke (1911; translated in Hess, 1972) reported that “in general,” for healthy individuals “every active intellectual process, every psychical effort, every exertion of attention, every active mental image, regardless of content, particularly every affect just as truly produces pupil enlargement as does every sensory stimulus”, except for light. It is however noteworthy that the results
obtained by these German physiologists were not known in the US until the 60s, when Eckard Hess translated and reprised their works. Hess' research caused renewed interest in psychopupillary reactions among physiologists and psychologists, but also violent discussions concerning the methodological validity and replicability of his results. We will later review his data and the critics he was moved.

2. *Anatomy of the pupil*

The iris is the circular, pigmented tissue with a central opening named 'pupil' (Stern & Dunham, 1990), characterized by a slightly elliptical shape toward the nasal side. The light enters the eye through the pupil, thus pupil diameter size is shaped to control the amount of light reaching the retina. Pupil diameter size is determined by the joint action of the parasympathetic and sympathetic branches of the autonomous nervous system/ANS (Dodd & Role, 1991). Each branch of the ANS acts primarily on one of the two opposing sets of smooth muscles which compose the iris: The sphincter and dilator muscle.

Fibers forming the *dilator muscle* are radially oriented. The contraction of dilator muscle fibers results in the retraction of the iris and in the widening of the pupil. Although some data indicated also inhibitory cholinergic input to the dilator muscles (Ehinger, 1967), dilator muscle fibers are thought to be primarily activated by alpha-adrenergic input from the sympathetic nervous system. As a result, noradrenergic (NA) agonists and antagonists cause pupil dilation (mydriasis) and constriction (miosis), respectively. For example cocaine, a powerful uptake blocker, causes norepinephrine to accumulate in the synaptic gap, in turn triggering mydriasis (Beatty & Lucero-Wagoner, 2000).

On the contrary, fibers forming the *sphincter/constrictor muscle* are circularly oriented. The contraction of sphincter muscle fibers results in the expansion of the iris and in the consequent narrowing of the pupil. Sphincter fibers receive cholinergic input
from the parasympathetic nervous system. Therefore, acetylcholine (ACh) agonists produce pupillary constriction, while ACh antagonists produce pupillary dilation. As an example, tobacco, stimulating nicotinic ACh receptors, causes pupil diameter constriction (Lie & Domino, 1999).

In the figure below we can observe the reciprocal action of dilator and sphincter muscles in the cross-section of monkey iris, during miosis and up to moderate and extreme mydriasis.

Illustration 3.1: Cross-section of monkey iris, (1) in miosis, (2) in moderate mydriasis, (3) and in extreme mydriasis. Figure from Loewenfeld, 1993.

The reciprocal action of dilator and sphincter muscles is such that when the agonistic dilator muscles are activated, the motor output to the antagonist sphincter muscles is reduced (Beatty & Lucero-Wagoner, 2000).
3. **Some factors affecting pupil diameter size**

Apart from the amount of light reaching the eye, a number of factors influence pupil diameter size. Following there is a short list of few of these factors.

Twin studies (Joseph, Baker, & Sitaram, 1992) pointed out a strong genetic influence on pupil diameter response. Moreover, even if several researches reported a considerable inter-individual variability (e.g., Winn, Whitaker, Elliott, & Phillips, 1994), human resting pupil diameter of young healthy adults under the same laboratory conditions is highly stable across repeated same-day sessions and up to 6 weeks apart (White & Dupue, 1999). Taken as a whole, these data indicate that pupil diameter measurements are intra-individually stable while inter-individually variable.

Winn, Whitaker, Elliott, & Phillips (1994), using a large sample of participants, investigated the effect of several factors (age, gender, iris color, and refractive error) on pupil diameter size during different environmental light levels. They found that age had a strong linear effect on pupil diameter size, such that at each light condition older participants had smaller pupillary diameter size. The authors mentioned that the general clinical impression and some data (Alexandridis, 1985) have suggested that women and myopes might have larger pupils. Winn and colleagues data did not support this belief, indicating that gender and refractive error did not have a significant effect on pupil diameter size. Moreover, iris color did not affect pupil diameter size either. It is also noteworthy that when the authors covaried pupil diameter with age (thus removing variance caused by this factor), gender, refractive error, and iris color still did not affect pupillary diameter.

It must be remarked, however, that slightly different results were obtained by Bergamin and colleagues (Bergamin, Schoetzau, Sugimoto, & Zulauf, 1998) who investigated the effect of iris color on pupillary response to light flashes. Confirming Winn and colleagues results, they found that iris color did not affect initial pupil diameter, however several pupillary light reflex indices were influenced by iris color. In
particular, brown eyes showed greater agility in responding to light compared to blue eyes, as indicated by significantly greater amplitude, speed of contraction, and redilation speed after the response to light. Thus, in general iris color does not seem to affect basal pupil diameter values, that is to say, pupil diameter of brown and blue-eyed people do not differ significantly. On the contrary, iris color does influence pupillary agility in responding to luminosity changes, revealing that dark eyes are better suited (faster and stronger) for reacting to light flashes.

4. Pupillary movements

Four basic pupil diameter movements have been described thus far (Beatty & Lucero-Wagoner, 2000):

- Pupillary hippus/unrest;
- accommodation/near reflex;
- pupillary light reflex;
- psychosensory pupillary reflex (Loewenfeld, 1993), also called psychopupillary response (Hess, 1972).

It has been reported (Beatty & Lucero-Wagoner, 2000) that while some of these movements are primarily optical in nature (accommodation, light reflex), other are supposed to involve higher cortical processing concerning sensory, mental, or emotional events (psychopupillary response). We will be able to appreciate, however, that this distinction is not so rigid, and also “strictly optical” reactions - like the pupillary light reflex - can be modulated by high-level cognitive and emotional factors.

4.1. Pupillary hippus/unrest

Pupillary hippus indicates the rhythmic but irregular (typically < 0.04 Hz) occurrence of pupil constrictions and dilations, which happens independently of eye movements and illumination changes. These slow waves, in darkness, last from about 4 to 40 seconds and have an amplitude of about 0.5 mm. They can be observed together
with shorter lasting (0.5-1 second) and smaller (0.1-0.3 mm) fluctuations (Goldwater, 1972). The pupillary hippus is not a pathological sign and it is a normal condition of the human pupil. Still, it can be altered as a result of some nervous system diseases (Beatty & Lucero-Wagoner, 2000). It is important to notice that pupillary hippus enhances during sleepiness and fatigue (Goldwater, 1972; Loewenfeld, 1993; Stern & Dunham, 1990; Wilhelm, 1998). Indeed, any decrease in alertness is accompanied by a reduction in the central sympathetic activation, which in turn causes a diminished inhibition of the parasympathetic system nuclei (Edinger-Westphal nuclei). This central sympathetic system instability is thought to be the reason for these spontaneous slow waves of pupillary unrest (Barbur, 2004; Goldwater, 1972; Wilhelm, Giedke, Luedtke, et al., 2001). However, as a consequence of contradictory results in sleep research studies (Wilhelm, Giedke, Luedtke, et al., 2001) it is still under debate whether pupillary hippus and pupil diameter instability can be used as sleepiness measures (Ranjin & Lack, 1997).

4.2. Accommodation/near vision reflex

The accommodation response involves a series of movements that allow changes in lens curvature in order to control the depth of the field (Beatty & Lucero-Wagoner, 2000). That is to say, when we are presented with closer objects, the lens accommodates so that objects are properly focused right on the surface of the retina. Changes in lens curvature are accompanied by pupillary changes. The near vision pupillary reflex indicates the tendency of the pupil to constrict when focusing at a point near the subject (Stern & Dunham, 1990). The accommodation reflex is presumed to require three muscular adjustments (Loewy, 1990): 1) the contraction of the ciliary muscle, which increases the curvature of the lens; 2) the contraction of the iris sphincter muscle, which reduces pupillary diameter; 3) the contraction of the medial rectus muscle (through the oculomotor/III cranial nerve), which converges the eyes towards the object. While the afferent pathways are not known and presumably involve cortical areas lacking direct connections to the oculomotor complex (Beatty & Lucero-Wagoner,
the efferent pathways for the accommodation response are probably similar to those involved in the pupillary light reflex, and include the Edinger-Westphal nuclei.

To avoid the interference of the accommodation reflex on pupil diameter measurements, some authors recommend to position visual stimuli farther away from the participant (Goldwater, 1972; Stern & Dunham, 1990)

4.3. Pupillary light reflex

Responding to changes in environmental illumination is probably the most obvious function of the pupil: Everybody is aware that in bright light the pupil is small and constricted, while in dim light and in darkness it is dilated. The amount of light reaching the retina is proportional to the diameter of the pupil, approximately according to a 16 to 1 ratio. In humans, pupillary diameter ranges from 1 up to 9 mm (Beatty & Lucero-Wagoner, 2000). It is interesting that most of the times we are not aware of variations in luminance. Indeed, it has been reported that changes of average luminance by factors of 10, 100, or 1000 pass largely unnoticed, and only increases by much larger factors are noticed by the viewer (Barlow, 1972).

4.3.1. Timing of the pupillary light reflex

The pupillary response to illumination changes has a specific timing, which has been extensively investigated.

In the following figure we can observe the time course of the pupillary light reflex. The distance before A indicates the initial pupil diameter, before the presentation of the stimulus; the distance between A and B indicates the latency before the onset of the response to the light (pupil diameter constriction/miosis); C indicates the moment of maximal pupil diameter constriction (peak at which light reflex amplitude can be measured); D indicates the time at which 75% recovery after light reflex is attained (Bitsios, Szabadi, & Bradshaw, 1999; Wilhelm, 1998).
Illustration 3.2: Light reflex timing. Y-axis: pupil diameter (mm), X-axis: running time (s). Horiz. bar: light stimulus; 1 initial pupil diam.; 2 75% recovery; 3 pupil diam. at max constriction; A onset of light stimulus; B onset of response; C time of max constriction; D 75% recovery. Figure from Bitsios, Szabadi, & Bradshaw 1999.

In humans, pupillary light reflex latency typically ranges between 180 and 200 ms, albeit a great intra-individual variability such that repeated tests with the same subject do not necessarily lead to consistent results over successive sessions (Loewenfeld, 1993). A considerable inter-individual variability has also been reported (Bergamin, Zimmerman, & Kardon, 2003), and different subjects response to light can vary both in amplitude (base to peak difference) and in latency. However, as general rules, light reflex latency is longer for weaker stimuli (Loewenfeld, 1993), and longer latencies are usually associated with smaller amplitudes (Bergamin & Kardon, 2003). Moreover, despite the abovementioned variability, light response peak typically occurs after 1000 ms from stimulus onset (Beatty & Lucero-Wagoner, 2000; Bitsios, Szabadi, & Bradshaw, 1999; Clarke, Zhang, & Gamlin, 2003).

Different components of the light reflex waveform are believed to reflect the activation of different branches of the autonomous nervous system. In particular, while the parasympathetic branch is involved in the initial pupillary constriction, the
sympathetic nervous system is presumably activated during the successive pupillary redilation (Bitsios, Szabadi, & Bradshaw, 1999).

4.3.2. Neural pathways of the pupillary light reflex

A parasympathetic pathway provides the neural basis for the pupillary response to light and consists of a six-neuron arc (Beatty & Lucero-Wagoner, 2000; Loewy, 1990), schematically exposed in the following illustration.

Still in the retina, photoreceptors (rods and cones) synapse on bipolar cells which in turn synapse on a specific kind of retinal ganglion cells (W-type), responsive to changes in luminance level. All types of ganglion cells form the optic nerve/tract and send visual information to the cerebral cortex and subcortex. The optic tract is located close to the lateral geniculate nucleus but it is not yet clear how connections between the optic nerve, the lateral geniculate nucleus, and the occipital cortex might be organized (Wilhelm, 1998). It is however known that the optic tract receives cortical inputs from occipital visual areas V1 and V4, from the superior temporal sulcus, and from extrastriate visual areas (Wilhelm, Wilhelm, Moro, & Barbur, 2002). From the optic tract, W-type ganglion cells project to the olivary, medial, and posterior pretectal nuclei. Studies in animals (Clarke & Ikeda, 1985; Gamlin, Zhang, & Clarke, 1995; Trejo & Cicerone, 1984) and humans (Wilhelm, Wilhelm, Moro, & Barbur, 2002) pointed out the role of olivary pretectal nucleus neurons as ‘luminance detectors’ and that their stimulation is associated with pupillary constriction. Neurons from the olivary pretectal nucleus project bilaterally to both the Edinger-Westphal nuclei (in particular to the lateral visceral cell columns of each E-W nucleus) involved in the control of the parasympathetic nervous system. The simultaneous bilateral projections to both the E-W nuclei are the reason why direct and consensual pupillary light reflexes are equal in humans (Wilhelm, 1998). (Indeed, asymmetries in pupillary light reflex—anisocoria—are taken as index of retinal, optic nerve, or central disease (Bergamin, Zimmerman, & Kardon, 2003)). From the Edinger-Westphal nucleus, pre-ganglionic fibers project to the ciliary ganglion via the oculomotor III cranial nerve. After synapting at the ciliary
ganglion, post-ganglionic fibers forming the short ciliary nerve directly stimulate the sphincter muscle, causing pupillary constriction.

Illustration 3.3: Sketch of the six-neuron arc responsible for the sphincter muscle contraction in the pupillary light reflex. “Stars” highlight sites where synapses occur.

The Edinger-Westphal nucleus is also known as pupillomotor nucleus and it has indeed a main role in the control of the pupillary response to light. Neurons in the E-W nuclei have a high firing rate also in the absence of external stimuli (Barbur, 2004; Sillito & Zbrozyna 1973). For this reason each E-W nucleus constantly receives inhibitory input from two main brain areas, with the purpose of control and decrease E-W neurons firing rate. Main inhibitory inputs to the E-W nuclei come both directly from the locus coeruleus via noradrenergic fibers, and indirectly from the A1/A5 nuclei of the brainstem via the hypothalamus. As earlier mentioned, the main excitatory input to the E-W nuclei comes from the olivary pretectal nucleus. According to Barbur (2004), signal changes in these inhibitory pathways are the reason why several pupillary phenomena occur (e.g., miosis during sleep or anesthesia, progressive miosis with age).
4.3.3. The fear-inhibited light reflex

The “fear-inhibited light reflex” refers to an increase in pupil diameter and a reduction in pupillary light reflex during the anticipation of highly aversive events (in the original experiments threat of an electric shock which was actually never administered). This phenomenon has been observed first by Bitsios and colleagues (Bitsios, Szabadi, & Bradshaw, 1996).

In addition, these authors found that under the threat of an electric shock the amplitude of the light reflex negatively correlated with subjective anxiety ratings (Bitsios, Szabadi, & Bradshaw, 1996), so that participants who reported a high level of anxiety due to the shock threat had also smaller pupillary responses to light flashes. With the goal of investigating the relationship between anxiety levels and pupillary light reflex, Bitsios and collaborators (Bitsios, Szabadi, & Bradshaw, 1998) administered an anxiolytic drug (diazepam) to a group of healthy subjects who took part to a “threat-of-shock” study. To their own surprise, when participants anticipated the aversive event, the anxiolytic drug selectively affected pupillary light reflex, while it did not alter initial pupil diameter.

In another research Bitsios, Szabadi, and Bradshaw (2004) compared pupillary reactions during the anticipation of an aversive (threat of shock) and a non-aversive event (anticipation of a not-startling, acoustic 70 dB noise). In line with previous data (Bitsios, Szabadi, & Bradshaw, 1996, 1998), they found that pupil diameter increased when anticipating both aversive and not-aversive stimuli, however they also reported that pupillary light reflex decreased significantly only in the threat-of-shock condition.

Thus data from both researches seemed to suggest that initial pupil diameter and pupillary light reflex might be somehow dissociated during fear of an electric shock.

Based on these results, Bitsios and colleagues (Hourdaki, Giakoumaki, Grinakis, Theou, Karataraki, & Bitsios, 2005) hypothesized that while the fear-inhibited light reflex might be the result of both the inhibition of brain stem arousal centers and the
activation of attention allocation centers in the amygdala (sensitive to changes in light stimulus intensity), the initial pupil diameter increase might be the outcome of the activation of non-specific sympathetic arousal centers in the locus coeruleus.

To test this dissociation hypothesis, Bitsios and colleagues (Giakoumaki, Hourdaki, Grinakis, Theou, & Bitsios, 2005) investigated the effect of a peripheral sympathetic blockade (using the $\alpha_1$-adrenoceptor antagonist dapiprazole) on initial pupil diameter and pupillary light reflex. They predicted that only initial pupil diameter would have been affected by the sympathetic blockade. Results were not fully in favor of a complete dissociation between these two pupillary responses during the threat of shock. (Authors indeed suggested that fear of shock, presumably involving central sympathetic activation, might have interfered with the peripheral sympathetic blockade, making the effect of peripheral sympathetic blockade less clear).

In a further research (Bitsios, Szabadi, & Bradshaw, 2002) a group of healthy subjects completed the State/Trait Anxiety Inventory (STAI) within the same threat-of-shock paradigm. Bitsios and colleagues’ purpose was to examine whether the level of state or trait anxiety affected initial pupil diameter and/or pupillary light reflex. Previously mentioned data (Bitsios, Szabadi, & Bradshaw, 1996) indicated that anxiety ratings negatively correlated only with fear-inhibited light reflex. Bitsios and colleagues employed STAI questionnaires suggesting that while state anxiety (referred to the momentary arousal level) could be correlated with the initial pupil diameter, basic trait anxiety might be correlated only with the pupillary light reflex. They predicted therefore that state anxiety ratings would have correlated with initial pupil diameter values, while trait anxiety ratings would have been related only with the amplitude of the response to light. Data did not support this forecast: In the threat of shock condition state anxiety was correlated to both pupillary indices, while trait anxiety seemed to be weakly negatively associated with pupillary light reflex.

It is however also possible that in this study the manipulation of anxiety level was not effective in causing dissociation between pupillary components. That is to say,
healthy participants with high trait-anxiety ratings did not reach a sufficient level of defensive activation to prompt a differentiation in pupillary responses. In this line of reasoning, a different research group (Kojima, Shioiri, Hosoki, Kitamura, Bando, & Someya, 2004) examined pupillary light reflex and initial pupil diameter in a group of panic disorder patients. They found that while initial pupil diameter values were the same for patients and controls, pupillary reactions to light flashes were significantly smaller for the panic disorder group, thus bringing further support to Bitsios and colleagues' pupillary dissociation theory under threat of shock conditions.

Taken as a whole, these not completely compatible outcomes do not allow to blindly support the expected dissociation between threat-induced pupillary light reflex and initial pupil diameter increase.

In addition, it can also be remarked that all these studies employed exclusively affectively negative stimuli. Consequently, it is plausible to suggest that not only the anticipation of highly aversive, but also that of highly appetitive stimuli might result in pupillary increase and light reflex reduction. Indeed, this is a possibility which has not been ruled out by the same authors (Hourdaki, Giakoumaki, Grinakis, Theou, Karataraki, & Bitsios, 2005), who advised that this pupillary effect might be not limited to fearful stimuli, but extendable to motivationally relevant stimuli in general.

4.4. The psychosensory pupillary reflex/psychopupillary response

We already referred to what the German physiologist Oswald Bumke (1911) stated about pupillary response to “psychological” stimuli. Bumke wrote that except for light and accommodation reflexes, for healthy individuals “every active intellectual process, every psychical effort, every exertion of attention, every active mental image, regardless of content, particularly every affect just as truly produces pupil enlargement as does every sensory stimulus”. This is a considerably strong assertion which has been

7 Translation from German by Eckhard Hess, 1972.
reprised during the 60s by physiologist Irene Loewenfeld (1993) in her violent criticism towards Eckard Hess’ research.

Trying to follow the chronology of this long-lasting and vitriolic controversy concerning the use of pupillary measures in psychology research, we must first say that it was noticed really early (Mentz, 1895; Bumke, 1911) that not only sensory, but also mental stimuli caused pupillary dilation. All these early researches had been conducted by physiologists (for the most part Germans) in Europe. For linguistic reasons, therefore, most American psychologists were not aware of these findings until the beginning of the 60s when Eckhard Hess published his first studies (Hess & Polt, 1960) and translated part of these European findings. The most controversial of Hess’ hypotheses was the so-called bi-directional theory of pupillary response, according to which when his participants were presented with emotionally pleasant images they showed pupillary dilation, while when they were presented with unpleasant stimuli they showed pupillary constriction. Because the knowledge of Bumke’s assertion of pupillary dilation to any stimulus was not known among American psychologists, but given for granted among physiologists, Hess’ data generated considerable interest together with fiery debate. Hess’ results were not replicated by other laboratories performing more strict controls on stimuli perceptual features, and Irene Loewenfeld (1966) among physiologists, and several psychologists violently criticized Hess’ theory and results. Nonetheless, a positive effect of Hess’ investigations was that during the 60s and 70s there was a sudden increase in the number of studies employing pupil diameter measures. The bad side of it was that when it was evident that Hess’ results could not be unequivocally replicated, pupil diameter was employed in really few researches investigating the psychophysiological effects of emotionally engaging stimuli (Janisse, 1977).

What follows is a statement by Hakerem (1972), which seems able to convey the feelings of researchers involved in pupil diameter research after 12 years since Hess’ first publication (Hess & Polt, 1960). This statement was delivered during the 1972 convention of the American Psychological Association. “It was however, Eckhard Hess, who for better of worse, left an indelible imprint (pun intended) on the research on pupillary motility by some rather striking demonstration of the pupillary dilations as a correlate of...
We will later review some of Hess’ findings, followed by other researchers’ critics and by more recent and methodologically respectable research in the field of pupil diameter studies.

4.4.1. Timing of the psychosensory pupillary reflex

The timing of the pupillary response to stimuli involving emotional or mental activation is slightly slower than the reaction described earlier for the pupillary light reflex (Loewenfeld, 1958).

Steinhauer and Hakerem (1992) reported that in darkness a variety of cognitive processing tasks prompt pupillary dilation, which reaches its maximum after 1200 ms from stimulus onset. In addition, this late dilation has been observed independently from whether tasks are requiring or not requiring a motor response. In contrast, when tasks are carried out in the light, participants who are asked a motor response show an earlier pupillary dilation, peaking approximately after 600-900 ms (a no-motor-response condition causes again a later peak, 1200 ms after picture onset). In general, illumination increments prompt progressively earlier dilation peaks (corresponding to shorter psychosensory reflex latencies). As we will later examine, it must also be mentioned that increasing task demands result in greater psychosensory pupil diameter dilation together with stronger pupil light reflex reduction (Loewenfeld, 1958; Steinhauer, Condray, & Kasparek, 2000).

4.4.2. Neural pathways of the psychosensory pupillary reflex

It has already been mentioned that pupil diameter responses resulting from emotional and mental activation are slightly slower than the reaction described earlier for the pupillary light reflex (Loewenfeld, 1958). It seems that this slowing is due to the strong emotions. It was the right demonstration performed at the right time in an age where the general public and psychologists were looking for objective indicators of emotions, fears [...]. Hess’ experiments generated an enormous interest [...]. But as so often in psychological research, after a strong beginning things turned out to be not quite as simple as they appeared first. Contradictory and inconclusive results by other researchers led to controversies and polemics which were at time vitriolic to say the least."
time-consuming modulation of cortical inhibition (Barbur, 2004; Barbur, Harlow, & Sahraie, 1992; Tsujimura, Wolffsohn, & Gilmartin, 2001).

The psychosensory reflex is implemented by both sympathetic and parasympathetic inputs (Beatty & Lucero-Wagoner, 2000).

In 1992 Steinhauer and Hakerem proposed a model of pupillary control systems. The authors pointed out that the early dilation (peaking at 600-900 ms) observed during cognitive tasks conducted in illuminated environments should be the result of the active inhibition of main parasympathetic centers (by and large the Edinger-Westphal nuclei). This parasympathetic inhibition is presumably the result of both descending cortical inputs (see also Barbur, 2004) and ascending reticular inputs to the Edinger-Westphal nuclei. The parasympathetic inhibition causes the relaxation of the pupillary sphincter muscle, indirectly resulting in pupil diameter dilation.

On the contrary, according to Steinhauer and Hakerem (1992) the later pupillary dilation (after ca. 1200 ms) comes from direct activation of the sympathetic system via the hypothalamic pathways (through the cilio-spinal center of Budge; see also Beatty & Lucero-Wagoner, 2000). Here pupil diameter dilation is the direct result of sympathetic stimulation of the pupillary dilator muscle. It must also be remarked that when pupil dilation peak is reached through direct sympathetic activation, it takes longer to return to baseline values (Loewenfeld, 1958; Steinhauer & Hakerem, 1992). This most likely occurs because sympathetic neuromodulator norepinephrine has a longer action than the parasympathetic neuromodulator acetylcholine (Beatty & Lucero-Wagoner, 2000).

4.4.3. Eckhard Hess’ research

It seems mandatory, within a review of pupil diameter employment in psychology research, to briefly summarize the results of Hess’ main papers (see Janisse, 1977, for a more detailed review). As Janisse (1977) pointed out, even if “his contribution has not been without controversy, yet his work has been a starting point for virtually every psychologist who is interested in learning about the pupil”. In Hess’
publications pupil diameter had been used to investigate a variety of cognitive, motivational, and emotional processes. These are some of the topics Hess investigates, presented in chronological order of publication.

**Interest towards pleasant visual stimuli.** In the first and probably most cited of Hess’ publications (Hess & Polt, 1960) the authors made use of pupil size as a measure of participants’ interest towards 5 visual stimuli. Only pleasant images were displayed to 6 subjects (2 women). Hess and Polt results indicated gender differences, showing larger pupil diameter size for women looking at affectively positive stimuli.

**Mental arithmetic.** The second paper published by Hess and Polt (1964) was the first one systematically investigating the effect of progressively harder tasks on pupil diameter size. Five participants (1 woman) were presented with 4 multiplication problems of increasing difficulty. The same order of tasks (from the simplest to the hardest multiplication) was employed for all 5 subjects. Pupil diameter size, measured as mean percentage of pupil diameter change (baseline/peak), showed a linear correlation with problem difficulty.

**Interest towards images of men and women in hetero- and homosexual males.** In this study Hess and colleagues (Hess, Seltzer, & Shlien, 1965) compared pupillary reactions of 5 admitted heterosexuals to those of 5 admitted homosexuals while viewing 15 images (depicting “art” images -5 trials-, nude or seminude females -5 trials-, and nude or seminude males -other 5 trials-). Small changes indicated for heterosexual participants larger pupillary responses to images depicting females, while for homosexuals smaller pupillary responses were found.

**Bidirectional hypothesis (pupillary reactions to affectively positive and negative stimuli) and other pupillary findings.** Hess’ 1965 paper is a review of several experiments conducted by Hess and colleagues, containing also the already mentioned theory of different pupillary reactions to pictures of appealing and distasteful
stimuli. According to Hess the pupil response to motivationally relevant stimuli ranged from extreme dilation (to pleasing stimuli) to extreme constriction (to repulsive stimuli). In this same publication Hess referred to other data suggesting the ability of pupil diameter to respond in line with participants' interest. In particular, larger pupillary dilation was found: 1) in hungry subjects presented with pictures of food; 2) in participants presented with different kinds of music (according to Hess, in this case pupillary differences indicated individual preferences); 3) in participants presented with the picture of their favorite political leader. Additionally, Hess exposed data indicating that when males were presented with two pictures of the same woman without knowing that the only difference was that one representation had larger pupil diameter size, participants consistently indicated that the woman with larger pupils looked more attractive. In his opinion (Hess, 1975) this would point out the role of pupil diameter "as a signal between individuals, usually at an unconscious level."

**Taste differences.** Hess and Polt in 1966 published a study in which examined pupillary reactions from 16 subjects (4 women) tasting orange drinks or drinking water. According to the authors a larger response to orange drinks indicated participants' predilection towards these stimuli.

**Other applications of Hess' pupil diameter data.** In the light of his own results, Hess sustained that pupil diameter could be reliably used as preference indicator in marketing research and as mental abnormality indicator in clinical psychology research (Hess, 1975).

It appears also noteworthy that after years of Hess' opponents' discussions concerning his bi-directional hypothesis, in later publications Hess (1975) defended his theory specifying that the constriction response to distasteful stimuli was by and large

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9 Interestingly enough, in Hess' opinion when pupil diameter indicated preference for a political leader while participant verbally stated to be in favor of another leader, pupil diameter was the sign of the "true" inclination of the subject. According to Hess this discrepancy might have been due to the participant's desire not to express a political preference which was not in line with that predominant in the college campus.
an individual reaction, which was easily not visible when "data from more than 5 subjects" were pooled.

4.4.4. Methodological criticism to Hess' work

Janisse (1977) emphasized that while psychologists' reaction to Hess' first findings was rather mild, physiologists answered promptly and quite adversely. As brought up earlier, Loewenfeld (1966) soon questioned not only the accuracy of Hess' conclusions, but also his scientific credentials, his originality and his disapproved tendency to appear in the popular press.

It should be clear that the attacks against Hess had also a personal component, beyond the scientific respectability of his data (Janisse, 1977). However, strong methodological concerns were at the core of critics' doubts in Hess' results and led his opponents to be skeptical towards the majority of his conclusions.

- Since early pupil diameter studies (Bumke, 1911) no sensory or mental stimuli, except light, were known to prompt pupillary constriction (Goldwater, 1972; Loewenfeld, 1993), and the vast majority of attempts to replicate Hess' findings (for a review, see Goldwater, 1972) were unsuccessful.

- Most of Hess' results indicating pupillary constriction to unpleasant stimuli were obtained using pictures and drawings as visual stimuli (among others: Hess, 1965; Hess & Polt, 1960; Hess, Seltzer, & Shlien, 1965). Hess (1972) claimed that his control of visual stimuli was extremely strict, and he described a long list of tests and changes he performed on images before presenting them to participants. Still, several authors (among others Beatty, 1982; Goldwater, 1972) interpreted the use of images as the ultimate reason why Hess observed pupillary constriction when distasteful stimuli were displayed. For this reason, many of Hess' hypotheses detractors (Goldwater, 1972; Beatty, 1982) proposed to limit pupillary research to studies employing non-visual stimuli (like acoustically presented words, scripts...). It must however be borne in mind that in the 70s images were not yet available in
easily manageable computerized formats (like JPEG and BMP image formats\textsuperscript{10}), so Hess' images manipulation was handmade since current automatic image editing softwares (like the widely employed Adobe Photoshop software) could not be used.

\begin{itemize}
  \item It is also of great importance that most of Hess papers and chapters (Hess, 1965; Hess & Polt, 1960, 1964; Hess, Seltzer, & Shlien, 1965) did not report any of the statistics supposed to be in favor of the conclusions he drew.
  \item In most of his studies (e.g. Hess & Polt, 1960, 1964; Hess, Seltzer, & Shlien, 1965) Hess collected data from very few participants, allowing readers to wonder whether random individual variations might have been at the basis of obtained results. Concerning this aspect it should be recalled that Hess stated (1973) that he deliberately employed few subjects, considering that pupillary constrictions to distasteful stimuli were typically visible in only few participants and in certain trials (usually near the end of the experiment, see Janisse, 1977). In his opinion, therefore, data pooling would have removed the effect, so few subjects were preferable to larger samples. Indeed, if Hess' proposal were true (which does not actually seem the case) and only few participants were showing pupillary constriction during few trials, this would in any case dramatically undermine the possibility of extending the ‘constriction to unpleasant stimuli’ hypothesis to the entire population of individuals.
  \item It might be remembered that Hess (1965) affirmed that pupil diameter was able to reveal the “true” political inclination of his participants. In particular, Hess suggested that when pupil diameter enlarged to a political leader (thus, according to Hess, indicating preference) while subjects' written report did not express inclination towards that same leader, pupil diameter had to be taken as the true political preference indicator, suggesting that for some reason the subject was reluctant to reveal his/her true preference.
\end{itemize}

\textsuperscript{10} JPEG (Joint Photographic Expert Group) and BMP (Bitmap) formats are both compressed image file formats.
The consequence of this line of reasoning is that Hess’ theory cannot be falsified. That is to say, it is not methodologically scientific that a theory is not falsifiable and if the result of a study is A, then results are in favor of hypothesis A, but if the result of the study is non-A, then results are STILL in favor of hypothesis A (Popper, 1959).

Taken as a whole, the critiques we just exposed together with the fact that Hess’ results could not be replicated essentially challenged most of his conclusions regarding pupil diameter behavior.

4.4.5. The cognitive perspective on pupil diameter data: Pupil diameter as a measure of processing load

Not everything Hess reported was opposed by his contemporary and subsequent researchers. Indeed, his results (Hess & Polt, 1964) indicating that pupil diameter was sensitive to the task’s processing load were replicated, confirmed, and deepened (for a review, see Goldwater, 1972). This line of research was specifically investigated by Kahneman and colleagues (Beatty, 1982; Beatty & Kahneman, 1966; Kahneman & Beatty, 1966; Kahneman, Beatty, & Pollack, 1967) who time-locked pupillary measurements to responses in different mental tasks (involving, for example, mental arithmetic, reaction times, recall tasks). Pupil diameter appeared a reliable index of “processing load”, such that pupil dilation consistently followed the presentation of additional or harder material (“loading” phase). Moreover, as soon as participants began reporting the solutions to the task, pupil diameter constricted, suggesting a later “unloading” phase. Pupil diameter, therefore, seemed to closely and accurately respond to task’s difficulty (see also Beatty, 1982). However, all these tasks included a motor response, thus it has also been speculated (Goldwater, 1972) that pupil diameter might have been the result of the act itself or of the motor preparation to it (Reinhard & Lachnit, 2002).
Kahneman (1973) pointed out that one of the criterions to identify a physiological response as a measure of processing load was to be able to order variations in processing demands across qualitatively different mental tasks. Beatty (1982) reviewed pupil diameter results from several studies using memory, language, reasoning, and perception-related tasks. It seems remarkable that in Beatty's opinion, pupil diameter responses provided a reasonable ordering of the tasks according to their presumed processing load, thus bringing data in favor of pupil diameter capability of revealing the ongoing processing load.

Other researches indicated that pupil diameter might be able to detect individual differences among participants' skills to solve the task. Beatty and colleagues (Beatty, 1982; Beatty & Ahern, 1979) during four different mental tasks measured pupil diameter reactions of subjects who scored high or low in a psychometric “intelligence” test (Scholastic Aptitude Test). During the task participants with higher “intelligence” scores had reliably smaller pupil diameter compared to subjects who scored lower. This effect was interpreted as a sign that the same task was less demanding (namely, had a smaller cognitive load) for “more clever” participants, so this resulted in smaller pupil diameter values. It must also be noted, nonetheless, that these two groups did not differ during a control/no-task condition.

So far we examined participants' pupillary performances during tasks perceived as “solvable” by subjects. There are further data (Granholm, Asarnow, Sarkin, & Dykes, 1996; Peavler, 1974) hinting that in resource overload conditions pupil diameter stops increasing linearly with tasks demands, reaches an asymptotic level and then dramatically decreases when task requirements exceed normal human capacity. This drop in task performance and pupillary level has been interpreted as the point at which subjects “give up” finding the solution to the task.
4.4.5.1. Some implementations of processing load findings.

The discovery that different processing loads have specific pupillary correlates led some researchers to attempt to apply this finding to specific fields.

Here we report just few examples: 1) **Deception investigation.** Dionisio and colleagues (Dionisio, Granholm, Hillix, & Perrine, 2001) assumed that if a higher cognitive load corresponds to a larger pupil diameter, then in deception context (meaning, when participants are lying or pretending to lie) there must be a higher processing load and a corresponding larger pupil diameter. Previous literature commonly employed skin conductance as "deception measure". This study brought data in favor of pupil diameter ability of "detecting deceiving behavior". It must be remarked, however, as the same authors did, that the situation might be totally different and, to say the least, less easy to disentangle in a real-life criminal investigation, where "true crime suspects might face severe punishment if judged deceptive". In this event data might clearly be less reliable, since they could be easily confounded by strong motivational aspects. 2) **Absolute pitch possessors research.** Absolute pitch possessors are rare individuals, commonly high-level musicians, who are able to correctly identify a note when they hear it. It has been found (Schlemmers, Kulke, Kuchinke, & Van Der Meer, 2005) that during a note identification task absolute or near-absolute pitch possessors have consistently smaller pupil diameter values compared to non-possessors, presumably indicating that the task is easier, less demanding for absolute pitch possessors. It should however be mentioned that some methodological flaws (specifically, a considerably larger amount of discarded trials in one of the two groups) recommend caution and the necessity to replicate these results before drawing any conclusion. 3) Applications in clinical populations: **Schizophrenic patients.** It has been stated elsewhere (Steinhauer & Hakerem, 1992) that "schizophrenia, instead of being a chronically manifest disorder, is episodic in nature, but that the vulnerability to the disorder persists." Using a variety of tasks schizophrenic patients showed specific
patterns of pupillary response compared to controls (for a review, see Steinhauer & Hakerem, 1992), suggesting that pupil diameter might be a reliable physiological correlate of this clinical disorder.

4.4.5.2. Not only cognition...

It could have been noticed that this more recent approach to pupillometry gave strictly cognitive explanations to pupil diameter data, even if occasionally motivational interpretations might have also been proposed\footnote{As an example, when participants are presented with harder trials, they might not only struggle to find a solution, but also feel more nervous and anxious compared to when they are solving easier trials. It is thus possible that both these factors combine in prompting a larger pupil diameter size. This firmly cognitive perspective is probably also the result of deep skepticism towards the use of pupil diameter in motivational/emotional research after Hess’ investigations.}.

Only a couple of studies, to the best of our knowledge, attempted to join contributions from the cognitive and the motivational perspective on pupil diameter data.

In 1971 Johnson employed a short-term memory task to investigate pupillary behavior while subjects tried to remember lists of words. In addition to the usual task demands, for one group of subjects a tone signalled that they had to forget all words they heard up to that point. On the contrary for the other group the same tone did not have any meaning. Besides the expected effect on pupillary values of processing loading/unloading, Johnson also found a specific pupillary effect of the tone only for the group which attributed a meaning to that tone. The author suggested that this effect, in his opinion not explainable by a cognitive approach, could have indicated participants’ surprise in hearing the tone. According to his view, here pupil diameter values were caused by the interference of arousal/anxiety components. In Johnson’s own words, “although the evocation of such interpretations is probably appropriate in many cases, such interpretations are very unsatisfying to those who are eager to substantiate the pupil as an accurate index of cognitive processing, per se. Nevertheless, it is probably safe to conclude that pupil changes which are observed during information-processing tasks are seldom produced exclusively by processing activities. Rather, it seems that in
many cases such pupil changes reflect the influence of information processing confounded with motivational effects”.

Few years later Stanners, Coulter, Sweet, and Murphy (1979) conducted a series of experiments and reached similar conclusions, finding evidences supporting both the arousal and the cognitive interpretation of pupillary reactions. They recorded both pupil diameter and skin conductance response during a no-task, a digit transformation task, and a no-task with threat of shock condition. In line with a cognitive perspective the authors observed increases in both pupil diameter and skin conductance when task difficulty increased. However, in line with a motivational perspective, they also found that both pupillary and skin conductance response augmented when participants were threatened with a shock while no task was given. Stanners and colleagues (1979) guessed that these data might mean that in conditions of minimal cognitive demands it is possible to see arousal effects on pupil diameter, while in conditions of high-level cognitive activity pupil diameter is influenced solely by processing loads.

4.4.6. The motivational perspective on pupil diameter data

AFTER Hess: Pupil diameter as a measure of emotional processing

To complete this quick review of psychopupillary reactions, we will now examine some data concerning the effect on pupil diameter data of motivationally relevant stimuli, but obtained outside Hess’ perspective.

4.4.6.1. Studies employing affective words

Stelmack and Mandelzys (1975) used groups of words which they labeled as “affective, taboo, or neutral”, according to whether they had a slightly disgusting, embarassing (sexually-related), or not affectively relevant content. Stimuli were presented acoustically. Only males were recruited for this research and assigned to three different groups depending on whether they were identified as introverts, ambiverts, or extraverts after filling out some questionnaires (Eysenck Personality Inventory,
extraversion dimension, form A). Based on Eysenck’s extraversion theory (1967), introverted individuals were expected to show greater physiological reactivity compared to other participants. Indeed, in general introverts had the largest pupil diameter reactions for all kinds of stimuli. Moreover, they had the greatest pupil diameter change for taboo compared to neutral words. Nonetheless, in general, pupillary reactions to affective (slightly disgusting) words did not differ from pupil diameter responses to neutral words. This last result and a closer examination of employed stimuli suggested us that taboo words might have been simply more arousing compared to affective (slightly repulsive) words, so that observed pupillary differences between taboo and affective stimuli were quantitative and not qualitative in nature.

More recently, Siegle, Granholm, Ingram, & Matt (2001) investigated pupil diameter response in depressed and non-depressed individuals while they were acoustically presented with affective and non-affective words. In doing so, Siegle and colleagues referred to some cognitive theories of depression (Macleod & Mathews, 1991) hypothesizing that depressed individuals are characterized by a bias in emotional processing and tend to pay more attention, interpret, and remember emotionally information differently from non-depressed individuals. Consequently, depressed individuals were expected to process more extensively negative stimuli and, accordingly, show larger pupil dilation compared to controls. Data did not support this theory and depressed individuals had larger pupil diameter reactions to both positive and negative emotional compared to neutral stimuli. However, in line with Siegle and colleagues' predictions, depressed patients reacted to emotional stimuli to a greater extent than controls.

Taken as a whole, these few studies point out that when negatively and positively affective words are matched for their level of activation, they prompt a comparable amount of pupil diameter change. Moreover, overall pupillary response to emotional stimuli is larger than that observed when neutral words are presented. However, further researches are needed to confirm and deepen these results.
4.4.6.2. Studies employing affective passages

In some other studies, researchers used verbal passages (small scripts) in the attempt to convey and prompt different emotions.

White and Maltzman (1978) presented affectively positive, negative, and neutral acoustic passages to a group of 12 healthy males while pupil diameter values were recorded. Only three passages were employed, lasting 120 seconds (2 minutes) and portraying a sexually erotic situation, a lynching mutilation, and a neutral passage. Arousal, interest, and valence ratings were also obtained. Arousal and interest ratings did not differ significantly between “sexual” and “mutilation” contents. Despite large pre-stimulus differences (which were not explained by the authors), pupillary data indicated a similar trend during “sexual” and “mutilation” scripts, showing a progressively larger pupil diameter. On the contrary, neutral passages were followed by a gradual decrease in pupil diameter. It must be however remarked that pupil diameter data from this study should be taken with caution, considering that data were averaged over huge chunks of 15 s intervals and that each stimulus condition included just one trial.

A more recent research conducted by Dabbs (1997) did not include numerous trials as well, but compensated with large samples of participants. Scripts described one sexual, one aggressive, and two neutral situations. Pupil diameter was larger for the sexual script compared to both the aggressive and the neutral script. It might be the case, however, that the sexual script was simply more arousing compared to the aggressive situation\(^\text{12}\). If this hypothesis were true, observed pupillary differences between sexual and aggressive stimuli would have a quantitatively and not qualitatively

\(^{12}\) This is how the same author (Dabbs, 1997) described the stimuli he selected. “The aggressive stimulus was a heated argument between a girlfriend and a boyfriend about unfaithfulness. The sexual stimulus was a vocal and noisy episode of a sexual intercourse between girlfriend and boyfriend. One control stimulus was a rambling conversation between two cowboys in the movie *Lonesome Dove*, and the other was an airline hostess' greeting to passengers at the beginning of a long flight”. The actual scripts were not provided.
different nature. As a result, the lack of control over the actually selected stimuli does not allow drawing definitive conclusions.

4.4.6.3. Studies employing affective sounds

The presentation of a sound reliably provokes pupillary dilation (Janisse, 1977). Pupil diameter does not, however, increase in a linear fashion to progressive increases in stimulus intensity. Indeed pupil diameter does not vary considerably after changes in intensity from 64.2 to 74.2 and 84.2 dB. However, it shows a significantly larger response after a 94.2 dB tone (Nunnally, Knott, Duchnowski, & Parker, 1967). As it is probably recalled from the first chapter, the latter is an intensity at which participants consistently perceive an acoustic stimulus as highly aversive and unpleasant (Bradley, Lang, & Cuthbert, 1993). Thus, these data can be interpreted as indicating that pupil diameter dilates following the presentation of loud aversive tones.

In a recent study Partala and Surakka (2003) used 30 stimuli taken from the IADS catalogue (Bradley & Lang, 1999a) to investigate pupillary reactions to pleasant, unpleasant, and neutral stimuli. The authors did not include erotic stimuli in the final set, however affectively positive sounds did not differ from negative stimuli for SAM arousal ratings (SAM/Self-Assessment Manikin; Lang, 1980). Each stimulus was presented for 6 s, with a 2 s ITI. Results indicated that pupil dilation was larger for emotional compared to neutral stimuli. Moreover, affectively relevant sounds kept on prompting larger pupillary reactions, even after stimulus offset.

4.4.6.4. Studies employing affective images

Up to now, only a couple of researches investigated pupil diameter behavior while viewing affective and non-affective pictures beyond Hess’ bi-directional hypothesis.

In 1973 Libby and the Laceys (Libby, Lacey, & Lacey, 1973) investigated pupillary behavior in 34 males, while participants were viewing 60 pictures, 30 of
which were experimental slides, representing pleasant and unpleasant contents. The authors tested also additional characteristics of the images (other than hedonic valence), which might have contributed in affecting pupil diameter (e.g. brightness, complexity, interest). Right after picture onset pupil diameter briefly constricted and then redilated and stabilized. Libby and colleagues found that unpleasant stimuli prompted larger pupillary dilations than pleasant stimuli.

Some years later Steinhauer and colleagues (Steinhauer, Boller, Zubin, & Pearlman, 1983) presented some preliminary data further investigating the relationship between pupil diameter and visually presented emotional stimuli. As in Libby’s experiment (1973) picture onset caused a short-lasting pupillary constriction and a subsequent redilation. A significantly wider pupillary redilation was observed only during the display of highly aversive or highly pleasant stimuli, while neutral images caused dilation to a lesser degree. Interestingly, these results are in line with those obtained by White and Maltzman (1978) using acoustically presented verbal passages.

More recently, Aboyoun and Dabbs (1998) presented uniquely pleasant images depicting clothed and unclothed individuals of both genders to male and female participants. The goal of the authors was to investigate whether men and women show pupillary reactions only to opposite-sex images or, on the contrary, show pupillary dilation to any picture of naked individuals, regardless their gender. Aboyoun and Dabbs' hypothesis was that in the first case pupillary response would have indicated sexual arousal, while in the latter it would have suggested a generic arousal response. Pupil diameter data supported the general arousal hypothesis: Both males and females had larger pupillary dilation to naked individuals, regardless their gender.

4.4.6.5. summarizing

Summing up, all experiments investigating pupillary behavior during the display of affectively relevant stimuli indicated that pupil diameter might be more responsive to emotional compared to neutral stimuli. Moreover, this effect does not appear to depend on selected material, since it has been suggested in studies employing sounds, words,
verbal passages, and pictures. The use of affective stimuli taken from the catalogues developed by Lang and colleagues (Lang, Bradley, & Cuthbert, 2005 for the IAPS catalogue of affective visual stimuli; Bradley & Lang, 1999a for the IADS set of affective sounds; Bradley & Lang, 1999b for the ANEW catalogue of affective words) would be capable of further investigating the pupillary reaction to emotional material, allowing a more strict control of the stimuli but also facilitating the replication of the results.
4 FIRST STUDY

In the first study we investigated ocular and pupillary behavior while young, healthy subjects viewed neutral and emotionally evocative pictorial stimuli presented one at a time.

4.1 Rationale

Ocular Behavior. Loftus & Mackworth (1978) were among the first researchers observing larger number of fixations on semantically more informative visual stimuli. In their manipulation, highly informative images were those including an object which was not consistent with the rest of the scene. These results were recently replicated by Henderson, Weeks, and Hollingworth (1999). Christianson and colleagues (Christianson, Loftus, Hoffman, & Loftus, 1991) presented images perceptually similar to each other, but depicting neutral, unusual, or emotionally unpleasant situations. Here, both affectively negative and unusual images were regarded as more informative compared to neutral stimuli. Interestingly, a larger number of fixations was observed only when unpleasant stimuli were displayed, in turn suggesting that from an evolutionary perspective emotional cues could have a greater importance compared to highly informative but not motivationally relevant images. Within the same perspective, presenting pairs of pictures, one depicting a neutral and the other an unpleasant image, Hermans and colleagues (1999) found that a larger proportion of first fixations fell on emotionally negative stimuli. Additionally, a considerable amount of data indicated that threatening cues are detected faster than neutral cues (Mogg & Bradley, 1999; Oehman, Flikt, & Esteves, 2001; Oehman, Lundqvist, & Esteves, 2001). Based on these data it
has been proposed that to facilitate the survival of the individual and its specie, life-threatening stimuli might be accessed more quickly than not-informative stimuli (Oehman, Flykt, & Esteves, 2001; Oehman, Lundqvist, & Esteves, 2001). Recent studies, however, investigating ocular behavior when either unpleasant or pleasant stimuli were presented coupled with neutral stimuli (Calvo & Lang, 2004, 2005), indicated that not only unpleasant, but emotional cues in general drew viewers’ attention earlier compared to neutral images.

It is important to notice, however, that the simultaneous presentation of pairs of stimuli implies a very specific condition, where emotional and non-emotional cues have to compete for the same limited amount of attentional resources. As a result, ocular behavior during single target presentation, when attention can be completely allocated to one stimulus, might be different (Calvo & Lang, 2004).

To investigate this issue, in the first study we presented motivationally relevant (both pleasant and unpleasant) and neutral pictures taken from the IAPS catalogue (Lang, Bradley, & Cuthbert, 1999) in a free-viewing condition, displaying one image at a time.

When we earlier exposed some methodological issues in research about ocular behavior during scene viewing (see section 6.2., in “ocular movements” chapter), we mentioned the concern about comparing data obtained using different kinds of pictorial stimuli. As an example, De Graef and colleagues (De Graef, Christiaens, & d’Ydewalle, 1990) could not replicate Loftus & Mackworth (1978) results, even if they both employed line-drawings as stimuli, and Henderson (Henderson and Hollingworth, 1998) suggested that this difference might have been due to the way information contained in the image was drawn. Specifically, Henderson proposed that the fact that objects in Loftus and Mackworth’ experiment were drawn more scattered and less detailed than in De Graef’s experiment might have explained why results from these two studies did not match. These data, therefore, imply that the way visual information is displayed on selected pictorial stimuli strongly affects viewers’ ocular behavior. For
this reason, in this study we also distinguished among images which depicted perceptually simpler figure/ground configurations and scenes including a greater number of elements, with the purpose of exploring how images perceptual composition influenced eye movements.

In the general introduction of the present inquiry we already remarked that there is no standard approach to investigate ocular behavior (see section 6.7., in “ocular movements” chapter). In particular, we referred to the fact that to date there is not a preferable and shared way to report eye movements variables. In the present study, in order to investigate ocular behavior during affective picture viewing, we decided to examine the absolute number of fixations participants made throughout pictures viewing. Moreover, after data collection we imposed over each picture an 8 X 6 matrix identifying 48 equally-sized cells (regions), with the aim of computing the amount of regions participants explored during each trial. In doing so, we assumed that while the absolute number of fixations could be regarded as a measure of the general amount of information contained in the pictures, the number of regions was considered as an indication of how much that same information was spread over that picture.

Pupillary Behavior. In one of the preceding chapters we briefly exposed data concerning pupil diameter emotional modulation. It is probably recalled that during the 60s and 70s there have been a considerable debate after Eckhard Hess first reported a pupillary bidirectional response to emotional stimuli (Hess & Polt, 1960). Hess' data indicated that pupil diameter dilated during pleasant stimuli, while it constricted during unpleasant stimuli. Several researchers advanced strong critiques to Hess results and failed to replicate his data (Beatty, 1982; Goldwater, 1972; Loewenfeld, 1993). As a result of methodological and theoretical flaws in Hess' hypothesis, pupil diameter recently has not been employed quite often as a measure of emotional modulation. Additionally, to the best of our knowledge, even fewer studies made use of emotional images with both positive and negative affective contents to investigate pupillary
behavior while viewing emotional and neutral visual stimuli (Libby, Lacey, & Lacey, 1973; Steinhauer, Boller, Zubin, & Pearlman, 1983). Moreover, results emerging from these two researches are not consistent: While Libby and colleagues (1973) found a larger pupil diameter response during the presentation of affectively repulsive stimuli, Steinhauer and co-workers (1983) reported a significant pupil diameter dilation during both pleasant and unpleasant images. Regrettably, the lack of access to the specific pictures included in each of these studies results in the impossibility of assessing whether the discrepancy in obtained results might have been due to differences in the chosen stimuli. Therefore, we were not able to make detailed predictions about the present research pupil diameter data. On one hand it could be expected that equally emotional pleasant and unpleasant stimuli prompt the same amount of pupillary dilation (given that perceptual differences are ruled out) (Loewenfeld, 1993), on the other hand, based on Libby and colleagues' data we could predict that negatively affective images might prompt larger pupil dilations.

Therefore, keeping in mind the necessity of carefully controlling affective material, we made use of digitized visual stimuli taken from the International Affective Pictures System (Bradley, Cuthbert, & Lang, 1999). The employment of IAPS stimuli allowed us to work on large number of visual stimuli, while accurately controlling selected images' valence and arousal levels referring to SAM standardized normative ratings (Self-Assessment Manikin, Lang, 1980).

4.2 Method

4.2.1 Participants

Thirty-eight University of Florida students (19 females; average age 19.6) took part in this study between April and the end of July 2003 and received course credits for their participation. Data from six subjects were excluded from analysis due to problems in the eye-tracker recording.
4.2.2 Material and design

Stimuli included 192 pictures taken from the IAPS catalogue (Lang, Bradley, & Cuthbert, 1999). Pictures were presented in a 16-bit color. Selected images were divided into three subgroups, each one including an equal number of pictures (64) identified as affectively positive, negative, or neutral, based on normative affective ratings (Lang, Bradley, & Cuthbert, 1999). Pleasure ratings were 6.98 for pleasant, 4.92 for neutral, and 2.76 for unpleasant pictures. Normative arousal values for emotional pictures were also controlled so that they did not significantly differ for pleasant and unpleasant stimuli (arousal values were equal to 5.01 and 5.75, respectively). Moreover, the set was organized so that half of the pictures depicted simpler figure/ground configurations (with single figures against a constant background), while the other half contained more complicated scenes. Additionally, pictures included in the final set were not significantly different in brightness among the different valences. (See Appendix for more details about stimuli employed in the first study).

Pictures were displayed one at a time for 6 seconds (with an inter-trial interval of 2.5 second). Two different pictures orders were alternated between subjects. Each order was divided into two blocks, balanced for picture valence and perceptual composition. The period between the first and the second block consisted of few seconds during which the subject simply waited for the next picture to appear.

4.2.3 Apparatus

Picture presentation was controlled by an IBM-compatible computer running VPM (Cook, 1997), and projected using a Proxima projector located in the control room. Pictures were displayed on a 127 by 95.5 cm screen set at a distance of 250 cm from where the subject was seated, and horizontally subtending 22.88 degrees of visual angle. The ASL model 504 eye-tracker system (Applied Science Laboratories, Bedford, MA), allows free movement of the head, and consists of a video camera and an infrared light source pointed at subject’s right eye. A magnetic sensor, attached to a headband
and located above subject's right eye, tracked and adjusted for head movement. Eye movement and pupil data were acquired using an ASL E5000 software (Eyenal software, version 1.47; ASL, copyright 2001). The eye-tracker independently monitored the corneal reflection and pupillary diameter, with a sampling rate of 60 Hz and a spatial accuracy of 0.2 mm. The eye-tracking video camera was located into a wood box in front of the subject and a red transparent screen prevented the participant from clearly recognizing the apparatus behind it. Moreover, two small Beckman sensors were placed on the left and right participant's forearm. These sensor did not record any physiological signal, but had the goal of distracting the participant's attention from measurement of the eye movements.

4.2.4 Procedure

After signing the informed consent form, the participant was accompanied to the laboratory where she sat on a recliner in a small dimly lit room. Once the sensors were attached, the experimenter went to the control room and calibrated the eye-tracking equipment by asking the participant to look at 9 pre-set locations on the screen. This procedure resulted in a system of horizontal and vertical coordinates which remained stable among different participants.

Next, participants were instructed that a series of pictures would appear on the screen and that their task was to look at each picture the entire time that it was on the screen. Whenever no picture was displayed, participants were asked to comfortably focus on a dot located in the middle of the screen.

Following two practice trials the set of 192 pictures was presented while eye movements and pupil diameter data were collected. Then, the sensors were removed and the participant was asked to fill out a post-experimental questionnaire. The experimenter subsequently debriefed, paid credits, and thanked the participants.
4.2.5 Data reduction & statistical analysis

Eyenal software (version 1.47, ASL, 2001) was employed to reduce eye movement data. Each trial corresponded to a 8.5 second recording which was segmented offline into a 2 second pre-pictures baseline period and a 6 seconds picture presentation period. Only the 6 s during which IAPS pictures were presented were included in data analysis.

Each fixation was computed as the amount of time during which the eye didn't move more than 1 degree of visual angle. Afterward, for each trial we computed the absolute number of fixations made by each participant. Moreover, when reducing eye movement data, an 8x6 matrix (48 cells) was imposed over each picture, thus identifying 48 smaller regions (see illustration below). Subsequently, for each trial we computed the number of regions explored by each subject. If the same region received more than one fixation, that region was still contributing with only one unit to the overall number of regions. Whenever a fixation was identified by the data reduction software, also the corresponding pupil diameter value was stored, so that pupillary values were provided during each progressive fixation. Accordingly, we could analyze pupil diameter trend during picture viewing.

Illustration 4.1: Matrix imposed offline over each picture to divide it into 48 (8 x 6) regions.

Absolute number of fixations, number of regions explored, and pupil diameter
values were each analyzed within a mixed-model analysis of variance (ANOVA), which included gender as between-subject factor, and hedonic valence and perceptual composition as within-subject factors. Statistical significance was evaluated at the .05 level.

4.3 Results

All our statistical analyses indicated that gender did not affect any of our dependent variables. Accordingly, gender effects will not be reported.
4.3.1 Absolute number of fixations

When the absolute number of fixations was examined, a significant main effect of hedonic valence was observed ($F(2,60)=39.8, p<.001$), such that emotional pictures, either pleasant or unpleasant in hedonic valence, consistently had a greater number of fixations compared to neutral pictures ($F(2,60)=86.9, p<.001$). The significant two-way interaction between hedonic valence and perceptual composition ($F(2,60)=4.3, p<.05$), exposed in the following graph, indicated that for both simpler figure/ground configurations and for more complicated scenes there was a significantly higher number of fixations for emotions compared to neutral images (for perceptually simpler images $F(1,30)=78.1, p<.001$; for more complicated scenes $F(1,30)=29.9, p<.001$).

Moreover, for neutral and unpleasant pictures, there was a significant effect of perceptual composition, such that more complicated scenes consistently had a greater number of fixations compared to simpler configurations (for neutral images, perceptual composition effect $F(1, 30)=16.6, p<.001$; for unpleasant images, perceptual composition effect $F(1, 30)=8.9, p<.01$).
Table 1: Absolute number of fixations, interaction hedonic valence by perceptual composition. Emotional images have consistently a greater number of fixations compared to neutral contents. Moreover, for perceptually more complicated scenes unpleasant and neutral pictures receive a larger amount of fixations.
4.3.2 Number of regions explored

For the mean number of different regions that were explored, a significant main effect of perceptual composition (F(1,30)=197, p<.001) indicated that a larger number of regions was explored whenever perceptually more complicated scenes were displayed. The effect of perceptual composition was consistent for pleasant (F(1,30)=36.6, p<.001), unpleasant (F(1,30)=73.4, p<.001), and neutral pictures (F(1,30)=158.4, p<.001).

In addition, hedonic valence had a significant main effect on the number of explored regions (F(2,60)=67.6, p<.001), and this effect was found for both perceptually simpler configurations (F(2,60)=93.8, p<.001) and for more complicated scenes (F(2,60)=11.7, p<.001): In both conditions emotional images, either pleasant or unpleasant, were explored more broadly than neutral pictures (for simpler images, emotional vs. neutral: F(1,30)=200.5, p<.001; for more complicated scenes: F(1,30)=17.7, p<.001).
Table 2: Number of regions explored, interaction
hedonic valence by perceptual composition. Perceptually
more complicated and motivationally relevant images
are consistently explored more widely than
figure/ground and neutral pictures, respectively.
Moreover, when viewing scenes, unpleasant images have
the larger amount of regions explored.

The significant two-way interaction between hedonic valence and perceptual
composition (F(2,60)=27.4, p<.001) shown in the previous graph, indicated that when
participants were looking at more complicated scene, affectively negative images were
explored more extensively than either affectively positive (F(1,30)=4.7, p<.05) and
neutral pictures (F(1,30)=23.9, p<.001).
4.3.3 Pupil diameter response

As shown in the graph below, immediately after the picture was displayed on the screen, a dramatic decrease in pupil diameter size was observed.

![Graph showing pupil diameter response](image)

Table 3: Absolute pupil diameter trend across 15 progressive fixations from the beginning of picture viewing.

This response began after the 1st fixation (lasting on average 290 ms) and reached its peak during the 3rd-4th fixation (after approximately 790-1070 ms from picture onset). The timing of this response was consistent with that of the pupillary contraction (myosis) due to increased illumination or the “pupillary light reflex” (see section 4.3.1, in “pupil diameter” chapter). Confirming this, the size of this pupil diameter decrease (computed as the difference between the peak of this response – during the 3rd fixation- and the pupil diameter value just before this response began – during the 1st fixation-) was highly correlated with the IAPS brightness scores (R²=0.61,
F(1,190)=304.5, p<.001), indicating that the brighter the pictures were, the greater was the response to the light. See the following graph.

Table 4: Correlation between pupil light reflex size, and IAPS brightness scores, ranging between 0 (black/no brightness picture), and 1 (white/maximum brightness picture). The highly significant linear correlation indicates that low brightness images were associated to small pupillary light reflexes.

4.3.3.1 Pupil diameter change scores from the peak in the pupillary light reflex

In order to exclude from our analysis the effect of the pupillary light reflex, pupil diameter during picture viewing was computed as a change from the peak in the response to increased illumination (pupil diameter values during 3rd fixation).

Looking at the pupillary response to pictures, a significant two-way interaction between hedonic valence and perceptual composition was observed (F(2,60)=3.9, p<.05). For either perceptually simpler images or more complicated scenes, emotionally
negative pictures prompted the largest pupil diameter change (unpleasant larger than other hedonic contents: for simpler configurations, $F(1,30)=7.6, p<.01$; for more complicated scenes, $F(1,30)=25.1, p<.001$). Moreover, when perceptually more complicated images were presented, those which were emotionally relevant caused a larger pupil diameter change compared to neutrals ($F(1,30)=28.7, p<.001$).

Perceptual composition had an impact on pupil diameter change only when subject were looking at neutral pictures ($F(1,30)=11.2, p<.01$): Only in this condition a larger pupil diameter change was observed for simpler figure/ground configurations.

![Pupil diameter change from baseline, interaction hedonic valence by perceptual composition. Unpleasant images are consistently associated with larger pupil diameter size. Only neutral images are affected by perceptual composition.](image)

Table 5: Pupil diameter change from baseline, interaction hedonic valence by perceptual composition. Unpleasant images are consistently associated with larger pupil diameter size. Only neutral images are affected by perceptual composition.
4.3.3.2 Absolute pupil diameter values computed as residuals from IAPS brightness scores

In order to further support the results obtained deviating pupil diameter values from the peak in light reflex baseline, an analysis of covariance was conducted on absolute pupil diameter using the IAPS brightness scores as covariate. Hedonic valence had a marginally significant effect on pupil diameter residuals from IAPS brightness scores (F(2,186)=2.4, p=.09). The pattern of this effect, see graph below, was consistent with that found using pupil diameter changes from baseline, and it indicated that emotionally negative pictures elicited the greatest pupil diameter response compared to both neutral images (F(1,186)=3.9, p=.05) and emotionally positive images (F(1,186)=3.3, p=.07).

Table 6: Pupil diameter residuals from IAPS brightness scores, main effect of valence. Again, we obtained a larger pupillary response for unpleasant compared to neutral and pleasant images.
4.4 Discussion

According to the bi-dimensional approach to emotion, we expected motivationally relevant stimuli to affect eye movements and pupil diameter variables more strongly compared to neutral, motivationally non-relevant pictures. In line with this hypothesis we found that emotional stimuli prompted a larger amount of fixations, an increase in image visual scanning (as measured by number of regions explored), and larger pupil diameter responses. In addition, we observed that motivationally unpleasant pictures tended to induce larger pupillary reactions compared to both pleasant and neutral stimuli. Moreover, when perceptually more complicated scenes were displayed, unpleasant pictures resulted in a larger number of fixations and a greater number of regions explored compared to neutral and pleasant stimuli.

Taken as a whole, data from this first study seemed to indicate a greater pupillary and ocular reactivity to affectively negative stimuli.

Based on previous literature analyzing ocular behavior during scene viewing, we expected that pictures labeled as perceptually more complicated would have caused more frequent fixations. Additionally, we hypothesized that more complex pictures might have been more widely examined compared to perceptually simpler images. These hypotheses were also confirmed: When more complex scenes were presented participants showed a greater amount of fixations and explored a larger number of regions. Moreover, the amount of regions explored seemed to respond more accurately than the absolute number of fixations to pictures' perceptual composition, easily distinguishing between perceptually simpler and more complicated images.

Perceptual composition did not affect pupil diameter behavior when emotional, images (either pleasant or unpleasant) were presented. Indeed, only when participants viewed non-affective neutral images, perceptual composition affected pupil diameter response.
5 SECOND STUDY

In the first study included in this investigation, eye movements, as measured by the overall amount of fixations and number of regions of interest explored, responded to both affective and perceptual composition picture content. On the contrary, pupil diameter reacted more specifically to the emotional value of the images. Taken as a whole, therefore, these results seemed promising in terms of pupil diameter and ocular movements capability of responding to pictures' emotionality and perceptual composition.

5.1 Rationale

We realized that some physical features of the stimuli included in this first experiment might have influenced and possibly biased the results we obtained.

First of all, selected pictures were presented in color. Hence, it is possible to hypothesize that some specific colors (e.g., red of blood in pictures depicting mutilations and pink of skin in images portraying naked individuals) might have been stronger “emotional cues”, more capable of inviting the viewer to a wider visual exploration of the stimulus compared to chromatically less homogeneous neutral images. Accordingly, strictly physical stimulus characteristics might have accounted for the larger visual exploration observed when participants viewed emotional compared to neutral images. Moreover, also pupil diameter has been shown to respond to changes in color (Drew, Sayres, Watanabe, & Shimojo, 2001).

For this reason in our second study we displayed only grayscale images.
Second, in the final set of pictures constituting the first study we included some images which were displayed vertically. As it can be seen in figure below, since the screen on which pictures were presented had a constant size, the vertical display of a picture resulted in two darker vertical bars on both sides of the image. This might have biased the results of our first study in two ways: On one side, vertical images had necessarily a smaller amount of regions to be explored compared to images which covered the whole screen, on the other side, the presence of wide darker areas might have affected pupil diameter values, making pupil diameter react to the contrast of darker areas included in these images more than to the emotional content of these pictures.

Illustration 5.1: Example of vertical pictures replacement. On the left, vertically-displayed IAPS picture no. 2280, employed in first study. It is possible to notice the large lateral black bands. On the right, IAPS picture no. 2240, used in the second study to replace IAPS no. 2280.

Keeping this in mind, in this second study we did not include pictures that were displayed vertically in the first experiment, replacing each vertical image with another
one from the IAPS catalogue which had the same emotional content and perceptual composition. We made also additional changes to other pictures until all images covered the same area of the screen.

Third, in our first experiment we did not monitor the amount of luminosity associated with each stimulus picture. Accordingly, we considered that pupil diameter results might have been due to random luminosity variations within each hedonic and perceptual composition category. For this reason in our second study we digitally controlled the luminosity associated with each image and, whenever it was necessary, we modified it. After these adjustments, the distribution of luminosity values was the same for each of the 6 cells obtained covarying “hedonic valence” and “perceptual composition” factors. That is to say, the distribution of luminosity values was the same for “figure/ground pleasant”, “perceptually more complicated pleasant”, “figure/ground neutral” images and so forth.

The common goal of the above-mentioned adjustments to stimuli physical features was to verify the results obtained in the first study, in an attempt to rule out eventual perceptual confounds.

A further goal of this second study was to investigate electromyographic/EMG blink, skin conductance, and pupillary reactions to acoustic startling probes delivered during picture viewing.

As it is probably recalled from the general introduction of this investigation (chapter 1, section 3.3.1.4 “Motivational priming: Reflexive reactions to aversive startling probes”), the presentation of acoustic startling probes (usually binaurally presented loud bursts of white noise), prompt a cascade of physiological defensive reflexes. In humans, the “startle reflex” is typically monitored through the electromyographic/EMG blink response to the probes. Lang and colleagues (Bradley, Cuthbert, & Lang, 1999; Bradley, Lang, & Cuthbert, 1993; Lang, Bradley, & Cuthbert,
1990, 1997) repeatedly observed that blink size diminishes when probes are delivered while participants view affectively pleasant images, while it enhances if it is administered during unpleasant images. The EMG response size to probes presented during affectively neutral stimuli falls in between.

In 1995 Vrana reported that acoustic startle probes caused also a motivationally modulated response in skin conductance. Within the context of an imagery paradigm, skin conductance response was significantly greater when probes were delivered during affectively unpleasant compared to neutral and pleasant texts. As previously exposed in the present work, any electrodermal reaction involves the activation of the sympathetic nervous system.

It might be recalled that also psychosensory pupil diameter dilation is presumed to result from sympathetic nervous system activation (Barbur, 2004; Steinhauer & Hakerem, 1992). Studies employing not aversively loud affective sounds (Partala & Surakka, 2003) found that pupil diameter was significantly larger during emotional compared to neutral sounds taken from the IADS catalogue (Bradley & Lang, 2000). In addition, earlier studies (Nunnally, Knott, Duchnowski, & Parker, 1967) reported that tones with an intensity sufficient to be perceived as aversive (94.2 dB) prompted a significantly larger pupillary reaction compared to less loud, non-aversive tones.

Based on these data in the present experiment we presented loud, aversive, startling noises while participants viewed affective and non-affective pictures taken from the IAPS catalogue (Lang, Bradley, & Cuthbert, 1999). We recorded pupil diameter, skin conductance, and EMG blink responses to the probes. We hypothesized that the pupil diameter response to startle probes might be modulated by the hedonic valence of the foreground image. Moreover, considering that the presentation of startling probes should cause sympathetic activation in both the pupillary and electrodermal system, we expected these psychophysiological systems to react in an analogous manner. The additional recording of the EMG blink response allowed us to verify that affective modulation took place as expected.
5.2 Method

The methodological procedure of this second experiment is in large part the same as the one reported for the first study of this investigation.

5.2.1 Participants

Thirty-six University of Florida students took part in this study between December 2004 and January 2005 and received course credits for their participation. Due to a programming error, eye-tracker and peripheral data from 9 subjects could not be used for data analysis purposes. Accordingly, the final set of participants comprised twenty-seven students (11 females). An experimenter error resulted in the loss of EMG blink data from one subject (a female) (startle blink reflexes, n = 26; all other dependent variables, n = 27).

5.2.2 Material and design

All visual stimuli were taken from the IAPS catalogue (Lang, Bradley, & Cuthbert, 1999) and consisted of 96 black-and-white pictures. Pictures were presented in a 16-bit grayscale.

Selected images were divided into three subgroups, each one including an equal number of pictures (32) identified as affectively positive, negative, or neutral, based on IAPS normative affective ratings (Lang, Bradley, & Cuthbert, 1999). Pleasure ratings were 6.96 for pleasant, 4.94 for neutral, and 2.38 for unpleasant pictures. Normative arousal values for emotional pictures were also controlled so that they did not significantly differ for pleasant and unpleasant stimuli (arousal values were equal to 5.53 and 5.89, respectively). As in the previous study, the set was organized so that half of the pictures depicted simpler figure/ground configurations (representing single figures against a constant background), while the other half contained more complicated scenes.

As mentioned before, in order to keep under control pictures' brightness we
excluded from the new set images of the previous experiment that did not cover the whole screen. Then we replaced these pictures with others that had the same hedonic and perceptual composition content. Next, we made use of an image editing software (Graphic Converter, version 4.5.2; Lemke Software, Peine, Germany) to convert all pictures into grayscale and, subsequently, we employed another image editing software (Adobe Photoshop, version 7.01; Adobe Systems Inc., San Jose, CA) to record the average luminosity value of each image. Afterwards, utilizing the same software we modified the luminosity of some images until the distribution of luminosity values was the same for each level of the two independent variables: Hedonic valence (3 levels) and perceptual composition (2 levels). That is to say, as already explained, the distribution of luminosity values across simpler unpleasant images was the same as that of perceptually more complicated unpleasant images, as well as that of more complicated pleasant pictures, and so forth. (See Appendix for more details about employed stimuli). A uniformly gray image with the average Adobe Photoshop luminosity values of all selected images was created in order to be displayed on participants' monitor during the inter-trial interval.

Two different orders of picture presentation were alternated between subjects.

Each trial began with a 2 s baseline followed by a 6 s period during which pictures were presented to the participant. Startle probes were presented halfway through picture viewing. Next, there was a 10 s inter-trial interval. In total each trial consisted of 18 s.

The acoustic startle probe consisted of a 50-ms presentation, 97.5 dB burst of white noise with instantaneous rise time. The startle stimulus was generated by a Coulbourn S81-02 white-noise generator and binaurally presented over matched headphones. During picture viewing startle stimuli were delivered after 3 s, 3.5 s, 4 s, or

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13Adobe Photoshop (version 7.01; Adobe Systems Inc., San Jose, CA) can provide for each picture some luminosity information, indicating the average luminosity of the image, its standard deviation, together with a graphical plot representing the distribution of the total number of pixels (y axis) plotted against Adobe's luminosity units (x axis). Adobe Photoshop luminosity units range from 0 (black) to 256 (white).
4.5 s from picture onset.

### 5.2.3 Apparatus

Image presentation was controlled by an IBM-compatible computer running the Presentation software (Neurobehavioral Systems, 2003, San Francisco, CA). Pictures were displayed on a 19 in monitor (Samsung SyncMaster 191T) located in the control room, at a distance of 39 in (99.06 cm) from where the subject was seated. The monitor horizontally subtended 22.88 degrees of visual angle.

Another IBM-compatible computer running the e-Prime stimulus presentation software, version 1.0 (Psychology Software Tools, Inc., 2002, Pittsburgh, PA), guided the participant before the beginning of the experiment through the eye-tracker calibration procedure, displaying eye-tracker calibration stimuli on subject's monitor.

The ASL model 504 eye-tracker system (Applied Science Laboratories, Bedford, MA) provided ocular data recording. Eye movement and pupil diameter data were acquired independently using an ASL E5000 software (Eyenal software, version 1.47; ASL, copyright 2001). Corneal reflection and pupil diameter were monitored with a sampling rate of 60 Hz and a spatial accuracy of 0.2 mm. As in the first study, the eye-tracking video camera, located into a wood box in front of the subject, was placed behind a red transparent screen that prevented the participant from clearly recognizing the recording apparatus.

Another IBM-compatible computer run VPM software (Cook, 1997) for psychophysiological data acquisition and reduction.

Skin conductance activity was recorded using two large Beckman sensors placed adjacently on the hypothenar eminence of the right palmar surface after being filled with 0.05-m NaCl Unibase paste. A Coulbourn S71-22 skin conductance coupler (Coulbourn Instruments, Allentown, PA) sampled electrodermal activity at 20 Hz. Digitized values (expressed in A/D units) were later converted back to conductance values (in microSiemens). Blink reactions to startle probes were recorded from two Beckman miniature electrodes placed on the orbicularis oculi muscle, beneath the right
eye, following Fridlund and Cacioppo (1986) recommendations. The recording of EMG activity over the orbicularis oculi muscle allowed us to measure the eyeblink component of the startle response. The eyeblink component of the startle response was measured by recording EMG activity over the orbicularis oculi muscle of the left eye. The raw EMG signal was amplified (x5,000), although only a 40% of that signal was already taken, and frequencies below 8 Hz and above 1000 Hz were filtered out with a Coulbourn S75-01 bioamplifier. The raw signal was rectified and integrated with a Coulbourn S76-01 contour-following integrator, with an actual time constant of 20 ms. Activity in the orbicularis oculi muscle was sampled at 20 Hz during baseline and picture viewing, with an increase in sampling rate to 1,000 Hz for 50 ms before the onset of the startle probe and 250 ms after probe onset.

5.2.4 Procedure

As in the previous experiment, after signing the informed consent form, the participant was accompanied to the laboratory where she sat on a recliner in a small dimly lit room. Once the sensors for ocular and peripheral signals were attached, the experimenter went to the control room and calibrated the eye-tracking equipment by asking the participant to look at 9 pre-set locations on the screen. This procedure resulted in a system of horizontal and vertical coordinates which remained stable among different participants.

Next, the participant was instructed that a series of pictures would appear on the screen and that she should look at each picture the entire time that it was on the screen. Whenever no picture was displayed, participants were asked to comfortably focus on a dot located in the middle of the screen.

Following three practice trials the set of 96 pictures was presented while eye movements, pupil diameter, skin conductance and EMG blink data were collected. The experiment lasted 30 minutes. Afterwards, the sensors were removed and the participant was asked to fill out a post-experimental questionnaire. The experimenter subsequently debriefed, paid credits, and thanked the participants.
5.2.5 Data reduction & statistical analysis

ASL Eyenal software (version 2.21; copyright 2001-2004) was employed to reduce eye movement data. The eye-tracker recorded each trial for 16 s. This recording was segmented offline into a 2 second pre-pictures baseline, a 6 seconds picture presentation period, and a 8 s post-picture interval. Only the 6 s segment during which IAPS pictures were presented was included in data analysis.

Again, each fixation was computed as the amount of time during which the eye didn't move more than 1 degree of visual angle. Next, for each trial we computed the absolute number of fixations made by each participant. Moreover, when reducing eye movement data, an 7x7 matrix (49 cells) was imposed over each picture, thus identifying 49 smaller regions. Subsequently, for each trial we computed the number of regions explored by each subject. As in the first study, if the same region received more than one fixation, that region was still contributing with only one unit to the overall number of regions. Whenever a fixation was identified by the data reduction software, also the corresponding pupil diameter value was stored, so that pupillary values were provided during each progressive fixation. Accordingly, we could analyze pupil diameter trend during picture viewing.

Psychophysiological reactions to startle probes were computed for skin conductance, EMG blink, and pupil diameter as differences between average activity 1 s before the probe was delivered and activity after it was presented. Average skin conductance change was additionally log transformed (log [SCR + 1]) to normalize the data. The blink data were reduced off-line by using a program which employs an algorithm devised by Globisch, Hamm, Schneider, and Vaitl (1993) to score each trial for magnitude in analog-to-digital units. Moreover, blink magnitude data were converted into standardized t-scores, to take into account inter-individual variability in the size of the EMG blink response to the probes. Pupil diameter response to the startle was determined by subtracting pupillary activity observed for 250 ms 1 s before probe presentation from that observed for 250 ms, at the peak of pupillary reaction to the
probe (approximately 1 s after probe delivery).

Absolute number of fixations and number of regions explored were each analyzed within a mixed-model analysis of variance (ANOVA), which included gender as between-subject factor, and hedonic valence and perceptual composition as within-subject factors\(^{14}\).

Pupil diameter response to picture viewing was measured across progressive fixations. We first controlled whether our manipulation of stimuli physical features was effective. In order to do so, we compared the first study pupillary light reflex with the response to light observed in our second study, where we attempted to rule out perceptual confounds. In both cases the pupillary light reflex was computed as the difference between the peak in the pupillary response to light (occurring during the 3rd fixation) and the pupil diameter value at picture onset (1st fixation). Subsequently, we performed on absolute pupil diameter data a repeated measures analysis of variance (ANOVA), which included progressive fixations (14 levels) and hedonic valence (3 levels) as within-subject factors. In our statistical analysis we did not include “perceptual composition” as a factor, since we knew from our first study that it affected pupil diameter only while viewing neutral images.

A separate analysis was also conducted to assess effects of arousal content for emotional picture: For emotional images - either pleasant or unpleasant - we employed a median split of IAPS “arousal” normative ratings to identify emotional images with low and highly arousing contents. In this case we conducted a repeated measures analysis of variance (ANOVA), which included progressive fixations (14 levels) and arousal content (5 levels: low arousing pleasant, highly arousing pleasant, neutral, low arousing unpleasant, and highly arousing unpleasant) as within-subject factors.

For skin conductance, EMG blink, and pupil diameter reactions to the startle, we

\(^{14}\) As it will be later remarked, gender did not affect any of our dependent variables. Therefore, data from male and female participants were collapsed, and, accordingly, data were analyzed within a repeated measures analysis of variance, including only hedonic valence and perceptual composition as within-subject factors, was performed.
conducted separate repeated measures analysis of variance to assess effects of picture valence (pleasant, neutral, and unpleasant) on reactions to the startle. Moreover, for the electrodermal and pupillary response we independently tested the effect of the probe presentation itself: For skin conductance we compared the average response 1 s before probe delivery with the response after the probe was presented; for pupil diameter we confronted the response after a spontaneous blink to that observed after a startle-evoked blink.

Statistical significance was evaluated at the .05 level.
5.3 Results

None of the dependent variables here exposed indicated a significant gender effect. For this reason, data from male and female participants were collapsed.

5.3.1 Reactions to pictures

5.3.1.1 Absolute number of fixations

![Bar chart showing absolute number of fixations](chart.png)

Table 7: Absolute number of fixations. On the left, main effect of hedonic valence, indicating a larger amount of fixations for images with an emotional, rather than neutral, content. On the right, main effect of perceptual composition, pointing out a greater number of fixations for perceptually more complicated pictures.

The graph above (left side) shows that when the absolute number of fixations was examined, a significant main effect of hedonic valence was observed (F(2, 52)=6.4,
such that emotional pictures, either pleasant or unpleasant in hedonic valence, consistently had a greater number of fixations compared to neutral pictures \( (F(1,26)=14.3, p<.001) \). Moreover, there was a significant main effect of perceptual composition, such that more complicated scenes consistently had a greater number of fixations compared to simpler configurations \( (F(1,26)=7.5, p<.05) \), see graph above, right side. No significant interaction was observed between hedonic valence and perceptual composition.

### 5.3.1.2 Number of regions explored

For the mean number of different regions that were explored, a significant main effect of perceptual composition \( (F(1,26)=173.1, p<.001) \) indicated that a larger number of regions was explored whenever perceptually more complicated scenes were displayed. The effect of perceptual composition was consistent for pleasant \( (F(1,26)=77.9, p<.001) \), unpleasant \( (F(1,26)=53.3, p<.001) \), and neutral pictures \( (F(1,26)=67.4, p<.001) \).

In addition, hedonic valence had a significant main effect on the number of explored regions \( (F(2,52)=53.1, p<.001) \), and this effect was found for both perceptually simpler configurations \( (F(2,52)=51.6, p<.001) \) and for more complicated scenes \( (F(2,52)=13.6, p<.001) \). Moreover, in both conditions unpleasant images were explored more broadly than affectively positive pictures (for simpler images: unpleasant vs. pleasant \( F(1,26)=8.9, p<.01 \); for more complicated scenes: unpleasant vs. pleasant \( F(1,26)=17.5, p<.001 \)) and visually searched more widely than neutral pictures (for simpler images: unpleasant vs. neutral \( F(1,26)=69.7, p<.001 \); for more complicated scenes: unpleasant vs. neutral \( F(1,26)=20.4, p<.001 \)).

A significant two-way interaction between hedonic valence and perceptual composition \( (F(2,52)=9.01, p<.001) \) pointed out that when participants viewed more
complicated scenes, the number of regions explored was the same for affectively positive and neutral images. See graph below.

![Graph showing exploration of regions by affective valence](image)

**Table 8:** Unpleasant images were always explored more broadly than neutral and pleasant pictures. A larger number of regions was explored during more complicated scenes.

### 5.3.1.3 Absolute pupil diameter

In graph below we compared the size of the pupillary response to picture onset in the first and in the second study of the present investigation. The pupillary light reflex\(^\text{15}\) was significantly smaller during our second experiment, \(F(1,57)=106.7,\)

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\(^{15}\) Pupillary light reflex was computed as the difference between the peak in the pupillary response to light (occurring during the 3rd fixation) and the pupil diameter value at picture onset (1st fixation).
p<.001), suggesting that our manipulation of images perceptual features was effective in reducing pupil diameter reactions to light changes.

![Pupil light reflex size comparison](image)

Table 9: Comparison 1st and 2nd experiment pupil light reflex size. The size of the pupillary response to picture onset was significantly smaller during the 2nd study.

When we assessed the effect of picture valence on absolute pupil diameter, we found a significant main effect of pictures valence content (F(2,52)=18.9, p<.001), indicating a larger pupil diameter for emotional, either pleasant or unpleasant, compared to neutral images (F(1,26)=28.5, p<.001). The following graph exposes the significant two-way interaction between hedonic valence and fixations order (F(26,676)=5.02, p<.001), showing how emotional pictures caused a larger pupil diameter response throughout picture viewing (using a Bonferroni-corrected level of significance, the difference between emotional and neutral images was significant from the 4th to th 14th fixation).
Table 10: Absolute pupil diameter. Throughout picture viewing we observed a larger pupillary response to emotional compared to neutral images.

We conducted a separate analysis of pupil diameter data when viewing high arousing or low arousing emotional images, distinguishing among pleasant and unpleasant picture contents. The significant main effect of arousal content ($F(4,104)=18.5$, $p<.001$) is shown in the graph below.
Table 11: Main effect of arousal content for high-arousing pleasant, low-arousing pleasant, neutral, low-arousing unpleasant, high-arousing unpleasant images. The typical U-shaped response indicates that pupil diameter reacted depending on pictures arousal content. (See text for more details).

When we examined this response throughout picture viewing, we observed a significant two-way interaction between pictures arousal content and fixations order (F(52, 1352)=5.8, p<.001). This interaction (see graph below), confirmed the pattern emerged with the main effect of arousal content and pointed out 1) that the most arousing stimuli, regardless of the valence, prompted the largest pupil diameter response, 2) that high-arousing pleasant stimuli reliably caused a wider pupil diameter

16 Using a Bonferroni-corrected level of significance, the difference between highly arousing stimuli, either pleasant or unpleasant, and low-arousing negative stimuli was statistically significant from the 7th to the 13th fixation.
size compared to highly arousing unpleasant stimuli, 3) that low-arousing negative stimuli evoked a larger pupillary reaction compared to neutral stimuli, and 4) that throughout picture viewing low arousing pleasant and neutral stimuli, inducing the smallest pupil diameter response, elicited an equivalent pupil diameter reaction.

Table 12: Absolute pupil diameter. Interaction between fixations order and arousal content. Pupil diameter was larger during high arousing contents (pleasant > unpleasant images) compared to low arousing contents. Pupil diameter reaction to low arousing pleasant stimuli did not differ from that observed during neutral images.

17 The difference between highly arousing pleasant and unpleasant stimuli was significant during the whole picture presentation period.
18 With a Bonferroni-corrected level of significance, the comparison between low-arousing unpleasant and neutral images was significant from the 6th up to the 12th fixation.
5.3.2 Reactions to startle probes

5.3.2.1. Skin conductance change

Skin conductance significantly augmented whenever the startle probe was delivered \( (F(1,26)=14.7, p<.001) \). See graph below.

![Graph showing skin conductance change](image)

Table 13: Skin conductance change after startle probe delivery. Skin conductance significantly increased whenever a startle probe was presented.

However, this electrodermal response to startle probe was not modulated by affective valence (n.s. effect\(^{19} \)), despite visual inspection of skin conductance data.

\(^{19} F(2,52)=1.7, p=.19\)
seemed to suggest a larger reaction when hedonically unpleasant stimuli were presented (see following graph).

Table 14: Skin conductance response to startle probe presentation was not significantly modulated by picture hedonic valence. However, the pattern we observed was the same as that found by Vrana (1995).
5.3.2.2. Electromyographic blink response

In line with previous results, we found (see graph below) that hedonic valence modulated the EMG blink response to startle probes (hedonic valence effect: (F(2,50)=3.7, p<.05). Pairwise comparisons indicated that the size of the EMG blink was larger for unpleasant compared to pleasant stimuli (F(1,25)=5.4, p<.05).

Table 15: Blink magnitude to startle probe delivery, expressed in t scores. As expected, blink magnitude was larger for unpleasant compared to pleasant images.
5.3.2.3 Pupil diameter response

Pupil diameter tended to increase after a startle-induced blink compared to after a spontaneously-occurring blink (F(1,26)=3.1108, p=.08), suggesting that pupil diameter after the startle probe did not simply increase as a mechanical result of eyelid closure/re-opening (see following graph).

![Graph showing pupil diameter change](image)

*Table 16: Pupil diameter change. Pupil diameter after startle probe delivery (right bar) was significantly larger than after spontaneously occurring blinks (left bar).*
However, as can be seen in graph below, pupillary response to the startle probes was not significantly modulated by pictures valence content\textsuperscript{20}.

![Graph showing pupil diameter response to startle probes]

Table 17: Pupil diameter response to startle probes was not modulated by the ongoing affective state.

5.4 Discussion

The attempt of replicating our first study ocular movement findings while controlling for some perceptual features of our stimuli was successful. Both the absolute number of fixations and the number of regions explored were affected by pictures hedonic valence and perceptual composition. However, while affective valence influenced the absolute number of fixations independently from the stimuli perceptual composition, the amount of regions explored seemed to be more specific in responding to pictures' hedonic valence and perceptual content. In particular, as already emerged in our first experiment, the number of regions explored was generally greater when viewing more complicated scenes. In addition, wider areas of the stimulus were

\textsuperscript{20} (not significant hedonic valence effect: F(2,52)=1.2, p=.32)
examined when unpleasant compared to pleasant stimuli were displayed, and for perceptually more complicated images the amount of regions explored was the same for affectively pleasant and neutral pictures. In general, therefore, given that the absolute number of fixations is the same for motivationally appetitive and threatening stimuli, affectively negative stimuli were explored more broadly (i.e., more regions were explored) compared to neutral and positive stimuli. From an evolutionary perspective, this could possibly suggest that when human beings are presented with any motivationally relevant input there is a general increase in degree of visual inspection of the stimulus, regardless of its hedonic valence. However, when a threatening stimulus is presented, individuals tend to investigate wider areas, gathering as many additional details as possible, compared to when viewing neutral or pleasant stimuli. On the contrary, it appears that when positive, desirable items are shown, individuals make several fixations on the same areas, without gathering additional information from secondary regions of the scene.

Pupil diameter results from this second study, in which pictures' physical features were more carefully controlled, could provide us with more reliable hints about pupillary behavior while attending motivationally relevant stimuli. Contrary to what emerged in our first study, during picture viewing pupil diameter was larger to both pleasant and unpleasant compared to neutral images. To further deepen this result, based on arousal IAPS ratings we distinguished among high and low-arousing pleasant and unpleasant images, in the attempt of investigating the degree to which pupil diameter reaction was solely depending on picture arousal content. Data indicated that during the whole picture presentation period, highly arousing appetitive contents reliably provoked the largest pupil diameter response, followed by the reaction to threatening stimuli (either high or low in arousal value). On the contrary, the response to low-arousing pleasant images was the same as that to neutral pictures. Taken as a whole, these data could indicate that for pleasant stimuli only more specific high-arousing images (namely, erotica) have an emotional evocative power sufficient to
provoke pupil diameter reactions larger than to neutral and low-arousing stimuli. On the contrary, negative stimuli, either highly or less arousing, prompt pupillary responses larger than to neutral and low-arousing stimuli. This could indicate that a threatening content is effective *per se* in activating the defensive system of the individual, while a pleasing stimulus needs to be highly arousing to activate the appetitive system.

In this second experiment we also investigated skin conductance, EMG blink, and pupil diameter reactions to startle probes, with the explicit goal of assessing if picture emotional content modulated this response. The EMG blink size to the probes was wider when participants looked at unpleasant as opposed to pleasant contents, and this pattern was in line with that found in previous studies employing the same picture viewing paradigm. The additional recording of the EMG blink and the observation of the expected pattern allowed us to infer that selected pictorial stimuli were effective in prompting the activation of peripheral physiology necessary for observing emotional modulation of startle reactions. Skin conductance and pupil diameter response to the probes was not modulated by the emotional content of the foreground picture. It must be kept in mind that activity increased in both systems as a result of startle delivery itself, thus we could reckon probes were effective in causing defensive activation in both systems. For skin conductance, however, we also noticed that the range of response was quite small compared to typically observed values (compare our graph’s range with that showing skin conductance response to different picture contents, section 3.3.1.3, in “bi-dimensional approach to emotions” chapter), and this could have suggested that some of the participants were low-responders. Moreover, for both pupil diameter and skin conductance startle response a “ceiling effect” could be hypothesized, since both variables showed a considerable increase after picture presentation and an additional one after probe delivery, and it is possible that neither of the variable had “enough room” for a further increase.
Data from both studies were consistent with a bi-dimensional perspective on emotional organization, according to which behavioral and physiological responses to emotional stimuli are largely based on the activation of a defensive and an appetitive motivational system (Lang, Bradley, Cuthbert, 1997). Data also confirmed that our manipulation of the perceptual composition of the stimuli was effective: Eye movement behavior reliably distinguished among figure/ground images and more complicated scenes. However, it is noteworthy that this response to images perceptual composition appeared to be independent from the hedonic valence content of the pictures.

Eye movement data. In the first study, for both the overall amount of fixations and the number of examined areas we observed a tendency towards a larger visual exploration of emotional and more complicated images. Interestingly enough, for both ocular behavior indicators, when more complicated scenes were displayed there was a larger response for affectively negative images. Therefore, the eyes moved to a greater extent and made a wider visual search when emotional and perceptually more complex stimuli were displayed, additionally suggesting also that unpleasant images prompted a stronger ocular reaction compared to pleasant and neutral stimuli. We ran our second study to control more carefully for possible perceptual confounds and ascertain that our eye movement data could not have been explained by low-level visual features. For this reason we excluded from our set vertically-displayed images (that hence had lateral black bands that reduced the number of regions that could actually be explored) and
presented grayscale images\textsuperscript{21}, expecting that after these manipulations, results from this second experiment would be more reliable than those from the first one. Data from our second study concerning ocular behavior indicated that the overall amount of fixations was reliably increasing when more complicated scenes and emotional images, either pleasant or unpleasant, were presented. Moreover, the way hedonic valence affected eye movements was independent from the effect of perceptual composition. Therefore, for each perceptual composition category emotional pictures received a greater amount of fixations compared to neutral contents. The number of regions explored, on the other hand, was consistently larger for affectively negative compared to positive or neutral pictures, and when more complicated images were displayed participants looked at the same amount of areas in pleasant and neutral images.

Thus, while the overall amount of fixations was the same for affectively positive and negative images, viewers tended to search wider areas of the picture when they were looking at unpleasant images. Our data therefore seem to suggest that participants implement different viewing patterns when they are presented with unpleasant compared to pleasant images: When viewing unpleasant negative images additional fixations are made on new, unexplored areas, while when viewing pleasant images several re-fixations are made on the same areas.

These results can be interpreted from an evolutionary perspective. Threatening stimuli could be more widely explored because the viewer needs to gather as many information as possible about the object which is perceived as a menace to the individual and its specie. From this point of view it is wise to investigate also secondary details of the feared stimulus, since the individual needs to understand as quickly as possible which is the most suitable reaction to it (freeze, fight, or flight). On the contrary, when appetitive stimuli are presented, the viewer does not necessarily need to take an extremely prompt action. Accordingly, the observer can focus on the same pleasant details, making several fixations on the same appetitive areas, without the need

\textsuperscript{21} This factor was not expected to affect ocular behavior and was in fact introduced to more carefully control pupil diameter data.
of taking into consideration other secondary and not motivationally relevant aspects of the stimulus.

Based on these data and on this latter hypothesis, it appears also worth reconsidering Henderson and Hollingworth “saliency map model” predictions (Henderson, 1992; Henderson, Weeks, & Hollingworth, 1999). It is most likely recalled that their saliency map model of scene perception assumed that “saliency weights” determine fixation placement, so that when an area has been successfully processed its saliency weight decreases and other areas are fixated, whereas whenever processing is not successful refixations occur.

Our data concerning ocular behavior while viewing pleasant stimuli and suggesting that participants made several refixations on the same areas cannot be interpreted as indicating that some details of these appetitive stimuli were not successfully processed by the viewer\textsuperscript{22}. Therefore, when emotionally-relevant stimuli are presented, refixations to the same areas or fixations to new areas appear as the result of an active choice of the individual, expression of the specific strategy activated by the appetitive or defensive meaning of the stimulus. Hence motivational stimuli seem to be a special kind of perceptual stimuli, that can give further hints concerning ocular behavior during complex scene viewing\textsuperscript{23}. For this reason pictures from the IAPS catalogue (Lang, Bradley, & Cuthbert, 2005), could be an extremely valuable tool for the investigation of ocular behavior when looking at natural, real-world scenes.

\textit{Pupil diameter data.} Data from our first study, where we did not control for the luminosity of the pictures and where stimuli were displayed in color, indicated a larger pupil diameter for unpleasant compared to neutral and pleasant stimuli. Being aware

\textsuperscript{22} The fact that pleasant stimuli details were not more difficult to process (thus needing refixations according to the “saliency map” hypothesis) compared to details of unpleasant stimuli is further confirmed by previous investigations indicating that skin conductance and cortical potentials response is equivalent for motivationally positive and negative stimuli. Typically, skin conductance and cortical potentials would react more to pleasant stimuli if they were more difficult to process.

\textsuperscript{23} In this context it must also be kept in mind that the same Henderson (Henderson & Hollingworth, 1998) remarked that up to date there were no studies examining the effect of specific contents on ocular behavior during scene viewing. Accordingly, the same authors did not exclude the possibility that picture meaning might affect ocular movements.
that these non-motivationally-related aspects of the stimuli might have explained the results we obtained, we attempted to control low-level perceptual features (pictures color, pictures luminosity, and the above-mentioned black bands in some vertically-displayed images) that might have eventually biased our results. Results from our second study did not replicate the larger response for motivationally negative stimuli, pointing out, instead, an equally larger pupil diameter response for emotional, either pleasant or unpleasant, compared to neutral stimuli. Pupillary diameter, therefore, appeared to react to the arousing, activating content of the images, more than to their specific hedonic content. To further deepen this hypothesis, we distinguished between high and low-arousing pleasant and unpleasant images. Pupil diameter reacted the most to highly arousing pleasant images, which prompted a larger response compared to highly arousing affectively negative pictures. In general, negative images, either high or low in arousal, caused a greater response compared to neutral and low-arousing pleasant stimuli. Despite the difference in the reaction to high-arousing pleasant and unpleasant images, the U-shaped response to the stimuli – larger for highly activating, smaller for more neutral items – is typical of any physiological system reacting to the arousal more than to the hedonic content of the pictures (see figure representing U-shaped skin conductance response in section 3.3.1.3 in the “bi-dimensional approach to emotions” chapter). This outcome is therefore in line with preliminary data observed by Steinhauer et al. (1983), suggesting a larger pupil diameter response to more arousing compared to neutral pictures. On the contrary, our results did not confirm Libby and colleagues' hypothesis (Libby, Lacey, & Lacey, 1973) of a greater pupillary reaction to hedonically negative compared to positive and neutral images. The most intuitive explanation for the discrepancy in these results lies in the specific stimuli employed by these researches, which in Libby et al. (1973) investigation probably included more activating negative pictorial stimuli. Consequently, once again it is possible to appreciate the advantage of employing the IAPS catalogue, providing unlimited access
to a large database of standardized affective stimuli, which can be easily controlled and reliably used worldwide for research in motivated attention.

In the general introduction of this investigation we briefly reviewed the sympathetic and parasympathetic inputs that are supposed to cause the psychosensory pupillary dilation (see "neural pathways of the psychosensory pupillary reflex", in section 4.4.2, second chapter of this investigation). On the one hand, hypothalamic pathways can directly activate the sympathetic branch of the autonomous nervous system, resulting in pupil diameter dilation (Steinhauer & Hakerem, 1992). On the other hand, psychosensory stimulation can indirectly inhibit the parasympathetic system, and this is also resulting in pupil diameter dilation. Main parasympathetic centers (mostly the Edinger-Westphal nuclei) can be inhibited by ascending inputs from reticular centers and descending inputs from the cortex (Barbur, 2004; Steinhauer & Hakerem, 1992). Loewenfeld (1958) pointed out that the additional involvement of the cerebral cortex is the main reason for the slower pupillary dilation after emotional and mental activation compared to after light stimulation. Considering that cortical activation is one of the components contributing to the pupillary dilation during emotional stimuli, it seems noteworthy that brain potentials also show a larger response to emotional compared to neutral stimuli (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Schupp, Cuthbert, Bradley, Hillman, Hamm, & Lang, 2004). Hence, both cortical potentials and pupil diameter show the largest reactions to the most arousing stimuli. It still needs to be investigated whether these two physiological systems simply correlate with each other in the way they respond to highly activating stimuli or whether brain cortex activity might have an active role in determining pupil diameter response to emotional stimuli.

*Stronger ocular and pupillary reactions to motivationally-relevant stimuli.*

Overall, both experiments pointed out stronger ocular and pupillary reactions to emotionally-relevant compared to neutral stimuli. Results from our second study, in
particular, indicated that ocular movements (as indicated by the absolute amount of fixations) and pupillary diameter reacted the most to motivational compared to emotionally non-relevant stimuli. We tried to consider also alternative ways of explaining these data. Affective stimuli might have been simply characterized by a greater cognitive load (Beatty, 1982; Beatty & Kahneman, 1966; Kahneman & Beatty, 1966; Kahneman, Beatty, & Pollack, 1967) compared to non-arousing items, and this higher cognitive load might have caused both pupillary and ocular reactions. However, there are some facts in favor of our motivational interpretation. 1) In classical studies of pupillary behavior during increasingly difficult tasks (Beatty & Kahneman, 1966; Kahneman & Beatty, 1966; Kahneman, Beatty, & Pollack, 1967), pupil diameter progressive dilations were interpreted as a reliable index of the greater processing load associated to harder tasks. If we do not rule out the possibility that pupillary dilations while viewing emotional stimuli might be due to greater cognitive load, we cannot also rule out the hypothesis that a greater level of anxiety associated with more difficult conditions might have caused pupil diameter enlargement. As Johnson (1971) pointed out, it is not always possible to easily disentangle the effect cognitive and emotional factors on pupil diameter reactions (see section 4.4.5.2 in pupil diameter chapter). 2) In our second experiment, the additional recording of other physiological variables like the startle EMG blink response indicated the presence of affective modulation during picture viewing. 3) For the absolute amount of fixations, if the hypothesis of a higher cognitive load for affective stimuli were true, we should expect perceptual composition, that distinguishes between figure/ground images and more complicated scenes, to reliably indicate emotional images as more complicated compared to neutral pictures. The lack of interaction between perceptual composition and hedonic valence factors is in favor of an independent effect of hedonic valence on the absolute number of fixations.

*Larger ocular responsivity to affectively negative stimuli.* In both our studies, we found that participants consistently explored unpleasant images more diffusely than
pleasant and neutral stimuli, thus suggesting a greater ocular responsivity when viewing affectively negative stimuli. Within the bi-dimensional approach to emotions a defensive and an appetitive motivational system are supposed to underlie physiological and behavioral reactions to emotional stimuli (Lang, Bradley, Cuthbert, 1997). Several investigators supporting this perspective hypothesized that if one of the two motivational systems might be dominant over the other, it would most likely be the defensive system (Bradley, 2000; Cacioppo, Berntson, Larsen, & Poehlmann, 2000; Oehman, Flikt, & Esteves, 2001; Oehman, Lundqvist, & Esteves, 2001). From an evolutionary perspective, this eventual dominance of the defensive system would be explained by the obvious advantage of paying attention to dangerous more than to appetitive stimuli (put it simply, it is clearly important to find a mate to ensure the offspring, it is even more important to react appropriately and remain alive after confronting a life-threatening situation). Since Miller's conflict theory (1951; 1959), showing a steeper gradient when the organism was avoiding instead of approaching a goal, a considerable amount of data have substantiated the "negativity bias" hypothesis (Cacioppo & Berntson, 1994; Ito, Cacioppo, & Lang, 1998). It is noteworthy that IAPS affective ratings, indicating more extreme ratings for unpleasant compared to pleasant stimuli, appear to closely parallel the approach/avoidance pattern of response observed in Miller's animal studies (Bradley, 2000; Lang, Bradley, Cuthbert, 1997). More recently Cacioppo and colleagues (Cacioppo, Berntson, Larsen, & Poehlmann, 2000) reported that there tends to be a greater psychophysiological reactivity to negative compared to positive cues.

In our experiments, wider areas of a scene were fixated whenever threatening stimuli were displayed. As earlier mentioned, the fact that instead the overall amount of fixations was the same for pleasant and unpleasant stimuli can be interpreted as a further support to the hypothesis that the observer actively implemented different viewing strategies based on the appetitive or defensive meaning of the motivational cue.
Electromyographic blink, skin conductance, and pupil diameter response to startle probes. In the context of the bi-dimensional approach to emotions, the "motivational priming hypothesis" has been advanced by Lang and colleagues (Bradley & Lang, 2000a; Lang, Bradley, Cuthbert, 1990, 1997) to predict the behavioral and physiological outcomes of the activation of the defensive or the appetitive motivational system. After one of the two motivational systems is activated, "a network of associations, representations, memories, and action programs" is primed and has, therefore, a higher probability to be accessed compared to the non-activated motivational system. As a result, reactions to appetitive or defensive cues augment/diminish according to a motivational match/mismatch with the ongoing motivational state. Electromyographic blink reactions to startling probes (typically rated by participants as the highly unpleasant events, Bradley, Cuthbert, & Lang, 1993) repeatedly supported the motivational priming hypothesis, showing that blink size augmented when probes were delivered while participants viewed unpleasant images -match condition-, while diminished during pleasant pictures -mismatch condition- (see section 3.3.1.4 of the "bi-dimensional approach to emotions" chapter). Our data confirmed the expected pattern, indicating that the EMG blink to the probes was modulated by the affective state elicited by the stimuli. The electromyographic blink is only one of the physiological reactions resulting from the presentation of a startling probe. Accordingly, we could expect other physiological variables to be affectively modulated as well. In 1995, Vrana delivered acoustic startle probes during an affective imagery paradigm. He found that skin conductance reactions to the probes were larger during unpleasant compared to pleasant and neutral scripts.

In the present research, using a picture viewing paradigm, we investigated whether electrodermal and pupillary responses, both primarily affected by the sympathetic branch of the autonomous nervous system, did indicate emotional modulation of startle probe reactions. Our skin conductance and pupil diameter data did not replicate the pattern observed by Vrana (1995), even if both systems increased after
the probes were delivered, suggesting that startle presentation was effective in activating the defensive system.

In our study skin conductance reacted within a really narrow range compared to typically observed values (see second study results and discussion), thus we hypothesized that one of the reasons why we did not find affective modulation was that some low-responders were included in the participants sample. Moreover, to explain pupil diameter lack of startle response affective modulation we conjectured a "ceiling effect", such that the pupillary system could not react further after it responded to picture content and to probe presentation (see also Giakoumaki, Hourdaki, Grinakis, Theou, & Bitsios, 2005). It is plausible that the iris allowed the pupil to react to picture content and to the startle probe, but its mechanical limitations impeded the observation of affective modulation of pupillary responses to the probe. Thus, it is possible that an earlier startle probe delivery might have been be sufficient for emotional modulation to occur.

However, as expected, the electrodermal and pupillary systems reacted to the probes in a comparable fashion (namely, their activity increased without showing affective valence modulation), thus we must also consider the possibility that these systems simply do not show affective modulation. As it might be recalled from the general introduction of this investigation, several animal studies suggested that while the non-specific physiological activity resulting from startle probe delivery is presumed to be mediated by a primary startle circuit (Davis & File, 1984; Davis, Gendelman, Tischler, & Gendelman, 1982), the affectively-modulated physiological response to the probes most likely involves a more complex fear-learned secondary startle circuit (Bradley, Cuthbert, & Lang, 1999; Davis & Lang, 2003). Our data therefore seem to suggest that skin conductance and pupil diameter responses may be mediated by activity in the primary startle reflex circuit, not responsive to foreground emotion. In addition, if these results were confirmed, Lacey's concept of "stimulus specificity" (1967), stating that specific stimuli and tasks are associated with specific physiological reactions, could
explain the fact that reactions to startle probes were emotionally modulated using an imagery paradigm, but not using a picture viewing paradigm.

Hence further data are needed to investigate the electrodermal and pupillary components of the startle reflex.

Possible future research. We just touched on some of the issues that still need to be solved concerning pupillary and electrodermal reactions to startling probes. There are also several aspects of ocular and pupillary behavior while viewing motivationally relevant stimuli that could be examined to a greater extent.

The present research pointed out the existence of different ocular behaviors, as revealed by the absolute number of fixations and the relative number of regions explored, when attending motivationally relevant compared to neutral stimuli. In addition, distinct ocular patterns for images exploration were found for emotionally unpleasant compared to pleasant pictures. It would therefore be helpful to look into the effect of specific picture content on ocular behavior, distinguishing between images depicting mutilations, threats, erotica and so forth. Using the simultaneous presentation of two images taken from the IAPS catalogue, Calvo and Lang (2005) already observed different viewing patterns when participants looked at “mutilation” compared to “threatening” contents.

Several studies by Bitsios and colleagues (Bitsios, Szabadi, & Bradshaw, 1996, 1998, 2002, 2004; Hourdaki, Giakoumaki, Grinakis, et al., 2005) reliably reported that pupillary light reflex size is decreasing when a flash light is presented during affectively negative compared to neutral conditions (this phenomenon has been labeled the “fear-inhibited light reflex”, see section 4.3.3 of the “pupil diameter” chapter). However, in Bitsios' investigations motivationally relevant stimuli were always exclusively represented by unpleasant cues and this, by admission of the same authors (Hourdaki, Giakoumaki, Grinakis, et al., 2005), leaves open the possibility that also affectively pleasant cues might prompt the same pupillary reaction. In the present studies our
control of pre-stimulus luminosity was not strict enough to enquire this issue without
doubting that "baseline effects" might have occurred. However, in our second study the
peak of the pupillary light reflex after picture onset looked smaller for emotional
compared neutral stimuli. Hence, further data are needed to understand whether the
pupillary light reflex to emotional stimuli is a fear-inhibited or a more generic
motivationally-inhibited phenomenon.

In our research pupil diameter reacted to images arousal content, showing the
largest pupillary dilation during highly activating stimuli, independently from the
emotional valence of the picture. These results are in line with both preliminary data
observed by Steinhauer and colleagues (1983) using a passive, free-viewing picture
paradigm, and with data found by Partala and Surakka (2003) who employed acoustic
affective stimuli taken from the IADS catalogue (Bradley & Lang, 1999a, 2000b).

Overall, results from these investigations seem to suggest that pupillary response
to emotionally relevant cues might not depend on the specific context in which the
pupillary reaction is prompted. Parallel studies enquiring pupil diameter behavior in
different contexts might give the last word about this hypothesis, and affective stimuli
taken from the IAPS (Lang, Bradley, & Cuthbert, 2005), IADS (Bradley & Lang,
1999a, 2000b), and also ANEW (Bradley & Lang, 1999b) catalogues might be
extremely useful for this purpose.
Abbreviations


the threat of an electric shock: A potential laboratory model of human anxiety,
Journal of Psychopharmacology, 12, 279-287.

reflex by the threat of an electric shock: A potential laboratory model of human

light reflex to diazepam, Psychopharmacology, 135, 93-98.

Bitsios, P., Szabadi, E., & Bradshaw, C. M. (1999). Comparison of the effects of
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Psychopharmacology, 143, 286-292.

light reflex’ to the level of state/trait anxiety in healthy subjects. International

Bitsios, P., Szabadi, E., & Bradshaw, C. M. (2004). The fear-inhibited light reflex:
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Psychophysiology, 52, 87-95.

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G. Berntson (Eds.), Handbook of Psychophysiology. Cambridge, MA: Cambridge University Press.


8 APPENDIX

In this appendix it is possible to find additional details concerning specific pictures selected from the IAPS catalogue (Lang, Bradley, Cuthbert, 1999) to be included in the final set of study 1 and study 2 images.
Study 1:
List of 192 employed color pictures taken from the IAPS catalogue (Lang, Bradley, Cuthbert, 1999). For each picture it is reported its normative description, information about ‘hedonic valence’ and ‘perceptual composition’ categories, normative ratings for valence and arousal (mean and standard deviation), and luminosity values.

<table>
<thead>
<tr>
<th>IAPS number</th>
<th>IAPS description</th>
<th>hedonic valence</th>
<th>perceptual composition</th>
<th>SAM valence ratings mean</th>
<th>SAM valence ratings std. dev.</th>
<th>SAM arousal ratings mean</th>
<th>SAM arousal ratings std. dev.</th>
<th>luminosity values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1050</td>
<td>Snake unpleasant figure/ground</td>
<td>3.46</td>
<td>2.15</td>
<td>6.87</td>
<td>1.68</td>
<td>0.114928</td>
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<td></td>
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<td>1051</td>
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<td>3.8</td>
<td>1.75</td>
<td>5.95</td>
<td>1.98</td>
<td>0.143143</td>
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<tr>
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<td>Snake unpleasant figure/ground</td>
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<td>1.93</td>
<td>6.93</td>
<td>1.68</td>
<td>0.222502</td>
<td></td>
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<tr>
<td>1280</td>
<td>Rat unpleasant scene</td>
<td>3.66</td>
<td>1.75</td>
<td>4.93</td>
<td>2.01</td>
<td>0.273851</td>
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<td></td>
</tr>
<tr>
<td>1300</td>
<td>Pit Bull unpleasant figure/ground</td>
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<td>1.78</td>
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<td>1303</td>
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<td>2.11</td>
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<td>2.04</td>
<td>0.230467</td>
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<td>1340</td>
<td>Women pleasant scene</td>
<td>7.13</td>
<td>1.57</td>
<td>4.75</td>
<td>2.31</td>
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<td>1500</td>
<td>Dog pleasant figure/ground</td>
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<td>1.88</td>
<td>4.12</td>
<td>2.5</td>
<td>0.270425</td>
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<tr>
<td>1610</td>
<td>Rabbit pleasant figure/ground</td>
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<td>1850</td>
<td>Camels pleasant scene</td>
<td>6.15</td>
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<td>Porpoise pleasant scene</td>
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<td>1.48</td>
<td>4.27</td>
<td>2.53</td>
<td>0.533549</td>
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<td>Metric 2</td>
<td>Metric 3</td>
<td>Metric 4</td>
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The table shows the response data for different descriptions with their corresponding probabilities.
Study 2:

List of 96 employed grayscale pictures taken from the IAPS catalogue (Lang, Bradley, Cuthbert, 1999). For each picture it is reported its normative description, information about ‘hedonic valence’ and ‘perceptual composition’ categories, normative ratings for valence and arousal (mean and standard deviation), and Adobe Photoshop luminosity values. The distribution of luminosity values was the same for each level of the two independent variables: Hedonic valence (3 levels) and perceptual composition (2 levels). In other words, the luminosity of selected pictures did not differ as a function of either emotional valence or picture composition.

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