IMPACT OF AGING ON THE BEHAVIORAL PERFORMANCE OF RATS
AND ELECTROPHYSIOLOGICAL CORRELATES OF AMYGDALA
NEURONS DURING EFFORT-BASED DECISION MAKING

By

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ABSTRACT

Healthy aging is associated with a progressive decline in several cognitive functions including working memory and processing speed. Age-related alterations of dopaminergic as well as serotonergic systems are known to contribute to deficit in associative learning. Rewards are used to mediate goal-directed behavior in appetitive conditioning. Here the effects of aging on effort-based decision making have been assessed. Aged and young rats were trained on an effort-discounting task in an operant chamber. Responding once on the low reward lever always delivered a small amount of reward, whereas selection of the other lever always delivered a larger amount, but only after performing a pre-specified number of lever presses. Our results showed that aged rats were generally affected by effort later in training, leading to a preference for the lever associated with the smaller reward at a lower cost. Electrophysiological recordings from the basolateral complex of the amygdala (BLA), a structure implicated in cost/benefit decision making were also assessed in this study. Our results confirm that amygdala neurons respond to the light-predicting cue, rewards, lever presses as well as to the anticipation of reward. In addition, our results demonstrate the shunting effect that putative interneurons have on principal cells of the amygdala in freely behaving rats. Our results also show the gradual increase and decrease of BLA neural activity during multiple lever presses, in anticipation of rewards. Because dopamine is known to modulate BLA interneurons activity, our findings suggest a mechanism by which age-related changes in emotional processing might be occurring.
INTRODUCTION

Amygdala
The amygdala is an almond shaped group of nuclei which lies in the medial temporal lobe of the brain. This structure is associated with a range of cognitive processes, which include emotion, reward, motivation, learning, memory and attention (Murray 2007). The amygdala is best known for its association with fear and fear conditioning (LeDoux et al., 1990). However, studies of neuronal activity in monkeys have shown that amygdala also plays a role in positive affect (Murray 2007). More specifically, the basolateral nucleus of the amygdale (BLA) is important in forming stimulus-outcome associations, via its extensive afferent input from sensory cortical areas and the central nucleus is important in acting as a mediator for conditioned responses, via its projections to midbrain and brainstem autonomic and behavioral centers (Seymour and Dolan 2008; Kapp et al., 1992). In order to carry out its reward related functions, it interacts with the basal forebrain cholinergic system, the midbrain dopaminergic system, the prefrontal cortex, cortical and subcortical structures (Baxter and Murray 2002). The amygdala’s interaction with most of the neocortical cell fields illustrates its critical positioning within the telencephalon.

Pavlovian conditioning
The amygdala plays an important role in Pavlovian conditioning. This form of associative learning, also known as classical conditioning, refers to a form of association that occurs between a stimulus and some positive or negative outcome. A typical procedure involves the presentation of a neutral stimulus paired with a stimulus of significance to the animal that normally induces an innate and reflexive response. As a result of pairing the neutral stimulus (conditioned stimulus, CS) with the stimulus of significance (unconditioned stimulus), the CS comes to elicit a conditioned response
(Cardinal et al., 2002). Studies have shown that lesions of the amygdala in rodents block responses to CSs that were previously paired with aversive outcomes (Balleine, 1992) and rewarding stimuli (Cardinal et al., 2002).

**Instrumental conditioning**

Instrumental conditioning refers to arranging a contingency between the animal’s behavior and an outcome that reinforces it (Thorndike 1998). In instrumental conditioning, animals learn in a response-outcome fashion as opposed to Pavlovian conditioning where animals learn in a stimulus-outcome fashion (Savage and Ramos 2009). Pavlovian procedures mediate instrumental behavior by adding affective/motivational and sensory/discriminative properties (Savage 2001). Indeed, goal-directed behaviors cannot be fully dissociated from the Pavlovian processes as they will always occur in a particular context, associated with a defined set of sensory stimuli. The Pavlovian-instrumental interaction involves a contingency between a stimulus (S), a response (R) and an outcome (O) (see Figure 1A). The interaction between Pavlovian-instrumental conditioning is known as the two process theory; the theory that associative learning is guided by overlapping Pavlovian and instrumental processes (Savage and Ramos 2009, Mowrer 1947, 1956). This interaction involves a Pavlovian conditioned expectancy state as an intermediary between the stimulus and the instrumental response (Figure 1B; Overmier et al., 2001). Reward expectation leads to an increase in activity of dopaminergic neurons which project to numerous brain regions including the amygdala.
Figure 1. Associative mechanisms that modulate goal-directed behavior. (A) Associative relationships in Two-Process Theory where S is the stimulus, R is the response and O is the reward outcome. (B) 1. Initially, the stimulus cue solely drives behavior. 2. Several couplings later, the stimulus cue evokes reward expectancy then modulates goal-directed behavior. Adapted from Savage and Ramos, 2009.

Role of amygdala in appetitive conditioning

Previous research surrounding the amygdala has focused on emotions and its influence on perception and cognition. In their experiment, monkeys that were conditioned via Pavlovian procedure, formed associations between stimuli and reward (Paton et al., 2006). Similar experiments using imaging studies have reported the increased activation of the amygdala when rats choose options associated with larger reward magnitudes, delayed rewards or uncertain rewards (Smith et al., 2009). Moreover, the amygdala’s role in reward-related associative learning has also established its involvement in decision making (Seymour and Dolan 2008, Hikosaka et al., 2008; Paton et al., 2006).
**Effort Discounting**

Previous animal decision making studies have assessed mechanics through which action selection for potential rewards are associated with costs. In these experiments animals choose between more costly larger rewards or easily obtainable smaller rewards. Effort discounting is a principle in which the value of reward is inversely related to the amount of effort required to obtain it. Floresco and his colleagues have shown the “discounting” of larger rewards due to its costs and therefore the increasing preference for smaller, low-cost rewards (Floresco et al., 2008b). Experiments using inactivation of the basolateral complex of the amygdala (BLA) showed that BLA-inactivated rats have a greater sensitivity to effort costs as compared to control, being more likely to work for the easier-smaller reward over the large reward, which requires performing multiple lever presses (Floresco and Ghods-Sharifi 2007, Ghods-Sharifi et al., 2009). Similarly the inactivation of BLA also rendered rats to less likely choose delayed large rewards over immediate small rewards, suggesting greater impulsivity in BLA-inactivated rats as compared to control rats (Winstanley et al., 2007).

**Dopamine**

Dopamine (DA) is a monoamine neurotransmitter which activates five types of dopamine receptors known as D1, D2, D3, D4 & D5. There are three main dopaminergic nuclei in the rat midbrain: substantia nigra, ventral tegmental area and the arcuate nucleus. Cells in the substantia nigra project to neostriatum to form nigrostriatal DA system (Nestler et al., 2001). Cells in ventral tegmental area project to limbic structures forming the mesolimbocortical DA system. Moreover, cells in the arcuate nucleus affect the pituitary gland and make up the tuberoinfundibular DA system. The neostriatal DA system regulates motor control and learning of motor programs and habits. The mesolimbocortical DA system is responsible for modulating cognitive and emotional functions. Dopamine has numerous important functions in the brain including its roles in behavior and
cognition, motivation, learning and reward (Nestler et al., 2001). More importantly dopamine has a close association with behaviors involving reward-seeking. Research has shown that as a consequence of anticipation of reward, the firing of dopaminergic neurons leads to stronger motivation to seek reward, hence the well known association of dopamine with drugs of abuse (Nestler et al., 2001).

Neurobiological experiments that focus on different kinds of decision making in animals, place a large emphasis on DA in mediating such functions. In these studies, animals assess the cost of response for a potential reward (Floresco et al., 2008a). These responses vary based on the effort required to receive a reward. In such decision making tasks, administration of DA receptor antagonists induce impulsive choice in rats which lead to reduced preference for larger, higher-effort or delayed rewards (Cardinal et al., 2000; Floresco et al., 2008a; Denk et al., 2005).

**Serootonin**

Serootonin is also a monoamine neurotransmitter, and it is produced by several brain stem nuclei located in the rostral and caudal clusters of the raphe nuclei. The rostral nuclei innervate most of the brain, including the cerebellum, whereas the caudal nuclei innervate cerebellum, brain stem and spinal cord (Nestler et al., 2001). Serotonin receptors are known as 5-hydroxytryptamine receptors (5 HT receptors). Fourteen different serotonin receptors have been discovered, each with unique structure, pattern of expression, pharmacology, and second messenger effector (Nestler et al., 2001).

In spite of extensive studies on serotonin, its precise roles in the brain are still unclear. It is involved in various functions, ranging from development of the brain (Bonnin et al., 2007) to social behaviors (Edwards and Kravitz 1997; Hikosaka et al., 2008; Deakin and Graeff 1991). It is best known for its involvement in depression, aggressivity and punishment. This association with negative
emotions led to the theory that serotonin functions as a negative reward signal, opponent to
dopamine signals (Daw et al., 2002). The latter, has however not been confirmed, and more recent
findings suggest that it acts in a complementary fashion to dopamine in goal-directed behaviors.
Unlike dopamine neurons, serotonin neurons are known to fire differentially when expecting reward
and also when receiving a reward (Nakamura et al., 2008). Studies have reported that manipulations
to reduce serotonin’s function results in an increase in frequency with which animals choose an
immediate small reward over a larger delayed reward (Bizot et al., 1999; Doya 2002; Hikosaka et al.,
2008). Analogous studies have found that serotonin is crucial for delay-based decisions, but not for
effort-based decisions (Denk et al., 2005). Comparatively, dopamine has been found to be important
for both effort-based and delay-based decisions (Denk et al., 2005). These studies combined suggest
a role of both dopamine and serotonin in goal-directed behavior and decision making.

Aging
Healthy aging is accompanied by cognitive changes that arise from numerous neuroanatomical and
neurobiological modifications. Aging results in a progressive decline in cognitive changes which
include episodic memory, working memory, and processing speed (Marschner et al., 2005).
Neurotransmitter systems including dopaminergic system and serotonergic systems undergo
substantial decline during the course of aging.

A negative relationship has been found between dopamine and adult age (Marschner et al., 2005).
Studies show strong evidence in age-related losses of pre- and postsynaptic biochemical markers of
the nigrostriatal dopamine system (Mohr et al., 2009). Both PET and SPECT studies on pre-synaptic
mechanisms show age-related losses of dopamine transporter in the striatum (Erixon-Lindroth et al.,
2005). Moreover, molecular imaging studies on post-synaptic mechanisms show age-related losses of
striatal D1 receptor densities (Suhara et al., 1991, Mohr et al., 2009). Similar age-related declines are
observed in mesocortical and mesolimbic pathways (Mohr et al., 2009). D2 receptor losses due to age have been observed throughout neocortex, amygdala, thalamus, and hippocampus (Inoue et al., 2001; Kaasinen and Tinne, 2002; Mohr et al., 2002).

Similarly, the serotonergic system experiences changes due to normal aging. A PET study provided in vivo evidence for reduced cortical serotonin binding sites due to age-related decline (Wong et al., 1984). Furthermore, studies of Alzheimer’s disease have documented abnormalities of the serotonergic systems with evidence suggesting that these alterations are associated with non-disease aging (McEntree and Crook, 1991; Mohr et al., 2002). Comparatively, there are limited data on the effects of aging on serotonergic system compared to dopaminergic systems.

Significant declines in both dopaminergic and serotonergic systems suggest that these alterations may contribute to changes in behavior or reasoning over the adult lifespan. A study involving a gambling task showed that older adults have reduced risk-taking behavior and a tendency to make poorer decisions (Deakin et al., 2004). Based on previous studies, it can be hypothesized that aged rats will show preference for smaller rewards as opposed to larger, effort cost associated rewards.
METHOD

Animals

Behavioral and neurophysiological studies were conducted on 17 Fisher 344 male rats, 11 young and 6 old. The young rats ranged from 11-14 months and the old from 25-31 months. The rats were housed individually in guinea pig cages and maintained on 12 h light/dark cycles. Both behavioral and neurophysiological recordings took place during the dark phase of the cycle. Prior to any experimentation, the spatial and visual discrimination abilities of each rat were tested on the Morris swim task (for details, see Shen and Barnes 1996). This task ensured that physical or visual limitations did not affect the performance of each rat by assessing visual and motor functions of the animal. The corrected integrated path lengths (CIPL) were obtained in addition to the latency to find the platform. Aged animals swim more slowly than young animals. In order to test animal’s spatial memory independent of the animal’s swim speed, CIPL is an ideal measure. Moreover, the rats were handled a week prior to the experiments.

Behavioral training and procedures

During behavioral tasks, animals were food deprived to 85% of their *ad libitum* weight. The rats were weighed daily prior to their tasks. The behavioral training took place in specially designed operant conditioning chambers (see Figure 2). Chambers were equipped with two levers, which upon pressing expelled the reward they were associated with. For the initial training, rewards were 10% sucrose and 15% maltodextrose. The percentage dilution of maltodextrose was increased due to rats' slight preference of sucrose over maltodextrose. Rats were trained to press levers for 12 days, on a gradually increasing random ratio schedule of reinforcement (RR1 to RR5) before participating in any tasks. Finally, after the rats were trained to press levers, a young rat was chosen for surgery.
involving the implantation of electrodes. Rats then participated in Reward-Magnitude Learning followed with an Effort-Discounting task.

![Figure 2](image)

**Figure 2.** Operant chamber designed for tasks involving light cue, levers and reward.

**Reward-magnitude learning**

Each session consisted of 36 different choice trials, separated into three blocks (see Figure 3). Each block consisted of twelve trials, two forced-choice trials and ten free-choice trials. Each trial lasted 40 sec. The two forced-choice trials involved the extension of one lever and the free choice trials involved the extension of both levers. One lever was designated as the low-reward (LR) lever and the other was designated as the high-reward (HR) lever. The trials began with the illumination of one or both cue lights placed above each lever and was followed by the extension of one or both levers 5 sec later. Failure to choose or complete the HR lever presses in 25 sec resulted in the retraction of the levers along with the turning off of the cue lights. In this case the trial was terminated. Selection of LR lever was immediately followed by turning off of the cue lights and retraction of the LR and HR lever. In addition, selection of low-reward lever was also immediately followed by delivery of 0.5 sec of Vanilla Ensure. Selection of HR lever was immediately followed by turning off of the cue lights and retraction of the HR and LR lever. Selection of high-reward lever
was also immediately followed by delivery of 2 sec of Vanilla Ensure. The rate of high-reward and low-reward lever presses were recorded along with breaks in the IR-beam located at the site of reward administration.
Figure 3. Reward magnitude task design. Learning the association of levers to high and low reward values and format of a single forced or free-choice trial of reward-magnitude learning task.

Effort-discounting

Each session consisted of 48 different choice trials, separated into four blocks (see Figure 4). The procedure is the same as in the reward magnitude training. The only difference is that, selection of the HR lever was immediately followed by the retraction of low-reward lever only, whereas the HR lever was retracted only after the completion of a fixed amount of lever presses (1, 2, 5, 10 and 20). The trials for behavioral rats were 40 sec long (and 100 sec long for the young rat undergoing electrophysiological recordings). The rate of high-reward and low-reward lever presses were recorded along with the reward delivery times and breaks of the infrared-beam located at the site of reward administration.
Figure 4. Effort discounting task design. Cost associated with responding on either lever and format of a single forced or free-choice trial of effort discounting task.
Surgical Implantation

The surgery involved the implantation of "hyperdrive" containing an array of 14 separately movable microdrives (see Figure 5). The tetrodes were built by twisting together four strands of insulated 13 μm nichrome wire (H. P. Reid). Two of the tetrodes had their wires shorted together to serve as a reference and a probe for recording EEG. A full turn of screw advanced the tetrode by 330 μm.

![Figure 5](image)

**Figure 5.** Hyperdrive recordings. **A.** Illustration of a recording tetrode in the brain. Tetrodes are made of 4 wire twisted together, and can record from numerous neurons at the same time (figure from Buzsaki, 2004). **B.** Diagram of a hyperdrive, which holds the recording tetrodes and allows lowering them independently.

All surgical procedures followed the National Institutes of Health and University of Arizona Institutional Animal Care and Use Committee guidelines. Rats were anesthetized by inhaling Isoflurane anesthesia during the surgery. The hyperdrive was implanted in a craniotomy positioned 2.9 mm posterior and 5.0 mm lateral to bregma. The implant was cemented in place with dental acrylic which was anchored by small screws. Post surgery, rats were orally administered 26mg of acetaminophen along with receiving 2.7mg/ml acetaminophen in their drinking for 1-3 d after surgery. They also received oral ampicillin on a 10 d on/ 10 d off regimen for the duration of the experiment.
**Neurophysiology**

The recording of extracellular spike signals has been described in detail previously (e.g., Maurer et al., 2006). After the surgery, the tetrodes were lowered in 600 um increments for the first week and between 100 and 300 um on the second week, such that the last days of tetrode lowering involved small travel distances to the basolateral complex of the amygdala. During this time, the animal did not continue its behavioral experimental task. The reference electrodes were positioned in or near the corpus callosum. The four channels of every tetrode were attached to a 50-channel unity-gain headstage (Neuralynx). Headstage is connected to digitally programmable amplifiers via a multiwire cable (Neuralynx). The extracellular spike signals of multiple single cells were amplified by a factor of 1000-5000, bandpass filtered between 600 Hz and 6 Hz, and transmitted to Cheetah Acquisition system. These signals were digitized at 32 kHz and the events reaching a predetermined threshold were recorded for duration of 1 ms.

**Histology**

Following testing, the final tetrode position was marked by passage of a 5 μA current through each microwire for ~10 s to create a small iron deposit. Immediately prior to perfusion, rats were given an overdose of pentobarbital and prepared for perfusion. The rats were then perfused intracardially with 4% formaldehyde. Brains were removed from the skulls and stored in 4% formaldehyde for several days. Two days prior to sectioning, brains were transferred to a 30% sucrose solution. The brains were then sectioned on a freezing microtome, and coronal sections (40 μm) were collected through the amygdala. Sections were mounted on glass slides, stained with Nissl and Prussian blue (ferric hexacyanoferrate diluted in hydrochloric acid) (see Figure 6). The Prussian blue staining was performed by incubating sections for 20 minute in the 5% potassium ferrocyanide-HCl solution.
Slides were coverslipped with Permount. Lesion and electrode placements were verified under a light microscope.

**Figure 6.** Recoding tetrode placements in the amygdala. A Nissl & Potassium ferrocyanide staining confirms the placement of a recording tetrode in amygdala. The slides presented here show tetrodes (dashed circles) ending in the basolateral complex of the amygdala. B Figure from Paxinos, 1998 illustrating the anatomy of the brain at the level of the amygdala.
RESULTS

Morris swim-task

Spatial task

Corrected integrated path length (CIPL) was measured for the 24 spatial trials. Acquisition of swim task is presented in Figure 7. The performance of old and young rats was similar on the first two trials of the spatial task. However, the young rats showed significant improvement in performance from the forth trial onwards. Although the old rats showed a slight improvement, their overall performance suggests comparatively impaired learning of the task (repeated measure anova, df1, p=0.01, F=22.0). There was an overall effect of training (repeated measure anova, df23, p<0.0001, F=3.95).

Figure 7. Acquisition of the spatial task by young and old rats. The average of the CIPL is plotted for 24 trials. The bars indicate standard error for CIPLs. Significant age difference found over 24 trials.
Behavioral study

Reward-Magnitude Learning

Initially, 17 rats were trained on the reward-magnitude learning task (see Figure 8). Two of these animals were excluded from data analysis; one young and one old. The old rat was excluded due lack of its ability to press levers and the young rat was excluded because it showed no distinguishable learning between the high and the low-reward levers. The remaining rats were trained for four days before displaying stable pattern of choice behavior. Each day consisted of a session in which there were three blocks, each of twelve trials. As indicated in Figure 8, there was no significance between age groups (repeated measure anova, df1, p>0.05, F=0.024) although there was a main effect of training (repeated measure anova, df11, p<0.001, F=6.336). Thus, in this task old and young rats learned to discriminate between low and high rewards at a similar rate.

![Figure 8. Reward-magnitude learning. Data are plotted as a function of the percentage choice of large reward obtained in a block per day (x-axis). There were three blocks in a session per day. The lines extending from the data points indicate standard error for the individual blocks. The diamond-shaped data points refer to the old rats and the squares refer to the data points for the young rats. Young and old rats learned to discriminate between small vs. large reward at a similar rate.](image)
Effort Discounting

A total of 10 rats were trained on an effort discounting task, 3 old and 6 young rats. The training took place for six days with one session per day. Each session included four blocks consisting of twelve trials. The fixed ratio of lever presses for obtaining a large reward were 1, 5, 10 and 20 for four blocks respectively. Because aged rats are known to take longer to adapt their behavior to changes in stimulus-reward contingencies, we first assessed possible age differences early in training (Figure 9a). During the first two days of the task, the acquisition of effort is similar between old and young rats as shown by the overlapping error bars (ttest; p = 0.097 on block 3). In contrast, the average presses by old and young rats for a large reward in the last two days of task along was different between age groups, but did not reach statistical significance (see Figure 9b; repeated measure anova, df1, p >0.05, F = 0.598). Indeed, in the later days of training, the old rats were generally affected by effort and decreased the overall proportion of lever presses for large rewards in all blocks. Moreover, young and aged rats choose to press less often for the larger reward when 10 or 20 presses were needed (repeated measure anova, df3, p <0.05, F=6.54). The change in behavior across days was not assessed here due to the greater variability early in training. Studies generally only report group differences based on later days in training, once behavioral stability has been reached (Ghods-Sharifi et al., 2009).
Figure 9a. Average of the first two days of effort discounting. Data are plotted as a function of choice of large-reward lever presses per block (x-axis). There were four blocks in a session with a fixed ratio of 1, 5, 10 and 20 required lever presses per block inorder to obtain a large reward. The vertical lines extending from the data points indicate standard error for the individual blocks. The blue line refers to old rats and the red refers to the young rats. Early in the training, the acquisition of effort discounting appears to be similar between old and young rats.
Figure 9b. Average of the last two days of effort discounting. Data are plotted as a function of choice of large-reward lever presses per block (x-axis). There were four blocks in a session with a fixed ratio of 1, 5, 10 and 20 required lever presses per block inorder to obtain a large reward. The vertical lines extending from the data points indicate standard error for the individual blocks. The blue line refers to old rats and the red refers to the young rats. Later in training, compared to the young rats, the old rats decreased the proportion of lever presses for large reward throughout all blocks.

Electrophysiological study

Data analysis and presentation format

In the following section, we investigated the types of neural responses presented by amygdala neurons to the predicting light cue, the lever presses and the reward delivery. These data are presented as Peri-Events Time Histograms (PETHs). For the reward PETHs, the histograms are centered at the Ensure delivery, which occurs about 2 seconds before the rat reaches the food port. For thelever press histograms, these are centered at the release of the lever. This is to minimize the variability in lever presses length, such as a rat maintaining a lever pressed for an extended period of time. Finally, the light cue PETHs are centered at the onset of the light cue. In this experiment, left lever presses always resulted in a small reward delivery whereas pressing on the right lever always led to a large reward delivery. The PETHs figures are always ordered as follows: Top left panel – free
choice trials for the left lever, light or small reward, whereas right panel – free choice trials for the right lever, light or large reward. Bottom left panel – forced choice trials for left lever, light or small reward and bottom right panel – forced choice trials for right lever, light or large reward.

**Reward Magnitude**

Prior to our effort discounting task, rats were trained to discriminate between a lever associated with a large reward and one associated with a small reward, over the course of 5 days. This reward magnitude training was recorded electrophysiological and behaviorally for the full duration of the experimental period. We found, neurons responsive to rewards, light cues and lever presses (see Figures 10a to 10c, and table 1).

![Graphs showing neuronal activity](image)

**Figure 10a.** Neurons displaying decreased activity to rewards and increased activity to lever presses and small rewards (Day 3)
Figure 10b. Neuron displaying increased activity to light cue (Day 3)

Figure 10c. Neuron displaying decreased activity to light cue (Day 4)
**Table 1:** Cell counts and proportions of cells that altered their firing rates to the various cues during the reward magnitude task

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<th>Number of cells</th>
<th>Proportion of recorded cells</th>
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<td>Light cue -increase</td>
<td>14</td>
<td>6%</td>
</tr>
<tr>
<td>decrease</td>
<td>24</td>
<td>10%</td>
</tr>
<tr>
<td>Lever Press - increase</td>
<td>23</td>
<td>10%</td>
</tr>
<tr>
<td>Reward - increase</td>
<td>19</td>
<td>8%</td>
</tr>
<tr>
<td>decrease</td>
<td>5</td>
<td>2%</td>
</tr>
<tr>
<td>Reward anticipation - increase</td>
<td>19</td>
<td>8%</td>
</tr>
<tr>
<td>No change in activity</td>
<td>154</td>
<td>67%</td>
</tr>
<tr>
<td>Total recorded cells</td>
<td>231</td>
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</table>

We also found neurons that showed anticipatory activity to rewards; being activated at the release of the lever and before reward consumption (see Figure 11). In this figure, the reward anticipation was present on the free trials only (when the rat could choose between either lever), whereas during the forced choice trials, the activity of amygdala neurons coincided with reward consumption.

![Figure 11. Neurons displaying increased activity to reward anticipation (Day 3)](image-url)
A subset of amygdala neurons was sensitive not only to rewards, but was differentially activated by small and large rewards (see Figure 12). In this figure, the forced trials were matched such that the rat was forced the same amount of times to press the left and right levers (bottom plots). This cell showed an increased activity about 2 seconds after lever pressing. In these PETHs, 0 stands for the release of the lever.

![Figure 12](image_url)

**Figure 12.** Neurons displaying increased activity to smaller rewards (Day 1)

Similarly, a subset of amygdala neurons showed differential anticipatory activity for the large over the small reward (see Figure 13). In this figure, there is no activity present on the top left plot (Free-Left Lever) because the rat never pressed on the left lever predicting small reward on that day (Day 4). Within 2 days, our rat had learned which lever predicted the large reward and would no longer press the lever predicting the small reward when given the option.
Effort Discounting

We then assessed the activity of amygdala neurons during an effort discounting task, in which the rat had to work increasingly harder in order to obtain his large reward. This task was separated into 5 blocks of 12 trials (see methods) and each block corresponded to a fixed amount of lever presses per block, varying from 1 to 20 lever press between blocks (1-2-5-10-20 presses). For example, the large reward in the fourth block required the rat to press 10 times to obtain it.

As in the reward magnitude training, we found neurons sensitive to the light cue, lever presses and rewards. To increasingly more difficult trials, we found neurons that decrease their activity to the large reward as the trials get harder (see Figure 14). In this figure, the activity following reward delivery is much greater in the earlier panels (or blocks) than in the last one (lower left panel).

Figure 13. Neurons displaying increased activity to larger reward anticipation Day 4
Figure 14. Activity of amygdala neurons to reward delivery at 0ms, during an effort discounting task. As trials get harder, this neuron displays a decrease in its activity to larger reward. In this figure, the activity following reward delivery is much greater in the earlier panels (or blocks) than in the lower left panel (or last block).

We also found neurons that displayed an increased activity to the predicting light cue as the task became more difficult (see Figure 15). In these figures, note the sharp light-correlated response that
is only present in the three later trials (Blocks 3 to 5). This neuron also showed a decrease in activity to lever presses, as nicely illustrated in the grouped figure on the lower right panel. This change in activity is also modulated by the amount of effort it predicts, with a greater inhibition in the easier trials and no noticeable change in later harder trials.

**Figure 15.** Example of an amygdala neuron differentially activated by the light cue on more difficult trials
A subset of amygdala neurons were not modulated by effort. Figure 16 shows an amygdala neuron during our effort-discounting task showing no difference in the amplitude of the reward-related activity between block types. It is interesting to note that this is the same neuron as presented in Figure 15, which showed differential activity in light cue activation for harder trials. This suggests differential plasticity mechanisms within neurons to various events. Please note that the peak in activation in the third block is related to the light cue, as presented in Figure 15.
Figure 16. Example amygdala neuron presenting no change in reward-related activity to easier and harder trials.

Amygdala neurons also could show either gradually increased or decreased firing rates following multiple lever presses (see Figure 17). In the left panels, the activity of two neurons to the light
predicting cue is shown. The top one, shows a decrease in activity to the light cue, followed by a ramping of activity in anticipation of the levers coming out and throughout lever presses (Right Panel) culminating with increased activity after the last press, before rewards receipt. The second neuron generally shows the opposite activity as the first one, with the exception that it does not show anticipatory activity to the levers extending in the recording chamber, yet it also shows the excitatory activity in anticipation of the reward, after performing the last press. This example also illustrates the distinct and differential activation pattern of certain amygdala neurons. Indeed, some amygdala neurons were activated by more than one cue and the pattern of activation was not consistent across neurons. As illustrated in Figure 17, a neuron that displayed activity increase to reward expectation could either show increased or decreased activity to the light predicting cue. Similarly, we found that neurons activated by rewards might or might not be, responsive to light cues.

**Figure 17.** Examples of two amygdala neurons showing gradual increase or decrease in activity during multiple lever presses. **A.** This neuron is activated by the light cue at 0ms. **B.** This neuron is activated by reward at 0ms.
DISCUSSION

Behavioral study
Old and young rats learned reward-magnitude discrimination at a similar rate. In addition, the acquisition of the effort discounting task was similar between the old and younger rats earlier in the training. We found a tendency for both old and young rats were less likely to work for large rewards when they had to press 20 times, as compared to a lower amount. Additionally, there was a general decrease in the overall preference towards the HR lever in aged rats, regardless of effort.

Earlier studies have reported a slower learning rate for aged rats, but these results are controversial (Marschner et al., 2005). Our original hypothesis was that reward-magnitude learning would be slower in aged rats, but instead we did not find significant differences in the acquisition phase of our reward magnitude or effort discounting tasks. One possible explanation for this could be that the batch of aged rats obtained was exceptional as shown by their performance during the Morris swim task when compared to the young rats. However, we found significant differences in CIPL scores between young and aged rats, suggesting that our aged rats had the working memory impairments known for this aged group and were therefore not abnormally well preserved. Thus, reward magnitude learning results suggest that the acquisition of changes in reward value is not impaired in aged rats across the reward levels examined. Because the number of aged rats tested was very small (n = 6), it is not possible to draw strong conclusions from the data. Moreover, prior to being trained on effort discounting tasks, the rats were trained on numerous other tasks which may have had an effect on the learning rate.

The results of effort discounting were unexpected and contrary to the hypothesis. The results showed no difference in old and young rats’ performance during the first two days of training.
Similar to the reward magnitude task, the acquisition phase was not affected in aged rats further strengthening our finding that aged rats are not impaired in lever-press to reward learning nor in adapting to changes in association contingencies. The differences in the performance of old and young rats later in the task may, although not significant, suggest that aged rats are more affected by effort. This is consistent with the generalized decrease in responding to large rewards regardless of task difficulty in aged rats. Effort discounting is a novel task and therefore there are minimal prior studies with its association to aging. A study by Levine and colleagues shows that older adults had lower performance and needed more trials to learn the stimulus response associations, as compared to younger adults (Levine et al. 1997). In contrast to this study, we did not find acquisition deficits, only performance deficits in aged rats.

**Electrophysiological study**

Consistent with the literature, we found amygdala neurons sensitive to the light predicting cue, to lever presses, to rewards as well as neurons anticipating rewards (Sugase-Miyamoto and Richmond, 2005; Wilson and Rolls, 2005; Belova et al., 2007). Likewise, we found neural responses modulated by reward-size (Pratt and Mizumori, 1998).

Our experimental results uncovered two additional findings. First, we confirmed \textit{in vivo} the shunting effect that putative interneurons have on principal cells of the amygdala. Indeed, as presented in Figure 10, the two example neurons were actually recorded from the same tetrode. These examples suggest that the relief of inhibition from the neuron presented on the bottom panel may have allowed the principal cell of the top panel to fire in response to the light cue. Principal cells of the basolateral complex of the amygdala are well known for being under the control fast-firing GABAergic neurons (Ehrlich et al., 2009). These cells have been shown to completely cover the soma of BLA principal cells, thereby being in prime position to exert maximal influence (Pare and
Smith, 1993). This effect of putative GABAergic neurons onto putative principal cells of the amygdala has not yet been reported during goal-directed behaviors. Interestingly, dopamine release and dopamine receptors are known to decrease with aging (Kaasinen et al., 2000; Nomura et al., 2004). Dopamine is known to regulate the activity of GABAergic neurons in the amygdala (Rosenkranz and Grace, 1999). It is possible some of the age-related changes in emotional processing occur through this indirect effect of dopamine on GABAergic neurons of the amygdala. Because, dopamine in the nucleus accumbens is known to be required in effort-based decision making (Bardgett et al., 2009), it may also play a similar role in the amygdala.

Second, we found anticipatory differential modulation of BLA neural activity during multiple lever presses, in our effort discounting task. Indeed, gradual increase or decrease in activity during multiple lever presses was observed. These data partially reproduce the recording data of Chang and colleagues, 1996 in the nucleus accumbens. However our amplitude differences were much greater than what was reported in their study (see Figure 17). Additionally, our lever press anticipation responses were associated with a sharp increase in excitation at the last lever press. This discrete activation was not observed in their recordings within the nucleus accumbens. This may be due to the different area that was recorded or to the amount of lever presses required to receive reward. Indeed, in the present study the rat pressed up to 20 times in order to receive reward, whereas Chang et al., (1996) only examined conditioning in which 10 presses were required to receive cocaine.

The reward evoked activity of amygdala neurons is known to decrease in amplitude as rewards become better predicted (Belova et al., 2007). It was not possible to assess this effect in our experiment. This investigation would require comparing expected versus unexpected reward delivery and we did not perform such a control. Adding unexpected rewards could have impacted our
results, due to the associated change in contingency. In the interest of reproducibility, the only change in contingency integrated to this study was the reward size during the first 5 days of testing and then effort for the remainder of the task.

Functional implications
The role of amygdala is becoming increasingly apparent in different forms of cost/benefit decision making. With the use of novel tasks like effort discounting, the present study provides insight into differences in performance between aged and young rats. In conjunction with other studies on aging, effort-discounting along with different tasks like delay-discounting and risk-discounting provides us with the knowledge of behavioral changes that are associated with normal aging, during goal-directed behaviors and decision making. Moreover, for future experiments, understanding the differences in the neuronal activity between aged and young rats during different tasks including effort discounting may provide us with additional information on age-related brain changes associated with normal aging. It is also important to obtain a more comprehensive understanding on how modification to dopaminergic and serotonergic systems lead to alterations in decision making that is observed in our aging community. Such knowledge will aid us in developing pharmacological and therapeutic interventions for preventing the decline of cognitive health associated with aging.
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