The role of the extracerebellar mechanisms in Pavlovian memory

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Abstract: - During the last decade an increasing number of observations have lead to a major change in the traditional view of the nature of Pavlovian conditioning. The following review paper will survey some of these critical findings which are responsible for this paradigm shift.

1 Introduction
The nictitating membrane (NM) reflex in the rabbit is currently regarded as an ideal animal model to study the neural mechanisms of associative memory. This claim rests on three main assumptions, which are: (a) all the sensory inputs and motor outputs are under total experimental control, (b) the learning is completely isomorphic with the eye blink response, and (c) the memory storage of this learning is mediated by the cerebellar cortex. The present paper will overview recent evidence in the literature which raises very serious questions about the validity of each of these assumptions.

(a) Stimulus control
Pavlovian conditioning of the nictitating membrane response (NM) in the rabbit (Gormenzano 1966) is probably the most powerful animal model of the neural mechanisms of associative learning. The movement of the NM occurs only in response to external stimulation. It does not occur spontaneously, nor is it mediated by any instrumental behaviour of the animal. Therefore, the conditioning can be standardized in all animals, which receive almost identical treatment. This ensures that there are minimal individual differences in both acquisition and performance of associative learning. It is claimed that these properties of Pavlovian conditioning allow the experimenter to have complete input control during learning.

To date few if any studies of Pavlovian conditioning have have investigated the sensory input pathways involved in the learning. Certainly no studies have exploited the full potential of Pavlovian conditioning by identifying the precise psychophysical parameters of the stimulus inputs that are used in NM conditioning. The almost universal use of either simple light flashes in a dark (or dimly illuminated) chamber or pulses of sound in a sound-shielded chamber (or one with a background of low-intensity white noise) as the CS has serious limitations. The use of such non-specific stimuli inevitably activates multiple afferent neural pathways involving multiple neural networks which converge on a final common efferent motor pathway. The failure to isolate specific CS input pathways will underestimate the sensory involvement in associative learning mechanisms and at the same time overemphasizes the role of the motor pathways (Hesslow and Yeo 2001; Morcuende et al. 2002; Thompson and Krupa 1994; Thompson...
We believe that a more detailed exploration of the CS input pathways is required to provide a more comprehensive and balanced view of the neural mechanisms involved in associative learning.

As knowledge of the neural mechanisms involved in sensory perception is most advanced in the visual system we used conditioning of the NM reflex to a specific visual pattern, e.g. a vertically oriented grating. There are two principal advantages to restricting conditioning to pattern information. First, pattern perception in the rabbit involves well-characterized discrete neural pathways that are known in great detail (Chow et al. 1971; Giolli and Guthrie 1969, 1971; Hughes 1971; Mathers and Mascetti 1975; Mathers et al. 1977; Steele-Russell et al. 1984). It involves exclusively the retino-geniculo-striate pathways to area 17 in both hemispheres (Chow et al. 1971, 1977; Hughes and Vaney 1982; Stewart et al. 1971, 1973). Visual control over the motor system is mediated by corticofugal projections to the pontine nuclei which serve as a relay to the cerebellum (Glickstein and May 1982). Thus using a specific visual pattern CS, access to the pontine-cerebellar pathways for cerebellar oculomotor control of the NM is only possible after cortical processing of the visual pattern information. In addition visual conditioning involving brightness changes (increase/decrease in overall energy level) would activate different neural pathways. They would utilize the retino-geniculo-collicular projections (Masland et al, 1971; Rose 1931; Rose and Woolsey 1943; Steele-Russell et al, 1987). Lesions of the visual cortex abolished pattern vision without any impairment of intensity or brightness discriminations (Steele-Russell et al, 1984).

The common practice of using self-indexing conditioned stimuli (CSs) in research on Pavlovian conditioning is a major source of experimental bias. The typical stimulus used is either a light square wave flash or sound pulse in a light/sound-shielded chamber. Under these conditions the onset characteristics of the CS signal totally predominate over the durational characteristic containing the pattern information. Thus a visual pattern presented as a CS in a dark chamber is confounded with a brightness change from darkness to light.

Evidence for this is provided by a recent report (Steele-Russell et al, 2006). The animals were conditioned with a brightness CS using a self-indexing signal paradigm. When tested for specificity of the conditioning, they showed complete transfer of learning to either a visual pattern or even an auditory CS. These findings indicated that the traditional conditioning paradigm is biased towards polysensory learning, i.e. any stimulus change. They further showed that specific sensory conditioning is critically dependent on selective attention mechanisms. When the onset characteristics of the CS signal were de-emphasized psychophysically by the use of equal energy background illumination both in the intertrial interval (ITI) and during conditioning; the animals were not able to feature extract either the onset or the durational component of the CS signal from the ITI background despite prolonged training. It was only by starting with conditioning that was initially anchored to the CS onset characteristics that a perceptual fade-in procedure would bias attention to feature extract the durational characteristics of the CS. Thus conditioning occurred only when the rabbit’s attention was directed to detection of the gratings display without any associated changes in visual albedo (Steele-Russell et al, 2006).

Finally when the animals were tested with the vertical-horizontal striation differentiation both stimuli were indistinguishable having the same onset characteristic. They both consisted of square-wave brief energy increase. Under these conditions (where the correct CS was identical to the incorrect one) animals could not predict the
US reliably, hence the loss of conditioning to the CS (Rescorla 1968). Thus using the conventional Pavlovian training paradigm the durational characteristics of the visual CS, i.e. the pattern information of the visual signal, is not incorporated in the sensory input as part of the learning. Conditioning using onset or offset of auditory stimuli as a CS (Hupka et al. 1969; Desmond and Moore 1991; Kehoe and Napier 1991) provides additional support to the conclusion that the onset characteristic of the CS is the critical feature extracted in conditioning.

Considered overall, the most parsimonious explanation of these findings is that conditioning occurs solely to the signal onset characteristic of the CS, independent of modality (Barlow et al. 1964; Levick 1967). Contrary to established claims and belief, this evidence clearly demonstrates that there is a total lack of control over the sensory information that is used by the animal during Pavlovian conditioning. Furthermore the demonstration that specific conditioning required selective attentional information processing, indicates the involvement forebrain structures rather than the cerebellum.

(b) Response isomorphism with the learning change
For many years research on the neural mechanisms of Pavlovian learning has focused exclusively on the cerebellum as the site of both Pavlovian memory and learning. A major rationale for this is the assumption that there is a complete isomorphism between learning and the changes in the eye-blink response or NM. The critical assumption here is that during Pavlovian conditioning all that is learned is an eye-blink, without any affective or cognitive component (Thompson et al, 1994; Hesslow & Yeo, 2001; Thompson, 2005). Lesions of lobus simplex in the vermis of the cerebellum have been long reported to interfere with eyeblink conditioning (Thompson et al, 1994). Also Purkinje cells are activated during smooth pursuit eye movements, indicating participating of this folia in oculomotor control (Suzuki et al, 1981). Accordingly the lobus simplex has been regarded as a region involved in attentional oculomotor movement control, but also the storage site of Pavlovian conditioning.

Current research seriously questions whether eye-blink conditioning of the nictitating membrane response (NM) in rabbit consists solely of motor learning or involves other extracerebellar control systems. The experimental procedures used a transfer design, to permit testing for retention of learning across different situations. The animals were first NM conditioned to a tone signal (CS) in the Pavlovian stock, where they typically show a discrete eye-blink response. Subsequently they were tested in a free environment on a continuous attention task to assess the range of their reactions to random presentations of the CS.

The results showed the presentation of the CS produced totally different behaviour in the free environment. The presentation of the CS produced: (a) a prolonged disruption of the constant attention task due to the presence of complex emotional reaction consisting of both retreating from and approaching to the sound source, (b) an absence of eye-blink responses. Control animals (that were not conditioned to the CS) were briefly distracted during the constant performance task (in the free environment) and also did not demonstrate any conditioned eye blink responses.

These findings provide strong evidence that Pavlovian learning involves the acquisition of a change in the meaning and emotional significance to the CS. This is expressed in differing situations by markedly different response patterns which unmasked a significant emotional component of the conditioning. They
demonstrate that NM conditioning consists of more than a change in a discrete cerebellar oculomotor reflex. The clear emotional component to the conditioning (Steele-Russell et al, 2008; Castiglioni et al, 2009) indicates the involvement of extra-cerebellar control mechanisms, possibly the amygdala (Lee T, Kim JJ, 2004; Boele et al, 2010).

(c) Cerebellar site of learning plasticity
Current research on Pavlovian conditioning suggests there are at least two distinguishable phases in the changes in neural processing that occur during learning (Holland, 1990; Holland & Gallagher, 1999, Weinberger, 2004). The first involves changes in the sensory pathways associated with the CS. This enables the formerly neutral stimulus to become capable of triggering a motor system to produce the conditional response (CR) (Weinberger 2004). The second results in modulation of the motor system that produces the unconditional response (UR) to the US.

Many observations point to the fact that the response that occurs to the CS is not a copy of the UR. Even in the training situation, the UR in eye-blink conditioning, has a temporal architecture typical of a short latency brisk phasic reflex response to the US; whereas the CR is a gradually incrementing anticipatory response to the CS, which is not tightly coupled to the CS onset (Steele-Russell et al, 2008). This difference in response morphology has been interpreted as reflecting attentional learning due to changes in the meaning of the CS. Such affective/sensory changes form a unique and essential role in conditioning independent of the plasticity changes in the motor system (Steele-Russell et al. 2006). It would also suggest that attentional conditioning could occur independently of the motor expression of conditioning.

In the previous section evidence was presented to make a powerful argument that the NM conditioning was independent of the eye blink motor response observed when the rabbit is confined to the restraining stock. In a free environment the animals display both a complex motor pattern of avoidance/approach behaviour indicative of emotional conditioning as opposed to a stereotype motor response. The present experiment examined the effect of traditional NM conditioning in animals that were unable to make an eye-blink response to the visual CS.

In the rabbit, section of the optic chiasma disconnects the direct retinal projections to the cerebellar oculomotor control system. At least 90% of the optic nerve fibres decussate in the optic chiasma in the rabbit (Giolli and Guthrie 1969; Giolli and Creel 1974). Similar to cat and monkey there is a clear retinal segregation between contralateral and ipsilateral projecting fibres. Contralaterally projecting ganglion cells are found throughout the rabbit retina; whereas ipsilateral projections arise solely from the temporal margin of the retina (Provis and Watson 1981).

Accordingly chiasma-section in the rabbit was used to disconnect the visual information processing from the cerebellum during NM conditioning. With only the ipsilateral ganglion cell projections remaining, the major retinal output would be mainly to V1 and extrastriate cortex (Hughes 1971; Hughes and Wilson 1969; Towns et al. 1977). Section of the optic chiasma of the rabbit will result of 90% of the retina, leaving only the small area centralis in the extreme temporal part of the retina (anakatabatic area). This area is the sole source of ipsilateral retinal pathways which via the thalamus project to the striate cortex on the V1-V2 boundary. They are binocular and subserve detailed pattern vision (Hughes et al, 1982). In contrast the contralateral fibres originate from the visual streak of the
rabbit’s retina and are responsible for movement vision. These projections provide the sole direct connections to the cerebellum, via the nucleus of the optic tract (NOT). Thus section of the optic chiasma will result in a complete disconnection of the retina from the cerebellum without any cerebellar damage. All other sensory cerebellar connections remain intact such as audition, tactile etc. This would result in a disconnection of the cerebellar occulomotor system such that conditioning of the eyeblink response to a visual CS would not be possible. Without the crossed fibre projection the rabbit would be unable to make anticipatory NM responses to a visual CS.

The purpose of the present experiment was to determine the extent to which visual conditioning was dependent on or independent of the motor expression of the eye blink response during training. Thus by comparing both normal and chiasma animals it is possible to determine the dependence/independence of conditioning on the motor expression of the eye blink response during training (Steele-Russell et al, 2008). The experiment used a complex design. All animals were first tested for any impairment in their visual capacity. This was essential to determine the extent to which the the chiasma transection produced any visual impairment. Chiasma sectioned animals could have a deficit in visual Pavlovian conditioning due to either: (a) due to the inability to access the cerebellar information processing mechanisms essential for such learning, or (b) due to a sensory deficit impairing their ability to see the visual CS.

Accordingly both normal and chiasma animals were given a battery of visual assessment tests to determine their visual capacity, using both brightness and pattern discrimination tests. Despite the loss of 90% of retinal ganglion cell fibres, no difference in visual capacities of the chiasma sectioned animals was seen. Their performance was the same as the normal control animals on both tests. More importantly sensory specificity tests clearly established that both the normal and chiasma-sectioned animals were using the same energy level differences for the brightness task and the same orientation disparities for the pattern task. There was no evidence that either normal or chiasma animals were solving these visual tests by the use of non-visual cues.

In the next phase all animals were trained on NM Pavlovian conditioning, first to a visual CS and then to an auditory CS. Two measures of conditioning were used. Firstly, motor measures from on-line NM recording was used to determine the development of CRs to the CS signals during training whilst confined in the stock. Secondly, sensory/affective measures from off-line testing were used to determine the change in emotional significance of the CS when presented in a non-restrained situation (Steele-Russell et al, 2008).

**Motor performance measures of Pavlovian conditioning**

All animals in both normal and chiasma-sectioned groups were tested on two Pavlovian conditioning tasks. The training procedure consisted of three consecutive phases. In phase 1 they were trained for 1,000 trials to a visual CS. In phase 2 to an auditory CS (500 trials) and in phase 3 retrained on the visual CS for a further 1,000 trials. Visual conditioning (phase 1) in the normal group was evident in the first training session and had reached a 90% detection rate by the end of the third session. Thereafter the hit rate tracked between 90 and 100%. No signs of any conditioning are seen in any animal in the chiasma-sectioned group, where no animal made a single conditioned response throughout the 1,000 training trials.

Different results were found for conditioning to an auditory CS. Both normal and chiasma-sectioned animals readily conditioned to the tone CS, with a slight acquisition advantage for the
normal animals. The superior normal performance on the auditory task probably reflects the usual serial learning facilitation of a second task compared with first task learning. In the chiasma animals the slower acquisition was typical of first task learning (Steele-Russell et al. 2008). Both groups, however, had detection rates at 90% or greater for the last three sessions of testing.

This failure of the chiasma-sectioned animals to show visual conditioning cannot be attributed to either a sensory (visual) or to a general associative learning impairment. These animals showed no deficits for either intensity or orientation difference perception in the visual capacity tests. Further there was no learning impairment for their conditioning to the auditory CS in phase 2 of the Pavlovian conditioning motor performance test.

Sensory recognition measures of Pavlovian conditioning

A continuous performance task (CPT) was used as an animal model of a continuous attention task used in human vigilance studies (Werka and Steele-Russell, 1982; Castiglioni et al, 2009). It provides an interference measure of differential attention to both neutral and/or conditioned stimuli. Animals are first trained continuously press a panel without pausing for food reward. Following the attainment of sustained and stable performance, the distractive effect of random presentations of different stimuli on performance is measured. Neutral stimuli (low salience) produce little change in terms of their ability to distract the animal from performing the baseline task. Stimuli with high salience (e.g. due to prior noci-ceptive conditioning) typically produce long distraction periods where the animal is reacting to the presentation of the CS signal and not performing the continuous attention task.

Before conditioning both normal and chiasma-sectioned rabbits showed equal amount of attention to the neutral stimuli. Both groups had a slight but non-significant tendency for greater attention to the neutral auditory signals over the visual ones. The normal animals had an average distraction time of 6.2 s to the auditory signal compared to 3.0 s time for the visual signal. Comparable scores were shown by the chiasma-sectioned group with an average distraction time of 5.0 s for the auditory signal and 3.2 s for the visual one.

Following NM conditioning there was a dramatic increase in distraction times to both auditory and visual signals by normal and chiasma-sectioned animals. Normal animals had an average distraction time of 79.2 s to auditory signals and 57.6 s to visual ones. Chiasma-sectioned animals were comparable showing 91.2 s distraction to auditory pain conditioned stimuli and 60.2 s to visual ones. This finding confirms the earlier observations of Yeo (1974) and Werka and Steele-Russell (1982) in both rats and rabbits using this same distraction paradigm.

The previous results (using motor response measures) showed that chiasma-sectioned animals had a total absence of NM motor conditioning to a visual CS but had entirely normal conditioning when an auditory CS was used. The present results (using sensory recognition measures) clearly indicate that these animals, despite the lack of motor learning to the visual CS, had normal sensory/affective conditioning to both the auditory and visual signals as a predictors of trigeminal stimulation (US). These observations show that although section of the optic chiasma disconnected the retinal output from the oculomotor expression of conditioning; it did not impair the cognitive or affective aspects of the conditioning which determine the acquisition of meaning to a formerly neutral stimuli. These findings also indicate the dangers of over-interpreting results based on a single response measure. They also establish beyond any doubt that the cerebellum
itself is not the neural site for learning and memory plasticity changes.

Conclusions

These findings shown that:

(a) The recent research has shown that the traditional claims of complete experimental control of input and output variables Pavlovian conditioning is open to serious question.

(b) The conventional methods of Pavlovian conditioning without any psychophysical control over the sensory inputs is seriously biased towards polysensory learning.

(c) The practice of only a single response measure has been both a very misleading and erroneous viewpoint of the nature of what the animal learns. It has taken now account of cognitive or affective compents of the conditioning.

(d) These findings show that NM Pavlovian conditioning involves a distributed neural network. This involves extracerebellar structures of which the amygdala may well a major component.

References:
4. KL Chow, RH Masland, DL Stewart. Receptive field characteristics of
16. A Hughes. Topographical relationships between the anatomy and physiology of the


